

When Puberty is Precocious

Scientific and Clinical Aspects

Edited by

Ora H. Pescovitz, MD

Emily C. Walvoord, MD

WHEN PUBERTY IS PRECOCIOUS

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SCIENTIFIC AND CLINICAL
ASPECTS

Edited by

ORA H. PESCOVITZ, MD

*Departments of Pediatrics and Cellular and Integrative Physiology, Riley Hospital
for Children, Indiana University School of Medicine, Indianapolis, IN*

EMILY C. WALVOORD, MD

*Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital
for Children, Indiana University School of Medicine, Indianapolis, IN*

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PREFACE

When puberty is precocious is a question in and of itself. The possibility that puberty is starting earlier in children of the 21st century has become a contentious topic during the past few years. It has received much attention in the medical literature as well as the lay press. As research methods and interpretation of data vary between the studies that have attempted to resolve this issue, we are pleased to present the reader with three very different approaches to addressing the same question. We are thrilled to have experts in the field of basic science share the most up-to-date understanding of the mechanisms involved in the complicated interplay of factors that initiate puberty in different species. Also reviewed in the book are the many fascinating causes of precocity and new observations regarding the influence of the *in utero* environment and the affect of adoption from a developing country on pubertal timing. We have also included cutting edge discussions of endocrine disruptors, and the often overlooked psychosocial ramifications of precocious puberty.

We believe that this book will be useful to primary care providers as well as specialists who deal with issues regarding abnormalities of puberty and reproduction. The topics covered in these chapters will provide the reader with a solid understanding of the diverse and fascinating causes of precocious puberty and different treatment options.

We wish to thank all of our contributing authors for sharing their time and talent to create such an outstanding work. We dedicate this book to our families and to our mentors who have inspired us over the years.

Ora H. Pescovitz, MD
Emily C. Walvoord, MD

CONTENTS

Preface	<i>v</i>
Contributors	<i>ix</i>
PART I: NEUROENDOCRINE CONTROL OF PUBERTY	
1 Control Puberty in Rodents: <i>Special Focus on the Female</i> <i>Sabine Heger, MD and Sergio R. Ojeda, DVM.....</i>	<i>3</i>
2 Control of Puberty in Non-Human Primates <i>Tony M. Plant, PhD.....</i>	<i>35</i>
3 Control of Puberty in Humans <i>Dennis M. Styne, MD and Melvin M. Grumbach, MD.....</i>	<i>51</i>
4 Genetic Regulation of the Variation in Pubertal Timing <i>Craig A. Hodges, PhD and Mark R. Palmert, MD, PhD.....</i>	<i>83</i>
PART II: SECULAR TRENDS AND THE ONSET OF PUBERTY	
5 Puberty Is Starting Earlier in the 21st Century <i>Marcia E. Herman-Giddens, PA, DrPH.....</i>	<i>105</i>
6 BMI and the Onset of Puberty <i>Paul Kaplowitz, MD, PhD.....</i>	<i>137</i>
7 Puberty Timing Remains Unchanged <i>Peter A. Lee, MD, PhD and Christopher P. Houk, MD.....</i>	<i>151</i>
PART III: ISOLATED BREAST DEVELOPMENT	
8 Premature Thelarche <i>Linda A. DiMeglio, MD, MPH and Adda Grimberg, MD.....</i>	<i>169</i>
9 Gynecomastia: <i>Epidemiology, Pathophysiology,</i> <i>and Management</i> <i>Samar N. Rahhal, MD and John S. Fuqua, MD.....</i>	<i>189</i>
PART IV: ADRENAL ABNORMALITIES AND PUBERTY	
10 Premature Adrenarche: <i>Harbingers and Consequences</i> <i>Alicia Belgorosky, MD, PhD, María Sonia Baquedano, PhD,</i> <i>Gabriela Guercio, MD and Marco A. Rivarola, MD.....</i>	<i>217</i>

11	Pubertal Consequences of Being Born Small for Gestational Age <i>Dyanne A. Wilson, FRACP, Wayne S. Cutfield, FRACP and Paul L. Hofman, FRACP</i>	247
12	Congenital Adrenal Hyperplasia <i>Lenore S. Levine, MD and Sharon E. Oberfield, MD, With editorial assistance of Lauren Antler, BA</i>	267
13	Polycystic Ovary Syndrome in the Peripubertal Period <i>Selma Feldman Witchel, MD</i>	285
14	Psychological and Social Problems in Children With Premature Adrenarche and Precocious Puberty <i>Lorah D. Dorn, PhD</i>	309

PART V: GONADOTROPIN-DEPENDENT PRECOCIOUS PUBERTY

15	Etiology of Gonadotropin-Dependent Precocious Puberty <i>Erik A. Imel, MD and Kathleen E. Bethin, MD, PhD</i>	331
16	Treatment of Gonadotropin Dependent Precocious Puberty <i>Zeina M. Nabhan, MD and Emily C. Walvoord, MD</i>	345

PART VI: GONADOTROPIN-INDEPENDENT PRECOCIOUS PUBERTY

17	McCune–Albright Syndrome: <i>Pathophysiology, Diagnosis, and Management</i> <i>Tamara S. Hannon, MD and Erica A. Eugster, MD</i>	365
18	Familial Male-Limited Precocious Puberty <i>Nerissa C. Kreher, MD, MSc</i>	383
19	Gonadal and Adrenal Tumors <i>Inessa M. Gelfand, MD and Nadine G. Haddad, MD</i>	403

PART VII: ENVIRONMENTAL EFFECTS ON PUBERTY

20	The Role of Endocrine Disruptors in Pubertal Development <i>Todd D. Nebesio, MD and Ora H. Pescovitz, MD</i>	425
21	Precocious Puberty in Internationally Adopted Girls <i>Grete Teilmann, MD, Anne-Simone Parent, MD, Niels E. Skakkebaek, MD, PhD and Jean-Pierre Bourguignon, MD, PhD</i>	443
	Index	461

CONTRIBUTORS

- MARÍA SONIA BAQUEDANO, PhD, *Laboratorio de Investigación, Hospital de Pediatría Garrahan, Buenos Aires, Argentina*
- ALICIA BELGOROSKY, MD, PhD, *Laboratorio de Investigación, Hospital de Pediatría Garrahan, Buenos Aires, Argentina*
- KATHLEEN E. BETHIN, MD, PhD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- JEAN-PIERRE BOURGUIGNON, MD, PhD, *Department of Pediatrics, University of Liège, Liège, Belgium*
- WAYNE S. CUTFIELD, FRACP, *Liggin's Institute, University of Auckland, Auckland, New Zealand*
- LINDA A. DiMEGLIO, MD, MPH, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- LORAH D. DORN, PhD, *Division of Adolescent Medicine, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH*
- ERICA A. EUGSTER, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- JOHN S. FUQUA, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- INESSA M. GELFAND, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- ADDA GRIMBERG, MD, *Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA*
- MELVIN M. GRUMBACH, MD, *Department of Pediatrics, University of California at San Francisco, San Francisco, CA*
- GABRIELA GUERCIO, MD, *Laboratorio de Investigación, Hospital de Pediatría Garrahan, Buenos Aires, Argentina*
- NADINE G. HADDAD, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- TAMARA S. HANNON, MD, *Department of Pediatrics, Divisions of Endocrinology and Weight Management and Wellness, Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, PA*
- SABINE HEGER, MD, *Hospital for Children and Adolescents, University of Leipzig, Leipzig, Germany*

- MARCIA E. HERMAN-GIDDENS, PA, DRPH, *North Carolina Child Advocacy Institute, University of North Carolina School of Public Health, Pittsboro, NC*
- CRAIG A. HODGES, PhD, *Department of Pediatrics, Case Western University School of Medicine, Cleveland, OH*
- PAUL L. HOFMAN, FRACP, *Liggin's Institute, University of Auckland, Auckland, New Zealand*
- CHRISTOPHER P. HOUK, MD, *Department of Pediatrics, Penn State College of Medicine, The Milton S. Hershey Medical Center, Hershey, PA*
- ERIK A. IMEL, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- PAUL KAPLOWITZ, MD, PhD, *Department of Endocrinology, Children's National Medical Center, Washington, DC*
- NERISSA C. KREHER, MD, MSC, *Serono, Inc., Rockland, MA*
- PETER A. LEE, MD, PhD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- LENORE S. LEVINE, MD, *Pediatric Endocrinology, Morgan Stanley Children's Hospital of New York-Presbyterian, Columbia University Medical Center, New York, NY*
- ZEINA M. NABHAN, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- TODD D. NEBESIO, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- SHARON E. OBERFIELD, MD, *Pediatric Endocrinology, Morgan Stanley Children's Hospital of New York-Presbyterian, Columbia University Medical Center, New York, NY*
- SERGIO R. OJEDA, DVM, *Division of Neuroscience, Oregon Regional Primate Research Center, Oregon Health & Science University, Beaverton, OR*
- MARK R. PALMERT, MD, PhD, *Division of Pediatric Endocrinology and Metabolism, Rainbow Babies and Children's Hospital, University Hospitals of Cleveland, Case Western Reserve University, Cleveland, OH*
- ANNE-SIMONE PARENT, MD, *Department of Pediatrics, University of Liège, Liège, Belgium*
- ORA H. PESCOVITZ, MD, *Departments of Pediatrics and Cellular and Integrative Physiology, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*
- TONY M. PLANT, PhD, *Departments of Cell Biology, Physiology, and Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA*
- SAMAR N. RAHHAL, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*

MARCO A. RIVAROLA, MD, *Laboratorio de Investigación, Hospital de Pediatría Garrahan, Buenos Aires, Argentina*

NIELS E. SKAKKEBÆK, MD, PhD, *Department of Growth and Reproduction, University Hospital of Copenhagen, Copenhagen, Denmark*

DENNIS M. STYNE, MD, *Department of Pediatrics, University of California at Davis, Sacramento, CA*

GRETE TEILMANN, MD, *Department of Growth and Reproduction, University Hospital of Copenhagen, Copenhagen, Denmark*

EMILY C. WALVOORD, MD, *Section of Pediatric Endocrinology and Diabetology, Department of Pediatrics, Riley Hospital for Children, Indiana University School of Medicine, Indianapolis, IN*

DYANNE A. WILSON, FRACP, *Liggins Institute, University of Auckland, Auckland, New Zealand*

SELMA FELDMAN WITCHEL, MD, *Department of Pediatrics, Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA*

I

NEUROENDOCRINE CONTROL OF PUBERTY

1

Control Puberty in Rodents

Special Focus on the Female

Sabine Heger, MD

and Sergio R. Ojeda, DVM

CONTENTS

INTRODUCTION

BASIC ASPECTS OF FEMALE SEXUAL DEVELOPMENT
IN RODENTS

PREPUBERTAL CHANGES IN GONADOTROPIN SECRETION

DEVELOPMENT OF STEROID FEEDBACK MECHANISMS

CENTRAL CONTROL OF GnRH SECRETION

NEUROENDOCRINE CHANGES AT THE ONSET OF PUBERTY

THE MECHANISM OF THE ONSET OF PUBERTY

PEROMONES AND RODENT PUBERTY

METABOLIC SIGNALS AND THE ONSET OF PUBERTY

ENDOCRINE DISRUPTORS

POTENTIAL DEFECTS IN NEURONAL–GLIAL CONTROL
THAT MAY LEAD TO CENTRAL PRECOCIOUS PUBERTY

CONCLUSIONS

ACKNOWLEDGMENTS

REFERENCES

Summary

The neuroendocrine control of the onset of puberty in rodents has been extensively reviewed over the years (1–7). In this chapter, we will provide both a brief account of the basic mechanisms underlying the pubertal process in these animals and an update of some recent developments in the field. Because the rat is the animal most extensively studied, we will discuss mainly results obtained in this species, and when available, we will offer the reader a comparison of these results with those obtained in mice.

Key Words: Hypothalamus; GnRH; Transsynaptic communications; Glia-to-neuron; Signaling; Growth factors; Female puberty.

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INTRODUCTION

Although rodent puberty has fundamental differences with puberty in primates, the basic mechanisms underlying the process of sexual maturation are well conserved across species. Such mechanisms include, among many others, those governing the transsynaptic and glial control of gonadotropin-releasing hormone (GnRH), the cellular underpinnings of steroid positive and negative feedback, and the hormonal/neural control of gonadal development. The most obvious difference between rodents and primates (including humans) is the apparent absence in rodents [but see (7)] of the gonad-independent, juvenile reduction in gonadotropin secretion that characterizes primate prepubertal development (8). Notwithstanding this difference, rats—like primates—exhibit a diurnal increase in pulsatile luteinizing hormone (LH) secretion at the end of juvenile development (9), indicating that rodents can serve as useful animal models to identify those primary neuroendocrine events that, by causing diurnal changes in episodic LH release, are ultimately responsible for setting puberty in motion.

BASIC ASPECTS OF FEMALE SEXUAL DEVELOPMENT IN RODENTS

Whereas in humans the neuroendocrine control of gonadotropin secretion (as assessed by the detection of pulsatile LH secretion) appears to be fully functional at birth, this is not the case of rats and mice, which are born very immature—at a developmental stage comparable with ~150 days of human gestational life (10). The gestational period of the rats and mice lasts 21–23 days. The first ovulation in most laboratory rats occurs 35–45 days after birth, but in mice it is highly variable taking place within several days, or even weeks, after vaginal opening (11,12). Canalization of the vagina, which normally is imperforated before puberty, occurs because of estrogenic stimulation and is the only phenotypic change observed in association with the onset of puberty. In rats, vaginal opening usually occurs a day after the first preovulatory surge of gonadotropins (2,3,13,14), and therefore, it coincides with the day of first ovulation; in mice, however, this association is much less evident. Because the first ovulation in mice may occur several days (4–20) after vaginal opening, assessing only this event is not a good indicator that puberty has actually taken place.

Postnatal development of the female rat can be divided into four phases, described in detail earlier (15). They are a *neonatal* period comprised between the day of birth and postnatal day 7, an *infantile* period (days 8–21), a *juvenile* period that ends around day 30–32, and a *peripubertal* period that culminates with the first ovulation (usually around day 38–40). The end of the juvenile period can be defined as the time when morning–afternoon differences in pulsatile LH release become established (9). Mouse postnatal development can also be considered as composed of the same stages, but in this case the peripubertal period may extend for several additional days because of the dissociation between vaginal opening and first ovulation (11,12).

PREPUBERTAL CHANGES IN GONADOTROPIN SECRETION

In both rats and mice, serum follicle-stimulating hormone (FSH) levels increase between birth and the second week of postnatal life and decline thereafter as the animal progresses through juvenile development (12,16,17). Plasma LH levels, on the

contrary, are less elevated, exhibiting instead sporadic bursts of release (18,19), which also disappear by the end of the infantile stage. These patterns of gonadotropin release appear to be determined by a changing GnRH output, which is more responsive to stimulation during the second week of postnatal life (20) but has a more rapid pulse frequency during juvenile days (21). Thus, as the reproductive hypothalamus matures during the first 2 weeks of postnatal life, GnRH appears to be released as infrequent discharges, which sustain FSH secretion but cause only bursts of LH release. Such a peculiar pattern of hormone secretion might be due to a “disorganized” activity of the GnRH neuronal network, which only sporadically becomes synchronized in response to incoming inputs. Conceptually, the infantile phase of development in rodents can be considered as the time when activity of the GnRH–pituitary axis first increases in response to regulatory inputs. This activation appears to condense the fetal and postnatal activation of the GnRH system in humans into a single event.

The important factors contributing to the enhanced output of FSH in infantile rats are the relative ineffectiveness of estradiol (E_2) negative feedback (22–25) and a facilitatory effect of 5α -reduced androgens produced in the anterior pituitary on GnRH-induced gonadotropin secretion (26). Throughout juvenile development, plasma FSH continues to decrease at a slower pace, with plasma LH levels remaining at low, but pulsatile, values (9,27,28). This pattern of FSH secretion is similar to that observed in humans during the infantile–juvenile transition, as in both cases plasma FSH levels decline. However, during the second half of the juvenile period, the patterns differ, as in humans FSH levels begin to increase again toward the high values characteristically seen at the time of puberty (29).

DEVELOPMENT OF STEROID FEEDBACK MECHANISMS

Estradiol Negative Feedback

In female rats, E_2 negative feedback develops after postnatal day 16 (22–25,30). This temporal pattern is due to the presence of high levels of α -fetoprotein, which binds estrogen avidly (31,32), in serum and tissues of infantile animals. It seems that at this time of development, the steroid negative feedback regulation of gonadotropin release is mostly exerted by aromatizable androgens instead of circulating estrogen levels (33) and that this androgenic control changes to a dual estrogenic–androgenic control during the juvenile period (5).

Estradiol Positive Feedback

The ability of E_2 to trigger a preovulatory surge of gonadotropins also develops during juvenile days in both rats and mice (34–36). The hypothalamic mechanisms underlying this developmental event remain to be identified. Although GnRH neurons do not contain estrogen receptor α ($ER\alpha$) (37,38), they express $ER\beta$ (39,40), indicating that, indeed, GnRH neurons are subjected to a direct estrogen control. Based on these observations, it has been suggested that estrogen acts directly on GnRH neurons via $ER\beta$ to suppress GnRH gene expression and indirectly via $ER\alpha$ (and/or $ER\beta$) located on neurons connected to the GnRH neuronal network to generate a preovulatory surge of GnRH secretion (41). A substantial fraction of the neurons mediating E_2 positive feedback is located in the anteroventral periventricular nucleus (AVPV) region of

the hypothalamus (41). Because a group of ER α -containing neurons in the AVPV expresses the *KiSS1* gene (42) (see section “The Transsynaptic Control”), and E₂ selectively increases KiSS1 expression in these cells (42), it has been postulated that KiSS1-containing neurons mediate the positive feedback of E₂ on GnRH release (43). This concept is complemented by the observation that kisspeptin (the processed product of the *KiSS1* gene, see section “The Transsynaptic Control”) is more effective in stimulating GnRH neurons in postpubertal than in juvenile mice (44).

CENTRAL CONTROL OF GNRH SECRETION

In contrast to humans, in which the so-called GnRH pulse generator is fully functional at birth, the capacity of the rodent hypothalamus to elicit pulsatile gonadotropin release becomes established during the infantile period. At this time, GnRH expression is low, increasing almost three-fold during juvenile days (45). As GnRH mRNA content accumulates, the positive feedback effect of E₂ on gonadotropin secretion develops (34), and the response of GnRH neurons to stimulatory inputs, such as excitatory amino acids (46) or prostaglandin E₂ (PGE₂) (47), increases. During juvenile development, the GnRH neuronal network becomes firmly subjected to a dual inhibitory/facilitatory transsynaptic control and a glial, growth factor-dependent regulatory input (6,7) (Fig. 1).

The Transsynaptic Control

The transsynaptic excitatory control of GnRH secretion is exerted by several neuronal systems, but most predominantly by neurons that use glutamate or kisspeptin for neurotransmission. It was earlier established that a major excitatory transsynaptic event prompting the initiation of puberty is an increase in glutamatergic neurotransmission (6,49), the primary mode of excitatory transsynaptic communication in the hypothalamus (50). The facilitatory control of glutamatergic neurons increases gradually during the juvenile period to become maximally effective at the time of the first preovulatory surge of gonadotropins [for a review, see (6,7)]. Activation of glutamatergic inputs increases GnRH secretion (51,52) and accelerates sexual maturation in both rats and monkeys (53,54). Glutamate acts both directly (55–58) and via regulatory neuronal subsets (59) to stimulate GnRH secretion. Although earlier studies had found that GnRH neurons are scantily innervated (6,60), recently Campbell et al. (61) used a dye-filling approach to demonstrate that GnRH neurons have long dendrites covered with spines, highly suggestive of an abundance of excitatory synaptic contacts. That indeed GnRH neurons are subjected to a strong excitatory amino acid control has been shown by electrophysiological studies demonstrating the presence of functional alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors in green fluorescence protein-tagged GnRH neurons (62–64). Very recently, a study using a transneuronal tracer transgenically expressed in GnRH neurons demonstrated that these neurons receive a profuse glutamatergic innervation from the lateral ventromedial and premammillary nucleus of the hypothalamus (65).

Kisspeptin (also known as metastin), a 54-amino-acid peptide product of the *KiSS1* gene (66,67), was recently shown to be a physiological ligand for the orphan receptor GPR54 (67,68). Proteolytic cleavage of the primary KiSS1 protein product originates the decapeptide kisspeptin-10, which is remarkably potent in eliciting LH release

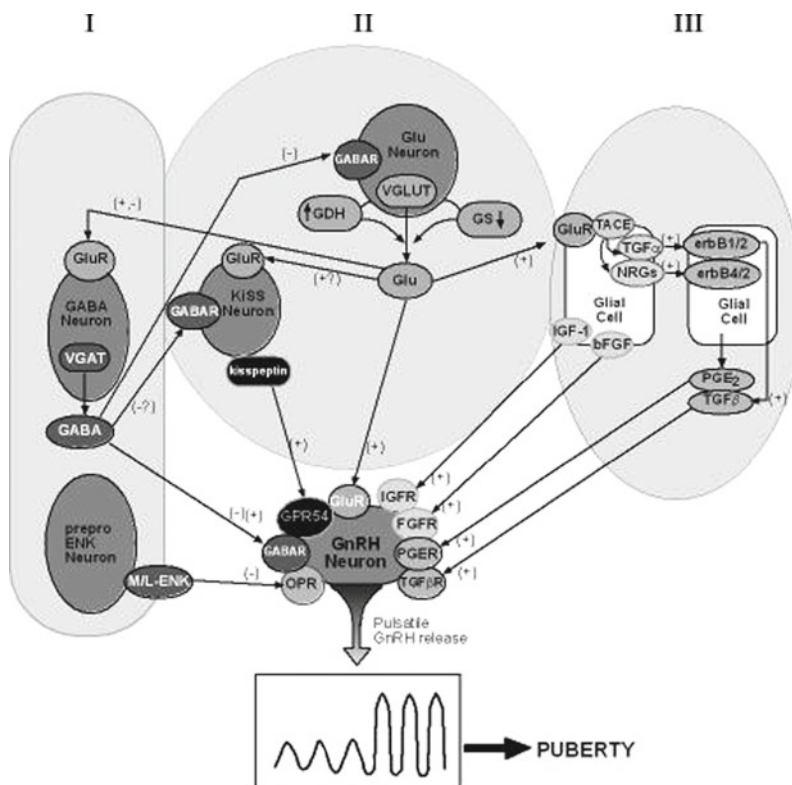


Fig. 1. Examples of genes involved in the transsynaptic and glial control of gonadotropin-releasing hormone (GnRH) neurons at the time of female puberty. These genes are postulated to function within a large interactive cellular network organized into the three indicated domains. Not all the potential cell–cell communication pathways are shown. VGLUT, vesicular glutamate transporters 1 and 2; VGAT, vesicular γ -aminobutyric acid (GABA) transporter 1; GDH, glutamate dehydrogenase; GS, glutamine synthase; Glu, glutamate; GluR, ionotropic and/or metabotropic glutamate receptors; GABAR, GABA receptor (A or B); M/L-ENK, met or Leu-enkephalin; OPR, opioid receptors; TACE, tumor necrosis factor alpha converting enzyme; TGF α , transforming growth factor α ; NRGs, neuregulins; erbB1, 2, and 4, receptors for TGF α (erbB1/2) and NRGs (erbB4/2); TGF β , transforming growth factor β ; TGF β R, TGF β receptors (I and III); IGF-I, insulin-like growth factor-I; bFGF, basic fibroblast growth factor; IGFR, IGF-I receptor; FGFR, FGF receptor; PGE $_2$, prostaglandin E $_2$; PGER, prostaglandin receptors; (+), stimulation; (–), inhibition; ?, not known. Notice that the direct GABA $_A$ receptor-mediated effects of GABA on GnRH neurons can be excitatory. Modified with permission (48).

(69–71). Because mutations of the *GPR54* gene result in hypothalamic hypogonadism in humans (72–74) and impaired sexual development in mice (73,75), it is now clear that activation of GPR54 receptors is a critical transsynaptic input to GnRH neurons required for the initiation of puberty. KiSS1 mRNA-containing neurons are mostly located in the AVPV nucleus of the preoptic region (42), and the periventricular and arcuate nucleus of the hypothalamus (42,69,70). Neurons containing kisspeptin/metastatin immunoreactivity have been described in other neuronal groups, including the dorsomedial nucleus of the hypothalamus and the nucleus of the solitary tract (76). Cells expressing GPR54 mRNA, on the contrary, are more widely distributed as they can be found not

only throughout the hypothalamus (69,77) but also in the adenohypophysis (67,68), suggesting that kisspeptin may also act at a pituitary level to stimulate gonadotropin secretion (78). That kisspeptin can act directly on GnRH neurons has been suggested by the detection of GPR54 receptors in these cells (77,79,80). Direct evidence for this direct site of action was recently provided by the demonstration of an excitatory effect of kisspeptin on the bursting activity of GnRH neurons (44). This study also showed that, although mouse GnRH neurons already display GPR54 receptors during early postnatal development, these receptors acquire the ability to excite GnRH neurons in response to kisspeptin only during the transition from the juvenile stage to adulthood (44).

The inhibitory transsynaptic control of GnRH neurons is mostly exerted by γ -aminobutyric acid (GABA)-producing neurons and neurons that utilize opioid peptides for neurotransmission. Push–pull perfusion experiments have shown that GABA release from the preoptic area of the hypothalamus (that contains most GnRH neurons) decreases during juvenile–peripubertal days (postnatal day 26–35) in female rats (81), suggesting that the initiation of puberty in the female rat might be preceded by a loss of GABA-mediated inhibition, similar to that shown in female monkeys (82). It is important to mention, however, that an inhibitory GABAergic tone exerted directly on GnRH neurons might be mainly mediated by GABA_B receptors (83), and not by GABA_A receptors, which have been shown to mediate an excitatory GABAergic influence on these neurons (84,85) (*Fig. 1*). For further discussion of this subject, see reference (7).

Although opioid peptides can suppress pulsatile LH release (86–88), their importance in inhibiting gonadotropin secretion before puberty is controversial (89). The discrepancy is perhaps due to the complexity of the opioid system, which may employ different opioid peptides and different receptors, in addition to different opioid-producing neuronal subsets, to control GnRH secretion at different developmental stages. Recent experiments suggest that enkephalin peptides produced by neurons of the lateral ventromedial nucleus of the hypothalamus do, in fact, inhibit female sexual development (90).

The Glial Control

Glial cells regulate GnRH secretion via plastic rearrangements and direct cell–cell communication. Both mechanisms require the participation of growth factors, such as transforming growth factor β 1 (TGF β 1) and TGF β 2, which act via serine–threonine kinase receptors, and others such as insulin-like growth factor-I (IGF-I), basic fibroblast growth factor (bFGF), and the family of epidermal growth factor (EGF)-related polypeptides, which exert their effects by activating tyrosine kinase receptors. Additional bioactive molecules are calcium, PGE₂, adenosine triphosphate (ADP), and glutamate [for a review, see (91)].

A role of TGF β s in the control of GnRH secretion was first suggested by experiments showing that TGF β 1 secreted from astrocytes stimulates GnRH release from immortalized GnRH neurons (92). E₂ enhances TGF β 1 release from hypothalamic astrocytes (93), implying that astrocytic TGF β 1 may also contribute to the mechanism by which E₂ induces the first preovulatory surge of GnRH. It appears, however, that the most important role of TGF β 1 is to regulate GnRH gene expression (92). Another important function of TGF β 1 is at the level of the median eminence, where it induces

plastic rearrangements of gliopendymal cells (tanycytes) (94). Tanycytes line the ventral surface of the third ventricle; their processes reach the external region of the median eminence (95) and prevent the direct contact of GnRH axons with the portal vasculature, via morphological specializations known as “end-feet.” At the time of the preovulatory surge of gonadotropins, this association is lost (96), and GnRH terminals become able to contact the pericapillary space of the portal vessels. In vitro treatment of tanycytes with TGF β 1 mimics this retraction, suggesting that the withdrawal of tanycyte processes seen in vivo may, at least in part, be due to TGF β 1.

Among growth factors acting via receptors with tyrosine kinase activity, bFGF appears to be important for migration and survival of GnRH neurons (97,98), effects that exert via activation of specific receptors (98–100). Basic FGF may also contribute to the preovulatory surge of gonadotropins because hypothalamic bFGF mRNA content increases in the evening of proestrus (101), due to an estrogen-initiated increase in astrocytic TGF α production (102). Although both TGF β 1 and bFGF can modify GnRH neuronal function, the role they may play in the control of puberty remains to be defined.

By contrast, a role of IGF-I in the control of puberty appears well established. In both rats and primates (including humans), circulating IGF-I levels increase at puberty (103–105). Although IGF-I is produced in astrocytes, most of IGF-I present in the median eminence derives from peripheral sources (106). Tanycytes take up IGF-I from the blood stream and presumably deliver the peptide to its sites of action within the hypothalamus (107). This uptake increases at a time of the preovulatory surge of gonadotropins (108), likely due to an upregulation of biologically active IGF-I receptors (106). Although both central (106) and peripheral (109) administration of IGF-I advances puberty in rats and monkeys, sexual development is delayed in mutant mice with deficient IGF-I production due to a lack of growth hormone receptors (110). These and other observations (6,7) suggest that IGF-I is a peripheral metabolic signal capable of facilitating the advent of puberty.

The growth factors best characterized in regard to their role in the onset of puberty are the EGF relatives, TGF α and neuregulins (NRGs). Both are produced in hypothalamic astrocytes and stimulate GnRH secretion indirectly, via a cell–cell communication mechanism that requires the activation of erbB receptors also located on astrocytes (111). Whereas erbB1 receptors (which bind TGF α) are located in both hypothalamic astrocytes and tanycytes (94,112), only astrocytes contain erbB4 receptors (which recognize NRGs) (113). In both cases, however, ligand-induced activation of these receptors recruit an auxiliary receptor termed erbB2 (94,113). Because of this activation, astrocytes and tanycytes release PGE $_2$ (113,114), which stimulates GnRH release by binding to PG receptor subtypes coupled to calcium mobilization and 3'–5' cyclic adenosine monophosphate (cAMP) formation expressed in GnRH neurons (115). Astrocytes also respond to erbB receptor stimulation with release of glutamate and the growth factors bFGF and TGF β 1 (6,116).

Pharmacological and genetic approaches have demonstrated a physiological role of glial erbB receptors to the control of female sexual development. Thus, both blockade of erbB1 receptors in the median eminence (117) and a natural mutation of the *erbB1* gene (118) delay puberty. Conversely, female sexual development is accelerated in transgenic mice expressing the human *TGF α* gene under the control of an inducible promoter (119), and rats carrying intrahypothalamic grafts of cells genetically modified

to secrete TGF α (120). Hypothalamic astrocytes also produce NRGs, which are recognized by erbB3 and erbB4 receptors. Because hypothalamic astrocytes only express erbB4 receptors (113), NRG signaling in these cells is exclusively mediated by erbB4/erbB2 receptor complexes. The hypothalamic expression of both erbB2 and erbB4 increases first during juvenile development, driven by gonad-independent, centrally originated mechanisms, and then at the time of puberty stimulated by gonadal steroids (113).

The contribution of erbB2 to the onset of puberty was demonstrated by administering into the third ventricle of female rats an antisense oligodeoxynucleotide directed against erbB2 mRNA. The treatment markedly delayed the onset of puberty in these animals (113). The participation of erbB4 receptors was investigated in mutant mice that overexpress specifically in astrocytes a truncated erbB4 protein that, lacking the intracellular domain, acts as a dominant negative receptor to block signaling via the intact receptor (121). Astrocytes derived from these mice release less PGE₂ in response to NRGs and less GnRH in response to astrocyte-conditioned medium, demonstrating that the truncated receptor disrupts the ability of hypothalamic astrocytes to respond to NRGs with the production of substances able to stimulate GnRH release. As expected from these findings, pituitary gonadotropin release was reduced and puberty was delayed in erbB4-deficient animals (121). Additional experiments in which these animals were bred to mice carrying defective erbB1 receptors delayed even further the onset of puberty and seriously compromised fertility (122), indicating that the erbB1-signaling and erbB4-signaling systems work in a complementary manner to facilitate GnRH release during sexual development and thereby contribute significantly to the initiation of female puberty (Fig. 1).

NEUROENDOCRINE CHANGES AT THE ONSET OF PUBERTY

The Ovary-Independent Activation of GnRH and Gonadotropin Secretion

In the rat, the first hormonal manifestation of puberty is a diurnal change in the mode of LH release that occurs by the end of the fourth week after birth (9,27,123). This change is manifested as an afternoon increase in LH pulse amplitude, which does not require the ovary (124) and, more specifically, is not caused by E₂ (125). Under the influence of these LH secretory episodes, the ovary produces more E₂ (126), which elicits minisurges of LH secretion (125), capable of inducing further ovarian activation (126) (Fig. 2). Both the increase in LH pulsatile release and the minisurges of LH secretion are caused by changes in GnRH secretion, as shown by the *in vivo* detection of GnRH release from the median eminence in conscious, free-moving female rats using a microdialysis probe (128). These events can be considered as markers of a second activational period of gonadotropin release (the first occurs during infantile development) and are thought to result from the functional coupling of the circadian clock to the neuronal and glial subsets involved in the control of pulsatile GnRH secretion, including GnRH neurons themselves (see section "The Puberty Clock").

The Ovary-Dependent Activation of Gonadotropin Secretion: The First Preovulatory Surge of Gonadotropins

The timing of puberty in the rat depends on the acquisition by the ovary of the capacity to secrete sufficient E₂ for an adequate period. Estrogen acts on the hypotha-

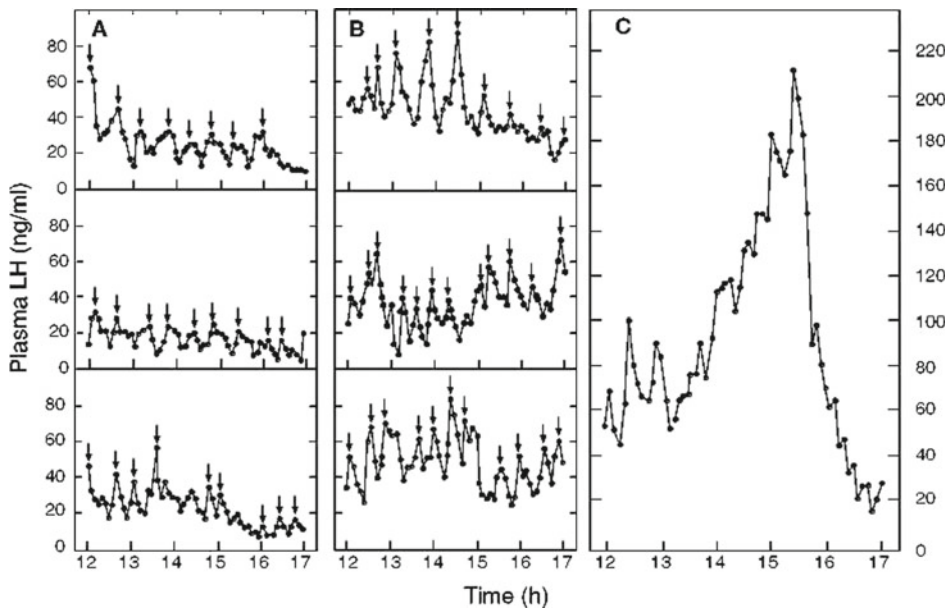


Fig. 2. A diurnal change in luteinizing hormone (LH) secretion signals the initiation of puberty in the female rat. (A) Representative afternoon plasma LH profiles from juvenile (27-day-old to 29-day-old) and (B) peripubertal (30-day-old to 38-day-old) female rats bled continuously for 5 h. Six individual profiles from a total of 16 are depicted. Pulses of LH secretion are indicated by arrows. (C) A minisurge of LH secretion occurring before the first preovulatory LH surge in the female rat. Modified with permission (127).

lamus to evoke a discharge of GnRH release (129), and on the pituitary, where it sensitizes the gonadotrophs to GnRH (130). Serum progesterone (P) increases two-fold to three-fold before proestrus (14,131–133), a change that facilitates the stimulatory effect of E_2 on GnRH release (134). The first preovulatory surge of GnRH and gonadotropins can be considered as the third and final activational period of neuroendocrine reproductive development in rodents.

The neuronal networks most relevant to the control of the preovulatory GnRH surge utilize excitatory amino acids and GABA as neurotransmitters (6). Recently, direct evidence for a direct involvement of the kisspeptin/GPR54 system in the genesis of the preovulatory surge was provided by the demonstration that immunoneutralizing monoclonal antibodies to kisspeptin abolish the proestrus LH surge when infused into the preoptic region of the brain (135). There are several other neurotransmitter systems that appear to participate in the process, either as targets of the glutamatergic/GABA-controlling system or as additional transsynaptic pathways. These systems include stimulatory neurons such as serotonergic, catecholaminergic, neuropeptide (NPY) yergic, and inhibitory opiategic neurons [for references and a more in-depth discussion, see (6,7)].

Glial cells also contribute to the first preovulatory surge of gonadotropins. Activation of glial erbB1 and erbB4 receptors is required for the correct timing of the surge (113,117). Activation of these receptors in astrocytes results in PGE_2 release; tanycytes in culture respond to $TGF\alpha$ -dependent activation of erbB1 receptors with both PGE_2

and TGF β 1 release resulting in changes in tanycyte morphology similar to those observed at the time of the preovulatory surge of gonadotropins (94). These findings suggest that the sequential increase in hypothalamic TGF α and TGF β 1 synthesis seen on the day of proestrus (101,117) causes retraction of tanycytic processes, allowing direct contact of GnRH nerve terminals with the portal vasculature (94). Proestrus levels of E₂ have been shown to stimulate glial production of TGF α (117,136), which then mediates an E₂-dependent increase in hypothalamic bFGF synthesis in the evening of proestrus (101,102).

THE MECHANISM OF THE ONSET OF PUBERTY

Pulsatile GnRH secretion increases at puberty due to an increase in neural activity, known as the “central drive” (5,8,137). Two complementary mechanisms appear to initiate this event: a loss of a tonic, transsynaptic inhibitory GABAergic tone (137), and activation of facilitatory inputs to the GnRH neuronal network (6,7) (Fig. 3). Whereas the importance of the loss in inhibitory tone, elegantly shown in nonhuman primates, remains to be demonstrated in rodents (6), the role played by facilitatory inputs to GnRH neurons is well established—and known to be of both transsynaptic and glial origin.

The Increase in Excitatory Transsynaptic Output

As indicated above, the transsynaptic facilitatory inputs are mostly provided by glutamatergic and kisspeptin neurons. Neurotransmitters such as norepinephrine (NE) and NPY appear to play secondary roles (Fig. 3).

GLUTAMATERGIC CONTROL

The physiological importance of glutamatergic transmission for the onset of puberty has been shown by several studies. For instance, activation of NMDA receptors via administration of NMDA increases GnRH secretion (51) and advances puberty in both rats and monkeys (53,54). Conversely, blockade of these receptors by a noncompetitive, use-dependent, antagonist delayed puberty in female rats (54). Other experiments have shown that the activity of hypothalamic NMDA receptors increases at the onset of puberty (46) and that this activation occurs in a gonad-independent manner (139). A puberty-related increase in the number of GnRH neurons expressing NMDA receptors has been demonstrated (140), and other studies have shown that a remarkable number (80%) of GnRH neurons in the medial preoptic area of adult rats express the mRNA encoding the obligatory NMDAR1 receptor subunit (55). Glutamatergic neurons also utilize kainate receptors for the regulation of GnRH secretion (52,141). During prepubertal development of the female rat, ~50% of GnRH neurons express the mRNA encoding the kainate-preferring glutamate receptor subunit KA2 (57). GnRH neurons also express GluR5 mRNA (58), which encodes a receptor subunit required for the heterodimeric assembly of high-affinity functional kainate channels (142). The importance of glutamatergic neurotransmission for the excitatory control of the onset of puberty is highlighted by the loss of GnRH pulsatility that results from the blockade of endogenous glutamate synthesis in the developing rat hypothalamus (143). The aforementioned findings notwithstanding, a very recent report (144) showed that female mice lacking NMDA receptors in GnRH neurons and other neurons of the limbic

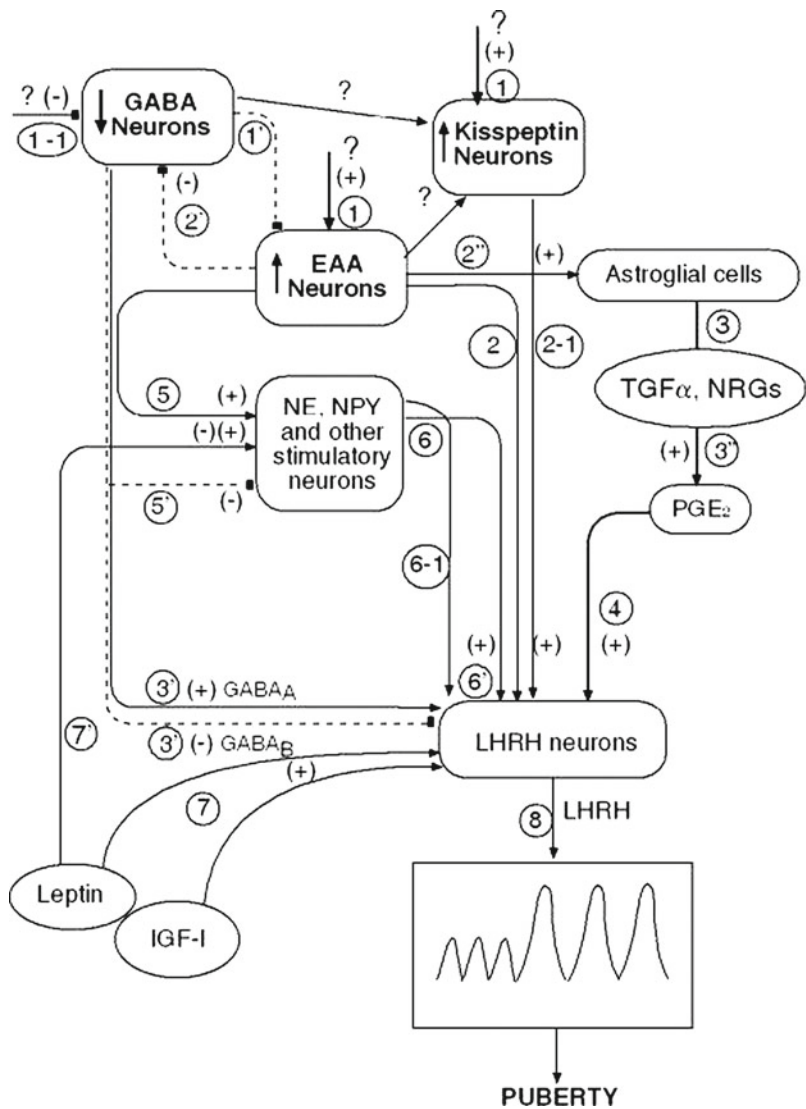


Fig. 3. The central events underlying the initiation of puberty. An increase in episodic gonadotropin-releasing hormone (GnRH) secretion is activated by an integrative mechanism (described in the text) involving both neuron-to-neuron and glia-to-neuron communication processes. The metabolic signals, leptin and insulin-like growth factor-I (IGF-I), further the process along by stimulating GnRH secretion either directly or via functionally connected neuronal networks. —, inhibition; (+), stimulation. Numbers in circles indicate the approximate temporal sequence of each event. Modified with permission (138).

system are fertile. By contrast, both male and female mice lacking the essential B subunit of the AMPA receptor showed impaired reproductive behaviors, such as pup retrieval and maternal aggression (in females) and mounting and aggression (in males). Like NMDA-deficient mice, AMPA-B-deficient animals had no discernible endocrine reproductive abnormalities.

KISSPEPTIN-DEPENDENT CONTROL

That an increasing activity of the kisspeptin–GPR54 signaling complex occurs at puberty was suggested by the findings that in both rats (145) and nonhuman primates (69), the hypothalamic content of KiSS1 and GPR54 mRNA increases at the time of puberty. Other studies supported this concept by showing that centrally administered kisspeptin advances vaginal opening in rats (71,146). The pubertal activation of the kisspeptin/GPR54 system appears to occur at two different, but complementary levels. On the one hand, there is a remarkable increase in the number of KiSS1 mRNA-containing neurons of the AVPV in adult mice as compared with juvenile animals (44), with no changes in the number of GnRH neurons expressing GPR54 mRNA or in the content of GPR54 mRNA per GnRH neuron (44). On the other hand, the number of GnRH neurons responding to kisspeptin with depolarization increases during the prepubertal–adulthood transition in the mouse (44), indicating that puberty is associated with an enhanced signaling capability of the receptor.

The Decrease in Inhibitory Transsynaptic Output

As indicated above, studies in the rhesus monkey have shown that a decrease in GABAergic inhibitory control plays a major role in restraining the onset of puberty in this species (82). It has been difficult to unveil such a predominant influence in the rat, which appears to respond to manipulations of the GABA system with either an increase or a decrease in GnRH/gonadotropin output, depending on the experimental conditions [for a review, see (6)]. Despite these inconsistencies, it appears clear that the preovulatory surge in the rat is indeed preceded by a decreased GABA output (147,148). The activity of the other major inhibitory neurotransmitter system, represented by neurons that use opioid peptides for neurotransmission and neuromodulation, also decreases during the days preceding the first ovulation (149). Importantly, this decrease appears to be more prominent in the afternoons of the peripubertal period (150).

The Coordination of Excitatory and Inhibitory Control Mechanisms

Although the importance of glutamatergic and GABAergic neurons in the control of puberty is unquestionable, it is unclear which change occurs first, the increase in glutamatergic outflow or a reduction in GABAergic inhibitory tone (Fig. 3). The two systems are tightly related because glutamate is the natural precursor for GABA synthesis. Because the synaptic activity of GABAergic neurons is extensively regulated by glutamatergic neurons (151–157), we favor the possibility that in the rat the activation of glutamatergic transmission precedes the loss in GABAergic inhibitory output. This concept is supported by recent findings showing that activation of either kainate or AMPA receptors in other regions of the rat brain results in the inhibition of GABA release (152–155).

If a glutamate-to-GABA hierarchical arrangement controls the initiation of puberty, it can be postulated that the neonatal–infantile activation of GnRH secretion is caused by the postnatal development of excitatory GABAergic synapses, an event that precedes the appearance of both mature inhibitory GABAergic synapses (158,159) and functional glutamatergic synapses (160,161). According to this concept, the infantile-to-juvenile decrease in GnRH secretion would be determined, to a significant extent,

both by the conversion of GABA neurotransmission from excitatory to inhibitory and by a glutamate-dependent, metabotropic receptor-mediated inhibition of excitatory GABAergic synaptic activity (151). It should be reiterated at this point that a direct excitatory GABA_A receptor-mediated input to GnRH neurons may remain operative throughout puberty (84,85,162) despite the switch from excitatory to inhibitory that occurs elsewhere in the brain by the end of infantile development. Despite the experimental robustness of these observations, other authors have claimed that the response of GnRH neurons indeed switches from depolarization to hyperpolarization at puberty (163,164). Further investigation is required to resolve this controversy. Another important issue that also awaits resolution is whether the changes in KiSS1 expression or the responsiveness of GnRH neurons to kisspeptin are influenced by glutamatergic/GABAergic inputs. It is entirely plausible that KiSS1 neurons receive glutamatergic and GABAergic innervation and are, therefore, regulated by these two major neurotransmitter systems (Fig. 3).

The Change in Glial Output

It is now clear that astrocytes play a major role in the process by which excitatory amino acids affect neuronal function (165). Glutamatergic neurons appear to use a glutamate-dependent, neuron-to-glia signaling pathway to coordinate the transsynaptic and glial activation of GnRH secretion at puberty (166). This study showed that hypothalamic astrocytes respond to glutamatergic stimulation with recruitment of erbB1 and erbB4 receptors and their respective ligands to the glial cell membrane, transactivation of the receptors via a mechanism requiring metalloproteinase activity, and increased erbB1 and erbB2 receptor expression. One of the metalloproteinase activities involved was recently identified as ADAM 17/TACE (167), a protease required for the ectodomain shedding of both TGF α and NRGs. These results indicate that the neuroendocrine brain uses a neuron-to-glia glutamatergic pathway as a basic cell–cell communication mechanism to coordinate the facilitatory transsynaptic and astroglial input to GnRH neurons during sexual development. Because astrocytes also potentiate GABAergic inhibitory synaptic transmission (168) and respond to GABA stimulation with depolarization (169,170), it appears clear that a dynamic bidirectional communication exists between astroglial cells and neurons that use excitatory and inhibitory amino acids as neurotransmitters (Fig. 3). The critical importance of these two neuronal systems in the developmental control of GnRH release, and the emerging evidence implicating astroglial cells in this process, indicates that such a tripartite communication system is a fundamental mechanism employed by the neuroendocrine brain to control the advent and progression of mammalian sexual maturation [for further discussion, see (6,7)].

The Integration of Neuron-to-Neuron and Neuronal–Glial Communication by Upstream Controlling Genes

As the reader already suspects, this is an area of research still in an embryonic stage. Studies in our laboratory have identified three genes as potential upstream regulators of the pubertal process. One of them is Oct-2, a transcriptional regulator of the POU-domain family of homeobox-containing genes (171). The *Oct-2* gene is expressed throughout the embryonic ventral forebrain, but after birth it remains expressed in

discrete hypothalamic neuronal subsets (172). Oct-2 proteins are, however, more abundant in cultured astrocytes than in neurons (173), suggesting that the *Oct-2* gene may be important for the transregulation of astroglial genes. *TGF α* has been shown to be one of these genes (174), as the transcriptional activity of its promoter is increased by Oct-2. The importance of this regulatory mechanism for the onset of female puberty has been made evident by several observations. For instance, hypothalamic Oct-2 mRNA levels increase during juvenile development in a gonad-independent manner, blockade of Oct-2 synthesis via antisense oligodeoxynucleotides reduced astrocytic TGF α synthesis, and delayed the age at first ovulation, and puberty-advancing lesions of the anterior hypothalamus activate both Oct-2 and TGF α expression in astrocytes near the lesion site (174).

The second candidate is another homeobox gene *TTF-1*. *TTF-1* is required for diencephalic morphogenesis (175); like *Oct-2*, it remains expressed after birth in discrete neuronal and glial populations of the hypothalamus, including GnRH and preproenkephalinergic neurons, and tanycytes of the median eminence (176). *TTF-1* acts on each of these cell types to regulate cell-specific functions. For instance, it enhances GnRH and *erbB2* promoter activity but inhibits preproenkephalin gene transcription (176). Conditional deletion of the *TTF-1* gene from hypothalamic neurons using the Cre-loxP system results in delayed puberty and decreased reproductive capacity (90). *TTF-1*-deficient animal showed increased preproenkephalin mRNA levels in the hypothalamus and a reduction in hypothalamic GnRH and *Kiss1* mRNA levels (90). These observations indicate that *TTF-1* acts coordinately on several components of the neuronal–glial circuitry regulating GnRH release. On the one hand, it enhances the transcriptional activity of genes required for the facilitatory control of puberty, such as GnRH, *erbB2*, and *Kiss1*; on the other hand, it represses the transcription of the preproenkephalin gene, which is involved in the inhibition of GnRH secretion.

We discovered the third candidate using cDNA arrays to interrogate the primate hypothalamus at the time of puberty (177). This gene was earlier known as chromosome 14 open-reading frame 4 (*C14ORF4*) (178). We have now termed it *EAP-1* (enhanced at puberty-1) (177). Hypothalamic *EAP-1* mRNA content increases during both monkey and rat puberty (179), suggesting an involvement in the control of the pubertal process. *EAP-1* encodes a nuclear protein, which—like *TTF1*—is expressed in neurons involved in the stimulatory and inhibitory control of GnRH secretion, including GnRH neurons themselves, in addition to glutamatergic, GABAergic, and proenkephalinergic neurons (179). *EAP-1* has dual transregulatory activity; it activates the promoter of genes involved in facilitating the advent of puberty (such as GnRH) and represses the preproenkephalin gene, which is inhibitory to the pubertal process. Knocking down hypothalamic *EAP-1* expression using siRNAs delayed puberty and disrupted estrus cyclicity, indicating that *EAP-1* is indeed necessary for the timely activation of GnRH secretion at puberty (179).

Based on these observations, a scenario might be envisioned in which a hierarchy of upstream genes, represented by *TTF-1*, *Oct-2*, and *EAP-1*, controls the pubertal process by coordinating the activity (and therefore the interactions) of several neuronal and glial subsets involved in the developmental control of the GnRH neuronal network (Fig. 4).

The Puberty Clock

In addition to the diurnal change in GnRH/LH release that occurs at the initiation of puberty (9), the norepinephrine (NE) (180) and NPY (181) systems also become activated in the afternoons. By contrast, the activity of the opiate and GABAergic system decreases at this time of the day (147,150). Although it would seem clear that these diurnal rhythms are controlled by a neural circadian clock, likely located in the suprachiasmatic nucleus (SCN) of the hypothalamus (182,183), neither the identity of the synaptic pathways conveying information from the circadian clock to the GnRH neuronal network nor the identity of the transsynaptic/cell–cell signaling molecules involved in the process has been defined. Whereas immunohistochemical and retrograde tracing studies have shown that GnRH neurons receive a substantial vasoactive intestinal polypeptide (VIP)-ergic input from the SCN (183,184), coculturing the SCN with preoptic area (POA) tissues showed that diurnal fluctuations in GnRH release coincide with release of arginine vasopressin (AVP) instead of VIP (185).

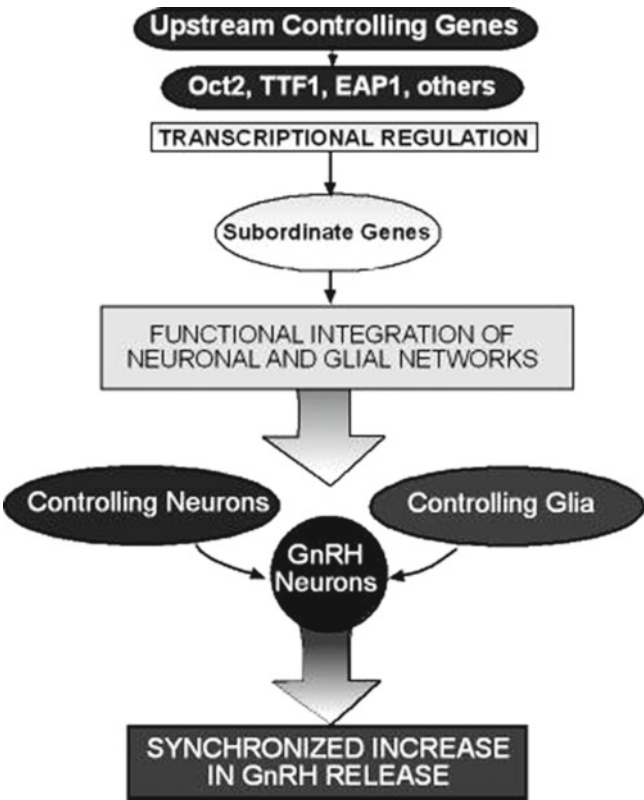


Fig. 4. The transcriptional control of the gonadotropin-releasing hormone (GnRH) neuronal network at puberty by upstream controlling genes. Changes in the secretory activity of GnRH neurons are specified by transsynaptic and glial inputs. These inputs are determined by subordinate genes (examples shown in *Figs 1* and *3*), which, in turn, are controlled at the transcriptional level by upper-echelon genes, such as *Oct-2*, *TTF-1*, and *EAP-1*. It is envisioned that this hierarchical arrangement is required to initiate and maintain an enhanced level of pulsatile GnRH secretion at puberty. Modified with permission (48).

In addition to AVP and VIP, excitatory and inhibitory amino acid neurotransmitters have been shown to generate a clock-dependent transsynaptic output from the SCN (186,187). Glial cells may play a role in this process because SCN astrocytes show a consistent oscillatory response to glutamate (186), the major neurotransmitter mediating the visual input to the SCN (188).

In addition to these conventional candidates, the photolyase-like proteins Cry1 and Cry2 were recently found in the SCN and in other tissues and have been shown to act as circadian photoreceptors able to detect light directly, thereby photoentraining the biological clock of different tissues (189). Because in their absence free-running locomotor rhythmicity is lost (189), they appear to be essential components of the SCN circadian clock. It is obvious that substantial efforts and new approaches will be required to unravel the mechanisms by which the SCN circadian clock entrains the diurnal changes in the activity of the GnRH pulse generator at the time of puberty. Identification of the transsynaptic and cell–cell communication pathways involved, and the functional characterization of the key molecules underlying this communication, remains a critical issue that awaits resolution. The recent finding that chromosomes 6 and 13 contain genes of potential importance of the time of puberty in mice (190) might shed new light into this issue.

PHEROMONES AND RODENT PUBERTY

It has been recently demonstrated that GnRH neurons in the mouse receive substantial input from both odor and pheromone-relay areas of the brain (such as the medial amygdala and posterocortical amygdalar nucleus) (65). Other authors independently reported that GnRH neurons are innervated by a projection pathway from the primary olfactory cortex that originates from neurons located in the main olfactory epithelium (191). These findings strongly suggest that the effects of pheromones on the onset of mouse puberty previously described (192) are, to a significant extent, mediated by transsynaptic pathways directly affecting GnRH neuronal function.

METABOLIC SIGNALS AND THE ONSET OF PUBERTY

The role of metabolic signals in the initiation of puberty has also been postulated (6,7). It now appears clear that IGF-I is indeed able to induce puberty on its own [for references and discussion, see (6,7)]. For a while leptin was considered as a potentially important peripheral hormone for the initiation of puberty. However, it is now clear that leptin is not the critical signal that times the initiation of puberty in rodents (or humans). Instead, leptin appears to play a permissive role important for the progression of the pubertal process (193).

ENDOCRINE DISRUPTORS

Environmental toxins can affect embryonic development and subsequent gonadal maturation, fertility, and the time of puberty. Many compounds from plant extracts, plastics, pesticides, and fertilizers have been shown to have endocrine disruptor activity and influence sexual maturation and the pubertal process (194). Females appear to be less sensitive than males, although human females exposed to such compounds have been shown to undergo precocious puberty (195). Estrogenic compounds such

as methoxychlor and phthalates can mimic the actions of estrogen; exposure to these compounds during fetal or neonatal life can alter the onset and progression of puberty (196–198). The plant extract genistein (199) and other compounds such as bisphenol A (200,201) and atrazine (202,203) also have similar effects. Of particular interest is the recent demonstration that fetal exposure to endocrine disruptors has transgenerational effects as it affects spermatogenesis for several generations after the initial exposure (204). Two observations in humans have raised the suspicion that endocrine disruptors might affect reproductive function by both accelerating the pubertal process and diminishing adult reproductive function. One observation is that foreign children adopted by Western European countries have an earlier onset of menarche as compared with the age at menarche of children born in either the foster country or the country of origin (195). The other is the decline in fertility rate observed in Europe since the 1970s (205) and in particular the drop in male fertility detected in Denmark during this period (206).

The mechanism underlying the advancement of puberty caused by endocrine disruptors has been postulated to involve an acceleration of hypothalamic maturation (207,208). Examination of this hypothesis in immature female rats demonstrated that the endocrine disruptor dichlorodiphenyltrichloroethane (DDT) accelerates pulsatile GnRH secretion and causes sexual precocity (209). Further studies are required to elucidate the molecular basis of this effect.

POTENTIAL DEFECTS IN NEURONAL–GLIAL CONTROL THAT MAY LEAD TO CENTRAL PRECOCIOUS PUBERTY

As our knowledge of the central mechanisms controlling the onset of puberty increases, it becomes more and more evident that defects at various levels of the regulatory neuronal–glial network may contribute to explaining cases of human idiopathic sexual precocity of central origin (*Fig. 5*).

Defects Within the GnRH Neuronal Network

A defect of any signaling pathway used by GnRH neurons to respond to multiple inputs of neuronal, endocrine, and glial origin might result in an autonomous increase in GnRH output, in the absence of primary alterations in GnRH synthesis itself. Because idiopathic precocious puberty affects predominantly females, such a molecular defect would become apparent only if it affects an imprinted gene selectively expressed in GnRH neurons (*Fig. 5*). A pertinent example can be found in the case of *UBE3A*, an imprinted gene affected in Angelman syndrome (211). The *UBE3A* gene, which encodes a ubiquitin ligase, is expressed biparentally in all cells except for Purkinje, hippocampal, and olfactory mitral neurons (211), in which only the maternal allele is expressed. Mutations of the expressed maternal allele lead to the neurological symptoms characteristic of Angelman syndrome. A problem with this scenario in the case of GnRH neurons is that the imprinted gene would have to be a gene specifying a sex-dependent mechanism, because defects of imprinting affect boys and girls equally and central sexual precocity of idiopathic origin is much more predominant in girls than in boys.

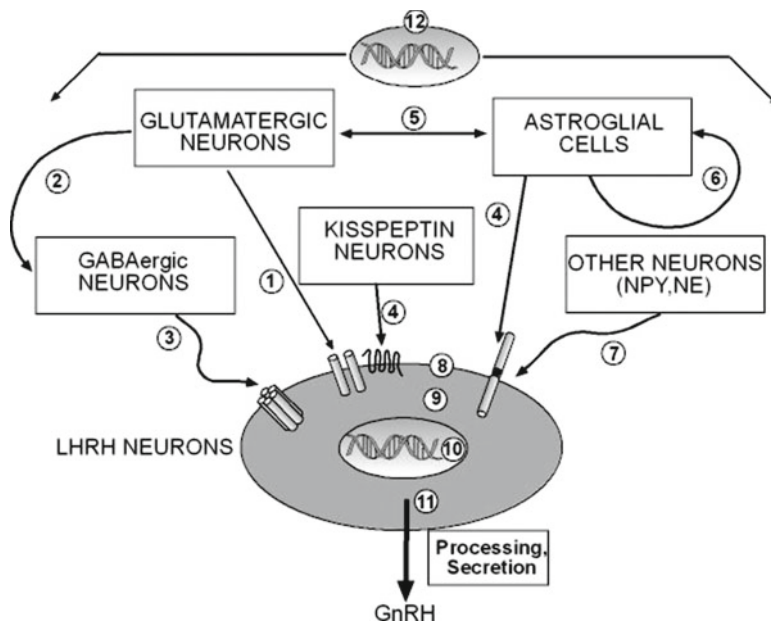


Fig. 5. Potential sites of alteration in the regulatory pathways controlling gonadotropin-releasing hormone (GnRH) secretion that may result in idiopathic precocious puberty of central origin. Each defect (denoted by a number) may occur individually or be accompanied by defects affecting other components of the system. The numbers do not reflect any hierarchical order. **1** and **2**, defect in the synaptic release of glutamate and/or lack of appropriate synaptic connectivity; **3**, defect in the synaptic release of γ -aminobutyric acid (GABA) and/or lack of appropriate synaptic connectivity; **4**, defects in kisspeptin secretion or GPR54 signaling; **5**, defect in reciprocal glial-neuronal or glia-to-GnRH neuron communication; **6**, defect in glial cell juxtacrine/paracrine communication (receptors/intracellular signaling, etc.); **7**, defect in the synaptic release of other neurotransmitters affecting GnRH secretion, such as NPY and NE, and/or inappropriate synaptic connectivity of these systems to GnRH neurons and/or other neuronal networks; **8**, defects in membrane receptors for neurotransmitters and/or growth factors; **9**, defect in intracellular signaling pathways mediating the actions of neurotransmitters/growth factors and/or adhesion molecules on GnRH secretion/GnRH neuronal function; **10**, defect in imprinted genes controlling GnRH neuronal function in a sex-dependent manner; **11**, defects in processing/exocytosis/delivery of the GnRH decapeptide to the portal vasculature; **12**, defects in genes involved in the upstream transcriptional control of the pubertal process. Modified with permission (210).

Defects in the Transsynaptic Control of GnRH Secretion

DEFECTS CAUSING PRECOCIOUS PUBERTY

A defect linking precocious puberty and a genetic abnormality that affects amino acid-mediated synaptic transmission has been described (212). Nonketotic hyperglycinemia is a metabolic abnormality caused by an inability to metabolize glycine, an amino acid that helps glutamate to activate NMDA receptors. A patient suffering from this disease exhibited signs of sexual development within the first year of postnatal life (212). Because glycine alone can stimulate pulsatile GnRH release in vitro (212), the advancement of puberty seen in this patient may have been caused by an excessive glycine/NMDA-mediated stimulation of GnRH secretion.

Another example was recently provided by a study implicating the kisspeptin/GPR54 signaling complex in the genesis of sexual precocity of central origin. This study showed that several individuals with idiopathic precocious puberty have mutations of GPR54 that enhance the signaling capability of the receptor (213) (*Fig. 5*).

DEFECTS CAUSING DELAYED PUBERTY

Defects in GABAergic transmission can also lead to alterations in the onset of puberty, as seen in Prader–Willi syndrome (PWS) (214,215). A cluster of GABA_A receptor subunits ($\alpha 5$, $\beta 3$, and $\gamma 3$), located in chromosome 15, is deleted in most PWS patients (216), indicating that its absence contributes to the phenotype of PWS in these individuals. PWS patients are hypogonadal and exhibit elevated plasma GABA levels of CNS origin (217), suggesting that an increase in presynaptic GABA release occurs as a compensatory response to decreased GABA_A receptor function. Mice carrying mutations of the genes encoding the $\beta 3$ subunit of the GABA_A receptor show neurological disorders similar to those seen in PWS (218,219), suggesting that a defect in this GABA receptor subunit might be related, directly or indirectly, to the hypogonadal condition of PWS patients.

Finally, we have already mentioned that mutations of the *GPR54* gene results in hypothalamic hypogonadism in humans (72–74) and impaired sexual development in mice (73,75).

Defects in Glial–Neuronal Communication

The possibility that such defects might exist has been suggested by the presence of TGF α in astrocytes of two hypothalamic hamartomas associated with sexual precocity (220).

CONCLUSIONS

Puberty is initiated by an increase in pulsatile release of GnRH. This increase requires both changes in transsynaptic communication and the activation of glia-to-neuron signaling pathways. The transsynaptic changes involve neurons that utilize excitatory and inhibitory amino acids, and the peptide kisspeptin, for neurotransmission. The glial-to-neuron control is exerted via the production of trophic factors and small cell–cell signaling molecules. A coordinated increase in glutamatergic and kisspeptin-mediated transmission accompanied by a decrease in inhibitory GABAergic-opioid peptide tone appears to initiate the transsynaptic cascade of events leading to the pubertal increase in GnRH release. Glial cells facilitate GnRH secretion via communication pathways mainly initiated by members of the EGF and TGF β families of trophic factors. In turn, a neuron-to-glia signaling mechanism mediated by excitatory amino acids coordinates the activation of transsynaptic and glia-to-neuron communication required for the acquisition of sexual maturity. A higher level of control involves the transcriptional regulation of subordinate genes expressed in neurons and glia by upper-echelon genes that form part of the transcriptional machinery controlling female sexual development.

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Control of Puberty in Non-Human Primates

Tony M. Plant, PhD

CONTENTS

INTRODUCTION
THE HYPOTHALAMIC NEURAL NETWORK RESPONSIBLE FOR GENERATING PULSATILE GnRH RELEASE
THE ONTOGENY OF PULSATILE GnRH RELEASE
THE NATURE OF THE PREPUBERTAL BRAKE ON PULSATILE GnRH RELEASE DURING JUVENILE DEVELOPMENT
NEUROBIOLOGY UNDERLYING THE RESTRAINT OF GnRH FROM BIRTH UNTIL PUBERTY
TIMING OF THE DEVELOPMENTAL SWITCHES RESPONSIBLE FOR THE ON-OFF-ON PATTERN OF PULSATILE GnRH RELEASE FROM BIRTH TO ADULTHOOD
OTHER CONSIDERATIONS
CONCLUSION
ACKNOWLEDGEMENT
REFERENCES

Summary

This chapter provides an overview of the mechanisms that govern the onset of puberty in the rhesus monkey, a representative higher primate. Particular attention is paid to the neurobiology that underlies the on-off-on pattern of pulsatile gonadotropin-releasing hormone (GnRH) release by the hypothalamus from birth until puberty, because the timing of the resurgence in the secretion of this brain peptide at the termination of juvenile development determines the age of gonadarche in both humans and monkeys.

Key Words: Rhesus monkey; GnRH, Kiss-1; Kisspeptin; Glutamate; GABA, INPY.

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INTRODUCTION

Non-human higher primates comprise two families of mammals, namely the Old World monkeys (*Catarrhina*) and apes (*Simiidae*) (1). In the context of the endocrinology of puberty, the major qualitative difference between these two families is that adrenarche is only observed in the apes (2). Thus, for the most commonly studied non-human primate, the rhesus macaque (*Macaca mulatta*), upon which this chapter is largely based, the stage of development known as puberty that is initiated at the end of the juvenile phase of the life history is underpinned by gonadarche, alone (2). In the female rhesus monkey, puberty begins to unfold at approximately 24–30 months of age with menarche and first ovulation at approximately 30 and 42 months of age, respectively (2). In the male, gonadarche is initiated at approximately 30 months of age and adult testicular size is usually attained by 5 years (2). In contrast to humans, the physical changes associated with puberty in non-human primates have not been systematically staged. Although there are major quantitative differences in the duration of the delay from birth to puberty between the monkey and our own species, the fundamental endocrine, neuroendocrine, and neurobiological processes underlying gonadarche in these higher primates appear similar, and the monkey therefore provides an ideal paradigm for studying the integration of the physiological systems underlying the onset of reproductive competence in humans. Of particular note is the similarity in the development of the hypothalamic drive to the pituitary–gonadal axis that results in a characteristic “on–off–on” postnatal pattern in gonadotropin secretion from birth to puberty (2,3). The intervening hypogonadotropic state of juvenile primates (and children) guarantees the quiescence of the gonad during this stage of development and therefore the prolonged postnatal delay to puberty in these species. The primary purpose of this chapter is to review our understanding of the mechanisms that control the developmental pattern in pulsatile gonadotropin-releasing hormone-1 (GnRH-1) release in the monkey and that therefore dictate the timing and tempo of puberty in this species of higher primate. It should be noted that GnRH-II is also found in the monkey (and human) hypothalamus (4) and the gene encoding the GnRH-II receptor is expressed in the anterior pituitary (5,6). The role, if any, of GnRH-II signaling in regulating the ontogeny of gonadotropin secretion in primates is unknown, and the term GnRH is used throughout this chapter to describe GnRH-1.

THE HYPOTHALAMIC NEURAL NETWORK RESPONSIBLE FOR GENERATING PULSATILE GnRH RELEASE

The hypothalamus of the adult monkey contains approximately 2000 GnRH neurons that are diffusely distributed from the rostral (lamina terminalis) to caudal (mammillary bodies) boundaries of the hypothalamus (7). The majority of neuroendocrine GnRH neurons (i.e. those projecting to the hypophysial portal system in the median eminence) are found in the mediobasal hypothalamus (8). This framework of peptidergic neurons receives afferent information from both neurons and glial cells. Interestingly, in contrast to the dogma that GnRH neurons are scantily innervated, recent studies of rodents employing contemporary imaging techniques indicate that axo-dendritic synapses on GnRH neurons are abundant (9). Such architecture makes intuitive sense because

the release of GnRH may be modulated by a host of neuropeptides, neurotransmitters, and neuromodulators (10). The neurobiological mechanisms that underlie the pulsatile mode of GnRH release remain to be fully established. One school of thought holds the view that pulsatility is intrinsic to the GnRH neurons themselves, whereas another argues that afferent inputs impose pulsatile release of the decapeptide (11–14). In any event, a pulsatile mode of release is critical for sustained gonadotropin release (15) and therefore for the onset of puberty.

THE ONTOGENY OF PULSATILE GnRH RELEASE

In the monkey, as in other mammalian species, GnRH neurons have their embryonic origin in the olfactory area and migrate to the hypothalamus during fetal development attaining their adult pattern of distribution between days 50 and 135 of gestation (16). At this stage of fetal development, the hypothalamus appears to be providing a pulsatile hypophysiotropic stimulus to the pituitary gonadotrophs as reflected, in the fetus, by elevated levels of circulating luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which, in the male, may be amplified by castration (17). Following parturition and the loss of the inhibitory effect of high levels of fetoplacental steroids on the neonatal hypothalamus, robust pulsatile GnRH release, as reflected by a corresponding pattern of LH secretion, is again observed during infancy in both male and female monkeys (18). Within approximately 6 months of postnatal life, circulating LH and FSH concentrations decline to low levels indicating that pulsatile GnRH release has been restrained thereby guaranteeing the hypogonadotropic state of the juvenile monkey that, in turn, ensures the relative quiescence of the ovary and testis (19,20). The juvenile phase of development in the monkey is terminated when the brake on pulsatile GnRH is withdrawn at approximately 2 years of age in the female and 2.5 years of age in the male and gonadarche (puberty) is initiated.

Because experimentally imposed gonadotropin stimulation of either the testis or the ovary of the juvenile monkey will lead to the precocious onset of ovulation in the female (21) and spermatogenesis in the male (22), the brake imposed on pulsatile GnRH release late in infancy is the key component responsible for the protracted delay to puberty in this and other higher primates. It should be noted, however, that during infancy when LH and FSH secretions are elevated the testicular seminiferous cords and ovarian follicles are relatively unresponsive to gonadotropin stimulation (2,23,24). In the male, this occurs in the face of elevated testicular testosterone secretion that is produced by the heightened LH drive at this stage of development. The inability of the seminiferous cord of the infantile monkey testis to respond to this adult-like endocrine milieu appears to be related to impaired androgen receptor and FSH receptor signaling in the Sertoli cell (25,26).

THE NATURE OF THE PREPUBERTAL BRAKE ON PULSATILE GnRH RELEASE DURING JUVENILE DEVELOPMENT

As first demonstrated for humans by Grumbach and his colleagues more than 30 years ago when they described the pattern of gonadotropin secretion throughout postnatal development in agonadal humans (27), the characteristic on–off–on pattern of

GnRH release from birth until puberty is preserved in the monkey in the absence of the gonad (*Fig. 1*). Thus, it may be proposed that the mechanism that holds pulsatile GnRH release in check for the greater part of prepubertal development may be viewed as a “central” or neurobiological brake rather than as a gonadal restraint. From a mechanistic perspective, it should be recognized that the concept of a neurobiological brake does not imply that the reduction in pulsatile GnRH release from infancy to puberty is necessarily occasioned by the inhibition of the GnRH neuronal network. The same functional state might, of course, be produced by withdrawal of an excitatory input.

Because of the loss of inhibitory steroid feedback in the agonadal situation, the developmental pattern of gonadotropin secretion from birth until the time puberty would have been anticipated had the animals remained intact is amplified and reflects primarily developmental changes in GnRH drive to the pituitary gonadotrophs. In agreement with the earlier onset of puberty in the female, the resurgence in gonadotropin secretion

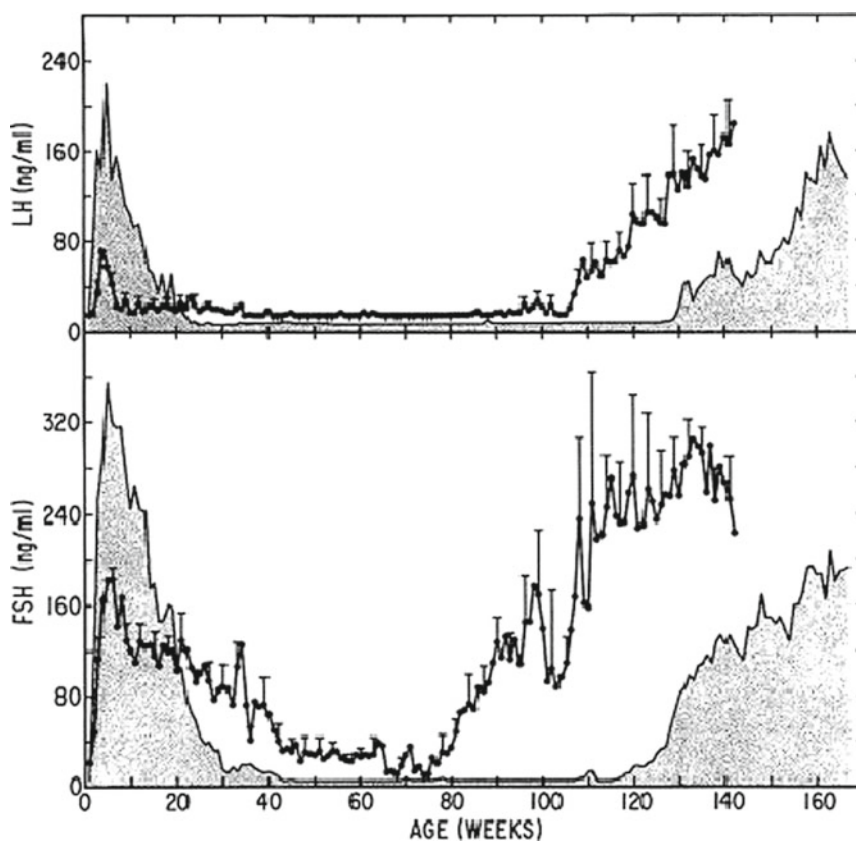


Fig. 1. The postnatal pattern of pulsatile gonadotropin-releasing hormone (GnRH) release in male and female rhesus monkeys, as reflected by the time course of circulating luteinizing hormone (LH) (above) and follicle-stimulating hormone (FSH) (below) in animals orchidectomized (stippled area) or ovariectomized (closed data points + SEM) at 1 week of age. Note the sex difference, which is particularly striking from the FSH profile, indicating that the neurobiological brake imposed on the activity of the hypothalamic–pituitary axis during juvenile development is applied with the greatest intensity and with the longest duration in the male. Reprinted with permission (2).

at the end of the juvenile phase of development in agonadal monkeys occurs at a younger age in the female compared with that in the male (*Fig. 1*). It may also be noted from the agonadal situation that, during the juvenile stage of development, the levels of circulating gonadotropin, and in particular those of FSH, are markedly higher in the female than in the male. Thus, it may be concluded that the intensity of the brake imposed on pulsatile GnRH release from infancy to puberty is most marked in the male and that the duration for which the brake is applied is longest in the male.

NEUROBIOLOGY UNDERLYING THE RESTRAINT OF GnRH FROM BIRTH UNTIL PUBERTY

The pattern of pulsatile GnRH release from birth to puberty in the monkey and other higher primates exhibits sex differences (see section immediately above), and this raises the possibility that the neurobiologic brake that restrains the hypophysiotropic drive to the pituitary–gonadal axis may be gender specific. At the present time, however, there is insufficient data to examine this notion, and for the present purpose, the assumption is made that the neural components of the brake are qualitatively similar in male and female.

Somewhat surprisingly, the expression of *GnRH-1* between infancy and puberty, the phase of development when pulsatile release of the encoded peptide is truncated, does not appear to be markedly down-regulated. In agonadal males, hypothalamic GnRH content remains unchanged throughout postnatal life (28,29), and GnRH mRNA levels at the time of the pubertal resurgence of GnRH release show only a modest increase (29), the magnitude of which is markedly dampened in intact animals (30,31). Additionally, the distribution of GnRH neurons and their peptide content, as reflected by immunocytochemical analysis, is comparable in infant, juvenile, and adult animals (2). Not only is GnRH expressed at high levels before the pubertal resurgence of pulsatile GnRH release, but trains of GnRH discharges with a periodicity and amplitude similar to that generated by the hypothalamus of the adult may be readily provoked from the brain of juveniles in response to repetitive electrical or neurochemical stimulation (22,32,33). These findings suggest that GnRH neurons in the juvenile hypothalamus are endowed with the molecular machinery required for generating an intermittent discharge of the peptide, providing a basis for the explosive resurgence in pulsatile GnRH release that is observed in the agonadal monkey (34). The “dormancy” of the hypothalamic GnRH network of the juvenile monkey could result from either a withdrawal of an excitatory input or the imposition of inhibitory signals, or a combination of the two.

With regard to the attenuation of an excitatory signal during juvenile development, kisspeptin and glutamate must be viewed currently as the most likely candidates. Kisspeptin, the 145-amino-acid prohormone of *KISS-1* (35), which is proteolytically processed in humans to a 54-amino-acid peptide termed metastin (36–38), has recently emerged as a major hypothalamic signal regulating GnRH release because of genetic studies of humans demonstrating an association between inactivating mutations of the G-protein-coupled receptor, GPR54 (39), and normosmic hypogonadotropic hypogonadism (40,41). In the monkey, GPR54 and *KISS-1* are expressed in the mediobasal hypothalamus, and expression of the ligand is increased in association with the pubertal resurgence of pulsatile GnRH release (42). Moreover, repetitive stimulation of hypothalamic GPR54 in the juvenile male monkey with metastin 45–54 elicits precocious

and sustained GnRH release, as reflected by the LH response, which is abolished by concomitant treatment with a GnRH receptor antagonist (43) (*Fig. 2*). As in other species (44), GnRH neurons of the hypothalamus of the juvenile monkey appear to express GPR54 (45), and therefore the site of the excitatory action of metastin is probably directly on the GnRH neuron, as has been empirically demonstrated in the mouse (46). Parenthetically, continuous administration of the ligand results in the down-regulation of GPR54 (47).

Hypothalamic glutamate release increases in association with the pubertal resurgence in GnRH secretion in the female rhesus monkey (48) (*Fig. 3*), and in the juvenile male monkey, repetitive activation of hypothalamic N-methyl-D-NMDA receptors elicits a sustained pulsatile discharge of GnRH (32), which, in the gonadally intact animal, results in a precocious and complete pubertal activation of the pituitary–testicular axis (22). Thus, it seems reasonable to propose that a combined synchronized action of amplified metastin and glutamate release represents a major upstream component of the hypothalamic drive for the pubertal resurgence of robust GnRH discharges.

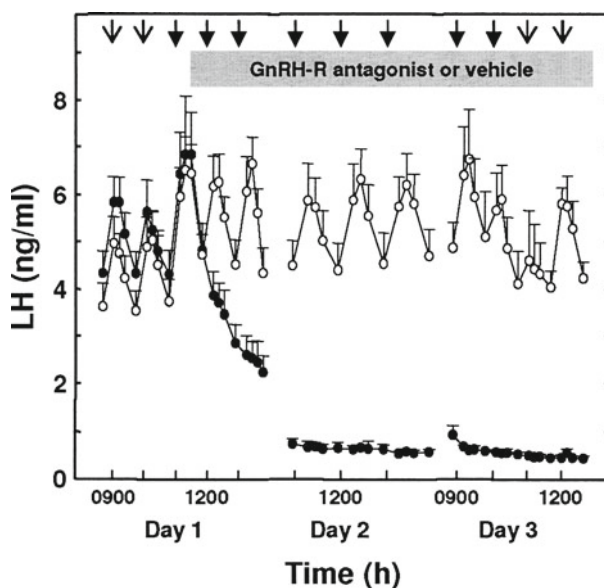


Fig. 2. Sustained pulsatile luteinizing hormone (LH) release induced in agonadal juvenile male monkeys by an intermittent i.v. infusion of human metastin 45–54 ($2\mu\text{g}/\text{animal}$ as a brief 1-min infusion every hour for 48 h) starting at 1100 hours on day 1 (*open data points*) was abolished by treatment with a gonadotropin-releasing hormone (GnRH)-receptor antagonist initiated immediately after the first pulse of the intermittent metastin infusion (*solid data points*). Although metastin was administered every hour, the LH response was tracked for only two or three pulses each day. Values are mean \pm SEM. The pituitary LH response to GnRH of the juveniles was enhanced before the experiment with a pulsatile infusion of synthetic GnRH ($0.15\mu\text{g}/\text{min}$ for 2 min every hour). The LH discharges in response to the last two priming pulses of GnRH at 0900 and 1000 hours on day 1 are shown, as are responses to reinitiation of GnRH priming at 1100 hours on day 3. *Solid arrows* indicate times of pulse infusions of metastin that were selected for tracking LH responses. *Line arrows* indicate times of GnRH pulse infusions. Reprinted with permission (43) and The Endocrine Society.

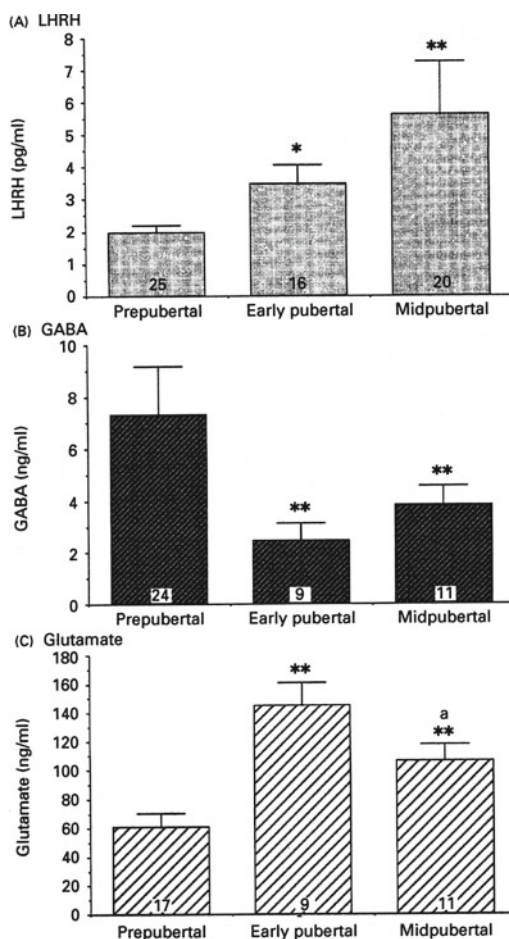


Fig. 3. Pubertal changes in hypothalamic release of gonadotropin-releasing hormone [GnRH (LHRH)], γ -aminobutyric acid (GABA), and glutamate in female rhesus monkeys as reflected in the concentration of GnRH (A), GABA (B), and glutamate (C) concentrations in “push-pull” perfusates of the hypothalamus of prepubertal, early pubertal, and mid-pubertal female rhesus monkeys. Note the pubertal resurgence in GnRH release is associated with an increase in glutamate release and a decrease in GABA release. Number of animals studied are indicated in individual bars. “*” and “**” indicate significantly different from prepubertal at $P < 0.05$ and $P < 0.01$, respectively. Reprinted with permission (48) and Blackwell Publishing. “a” indicates significantly different from early pubertal at $P < 0.05$.

By inference, a reduction in metastatin and glutamate tone would represent a major component of the neurobiological brake on GnRH release during juvenile development.

Turning now to inhibitory pathways, several lines of evidence indicate that neuropeptide Y (NPY) is an important component of the neurobiological brake restraining GnRH release during juvenile development (29). Central administration of this peptide inhibits pulsatile GnRH release in postpubertal monkeys (49–51), and changes in hypothalamic NPY expression and NPY content during the transition from the juvenile to pubertal phase of development are inversely related to those in pulsatile GnRH release (29). NPY has also been implicated in the stimulation of GnRH release

at the time of puberty (51–54), and the most parsimonious explanation to account for this paradox is to posit two subsets of NPY neurons (55). One subset comprises a component of the brake that holds the GnRH pulse generator in check during juvenile development. The other subset comprises a component of the GnRH pulse-generating neuronal network that is only manifest before and after the juvenile brake on the pulsatile GnRH release has been lifted.

γ -Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system, and studies of the female monkey by Terasawa's group has provided evidence that this amino acid plays an important role in the onset of primate puberty (56). The pubertal resurgence in GnRH secretion is temporally coupled to a decrease in hypothalamic GABA release (57), and inactivation of the GABA_A receptor or reduction of GABA tone in the prepubertal hypothalamus with either pharmacologic or molecular approaches elicited an immediate discharge of GnRH (57,58). Moreover, the pubertal decrease in hypothalamic GABA tone in the female is associated with, and may be the trigger for, an elevation in glutamate release at this stage of development (48) (Fig. 3). Finally, chronic repetitive GABA_A receptor inhibition in the juvenile leads to precocious puberty (59). Because the foregoing studies were conducted in ovarian intact animals, it is unclear whether GABAergic neurons represent a component of the fundamental neurobiological brake or the ovarian feedback mechanism that amplifies the restraint by the central brake (20). In this regard, there is no evidence for peripubertal changes in the expression of *GAD 65* and *GAD 67* in the male monkey (29,60), in which non-gonadal restraint is most profound.

Opioid peptides do not appear to be a major component of the neurobiological brake on GnRH release during juvenile development in the monkey. Administration of opioid antagonists such as naloxone and naltrexone has consistently failed to release the prepubertal brake on pulsatile GnRH release, and in the male, puberty appears to be associated with an up-regulation of the *POMC* gene in the hypothalamus (2).

Studies of the role of glial-derived growth factors, such as transforming growth factor α (TGF α), neuroregulins, and their cognate erbB transmembrane tyrosine kinase receptors in the control of puberty in the monkey are limited: TGF α mRNA in the hypothalamus of the female monkey is increased dramatically at the time of puberty (31), although in the gonadal male, expression of this gene is only marginally increased at a corresponding stage of hypothalamic development (29). Because hypothalamic lesions result in an astrocytic response that includes activation of TGF α and expression of erbB receptors (61,62), the precocity associated with hypothalamic lesions (63) may result from increased erbB signaling, rather than by interruption of inhibitory inputs to the GnRH network as was previously proposed (64). The actions of these growth factors on GnRH neurons are stimulatory and indirect, involving autocrine/paracrine signaling by factors such as prostaglandin estradiol (E₂) released from neighboring astrocytes (see chapter by Ojeda for further details).

Regardless of the relative importance of excitatory and inhibitory transynaptic and astroglial inputs that appear to underlie the pubertal resurgence of pulsatile GnRH release, it is not unreasonable to propose that this developmental event involves a remodeling of the neural substrate that governs the control of the neurons that release GnRH. This notion is consistent with the finding that markers of structural plasticity such as “embryonic” or polysialic acid neural cell adhesion molecule (PSA-NCAM)

are expressed in the mediobasal hypothalamus of the monkey (65). Indeed, quantitative ultrastructural studies have demonstrated that synaptic input to GnRH perikarya in this region of the hypothalamus declines in association with the pubertal resurgence in pulsatile GnRH release (66,67). Because thyroid hormone is critical for synaptogenesis, cell migration, and proliferation of glial cells during the late development of the fetal brain and is important for continued maturation of the postnatal brain, it is interesting to note that the induction of a hypothyroid state during juvenile development leads to a delay in the pubertal resurgence of GnRH release in the monkey (68).

Before closing the discussion of the neurobiological basis of the brake that holds pulsatile GnRH release in check from infancy to puberty, it should be noted that attention has focused almost exclusively on the resurgence in activity at the time of puberty. Of equal importance in the context of the timing of puberty in primates, however, is the mechanism responsible for timing the initiation of the restraint on pulsatile GnRH release during late infancy (69).

TIMING OF THE DEVELOPMENTAL SWITCHES RESPONSIBLE FOR THE ON-OFF-ON PATTERN OF PULSATILE GNRH RELEASE FROM BIRTH TO ADULTHOOD

As has been argued before, the characteristic pattern in hypothalamic GnRH pulsatility from birth to adulthood must be governed by one of the two general control systems (2). On the one hand, a central neural time-keeping mechanism that is capable of measuring age may be invoked, which would operate a gating system to interrupt GnRH pulsatility from infancy until the onset of puberty. On the other hand, gating for the developmental pattern of GnRH release could be achieved by a central neural growth tracking mechanism, a somatometer, that would co-ordinate the initiation of the pubertal resurgence in GnRH release with the pending attainment of an adult body size. The most compelling example of the somatometer notion is embodied in the earlier ideas of Frisch and her colleagues (70,71), who posited that the onset of puberty in girls is triggered by the attainment of a critical body weight or body composition. In this regard, circulating levels of the adipocyte hormone, leptin, have been reported in the female monkey to be greater at the initiation of gonadarche (approximately 24 months of age) than those observed at 14 months of age (72). In the male monkey, however, the relationship between the onset of testosterone secretion and plasma leptin levels is unremarkable (73) and suggests that gonadarche in the male is triggered in the absence of a rise in circulating leptin. Daily administration of recombinant human leptin in female monkeys, starting at 1 year of age, was associated with increased circulating LH concentrations at 14 months of age (72), and menarche was advanced by approximately 2 months. The mechanisms underlying this leptin associated premature amplification of endocrine activity in the pituitary-ovarian axis, however, are unclear. In a simpler experiment, a continuous 16-day intravenous infusion of recombinant human leptin to juvenile males did not trigger precocious activation of pulsatile GnRH release (74). Taking these findings together with those in humans and rodents, it seems reasonable to conclude that leptin, although obligatory for puberty to unfold, does not provide the signal that times the resurgence of pulsatile GnRH release and, therefore, is not the long sought after cue that terminates the prepubertal state (73).

The notion of a causal relationship between the neuroendocrine axis governing growth and that regulating gonadarche has been examined extensively in the female monkey by Wilson and his colleagues (75). They conclude that growth hormone (GH) is not involved in the timing of the pubertal resurgence in pulsatile GnRH release but does govern the tempo of pubertal development following activation of the critical neurobiological event triggering increased GnRH release. One mechanism whereby GH accelerates the tempo of pubertal development in the female appears to involve an action of insulin-like growth factor-1 (IGF-1) at the hypothalamic and/or pituitary level to impair the negative feedback action of E_2 secreted by the pubertal ovary on gonadotropin secretion (76).

OTHER CONSIDERATIONS

As in other mammalian species, multiple genes are likely to modulate the onset and tempo of non-human primate puberty, which represents a complex developmental process (77). Our understanding of the role played by genetics in regulating the timing of gonadarche in primates, however, comes primarily from studies of human puberty (3). Nevertheless, it is appropriate to note here that identification of genes that influence the timing of puberty may not necessarily contribute to our understanding of the basic biology of the neurobiological brake that holds pulsatile GnRH release in check during juvenile development (2). As previously argued (2), the term “puberty” gene should be restricted, in the case of the monkey and other higher primates, to those genes that are specifically involved in timing gonadarche and not used to describe those that are necessary in a permissive sense for this developmental event to unfold. Such putative “puberty” genes might dictate the resurgence of pulsatile GnRH release not only by triggering a hypothalamic signal at puberty but also by determining the duration of the prepubertal brake on pulsatile GnRH release or by gating the “turn off” of the GnRH release during infancy. The most likely, although theoretically not only, site for expression of such “puberty” genes would be the brain, and probably the hypothalamus.

Spontaneous disorders of pubertal development in non-human primates are essentially unreported (78) and, in contrast to the human situation, have not therefore contributed to our understanding of puberty in the monkey. However, the central precocity that may be observed in children because of early exposure to elevated androgen levels (3) may be reproduced experimentally in the monkey (79,80). Similarly, little information has been forthcoming from non-human primate models on the impact of fetal programming, post-natal nutrition and environment, and stress on the timing and tempo of the onset of puberty: areas of increasing contemporary interest. In the case of the latter, social dominance appears to be associated with earlier pubertal development in both male and female monkeys (81–83). The behavioral and neuroendocrine mechanisms responsible for this intriguing relationship between social dominance and the neuroendocrine axis governing reproductive function are unknown, but it is to be anticipated that modulation of the GnRH pulsatility is involved. Moreover, according to contemporary thinking social subordination before puberty represents one of several “pediatric” stresses (84), and as such, any modulation on the GnRH pulse generator would be the result of activation of the hypothalamic–pituitary–adrenal axis (85).

CONCLUSION

Puberty in the monkey, as in humans, may be viewed as manifesting itself because of the cascade of physiological and behavioral processes that are set in motion by the resurgence or reawakening of a pulsatile mode of hypothalamic GnRH secretion, extant during perinatal development but which has been held in check since infancy. The protracted brake on GnRH pulsatility from late infancy until puberty is imposed primarily by extragonadal mechanisms, although in the female and to a lesser extent in the male this is amplified by gonadal hormones. The molecular machinery of the GnRH neuron that is required for generating an adult pattern of pulsatile GnRH release appears to be extant throughout the juvenile (GnRH off) phase of development and may be precociously brought into play with surprising ease by experimentally activating GPR54 and glutamate receptor-signaling pathways within the hypothalamus prematurely. The pubertal activation of these and other upstream signaling pathways responsible for the pubertal resurgence in pulsatile GnRH release is associated with structural remodeling (plasticity) within the hypothalamus. The identity of the physiological control system that dictates the timing of the arrest and reawakening of the hypothalamic GnRH pulse generator during postnatal life, however, remains a fundamental mystery in developmental biology.

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Control of Puberty in Humans

Dennis M. Styne, MD
and Melvin M. Grumbach, MD

CONTENTS

INTRODUCTION
PATTERN OF GONADOTROPIN SECRETION
NEURAL CONTROL
TIMING AND ONSET OF PUBERTY
THE NEGATIVE FEEDBACK MECHANISM (GONADAL STEROID DEPENDENT)
“INTRINSIC” CNS INHIBITORY MECHANISM (GONADAL STEROID INDEPENDENT)
INTERACTION OF NEGATIVE FEEDBACK MECHANISM AND INTRINSIC CNS INHIBITORY MECHANISM
POTENTIAL COMPONENTS OF THE INTRINSIC CNS INHIBITORY MECHANISM
SLEEP-ASSOCIATED LH RELEASE AND ONSET OF PUBERTY
PITUITARY AND GONADAL SENSITIVITY TO TROPIC STIMULI
MATURATION OF POSITIVE FEEDBACK MECHANISM
CONCLUSIONS
REFERENCES

Summary

Puberty is a developmental milestone that involves the disinhibition or reaugmentation of the hypothalamic GnRH pulse generator and gonadotropin secretion. The hypothalamic GnRH pulse generator pituitary system in the human function during fetal life and early infancy is suppressed to a low level of activity during childhood (the juvenile pause) and is derepressed or reactivated during puberty. In this light, puberty does not represent the initiation or first occurrence of pulsatile secretion of GnRH or pituitary gonadotropins but the reactivation or disinhibition of GnRH neurosecretory neurons in the medial basal hypothalamus and the endogenous, apparently self sustaining, oscillatory secretion of GnRH after the period of quiescent activity during childhood. An increase in the pulsatile release of GnRH heralds the onset of puberty in the primate as well as other mammals. The CNS, and not the hypothalamic GnRH pulse generator, pituitary gland, gonads, or gonadal steroid target tissues, restrains activation of the hypothalamic pituitary gonadal system during the prepubertal years according to a large body of evidence. This

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inhibitory effect of the CNS appears to be mediated through the hypothalamus on the neurosecretory neurons that synthesize and secrete GnRH in a pulsatile manner.

Key Words: GnRH pulse generator; KiSS-1; GPR54; Leptin; LH; FSH.

INTRODUCTION

The neural component controlling gonadotropin secretion in human beings resides in the medial basal hypothalamus including the arcuate region (1). Its transducer gonadotropin-releasing hormone (GnRH) [or luteinizing hormone-releasing hormone (LHRH)] neurosecretory neurons, which are few in number (about 1500–2000 neurons), are dispersed and not segregated into a specific nucleus although they are functionally interconnected. The GnRH neurosecretory neurons become coordinated to comprise the GnRH pulse generator (the “final common pathway”) (2,3) that translates the neural signals into a periodic, oscillatory chemical signal, GnRH, which in turn drives and controls the pituitary-gonadal components. Pulses of GnRH are associated with episodic electrical activity within the hypothalamus of the same frequency (3–5). GnRH, a decapeptide, is synthesized as part of a larger precursor protein. The GnRH gene, which contains four exons and three introns (6), is located on the short arm of chromosome 8.

The GnRH neurosecretory neurons do not originate in the hypothalamus but rather migrate there in an orchestrated manner in early fetal life from the olfactory placode, in contrast to neurons containing other hypothalamic hormones [e.g., thyrotropin-releasing factor (TRF) or corticotrophin-releasing factor (CRF)] that arise in the hypothalamus itself. Thus, the distribution pattern of GnRH neurons is determined in the prenatal period, and the number of GnRH neurons, as well as the GnRH mRNA levels, does not change during pubertal development, judging from data from the non-human primate. Furthermore, the ability of the GnRH neuron to respond to electrical or neurochemical (e.g., glutaminergic and kisspeptinergic) stimuli does not change with pubertal development (7).

Aberrant migration of the GnRH neurons leads to delayed or absent pubertal development in several clinical situations. Kallmann syndrome 1 is due to a mutation in the KAL1 gene at Xp22.3 that codes for anosmin, a protein that has features of the extracellular matrix and encourages the outgrowth of axons from the olfactory bulb to the olfactory cortex (8). Kallmann syndrome 2 is due to a mutation in the autosomal fibroblast growth factor receptor 1 (FGFR1) gene at 8p11.2–p11.1, leading to haploinsufficiency of FGFR1. Anosmin-1 binds to heparin sulfate, which is important in the FGFR1-FGF system, and anosmin-1 and FGFR1 are coexpressed across normal development, suggesting interaction between these factors (9). Other postulated defects that might interfere with GnRH neuron migration are caused by mutations in the genes for neural cell adhesion molecules (NCAMs) and related proteins, such as tenascin, laminin, and phosphacan, as well as various glycoconjugates (10,11).

GnRH has been detected in human embryonic brain extracts by 4.5 weeks of gestation and in the fetal hypothalamus as well; furthermore, the fetal pituitary gonadotropes are responsive to GnRH (12). The hypothalamic–hypophyseal portal system is functional by 11.5 weeks of gestation (13,14). By 9 weeks, GnRH neurons are detectable

in the fetal hypothalamus, and by 16 weeks, axon fibers that contain GnRH are present in the median eminence and terminate in contact with capillaries of the portal system (12,15,16).

The GnRH neurosecretory neurons of the hypothalamic GnRH pulse generator exhibit spontaneous autorhythmicity (3,5,17,18), but the mechanism for the coordinated synchronous discharge of GnRH from neighboring but dispersed cells is uncertain. Current evidence (19) suggests that the autorhythmicity in the GnRH neurosecretory neurons (4,20,21) involves cAMP and cyclic nucleotide-gated cation channels associated with oscillatory increases in intracellular calcium, a hallmark of neurosecretion and gap junctional communication (22). In vitro studies suggest that the generation of the GnRH pulse is an intrinsic property of the GnRH neurosecretory neuronal network and that other factors modulate the fundamental autorhythmicity of the GnRH neuron (23–25). Cultured monkey GnRH neurons exhibit spontaneous pulsatile release of GnRH at a frequency similar to that observed in vivo, whereas patch-clamped primary GnRH neurons show disordered patterns of release that are sensitive to increased extracellular potassium. Firing activity can be stimulated by exposure to estrogen in a manner that appears to function through the estrogen receptor (26). The immortalized GnRH neuronal cell line contains neuronal nitric oxide (NO) synthase (27), and the NO generated by GnRH neurons may act as a neurotransmitter, an intercellular as well as intracellular messenger (28). Furthermore, GnRH acting as an autocrine factor may play a role in the synchronization mechanism (1,25).

GnRH synthesized in these hypothalamic neurons is released episodically from axon terminals at the median eminence into the primary plexus of the hypothalamic–hypophyseal portal circulation. The hormone is then transported by the portal vessels to the anterior pituitary gland. GnRH is essential for the release of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The amplitude and frequency of the pulsatile GnRH signal are modified by serotonergic and catecholaminergic neurons, through their effect on hypothalamic norepinephrine, dopamine, serotonin, opioid peptide, neuropeptide Y (NPY), leptin, galanin, corticotropin-releasing hormone, [gamma]-aminobutyric acid (GABA), and excitatory amino acid neuronal networks (29–35). In addition to these transsynaptic signals, astroglial cells may facilitate GnRH release through growth factor cell–cell signaling including the epidermal growth factor receptor (EGFR, also known as ErbB1) and its relatives. Thus, the hypothalamic–pituitary gonadotropin unit is influenced not only by gonadal steroids, inhibin, activin, and follistatin (36,37) but also by complex neural influences that integrate various intrinsic stimuli and environmental factors and cues.

In the mouse, in vitro and in vivo studies, using the GnRH green fluorescent protein model, demonstrate an increase in dendritic and somal spines in GnRH neurons of adult mice compared with those of juveniles, suggesting an increase in direct excitatory inputs to GnRH neurons across the time of puberty (38). This suggests increased glutamatergic stimulation of GnRH neurons with puberty (39). In the mouse, GABA_A receptor signaling occurs in the fetus, whereas glutamate signaling is detected only after birth. In the mouse, a switch from depolarizing to hyperpolarizing activity occurs only at the time of puberty in GnRH neurons (39).

Recent description of the role of KiSS-1, a human metastasis suppressor gene at 19p13.3, in pubertal development led to a flurry of subsequent investigations. KiSS-1

mRNA is found in the placenta, testes, pancreas, liver, small intestine, and brain, mainly in hypothalamus and basal ganglia (40,41). The product of the KiSS-1 gene is a 145-amino acid peptide, but the secreted product of the KiSS-1 gene is a 54-amino acid peptide known as metastin or kisspeptin that binds to an endogenous receptor GPR54. Spontaneous mutations in the GPR54 gene are very rare but instructive in elucidating the role of KiSS-1 in pubertal development. A consanguineous kindred with idiopathic hypogonadotropic hypogonadism (IHH) had a homozygous deletion of 155 nucleotides in the GPR54 gene encompassing the splicing acceptor site of intron 4–exon 5 junction and part of exon 5 in all affected family members (42). The deletion was present in only one allele and, not or absent in unaffected family members. Another Saudi Arabian kindred had an L148S mutation in GPR54, whereas another subject had two separate mutations, R331X and X399R, in the GPR54 gene (43). The latter patient had decreased amplitude of LH and FSH peaks but increased responsiveness to exogenous GnRH administration. Recently, more mutations have been described: of 30 normoosmic subjects with hypogonadotropic hypogonadism, one had two defects, a missense mutation in GPR54: cysteine 223 to arginine (C223R) in the fifth trans-membrane helix and an arginine 297 to leucine (R297L) in the third extracellular loop—the former had no activity and the latter manifested mildly decreased signaling ability (44).

Kisspeptin is the endogenous agonist for GPR54 (metastin receptor), a G protein-coupled receptor of the rhodopsin family. The receptor has a 45% homology with galanin receptors, but GPR54 does not bind galanin (42,45). GPR54 is highly conserved across species, with a 95% homology between the human receptor and those in mice (82%) and rats (95%). This receptor protein is found in the brain, mainly in the hypothalamus and basal ganglia, and in the placenta from where it was first isolated and sequenced. KiSS-1 mRNA is present in the primate in the medial arcuate nucleus and in the mouse in the arcuate, periventricular, and anteroventral periventricular nuclei (AVPVs) and in the regions important in reproductive function (46,47).

GPR54 coexpresses within GnRH neurons in the rat, in the medial and lateral sections of the arcuate nucleus and the ventral aspect of the ventromedial hypothalamus in mice (11) and primates (46). As GPR54 is also located in areas not involved in reproduction, it quite likely has other functions. Although the expression of GPR54 mRNA does not increase with development in the mouse, kisspeptin mRNA increases dramatically in the AVPV and the number of receptors responsive to kisspeptin increases with development in the mouse (48). The activation of GnRH neurons by kisspeptin at puberty in the mouse reflects a dual process involving an increase in kisspeptin input from the AVPV and a posttranscriptional change in GPR54 signaling within the GnRH neuron.

Animal studies support the role of KiSS-1 in pubertal development although data from rodents may differ from those from primates, and primate studies may not translate directly into relevance for human physiology. Mice transfected with mutant GPR54 genes exhibited hypogonadotropic hypogonadism although they had normal content of GnRH in their hypothalamus (43). Furthermore, they were responsive to GnRH or gonadotropin administration, suggesting normal function of the gonadotrope GnRH receptors and the gonadal LH and FSH receptors despite the mutation. The intact mouse also releases significant LH boluses after kisspeptin administration, an

effect that is abolished in the GPR54^{-/-} mouse that lacks the receptor (11). This demonstrates the importance of GPR54 and kisspeptin in the control of gonadotropin secretion.

Administration of kisspeptin-54 into the rostral preoptic area (RPOA), medial preoptic area (MPOA), paraventricular nucleus (PVN), and arcuate nuclei of the hypothalamus of male adult rats increased plasma LH and testosterone substantially (49). Furthermore, intracerebral kisspeptin administration to intact male rats stimulates the release of FSH, albeit at a far higher dose than needed to stimulate LH release (50); the release of FSH is abolished with the blockade of GnRH, demonstrating the necessary link of GnRH in the central actions of kisspeptin in the rodent. In the rat, the mRNA for kisspeptin and its receptor increases at puberty, and the administration of intracerebral injections of kisspeptin in prepubertal female rats caused large peaks of LH and advanced vaginal opening as a sign of pubertal development as well as premature ovulation (51,52). The ovulation elicited by peripheral kisspeptin in the prepubertal female rat is abolished by blocking GnRH (53). These data further support the role of kisspeptin and its receptor in reproductive function in the rat.

KiSS-1 and GPR54 mRNA expression are found in the region of the arcuate nucleus of the monkey as well as in rodents. KiSS-1 mRNA levels detected by real-time-polymerase chain reaction (PCR) increase with puberty in intact male and female monkeys: in intact females, GPR54 mRNA levels in the hypothalamus increased approximately three-fold from the juvenile to midpubertal stage but not in gonadal male monkeys. Administration of Kiss-1 through intracerebral catheters to GnRH-primed juvenile female rhesus monkeys stimulates GnRH release, but this release is abolished by infusion of a GnRH antagonist. These findings lead to a postulate that KiSS-1 signaling through the GPR54 receptor may be activated at the end of the juvenile pause and contribute to the pubertal resurgence of pulsatile GnRH release (46). This process may have significant relevance to the physiology of puberty in the human being. Remarkably, just as continuous infusion of GnRH will suppress GnRH release, continuous infusion of kisspeptin decreases the response of gonadotropes in the gonadal male monkey to boluses of kisspeptin. However, the release of FSH and LH after a bolus of NMDA or GnRH was maintained, demonstrating that the desensitization of the GPR54 receptors was selective to kisspeptin administration (54). This down-sensitization of the GPR54 receptors after continuous kisspeptin infusion may have a therapeutic function in central precocious puberty in the future just as GnRH agonists are used at present.

GPR54 mRNA is expressed in the pituitary gland, and there is evidence that kisspeptin can act directly on the gonadotrope (55) to cause LH secretion (56). In the sheep, kisspeptin colocalizes to a high proportion of GnRH receptor cells in the preoptic area, as well as various neuronal fibers within the external neurosecretory zone of the median eminence. This raises the possibility that both kisspeptin and GnRH are secreted into the pituitary portal system to affect the pituitary gland (57). There are also kisspeptin cells in the preoptic area, and the number rises with ovariectomy. Thus, spontaneous mutations in human beings augmented with studies of various animal species suggest that kisspeptin, acting through the GPR54 receptor, stimulates increasing amplitude, not the frequency of GnRH secretion.

PATTERN OF GONADOTROPIN SECRETION

There are two pulsatile secretory patterns of gonadotropins: tonic and cyclic. Tonic, or basal, secretion is regulated by a negative, or inhibitory, feedback mechanism in which changes in the concentration of circulating gonadal steroids and inhibin result in reciprocal changes in the secretion of pituitary gonadotropins. This is the pattern of secretion in the male and one of the control mechanisms in the female.

Cyclic secretion involves a positive, or stimulatory, feedback mechanism in which an increase in circulating estrogens, to a critical level and of sufficient duration, initiates the synchronous release of LH and FSH (the preovulatory LH surge) that is characteristic of the normal adult woman before menopause.

The secretion of FSH and LH is always pulsatile or episodic, whether the pattern is tonic or cyclic and regardless of age (i.e., in the fetus, infant, or child, during puberty, or in the adult) (13,19,58). Recent advances in ultrasensitive assays for serum gonadotropins demonstrate the low level of activity of the hypothalamic–pituitary axis (59–63) and the striking changes in the peripubertal period and during puberty. Although GnRH stimulates the release of both FSH and LH, the pulsatile secretion of immunoreactive FSH in normal adults is less prominent; this discordance in FSH and LH pulses is attributed in part to the longer half-life of FSH than LH, to differences in the factors that modulate the action of GnRH on FSH and LH release by the gonadotropes (especially gonadal steroids, inhibin, and possibly activin and follistatin), and to intrinsic differences in the secretory pattern of the two gonadotropins. For example, a change in the frequency of GnRH pulses can modify the ratio of FSH to LH released (2,64–67); midfollicular phase concentrations of estradiol (E_2) and adult male concentrations of plasma testosterone have a greater inhibitory effect on the response of FSH than on that of LH to pulsatile injections of GnRH (64,65,68).

The inherent oscillatory characteristic of gonadotropin secretion is a consequence of the pulsatile release of GnRH. However, the physiological significance of the episodic, rhythmic pattern of gonadotropin secretion was unclear until the classic studies of the rhesus monkey by Knobil and associates (2,3,69) revealed the essential nature of a periodic, oscillatory GnRH signal for the regulation of gonadotropin secretion and the inhibition of gonadotropin secretion that results from the continuous infusion of GnRH because of desensitization of GnRH receptors on the gonadotrope (70–72). This phenomenon is used to treat central precocious puberty with superactive GnRH agonists that act as a constant infusion of GnRH. On the contrary, intermittent, or pulsatile, administration of GnRH restored pulsatile release of LH and FSH in adult monkeys in which hypothalamic lesions obliterated the arcuate nucleus region and thus eliminated endogenous GnRH secretion (2,69). Pulsatile GnRH administration also re-established gonadotropin secretion in animals in which gonadotropin secretion had presumably been suppressed by the continuous infusion of GnRH. These studies provided evidence that the GnRH signal to the pituitary gonadotropes of the adult is frequency coded. Therapeutic pulsatile administration of natural GnRH has made possible the induction of ovarian or testicular maturation, including fertility, in patients with hypothalamic hypogonadism. The suppression of gonadotropin secretion is also possible by long-acting, potent GnRH analogs in boys and girls with true or central precocious puberty (73).

The human fetal gonad is affected first by placental gonadotropins and then by fetal pituitary FSH and LH [reviewed in (16)]. Fetal Leydig cells are unique in structure and function, but they regress to later be followed by the differentiation of adult-type Leydig cells (16,74–77). FSH and LH are detectable in the human fetal pituitary gland by 10 weeks of gestation and can secrete these hormones by 11–12 weeks; the content of FSH and LH increases until approximately 25–29 weeks of gestation (12,16,78,79). The fetal serum LH and FSH concentrations rise to peak levels by midgestation and then decrease; the values in umbilical venous blood at term are low. Because the hypothalamus in fetal sheep secretes GnRH in a pulsatile manner (80,81), the available data are compatible with the development of a human fetal hypothalamic GnRH pulse generator by at least the end of the first trimester. Consistent with this sequence of events, *in vitro* studies indicate that the human fetal pituitary gland is responsive to GnRH as early as 10 weeks of gestation (82); the GnRH-stimulated release of LH is greater in second-trimester fetal pituitary cells cultured from females than from males and is augmented by E_2 in both sexes (83). *In vivo* studies (84) during middle and late gestation demonstrate the stimulating action of exogenous GnRH on fetal FSH and LH release by 16 weeks of gestation with a striking sex difference in the FSH response and a fall in responsivity to GnRH in late gestation. The mean FSH and LH content of fetal pituitary glands and the concentration of fetal serum FSH are greater in female than in male fetuses at midgestation related to the higher concentration of plasma testosterone between 11 and 24 weeks in the male fetus (this, the only major difference in gonadal steroids between the male and female fetus, is due to the functional fetal testes) and fetal testicular inhibin (16). The decrease in both serum FSH and LH concentrations during late gestation has been attributed to the maturation of the negative feedback mechanism, the development of gonadal steroid receptors in the hypothalamic–pituitary unit (12,78,85) and the effect of inhibin (16,86).

The pattern of changes in FSH and LH concentration in the fetal pituitary glands and serum is consistent with a sequence of increasing synthesis and secretion due to relatively autonomous, unrestrained activity of the fetal hypothalamic GnRH pulse generator. This is followed by a decline after midgestation due to maturation of the negative feedback mechanism with increasing sensitivity of the hypothalamus and its GnRH pulse generator to the inhibitory effects of the high concentration of sex steroids, so that the hypothalamus secretes less GnRH (85) that persists to term (12,15,16). The increasing central nervous system (CNS) control of gonadotropin secretion seems to require the maturation of gonadal steroid receptors (intracellular or on the cell surface or both) in the fetal hypothalamus and in the pituitary gonadotropes (87).

In both sexes, the concentration of plasma FSH and LH is low in cord blood as a consequence of the inhibitory effect of the high levels of placental-derived estrogens. However, the hypothalamic regulatory mechanisms for pituitary gonadotropins, as for other pituitary hormones, are not fully developed at birth (15), and within minutes after birth in the male neonate, the concentration of LH increases abruptly followed by an increase in serum testosterone concentration during the first 3 h that persists for 12 h or more (88). FSH pulse amplitude is much greater in the female infant and is associated with a larger FSH response to GnRH throughout childhood, whereas LH pulses are of greater magnitude in the male. This striking sex difference is also present in agonadal male and female infants (15,89) and in infant rhesus monkeys (90,91). It is postulated

that this sex difference in the pattern of gonadotropin secretion in infancy is related to the effect of testosterone in the male fetus on the development and function of the hypothalamic–pituitary apparatus (16). The elevated gonadotropin concentrations are associated with a proliferation of Sertoli cells and gonocytes [and their transformation into spermatogonia (92)], a transient second wave of differentiation of fetal-type Leydig cells, and increased serum testosterone levels in male infants during the first few postnatal months (77) and increased E_2 levels intermittently elevated during the first year of life and part of the second year in females (93,94). The mean FSH concentration is higher in females than in males during the first few years of life. By approximately 6 months of age in the male and 2 to 3 years of age in the female, the concentration of plasma gonadotropins decreases to the low levels that are present until the onset of puberty. Thus, the restrained activity of the hypothalamic GnRH pulse generator and the suppression of pulsatile GnRH secretion (and thus LH release) do not attain the prepubertal level of quiescence until late infancy in boys and early childhood in girls (15,95).

NEURAL CONTROL

The neural control of puberty involves two major factors: the timing of puberty and the mechanisms involved in the control of the transition from the prepubertal, or sexually infantile state, through complete sexual maturation. The period of pubertal development is a time of remarkable changes in the anatomy of the CNS. The changes in cognition, emotion, and behavior during this time are accompanied by changes in brain morphology demonstrated by magnetic resonance imaging (MRI) scans. Total brain size peaks in girls at 11.5 years and in boys at 14.5 years (96). Gray matter, due to a process of synaptic pruning, follows a “U” shaped curve of increase until age 6 when 95% of maximal volume is reached followed by a loss in gray matter density later in puberty. White matter, however, increases because of an increase in myelination during development. Recent longitudinal studies utilizing dynamic mapping of human cortical development demonstrate that higher-order association cortices [e.g., those involved in executive function, attention, and motor coordination] mature after lower-order somatosensory, motor, and visual cortices mature and those areas phylogenetically older mature before new ones (97,98)]. Such changes in brain development may explain why some diseases, for example, depression or schizophrenia, may first manifest during puberty. Studies from experimental animals shed light on the influences of brain development at puberty; for example, evidence from the Syrian hamster demonstrates the effects of gonadal steroids on sexual differentiation of the perinatal brain setting the stage for pubertal refinement of structural and functional organization of the brain leading to adult responses to gonadal hormones (99). However, it is not yet possible to determine the effects of endocrine changes of puberty on brain remodeling or vice versa in human beings although studies on the subject are underway (100).

TIMING AND ONSET OF PUBERTY

The time of onset of puberty and its course are influenced by many factors. Clear genetic influences are reflected in the monogenetic disorders noted on pages 46 and 52 such as *KaL1* or *GPR54* mutations that can prevent pubertal development.

But genetic traits appear to influence the time of pubertal development in a complex manner that is now being analyzed by study of linkage analysis (quantitative trait loci are shown to relate to the age of menarche) (see Chapter 4) (101) and large-scale haplotype-based association studies. However, early data suggest that variation in GnRH1 and GnRH receptor proteins does not seem to be related to pubertal onset in a significant manner (102). Pedigree analyses have revealed relative risks of delay in puberty in kindreds with histories of constitutional delay compared with those without; for example, for first-degree relatives, the relative risk for a 2 SD delay in the onset of puberty is 4.8 fold increased (103). If KiSS-1 is truly a switch that turns on the pubertal process, a central question remains as to what influences KiSS physiology.

Genetic influences are postulated to exert between 50 and 74% of the influence on the age of pubertal onset with environmental factors operating through the CNS, including socioeconomic factors, nutrition, general health, geography, and possibly altitude accounting for much of the rest. Little is known about the effect of gene interactions (epistaxis) on this paradigm of complex traits, but it is an area of increased study (104–110).

Nutrition is an important factor in the timing of puberty as demonstrated by the earlier age of menarche in moderately obese girls (111), delayed menarche in states of malnutrition and chronic disease and following early rigorous athletic or ballet training, and the relationship of weight and diminished body fat to decreased gonadotropin secretion and amenorrhea in girls with anorexia nervosa (112), voluntary weight loss, and strenuous physical conditioning (113,114). Nutritional effects have changed the role of the pubertal individual to their society in the developed world. Early in the history of Homo Sapiens, in a simpler world, psychosocial maturation occurred at an age close to that of reproductive maturity. Later, in the developing world with increased population, the advent of agriculture and the growth of cities and later urban centers, menarche occurred later and the complexity of life led to a delay in the attainment of an adult role in society. Now, with improved nutritional status and improved health, the age of menarche decreased but the age of social adulthood remains later causing a discrepancy that may never have occurred before in human history (115). Further, a tendency towards earlier puberty in SGA babies added to the tendency for catch up growth to further advance the age of puberty becomes all the more significant in children from disadvantaged backgrounds raised in more affluent developed countries who are now reported with central precocious puberty. Although there are substantial changes in weight and body composition in early puberty and premenarche in girls (116,117), an early theory that postulated an “invariant mean weight” (48 kg) or degree of adiposity for the initiation of puberty (118) generated considerable controversy (119–123) and has not been substantiated by direct measurements (124–126). However, a longitudinal study of 469 girls followed for 5 years did demonstrate that a similar percentage of body fat was associated with the onset of puberty at different chronological ages (127).

The theory that weight or body composition affects the CNS restraints on pubertal onset and progression (128–130) appeared to gain support with the discovery of the genes encoding leptin, an adipocyte afferent satiety factor acting on the hypothalamus to suppress appetite (131–137), and the leptin receptor (138). Leptin is a highly conserved

167-amino acid cytokine-like protein produced mainly, but not exclusively, by adipose tissue. Its receptor, a member of the gp family of cytokine receptors, occurs in several isoforms. In the hypothalamus and other areas of the brain, only the leptin receptor splice variant with the long intracellular domain (Ob-Rb) contains the protein motifs that possess signaling properties. In the human, leptin circulates in both a free and a high-molecular-weight bound form (139). Leptin is secreted in a pulsatile manner (140) and exhibits a diurnal rhythm with a peak at night and a nadir in the morning (131,141,142). Leptin reflects body fat and hence energy stores and has an important role in the control of body weight and the regulation of metabolism (135,137,141). Well-designed studies reveal that leptin is not a metabolic trigger for the onset of puberty in the rodent but is one among several permissive factors (129,143).

In the male rhesus monkey, leptin levels were similar during the advancement of prepuberty to puberty (144,145). In a 3-year-old to 5-year-old peripubertal rhesus monkey fasted for 2 days, the administration of leptin prevented the decrease in plasma gonadotropins detected in the untreated animals (146). However, continuous infusion of leptin into the lateral ventricle of gonadal male monkeys failed to evoke an increase in GnRH on gonadotropin secretion (160).

Although an early longitudinal study in normal boys suggested that there was a brief rise in circulating leptin at the onset of puberty (147), two subsequent large cross-sectional studies (148,149) and a longitudinal study (150) of serum leptin levels in prepubertal and pubertal boys and girls showed leptin to increase gradually during the prepubertal years. During puberty, leptin continues to rise in girls, whereas in boys, leptin levels peak at Tanner stage 2 (148,149,151) and decreased to prepubertal concentrations by genital stage 5. The decrease is attributed to the effect of testosterone on leptin secretion (152,153).

Leptin binding to the high-affinity binding protein (the soluble leptin receptor) in serum is highest in childhood and decreases to relatively low levels during puberty and remains stable in the adult. It is postulated that the decrease in leptin binding activity in serum reflects a reduction in expression of truncated leptin receptors, thereby allowing leptin to bind to the full-length receptor, which is the form that transmits the biological signal for leptin (154). A study of 132 monozygotic female twin pairs and 48 dizygotic female twin pairs demonstrated a rise in leptin throughout puberty and confirmed a decrease in the soluble leptin receptor between stages 1 and 2 leading to a rise in the free leptin index between stages 1 and 2: there was a greater heritability to the soluble leptin receptor than leptin (155). The soluble leptin receptor appears to be higher in males than in females and is inversely related to leptin levels later in development in females (156). Free leptin is postulated to have more relevance to reproductive development than total circulating leptin levels (150).

Individuals with a homozygous mutation in the leptin gene (157) or the leptin receptor (158) not only have morbid obesity but have a striking delay in puberty owing to hypogonadotropic hypogonadism. A 9-year-old girl with congenital leptin deficiency following treatment with recombinant leptin lost weight and had an early pubertal pattern of LH release to the administration of GnRH, whereas there was no such pulsatile release before leptin treatment despite an advanced bone age of 13 years (159). These mutations indicate that the virtual absence of leptin or a functional leptin receptor leads to sexual infantilism due to hypogonadotropic hypogonadism, similar to

that in ob/ob and db/db mice. Remarkably, when leptin was administered to a 4-year-old male relative of this 9-year-old girl, he benefited from the metabolic improvement but did not undergo early pubertal development, indicating the permissive nature of leptin on puberty (160).

Serum leptin rises after the administration of pulsatile GnRH for 36 h but not after a single dose of Buserelin (GnRH agonist), suggesting again that rather than leptin triggering puberty, the pubertal increase in GnRH pulsatility leads to a further increase in leptin secretion (161).

These observations suggest that although a critical level of leptin and a leptin signal is required to achieve puberty, a rise in leptin is not required to trigger or initiate puberty (150,162). Boys with constitutional delay in growth and puberty can enter puberty without an increase in circulating leptin; these boys have lower mean levels of leptin than expected for weight. Furthermore, in two women with congenital lipoatrophic diabetes (Berardinelli–Seip syndrome), which is associated with the absence of both subcutaneous and visceral adipose tissues, the severe hypoleptinemia did not lead to a delay in menarche. One of the women had three unaffected children (153).

A major quantitative locus on human chromosome 2 is linked to serum leptin levels and fat mass (163) (the structural gene that encodes leptin is located on chromosome 7) (132), an additional indication that various factors affect leptin. Furthermore, in considering puberty as an energy-dependent process, a group of 9-year-old to 10-year-old boys were followed for 18 months. During the months leading up to an increase in morning salivary testosterone concentrations, they had a relatively constant basal metabolic rate (BMR)/lean body mass (LBM) ratio but an increase in the ratio of BMR/total daily energy expenditure. The suggestion is that a subtle energy-dependent process is in play, possibly related to an increase in brain BMR as a secondary phenomenon at the initiation of puberty or that a central rise in BMR is a signal for the onset of puberty (164,165). Although there is no known relationship between leptin and leptin receptor gene polymorphisms and constitutional delay of puberty, the presence of a short allele of leptin was associated with heavier weight, but those who were thin and had significant bone age delay and an increased frequency of parental pubertal delay were less likely to have this leptin short allele (166). In sum, accumulating evidence supports the function of leptin as a permissive factor (tonic mediator) and not a trigger (phasic mediator) in the onset of human puberty (167,168).

Ghrelin is a gastric 28-amino acid peptide that is the natural ligand for the growth hormone secretagogue receptor but also serves as an orexogenic signal that is increased after food deprivation and is usually negatively correlated with body mass index (169). Ghrelin administration can delay pubertal development in rats because of gonadal and central effects, suggesting a link between malnutrition and the decrease in reproductive development or function (170). Polypeptide YY (PYY) is an anorexogenic intestinal hormone that may influence hypothalamic GnRH release. In the male castrate rhesus monkey, it may have a suppressive effect (171) although in the intact female rhesus it apparently has a stimulatory effect (172). Thus, the decrease in PYY with fasting may be the reason for a decrease in reproductive function in this state, and this process could affect pubertal development as well.

Fetal and neonatal growth affect puberty; longitudinal studies reveal that low birth weight and rapid weight gain in infancy may lead to tall childhood stature and early

pubertal development (173,174). The age of menarche is a reflection of the age of onset of puberty but is several years removed. However, longitudinal and cross-sectional studies of age of menarche demonstrate a decrease of 3 months in White girls and 5.5 months in US Black girls between 1960 and 1990 (175). There is also a decrease in the age of pubertal development in Mexican-American boys and girls over the same period. Although there may be a decrease in the age of puberty of the Black population in the United States as well, there is no apparent change in the age of breast development in White girls (176). Various European populations show less or no secular trends unless considering a group that is nutritionally deprived (177). Teasing out the various factors involved in secular trends will require further long-term study and new methodological approaches (178) (see Part II: Secular Trends and the Onset of Puberty).

Two interacting mechanisms have been proposed to explain the prepubertal restraint on gonadotropin secretion, known as the juvenile pause (15,179). One is a hypothalamic–pituitary–gonadal negative feedback system in infancy and early childhood that is exquisitely sensitive to small amounts of gonadal steroids. The other is a steroid-independent mechanism that involves “intrinsic” CNS inhibition of the GnRH pulse generator in the medial basal hypothalamus (15,179,179) that is dominant throughout the childhood juvenile pause.

THE NEGATIVE FEEDBACK MECHANISM (GONADAL STEROID DEPENDENT)

The principal evidence for an operative negative feedback mechanism in prepubertal children is as follows (15,180): the pituitary of the prepubertal child secretes small amounts of FSH and LH, suggesting that the hypothalamic–pituitary–gonadal complex is operative in childhood but at a low level of activity. In the absence of functional gonads in the prepubertal child, as in patients with the syndrome of gonadal dysgenesis or other congenital or postnatal gonadal deficiency, secretion of FSH and, to a lesser degree, LH is increased. The elevated gonadotropin levels in infancy and early childhood in patients with gonadal dysgenesis suggest that even low levels of hormones secreted by the normal prepubertal gonad inhibit gonadotropin secretion (15,94,179,181). This inhibition supports the hypothesis that a sensitive, functional, tonic, negative feedback mechanism is active in infants and prepubertal children (15,180). The low level of gonadotropin secretion in childhood is shut off by the administration of small amounts of gonadal steroids. The suppressive effect supports the idea that the hypothalamic–pituitary gonadotropin unit is highly sensitive to the feedback effect of gonadal steroids (15,182). The prepubertal hypothalamic–pituitary unit appears to be approximately 6–15 times more sensitive than the adult system (180).

“INTRINSIC” CNS INHIBITORY MECHANISM (GONADAL STEROID INDEPENDENT)

The diphasic pattern of basal and GnRH-induced FSH and LH secretion from infancy to adulthood is similar in normal individuals and in gonadal patients, but in the latter, gonadotropin concentrations are higher, except during the middle childhood nadir (179,181). The high concentration of plasma FSH and LH in gonadal children between infancy and about age of 4 and the increased gonadotropin reserve reflect the absence

of gonadal steroid inhibition of the hypothalamic–pituitary unit by the low levels of plasma gonadal steroids (15). However, the striking fall in gonadotropin secretion between ages 4 and 11, with or without gonadal function, suggests the presence of a CNS inhibitory mechanism that, independent of gonadal steroid secretion, restrains the hypothalamic GnRH pulse generator during this pause (15). Thus, a steroid-independent inhibitory mechanism for suppression of the hypothalamic GnRH pulse generator seems to be the dominant factor in the restraint on puberty between ages 4 and 11 (15,183). Gradual loss of this intrinsic CNS inhibitory mechanism would lead to disinhibition or reactivation of the GnRH pulse generator at puberty. This inhibition is attributed to the inhibitory effects of GABA in the female primate in the prepubertal period and increasing glutamate stimulation of LH release as the influence of GABA wanes during the progression through puberty (174).

INTERACTION OF NEGATIVE FEEDBACK MECHANISM AND INTRINSIC CNS INHIBITORY MECHANISM

During the first 2–3 years of life, the gonadal steroid negative feedback mechanism seems dominant, as evidenced by the striking difference in gonadotropin secretion between the agonal and the intact infant and young child. At about 3 years of age, the intrinsic CNS inhibitory mechanism becomes dominant and remains so during the rest of the juvenile pause, as evidenced by the fall in FSH and LH levels between ages 3 and 10 despite the lack of functional gonads (179,181). As puberty approaches, the CNS inhibitory mechanism gradually wanes, initially during nighttime sleep, and the hypothalamic GnRH pulse generator becomes less sensitive to gonadal steroid negative feedback (15). After the onset of puberty, gonadal steroid negative feedback attains the set point characteristic of the adult and is again the dominant mechanism in restraining gonadotropin secretion (along with inhibin), as reflected in the increased gonadotropin concentrations characteristic of the adolescent with severe primary hypogonadism. Many neural, neurotransmitter/neuromodulator, hormonal, growth factors and metabolic factors (184–190), as well as exteroceptive influences and cues, can influence the activity of the GnRH pulse generator (174). In the rhesus monkey, despite the damping of the GnRH pulse generator during the juvenile pause (191), the content of hypothalamic GnRH and the GnRH messenger RNA during this phase is similar to that in the infant and adult monkey. Low-amplitude LH and FSH pulses are detectable by sensitive and specific immunometric assays during prepuberty in human beings (192–197); however, the end of the juvenile pause is marked by an increase in LH pulse amplitude most evident during the early hours of sleep (192,193).

POTENTIAL COMPONENTS OF THE INTRINSIC CNS INHIBITORY MECHANISM

Indirect evidence for an inhibitory neural network that arises or projects through the posterior hypothalamus and suppresses the GnRH pulse generator has been derived from studies of children with organic forms of central precocious puberty (15) and studies in the female and male monkey (198–201). Children with central precocious puberty associated with posterior hypothalamic neoplasms (usually a pilocytic astrocytoma), radiation of the CNS, midline CNS developmental abnormalities, such as

septo-optic dysplasia with deficiency of one or more pituitary hormones, or other CNS lesions provide indirect evidence for an inhibitory neural component located in or projecting through the posterior hypothalamus. As a consequence of these lesions, the neural pathway inhibiting the hypothalamic GnRH pulse generator is compromised, resulting in its disinhibition and activation (15). For example, a suprasellar arachnoid cyst can cause central precocious puberty by compressing and distorting the hypothalamus (15), but decompression of the cyst may stop the pubertal development by reversing the disinhibition of the CNS inhibitory mechanism. The GnRH-secreting hypothalamic hamartoma, a heterotypic mass of nervous tissue that contains GnRH neurosecretory neurons (202,203) attached to the tuber cinereum or the floor of the third ventricle, can cause central precocious puberty (204) by secreting GnRH in pulsatile fashion. We consider the hypothalamic hamartoma as an "ectopic GnRH pulse generator" that functions independently of the CNS inhibitory mechanism that normally restrains the hypothalamic GnRH pulse generator (15,204). Some rare, large hypothalamic hamartomas that cause central precocious puberty contain few or no GnRH neurosecretory neurons but do contain transforming growth factor alpha (TGF- α). Although the secretion of TGF alpha may interact directly or indirectly to stimulate GnRH release, these tumors are so large that compression or destruction of tissue involved in the CNS suppression of gonadotropin secretion may still be the etiology of precocious puberty in these cases. Precocious sexual maturation can also be induced in the juvenile female rhesus monkey by posterior hypothalamic lesions (198,199).

The CNS control of puberty is accomplished by noradrenergic, dopaminergic, serotonergic, kisspeptidergic, and opioergic pathways; inhibitory neurotransmitters (e.g., GABA), excitatory amino acids (e.g., glutamic and aspartic acids), nitroergic transmitters, other brain peptides, including neurotrophic and growth factors, and corticotropin-releasing hormone affect the hypothalamic GnRH pulse generator (34,66,184–190,205–213). A critical and landmark advance in our understanding of the nature of the juvenile phase and central inhibition of the GnRH pulse generator was provided by the studies of GABA, the most important inhibitory neurotransmitter in the primate brain including the hypothalamus, by Terasawa and her colleagues (174,192,193,214–217), showing that there is a potent inhibition by GABA and GABAergic neurons of the GnRH pulse generator in prepuberty. Glutamic acid decarboxylase (GAD) is the enzyme that catalyzes the conversion of glutamate to GABA, and both classes of GAD, GAD 65 and GAD 67, are present in mammalian brain in the mediobasal hypothalamus, the site of the GnRH pulse generator. Decreased GABA action by the administration of antagonists or inhibitors advances puberty (214,217), providing additional support for GABA, arising from interneurons, as the major intrinsic CNS inhibitory neurotransmitter during the juvenile pause of prepuberty (215). The effect of GABA quite likely has a direct action on the GnRH pulse generator neuron (218) that contains GABA_A receptors (219,220). GABA is the principal inhibitory neurotransmitter in the juvenile and adult brain, but early in brain development extending (at least in the experimental animal) through the postnatal period, GABAergic synaptic transmission is excitatory and increases intracellular calcium concentrations (221,222).

A persistent question has been how a single central signal can activate GnRH neurons to cause LH release and bring about ovulation by simultaneous suppression of GABA

and stimulation of glutamate release that converge in the AVPV. Recently, it was reported that nearly all neurons in the AVPV of female rats express vesicular glutamate transporter 2 (VGLUT2), a marker of hypothalamic glutamatergic neurons, as well as GAD and vesicular GABA transporter (VGAT), markers of GABAergic neurons. These dual-phenotype neurons are twice as prevalent in females than in males and are the main targets of E2 in the region (223). Moreover, dual-phenotype synaptic terminals contact GnRH neurons, and at the time of the surge, VGAT-containing vesicles decrease and VGLUT2-containing vesicles increase in these terminals. The investigators proposed that dual-phenotype GABA/glutamate neurons act as central transducers of hormonal and neural signals to GnRH neurons. In addition, the stimulatory effects of kisspeptin on the GPR54 receptor serve as another promoter of gonadotropin secretion at puberty as described on page 52.

GABA, through the activation of the GABA_A receptor, a chloride ion channel member of the pentameric ligand-gated ion channels, exerts rapid inhibition that is transient from the brief exposure to GABA, referred to as phasic inhibition. Recently, GABA was shown to exert tonic inhibition of activity of supraoptic neurosecretory neurons, which is modulated by the activity of glial GABA transporters (GATs). These differing effects may be activated by differing sources of GABA, possibly being activated by “spillover of GABA” during strong or synchronous afferent activity. Furthermore, there are different GABA_A receptors demonstrating different pharmacological properties. It seems possible that the transition from the dominance of the gonadal steroid-dependent negative feedback mechanism in infancy and early childhood to the dominance of the intrinsic CNS inhibitory mechanism is associated with the developmental switch of GABAergic synaptic transmission from excitatory to inhibitory as one factor. Some of these newly discovered phenomena may help explain some of the complex effects of GABA on the CNS control of puberty. Furthermore, NPY has been suggested as a component of the central restraint mechanism (171), at least in the castrate male rhesus monkey.

Excitatory *N*-methyl-D,L-aspartate (NMDA) amino acid neurotransmitter receptors are widely distributed throughout the CNS including the hypothalamus (226). NMDA stimulates LH release in neonatal (227) and adult (228) rats, fetal sheep (207,208), and prepubertal (229) and adult (230) rhesus monkeys by direct effects on the hypothalamus but not the pituitary gland (207,231,232).

The onset of puberty in the rhesus monkey is characterized by a decrease in GABAergic (and possibly NPY) inhibition of the hypothalamic GnRH pulse generator and the increased release of glutamate (174,233), the major excitatory amino acid neurotransmitter in the hypothalamus (212,229,234). The sensitivity to the stimulatory glutaminergic input into the GnRH pulse generator increases strikingly after the onset of puberty (235), but it is the reduction in GABAergic inhibition that is a critical factor in the disinhibition of the GnRH pulse generator (174). Furthermore, the increase in the stimulatory influence of kisspeptin on the GPR54 receptor, as described on page 52, plays a role in the onset of puberty. Repetitive intravenous administration of NMDA in both the prepubertal and the pubertal female rhesus induced the release of GnRH (174,233,236). Immortalized GnRH neurons contain ionotropic NMDA receptors that mediate the release of GnRH by NMDA (237).

In sum, with the onset of puberty, the disinhibition and reactivation of the GnRH pulse generator is associated with a fall in GABAergic inhibitory neurotransmission and a concomitant increase in the input of excitatory amino acid neurotransmitters (e.g., glutamate and kisspeptin) as well as other neurotransmitters (e.g., norepinephrine and NPY). Ojeda and Ma (238) have proposed that in addition to the transsynaptic mechanisms, astrocytes regulate GnRH neurosecretion through astroglial-derived growth factors [including transforming growth factors, IGF-I, basic fibroblast growth factor (FGF), neuregulins, and epidermal growth factor (EGF)-related peptides] that act directly or indirectly to stimulate GnRH release and, accordingly, may contribute to the reactivation of the GnRH pulse generator at puberty (238–242) (see Chapter 1). The GnRH pulse generator, the anterior pituitary gland as well as the gonads, and the gonadal steroid-dependent end organs are functionally intact in prepuberty (as well as in the fetus) and can be fully activated before puberty by the appropriate stimulus. Hence, the CNS restraint on puberty lies above the level of the autorhythmic GnRH neurosecretory neurons in the hypothalamus.

SLEEP-ASSOCIATED LH RELEASE AND ONSET OF PUBERTY

In pubertal children, Boyar and colleagues (243) described mainly the sleep-associated pulsatile release of LH in early and midpuberty; only in late puberty were prominent LH-secretory episodes detected during the day. Kulin and co-workers (244) also noted significantly increased excretion of urinary LH in prepubertal children at night versus during the day, although the absolute differences were small. In late puberty, the daytime LH pulses increase in amplitude but are still less than during sleep until the adult pattern is finally achieved. In boys, augmented LH release during sleep leads to increased testosterone secretion and a rise in the plasma concentration of testosterone at night (245). This pattern of sleep-associated LH secretion occurs in gonadal patients during the pubertal age period (243), suggesting that it is not dependent on gonadal function. Furthermore, augmented sleep-related gonadotropin release is demonstrable in children with idiopathic central precocious puberty (246) and in glucocorticoid-treated children with congenital adrenal hyperplasia who have an advanced bone age and an early onset of true puberty. Thus, sleep-enhanced LH secretion can be viewed as a maturational phenomenon related to changes in the CNS and in the hypothalamic restraint on GnRH release. Sleep-associated LH release in the peripubertal period correlates with the increased sensitivity of the pituitary gonadotropes to the administration of GnRH in the peripubertal period and puberty. The increased LH release at night in both sexes is evidence that the hypothalamic GnRH pulse generator initially is less inhibited during sleep even in prepubertal children. The much augmented LH pulse amplitude entrained during sleep is the neuroendocrine hallmark of the onset of puberty.

PITUITARY AND GONADAL SENSITIVITY TO TROPIC STIMULI

The release of LH after the administration of GnRH is minimal in prepubertal children beyond infancy, increases during the peripubertal period and puberty (15, 180), and is still greater in adults (depending on the phase of the menstrual cycle in women) (247,248). Sex differences in LH and FSH response to GnRH suggest that the

pituitary gonadotropes of prepubertal females are more sensitive to GnRH than those of prepubertal males, although the concentration of circulating gonadal steroids is very low in both sexes at this stage of maturation. Prepubertal girls have a larger readily releasable pool of pituitary FSH than prepubertal or pubertal males, related at least in part to the higher concentration of inhibin B in prepubertal boys. The sex difference in sensitivity to GnRH and releasable FSH and the low inhibin levels in the prepubertal female may be factors in the higher frequency of idiopathic central precocious puberty in girls and in the occurrence of premature thelarche (249).

The responses to GnRH in peripubertal children who do not yet exhibit physical signs of sexual maturation provide evidence that the self-priming effect (180) of endogenous GnRH augments pituitary responsiveness to exogenous GnRH and is an important factor in the increased gonadotropin secretion at puberty. This change in responsiveness of the gonadotropes is apparently mediated by increased pulsatile secretion of GnRH (15,180); the increased LH response to synthetic GnRH is one of the earliest hormonal markers of puberty onset. The prepubertal pituitary gland has a smaller pool of releasable LH and decreased responsiveness to the acute administration of synthetic GnRH. With the approach of puberty, the reactivation of the hypothalamic GnRH pulse generator and the increased pulsatile secretion of GnRH augment pituitary sensitivity to GnRH and enlarge the reserve of LH. Furthermore, inhibin and endogenous gonadal steroids may also affect this ratio through their action on the hypothalamus, the pituitary gland, or both sites. Responsiveness of the gonads to gonadotropins also increases during puberty. For example, the augmented testosterone secretion in response to the administration of hCG at puberty in boys is probably a consequence of the priming effect of the increase in endogenous secretion of LH (in the presence of FSH) on the Leydig cell (250,251).

MATURATION OF POSITIVE FEEDBACK MECHANISM

In normal women, the midcycle surge in LH and FSH secretion is attributed to the positive feedback effect of an increased concentration of plasma E_2 for a sufficient length of time during the latter part of the follicular phase (2,247,252). E_2 has both negative and positive feedback effects on the hypothalamic–pituitary system. Although the suppressive effect is probably operative from late fetal life on, the positive action of endogenous (or exogenous) E_2 on gonadotropin release has not been demonstrated in normal prepubertal and early pubertal children (15,180,253). Hence, acquisition of positive feedback, a requisite for ovulation, is a late maturational event in puberty and, from the present evidence, probably does not occur before midpuberty in normal girls (15,180,253,254). Among the requirements for a positive feedback action of E_2 on gonadotropin release at puberty (180) are ovarian follicles primed by FSH to secrete sufficient E_2 to reach and maintain a critical level in the circulation, a pituitary gland that is sensitized to GnRH and contains a large enough pool of releasable LH to support an LH surge, and (controversial in the human, but not in lower animals) sufficient GnRH stores for the GnRH neurosecretory neurons to respond with an acute sustained increase in GnRH release in addition to the usual adult pattern of pulsatile GnRH secretion.

The main site of action of E_2 is at the level of the anterior pituitary, but estrogen has dual sites of action (255), including a negative as well as a positive feedback

action on the hypothalamus. E_2 has a positive feedback effect directly on the pituitary gland in normal women, and prolonged administration of E_2 is accompanied by an augmented LH response to GnRH (248). The fact that the major positive feedback action on the pituitary gland is demonstrable in the absence of an increase in pulsatile GnRH secretion suggests that the failure to elicit a positive feedback action with the administration of E_2 into prepubertal girls could be related to the inadequate GnRH pulses or insufficient LH reserve, respectively, or by both components.

We visualize the process leading to ovulation as a gradual one in which the ovary [the Zeitgeber for ovulation (2)] and the hypothalamic–pituitary gonadotropin complex become progressively more integrated and synchronous until, finally, an ovary primed for ovulation secretes sufficient E_2 to induce an ovulatory LH surge (95).

Studies of basal body temperature (256) and plasma progesterone concentrations (257,258) suggest that as many as 55–90% of cycles are anovulatory during the first 2 years after menarche and that the proportion decreases to less than 20% of cycles by 5 years after menarche. A cyclic surge of LH occurs during some anovulatory cycles in adolescence, but the mechanism of ovulation seems unstable and immature and does not appear to have attained the fine-tuning and synchronization requisite for the maintenance of regular ovulatory cycles.

CONCLUSIONS

Our understanding of the complex maturational processes of the hypothalamic–pituitary–gonadal system in the control of the onset of puberty is incomplete. Puberty is not an immutable process; it can be arrested or even reversed. Environmental factors and certain disorders that affect the onset or progression of puberty mediate their effects by direct or indirect suppression of the hypothalamic GnRH pulse generator and its periodic, oscillatory signal, GnRH.

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Genetic Regulation of the Variation in Pubertal Timing

Craig A. Hodges, PhD
and Mark R. Palmert, MD, PhD

CONTENTS

INTRODUCTION
CURRENT DATA ON THE GENETIC CONTROL OF PUBERTAL ONSET
APPROACHES TO IDENTIFYING GENETIC FACTORS: <i>HUMAN STUDIES</i>
APPROACHES TO IDENTIFYING GENETIC FACTORS: <i>ANIMAL MODELS</i>
CONCLUSIONS
REFERENCES

Summary

“The timing of pubertal onset among humans is controlled by both environmental and genetic factors. Although the environmental factors are important, the high heritability of pubertal timing suggests that at least half of the variability of this trait is due to genetic variation within the population. Thus, identification of the genetic factors that control this trait will enhance our understanding of pubertal development. New research strategies in both humans and animal models are facilitating the identification of these genes. In this chapter, we will discuss data supporting the heritability of pubertal timing, investigative approaches, and progress that has been made in identifying the genetic factors that regulate pubertal timing.”

Key Words: Puberty; Genetics; Genomics; Linkage; Chromosome substitution; Complex trait.

INTRODUCTION

Puberty is a major developmental milestone marking the transition from childhood to adolescence. This transition is accompanied by many physiologic changes (e.g., increases in hormone levels, initial production of mature gametes, and development of secondary sexual characteristics) as well as emotional, behavioral, and social changes. Understanding the regulation of the timing of pubertal onset is important to the understanding of this fundamental developmental process and to the understanding

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of abnormalities of pubertal timing such as early, delayed, or absent puberty [e.g., hypogonadotropic hypogonadism (HH)].

The onset of puberty results from a complicated cascade of events during which increased secretion of gonadotropin-releasing hormone from the hypothalamus causes increased secretion of gonadotropins from the pituitary, which in turn increases the production of gonadal sex steroids leading to sexual maturation (see Chapter 3). The regulation of the hypothalamic-pituitary-gonadal (HPG) axis and the timing of puberty is complex and affected by both physiologic and environmental factors; however, the timing of puberty is also highly heritable, suggesting that much of the natural variation observed in the age at pubertal onset is due to genetic variation within the population. In fact, the available data indicate that up to 50–80% of the variation in the timing of pubertal onset is due to genetic rather than environmental factors [reviewed in (1–3)]. Thus, genetic background likely explains much of why one young woman begins puberty at 9 years and another at 12 years. It is important to note that high heritability does not preclude a role for environmental influences that have changed over time and lead to significant historical effects on population means. For example, the secular trend toward earlier puberty may be a result of improved nutrition and health status (4). However, within an individual population at a given moment in time, much of the variation in the timing of puberty is the result of genetic rather than environmental factors.

Investigation of the genetic factors that regulate the variation in the age of pubertal onset within the general population is relatively recent. In this chapter, we will discuss data supporting the heritability of pubertal timing, investigative approaches, and progress that has been made in identifying the genetic factors that regulate pubertal timing.

CURRENT DATA ON THE GENETIC CONTROL OF PUBERTAL ONSET

Evidence regarding the genetic regulation of the timing of puberty in humans comes from several lines of research including population, family, and twin studies. Although confounded by socioeconomic influences, studies have repeatedly demonstrated that the timing of puberty varies among population groups (5–11). As an example, Herman-Giddens and colleagues conducted a study describing the development of secondary sexual characteristics (breast and pubic hair) and the occurrence of menses in American girls. They observed that African-American girls were significantly advanced in pubertal timing when compared with White girls (breast development 8.87 ± 1.93 vs. 9.96 ± 1.82 ; pubic hair development 8.78 ± 2.0 vs. 10.51 ± 1.67 ; and menarche 12.16 ± 1.21 vs. 12.88 ± 1.20 years) (9). Several more recent studies have used data from the third National Health and Nutrition Examination (NHANES III) survey completed in the United States, where Tanner staging was assessed in girls aged 8–19 years, to compare sexual maturation characteristics within different racial groups. Chumlea et al. (8) found that the NHANES III data also demonstrated that non-Hispanic Black girls had an earlier time of maturation than both non-Hispanic White and Mexican-American girls. Boys have also been compared using the NHANES III data, and once again, non-Hispanic Blacks had earlier maturation than non-Hispanic Whites and Mexican Americans (10,11). All of these studies provide evidence of the heritability of the timing of puberty by showing consistent differences among population groups.

Family studies have also contributed to our knowledge of the importance of genetic factors in regulating the timing of puberty. Within individual families, there is a strong correlation between ages at which parents and their children undergo pubertal development. The age of menarche between mothers and daughters is highly correlated (12–14), indicating that a mother’s age of menarche is a good predictor of her daughter’s age of menarche (15). Genetic contribution from mother and father has also been highlighted in a case series (16) and pedigree analysis (17) where constitutional delay of growth and maturation (CD) was found to cluster among primary and secondary relatives of both genders and often to derive from autosomal dominant inheritance with variable penetrance. Similar data have subsequently been reported for idiopathic precocious puberty (18).

Perhaps the strongest evidence for genetic factors affecting pubertal timing comes from twin studies. To determine whether a trait has a strong genetic component, twin studies compare phenotypic concordance among monozygotic twins (MZ, with identical genomes) with that seen among dizygotic twins [DZ, on average 50% genetically identical (same as regular siblings)]. Higher concordance rates among MZ versus DZ twins indicate a genetic component for the studied trait. Twin studies on pubertal development have shown that peak growth velocities, skeletal maturation, and the development of various secondary sexual characteristics (e.g., breast development, development of sexual hair, and voice change) are all more concordant among MZ than DZ twin pairs (19–23). The age of menarche has been the most extensively studied trait in girls likely because of the ease and relative reliability of characterizing the timing of its occurrence by recall. Multiple studies having shown a higher correlation among MZ than DZ twins (19,22–31) (Table 1). One interesting physiological study has also reported that serum gonadotropin levels [luteinizing hormone (LH) in both sexes and follicle-stimulating hormone (FSH) in girls] were more highly correlated among pubertal MZ than DZ twins, indicating that high heritability can even be demonstrated in the underlying hormonal changes responsible for pubertal development (32). In all, these various studies suggest that up to 50–80% of the variation in pubertal timing is genetically determined.

Table 1
Results of selected twin studies with respect to age of menarche

<i>Study</i>	<i>MZ correlation</i>	<i>DZ correlation</i>	<i>% genetic contribution</i> ¹
Eaves et al. (24) ²	.89	.64	96
Kaprio (25)	.75	.31	74
Kirk et al. (26)	.51	.17	50
Loesch et al. (23)	.95	.58	95
Meyer et al. (27)	.65	.18	71
Mustanski et al. (28) ²	.82	.53	88
Sharma (29)	.93	.55	84
Snieder et al. (30)	.61	.18	45
Treloar et al. (31)	.65	.18	61–68
Fischbein (19)	.93	.62	Not reported

¹This percentage is the estimated percentage of phenotypic variance resulting from genetic effects.
²These two studies used several pubertal development phenotypes including age of menarche for their estimate.

APPROACHES TO IDENTIFYING GENETIC FACTORS: HUMAN STUDIES

Complex genetic traits, such as the timing of pubertal onset, are a result of the interaction between genetic variants and environmental exposures (33) where a one-to-one relationship between genotype and phenotype does not exist (34). Unlike classical Mendelian traits, complex traits display measurable phenotypic variation that results from multigenic influences and are often characterized by a broad distribution of phenotypes within the general population (35). Although difficult, identifying genetic factors that affect susceptibility to common diseases and the regulation of complex traits would greatly strengthen our understanding of the underlying physiologic mechanisms involved with these diseases and traits.

To date, three major approaches have been used to find genes underlying complex traits in humans: resequencing of candidate genes, association studies, and genome-wide linkage analyses. The effectiveness of each of these strategies in identifying causal genetic variants depends on whether the variants are common (i.e., exist in the population with a frequency of at least $\sim 1\text{--}5\%$) and on penetrance (i.e., how likely carrying a particular variant is to affect the phenotype) (1). Because the frequency and penetrance of variants that modulate pubertal timing remain unknown, all three are viable strategies (*Fig. 1*).

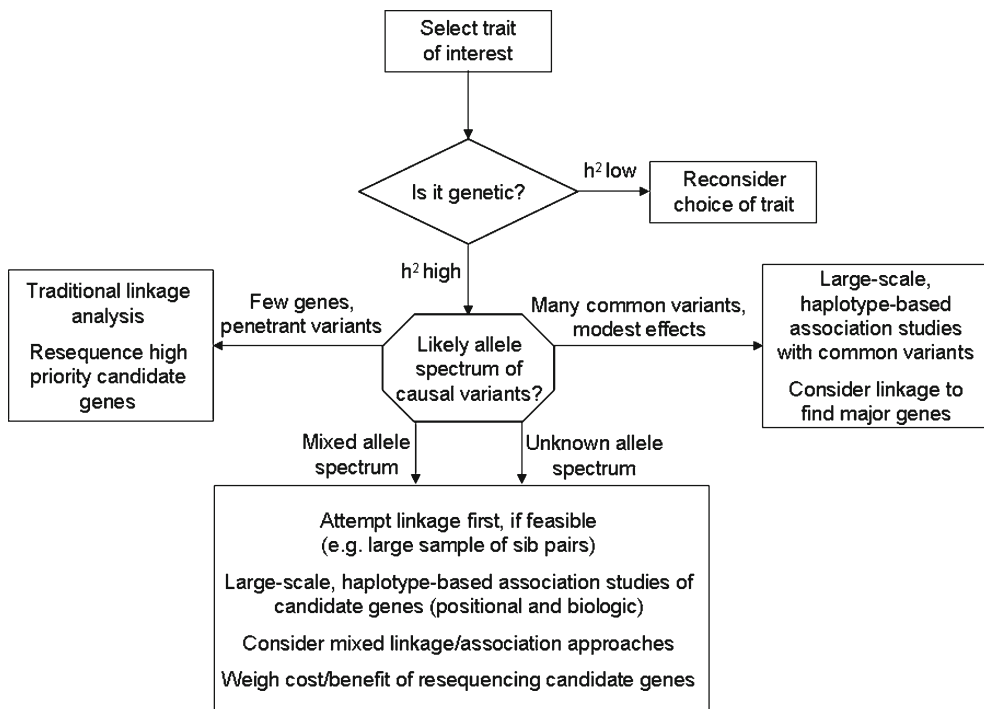


Fig. 1. Schematic of strategies for identifying genes that modulate complex traits. Heritability (h^2) refers to the fraction of trait variation that is explained by (additive) genetic effects; high heritabilities (50% or greater) are consistent with a major genetic component to the trait. See text for details of the depicted schematic. Reprinted from (1) with permission from Elsevier, copyright 2003.

Resequencing of Candidate Genes

One approach for identifying genetic factors affecting pubertal timing is the resequencing of candidate genes. This method is based on identifying and selecting candidate genes and determining whether sequence variants are present that can explain phenotypic variation. Although costs continue to decrease and efficiency continues to increase, this approach can be expensive and time consuming; therefore, it is best used when there is a high likelihood that the candidate gene harbors causal variants. Many single-gene disorders are explained by highly penetrant, rare variants (36), which is expected for diseases that are often early-onset and under negative evolutionary pressure (37). Many such disorders have been successfully investigated through the sequencing of candidate genes. Although, less commonly, complex traits may also be explained by rare genetic variants, examples include predisposition to early-onset breast cancer (38–43), risk of severe early-onset obesity (44), low levels of high-density lipoprotein cholesterol (a major risk factor for coronary atherosclerosis) (45), and possibly hyperandrogenism (46,47).

Candidate genes can be identified based on location in the genome or by their known or putative function. Genes identified based on location are referred to as positional candidates and are usually selected because they lie within a region that has been linked to a trait through genome-wide linkage analysis. Genes identified by function are referred to as biological candidates and often arise from single-gene disorders, known pathways, and experiments in model systems. There are several biological candidates that could account for the variation in pubertal timing seen in the general population. For example, genetic variation in genes identified as having a role in HH such as the gonadotropin-releasing hormone receptor (*GnRHR*) (48,49), *KAL1* (50,51), *GPR54* (52,53), leptin and leptin receptor (54–57), fibroblast growth factor receptor-1 (*FGFR1*) (58,59), dosage-sensitive sex-reversal adrenal hypoplasia congenital (DSS-AHC) critical region on the X chromosome (*DAX1*) (60,61), and prohormone convertase 1 (*PC1*) (62) could account for the variation in pubertal timing within the population. Genes that modulate growth (63) and timing of developmental processes (64) are also possible candidates.

Whether being a heterozygous carrier for disease-causing mutations or harboring important polymorphisms in these candidate genes causes the variation in pubertal timing in the general population is an area of active investigation. For example, we have resequenced several candidate genes in a population of individuals with late but otherwise normal pubertal development (65). No rare missense, frameshift, or nonsense mutations have been identified in these genes so far, but negative findings may not be surprising because traits that are on balance evolutionarily neutral or favorable may be explained largely by genetic variation that is common in the general population instead of rare variants (37,66–70). There are a growing number of precedents for common variants having modest effects on complex traits (71–82); however, resequencing of candidate genes is usually not required to detect these common variants because databases of single-nucleotide polymorphisms (SNPs) are readily available from dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>) and Celera (<http://www.celera.com>).

Linkage Analysis

Linkage analysis is another method used to identify genes involved in complex traits. One advantage to linkage analysis is that it does not require assumptions about which genes or pathways are likely to affect the trait because the method involves an unbiased genome-wide scan for causal genes. This method has been very successful in identifying the genes that underlie single-gene disorders (83) because the causative mutations cosegregate nearly perfectly with disease in families, allowing for easier identification of the responsible gene.

Genes involved in complex traits, on the contrary, have been harder to identify using linkage studies (84,85). Because complex traits result from the interaction of multiple genetic factors, variants involved likely only have modest effects on the trait, making them more difficult to detect and requiring that adequately powered studies involve many more subjects than would be required for a single-gene disorder (86). Environmental factors exert substantial effects on the phenotype of complex traits, which further limits the power of linkage studies by making the phenotype–genotype correlations variable. Even with these limitations, there are an increasing number of successful linkage studies for complex traits including inflammatory bowel disease (80,87–90), schizophrenia (81,82), and type 2 diabetes (91), demonstrating that this is a viable strategy.

No genes that modulate the timing of puberty within the general population have been identified thus far using linkage analysis. However, important progress has been made within the last year. Guo et al. used a genome-wide linkage analysis in 2461 Caucasian women to investigate the genetic basis for variation in the age of menarche. Statistically significant linkage to the genomic region 22q13 and two suggestive linkage peaks at 22q11 and 11q23 were identified (92). Candidate genes within these regions include sterol regulatory element binding factor 2 (*SREBF2*), catechol-*O*-methyltransferase (*COMT*), and the progesterone receptor (*PGR*). Another genome scan investigating the age of menarche and development of breast buds has been reported in abstract form, but only suggestive peaks were found in this study (93).

Association Studies

Association studies have also been used to identify genes that modulate complex traits. In association studies, the frequency of a genetic variant in an affected population and that in controls or the genotypes of individuals with extreme trait values are compared. If the frequency of a variant differs significantly between the comparison groups, an association is said to be present. An alternative strategy is to compare the mean trait values of individuals with different genotypes. One significant advantage to association studies is that they have better power for the analysis of common variants with modest genetic effects than linkage analyses (86).

On the contrary, there are several limitations of association studies that must be considered when either designing the study or interpreting the results. First, the studies depend on candidate genes; therefore, unlike linkage analysis, assumptions must be made regarding causative genes and pathways. In addition, association studies can suffer from inadequate power, multiple hypothesis testing, and testing of a marker that is not in strong linkage disequilibrium with the causal variant (33,94–96). Another possible limitation is population stratification or ethnic admixture (97). Population

stratification occurs when the two populations being compared (cases and controls) are not matched for ethnicity and have differences in allele frequency due to ancestry rather than association of genetic variants with a trait (97–99). This possibility is important in studying genes affecting pubertal onset because African Americans enter puberty earlier than Whites, so a simple study of early-versus late-maturing individuals would be confounded by the ethnic make-up of the two groups. Although matching cases and controls by self-reported ethnicity has been suggested to avoid false-positive associations (99,100), this may not be sufficient (101); another way to avoid population stratification is the use of a family-based method, transmission disequilibrium test (TDT). The TDT avoids population stratification by using trio data from an affected individual and his/her parents and relying on internal rather than unrelated controls (102).

In recent years, there has been an increase in the number of association studies designed to identify genes that modulate the timing of pubertal onset. Most of the published studies have used the age of menarche as a surrogate for age of pubertal onset. These studies have mostly focused on genes related to estrogen responsiveness such as metabolizing enzymes, receptors, and sex steroid-binding proteins. For example, cytochrome P450c17 α (*CYP17*), involved in estrogen biogenesis, and *COMT*, which is important in the metabolism of estrogen, have been assessed in several studies. Variants of *CYP17* have been variably associated with early menarche, but the publication of several positive reports (independent replication) greatly enhances the chance of a true association between genetic variants in this gene and the age of menarche (103–111). Conflicting reports have been published regarding an association between the age of menarche and variants in *COMT* (107,112), but it is interesting to note that the genome-wide linkage study mentioned in “Linkage Analysis,” performed by Guo et al., found linkage to the genomic region that contains *COMT* (92). To date, only conflicting reports without replication of the positive findings have been reported regarding association between the age of menarche and variants in estrogen receptor α (107,113–116), the sex hormone-binding globulin (*SHBG*) gene (117), and the androgen receptor (118,119). False positives and false negatives can occur fairly readily in association studies (73); it is too early to know to which category these initial studies belong.

If eventually proven accurate, the associations between genes involved in sex steroid metabolism and/or signaling and the age of menarche will have added greatly to our understanding of one feature of pubertal development. They will have demonstrated that variation in sex steroid pathways can affect age at first menstrual period. It is uncertain, however, whether the genes and variants identified within the sex steroid pathways simply regulate estrogen responsiveness or affect the maturation and central activation of the HPG axis that heralds the onset of puberty. That is, whether variants in genes are among the elusive regulators of the increased GnRH secretion that typifies the onset of central puberty remains to be seen.

In our work, we have used trio-based and case–control-based association studies to test for associations between variants in *GnRH* and the *GnRH* receptor (*GnRHR*) and late pubertal development. We found only modest, nominally significant associations between three SNPs in the *GnRHR* gene and late pubertal development (65). Thus, genetic variation in *GnRH* and *GnRHR* does not appear to be a substantial modulator of pubertal timing within the general population. Rather, our data suggest that the genes

that modulate the onset of central puberty are likely upstream regulators of GnRH secretion or downstream effectors of GnRH action.

Recent Advances

Each strategy for identifying genes involved in complex traits has advantages and disadvantages, but advances in genomics are making these strategies easier to accomplish. Sequencing of the human genome has facilitated the identification of putative candidate genes. This is important for resequencing and association studies and also enhances linkage studies by facilitating progression from a region of linkage to the identification of the gene(s) and sequence variant(s) that are responsible for phenotypic variation. In addition, the identification of over 9 million SNPs (dbSNP; <http://www.ncbi.nlm.nih.gov/SNP/>), including most of the genome's estimated ~ 11 million SNPs with minor allele frequencies of 1% or greater, and new genotyping technologies raise the possibility that future linkage studies may be done using dense panels of SNPs instead of the more widely spaced traditional markers (70,96,120,121). These whole-genome association studies should greatly advance our understanding of the genetic regulation of complex traits.

Finally, the recognition of patterns of human variation is also facilitating association studies. It is now known that most of the genome can be parsed into regions or "blocks" in which genetic variants are correlated with each other; that is, the alleles from neighboring variants fall into one of a few common patterns called haplotypes (122–125). On average, these haplotype blocks span from 11–22 kb and contain on average four to five common haplotypes (124). Because of the correlation between variants within blocks (linkage disequilibrium), a few well-chosen variants (usually SNPs) can serve as proxies for the remaining common variants in the block. Thus, by typing a few variants that tag the common haplotypes [referred to as haplotype tagging SNPs or htSNPs (126)], the vast majority of common variation can be efficiently assayed (124). A large-scale international effort has just been completed to determine the haplotype patterns on a genome-wide scale (127). This human haplotype map will make it much easier to comprehensively assay common variation in candidate genes.

Together, these recent advances may well lead to human studies that identify genes that regulate the variation in the timing of puberty in the general population.

APPROACHES TO IDENTIFYING GENETIC FACTORS: ANIMAL MODELS

Studying the genetic component of complex traits in humans can be difficult because of extensive genetic diversity, wide variation in environmental influences, and the inability to assemble large enough populations for linkage studies (or even well-powered association studies). Animal models, which involve analysis of a large population of genetically similar animals in a controlled environment, can circumvent these limitations and are often important adjuncts to human studies. The regulation of pubertal timing has been studied in many animal models, including sheep, ferrets, hamsters, rats, and mice (128,129–132) as well as non-human primate model systems (133–135). Although the study of pubertal timing in each of these models has its advantages and disadvantages, to date, the mouse has the most genetic information known and the best track record regarding mapping of genetic factors that regulate complex traits.

Advantages of Mouse Models

Mice have several advantages as a model system for the study of pubertal onset. The timing of puberty is known to vary among inbred strains of mice (136,137), indicating that pubertal onset is genetically regulated in mice; the mouse genome shares extensive homology with the human genome (138); and the discovery of quantitative trait loci (QTLs) is facilitated because matings can be designed and carried out within a controlled environment.

There are species differences in pubertal physiology (3,139), and these may represent disadvantages of a model system. First, although humans and non-human primates have a “juvenile pause,” a time of relatively quiescent gonadotropin secretion starting 6–24 months after birth and lasting until right before puberty, mice likely progress gradually and more continuously into puberty. Second, γ -aminobutyric acid (GABA), which plays an important inhibitory role in GnRH secretion in primates, has been reported to have both inhibitory and excitatory effects on GnRH secretion in mice (140). The importance of this temporary species difference is unclear, but it may stem from differential expression of *KCC2*, a potassium chloride cotransporter that permits the effects of GABA to switch from depolarizing to hyperpolarizing and that is expressed in GnRH neurons (141). Lastly, although mice may facilitate the mapping and identification of genes involved in murine pubertal onset, genomic differences between mice and humans, although uncommon, could conceivably lessen the relevance of a particular finding to human physiology (142).

However, the key components of the HPG axis are shared between humans and mice, and many genes have been identified as key regulators of the reproductive endocrine axis in both species. Indeed, several genes that cause HH in humans display phenotypic concordance in mouse models (143). Thus, it seems likely that a gene identified as modulating the timing of puberty in mice may regulate the timing of puberty in humans as well. Mouse studies also present the prospect of eventually performing *in vitro* or *in vivo* experiments designed to establish a causal relationship and mechanism for genotype–phenotype associations.

Another advantage of model systems is that genome-wide scans can be performed more readily in mice than in humans. Such scans are important in the study of complex traits because they do not require *a priori* assumptions about causative genes or pathways and may lead to the discovery of novel regulators. Even in advance of identifying specific genes, the chromosomal locations of QTLs and mouse: human synteny maps can be used to prioritize genes for candidate analyses in humans. Moreover, because the mouse and human genome sequences are both almost completely known, the identification of the gene(s) within a QTL should be facilitated.

Chromosome Substitution Strains

There are many strategies that can be used to perform genome-wide scans in mice (34,144), but one of the more powerful strategies is the use of chromosome substitution strains (CSSs). In CSSs, a single chromosome from a donor mouse strain is substituted in a homozygous fashion for the corresponding chromosome in a host strain (145,146) (Fig. 2). If the trait of interest differs between the donor and host strains, a full panel of CSSs can be phenotyped to identify which substituted chromosome(s) harbor(s) a gene or genes that modify the trait (Fig. 2).

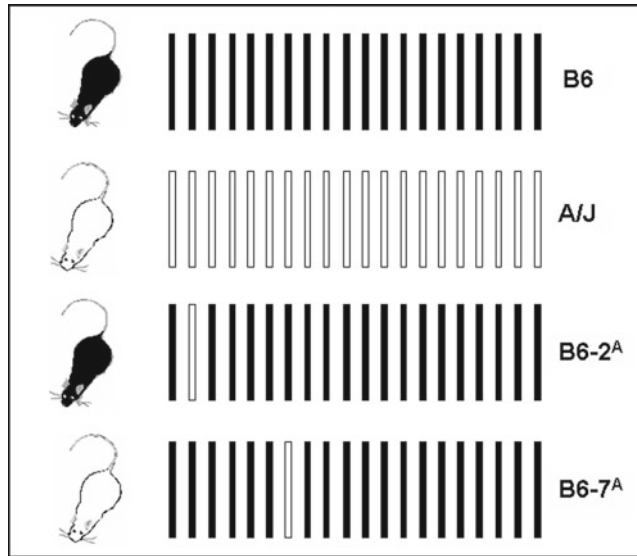


Fig. 2. Use of chromosome substitution strains (CSSs). In this theoretical example of the use of CSSs, the chromosome harboring gene(s) controlling coat color is identified. The CSS with A/J (white) chromosome 2 homozygously substituted on to the B6 (black) background (B6-2^A) has black fur, whereas the CSS with A/J chromosome 7 substituted (B6-7^A) is white, indicating that a gene(s) on chromosome 7 controls coat color. Note in this depiction that each bar represents a pair of chromosomes. See (147) for more detailed discussion. Reprinted with permission (146) and Lippincott, Williams and Wilkins, copyright 2004.

The CSS panel we have used to study pubertal timing was generated by collaborators at Case Western Reserve University and the Broad Institute of Massachusetts Institute of Technology and Harvard (147). In this panel, the donor strain is A/J and the host strain C57BL/6 (referred to as B6). The available panel partitions the genome into 22 strains (one for each of the 19 autosomes, the two sex chromosomes, and the mitochondria) that reside on a defined and uniform genetic background. Because the strains can be propagated, one can initially screen the full panel for phenotypic differences and then re-breed and iteratively phenotype genetically identical mice to verify the initial results.

Survey of CSS Panel and QTL Identification

Our initial studies demonstrated that the CSS progenitor strains, A/J and B6, have different ages of pubertal onset [as assessed by vaginal opening (VO)], with VO occurring on average 5–6 days earlier in A/J compared with B6 mice (148). This finding provided a unique opportunity to use CSS mice to determine which chromosomes harbor loci that exert substantial effects on pubertal timing. VO was assessed in 20 CSSs (the 19 autosomes and the X chromosome, referred to as B6-1^A, B6-2^A, B6-3^A, etc.) and compared with the B6 progenitor strain. The majority of CSSs had a timing of VO that was similar to the host strain. The exceptions were CSSs for chromosomes 6 and 13 (B6-6^A and B6-13^A), which both had a significantly earlier time of VO than B6 (on average 3 days earlier for B6-6^A and 4–5 days earlier for B6-13^A) (Fig. 3).

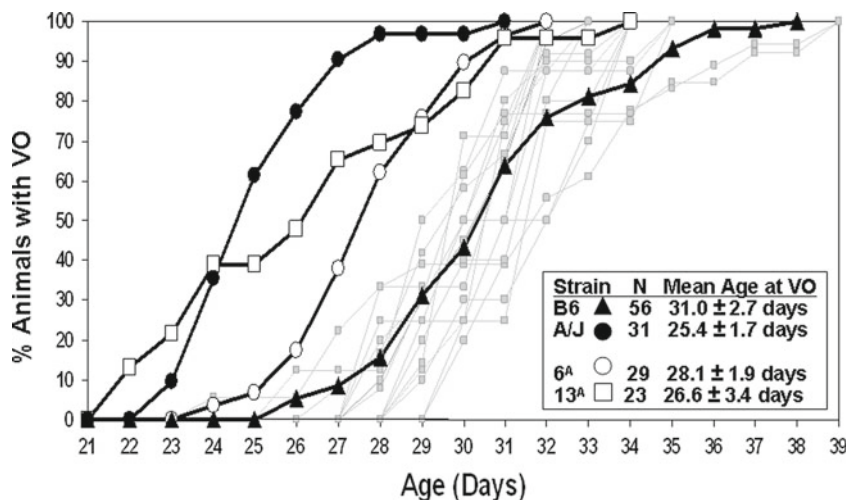


Fig. 3. Results of chromosome substitution strain (CSS) panel survey. Initial phenotyping revealed A/J mice (filled circles) having significantly earlier vaginal opening (VO) as compared with the B6 mouse (filled triangle). Analysis of complete CSS panel identified two strains with significantly earlier VO timing: B6-6^A (open circles) and B6-13^A (open squares). The rest of the CSS strains, denoted in faded lines, were not significantly different from the B6 mouse. These results indicated that chromosomes 6 and 13 harbored genes that altered pubertal timing in the mouse. Reprinted with permission (148) and copyright 2004.

These data suggest that chromosomes 6 and 13 harbor genes that affect the timing of puberty in mice (144).

The next step in the experimentation was the performance of linkage analysis designed to identify the location (QTL) of the genes that modulate pubertal timing. These studies are greatly facilitated in CSSs because, unlike traditional QTL analysis, only the variants on a single chromosome are segregating in the experimental crosses (149). To date, a QTL on the distal portion of chromosome 6 that affects the timing of VO has been identified (150).

Congenic Strains

QTLs identified in linkage analyses can be validated by studying congenic mouse strains (148). Unlike the CSS mice where an entire chromosome has been substituted, congenic mice contain only a segment of the chromosome that has been homozygously substituted (Fig. 4).

Using congenic animals to investigate a complex trait, such as the timing of puberty, has several advantages (151) (Fig. 5). For example, in our work, the panel can be used to verify linkage results by determining whether mice that contain the region of the A/J chromosome that harbors the QTL show earlier puberty than B6 mice. If the QTL is confirmed, congenic mice can be bred to contain smaller and smaller regions on the substituted A/J chromosome 6 (“subcongenic” strains), which will facilitate fine mapping of the QTL(s) and the eventual identification of the responsible genes.

We have used CSS as an example of the importance of animal models in the study of complex traits. However, many other strategies are also being used to identify genes

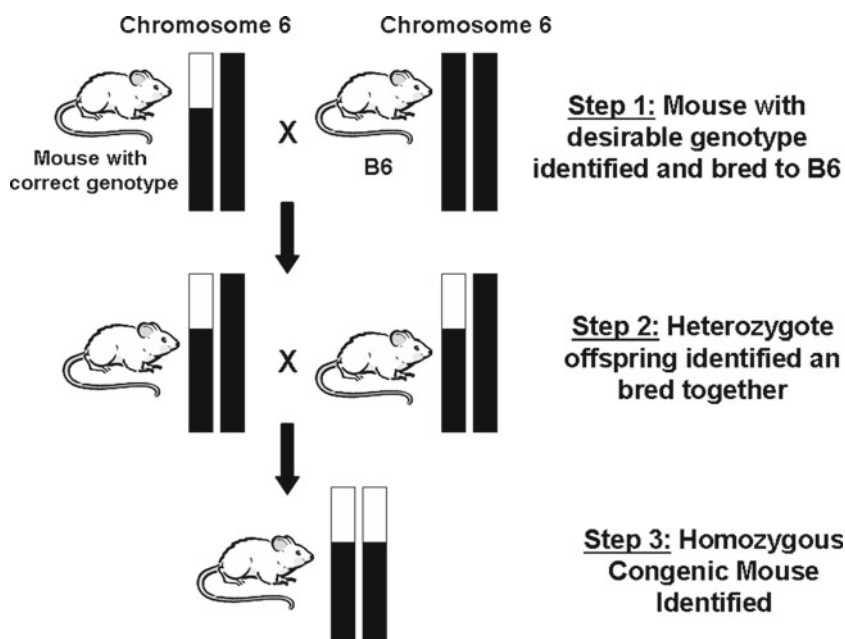


Fig. 4. Generation of the congenic mouse. The first step in generation of the congenic mouse is to identify mice that contain the desired portion of the A/J chromosome (depicted in white) (step 1). These mice are then bred with progenitor B6 mice (B6 chromosomes depicted in black), and offspring that are heterozygous for the desired segment of the A/J chromosome are identified and bred together (step 2). The resulting homozygous offspring are the congenic mice of interest that can be bred together to maintain the congenic strain (step 3). Note that in this figure, each bar represents a single chromosome (chromosome 6 in this example) in order to illustrate the homozygous and heterozygous states important in generating congenic mice. Modified with permission (151).

that modulate the timing of puberty in mice and in other species (see Chapters 1 and 2) (140). The combined results from these animal experiments should lead to the eventual identification of genes (and pathways) that are candidates for regulating the timing of puberty in humans.

CONCLUSIONS

Environmental and physiologic regulation of the HPG axis and the timing of puberty are superimposed upon substantial genetic control. As much as 50–80% of the regulation of pubertal timing in humans may be genetic according to data from population, family, and twin studies. In the near future, we expect that the identification of genes affecting variation in pubertal timing in the general population will come from both human studies (linkage, resequencing, and association studies) as well as animal experimentation. Identifying genes that regulate pubertal timing will provide new understanding of this fundamental developmental process and likely open new areas of research, such as investigation of gene–gene and gene–environment interactions that regulate the HPG axis.

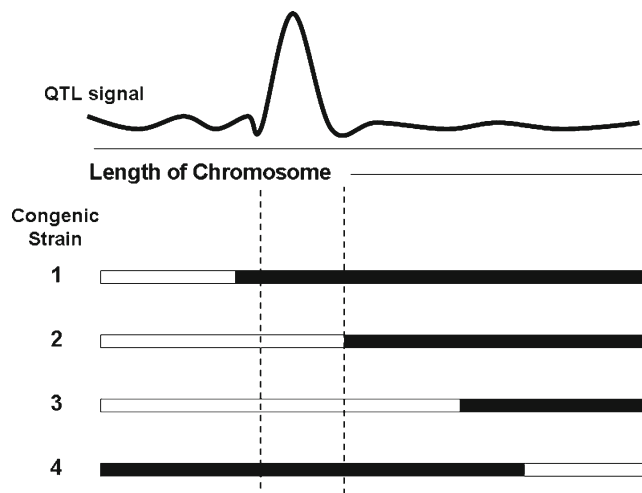


Fig. 5. Advantages of congenic mice. In this theoretical example, four strains of congenic mice are displayed, all with variable lengths of a homozygously substituted A/J (white) chromosome on to a B6 (black) background. A peak has been drawn at the top of the figure to illustrate the location of a hypothetical quantitative trait locus (QTL) identified by linkage analysis. When the trait of interest, for example, vaginal opening, is assessed, congenic strains 2 and 3 display earlier puberty as compared with the progenitor B6. In contrast, congenic strain 1 has pubertal timing similar to B6. Because congenic strains 2 and 3 contain the same region of the A/J chromosome (outlined by dotted vertical lines) and this region corresponds to the location of the QTL, such data would provide confirmation of the linkage results. The panel of congenic mice can also be used to identify regions that harbor QTL(s) not detected in linkage analyses. For example, if congenic strain 4 also had earlier timing of vaginal opening than B6, it would suggest that another QTL that modifies pubertal timing resides in the substituted A/J region. One could then design crosses of congenic strains 2 and 4 or 3 and 4 to test for interactions between the identified QTLs (152). Modified with permission (151).

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II

SECULAR TRENDS AND THE ONSET OF PUBERTY

5

Puberty Is Starting Earlier in the 21st Century

Marcia E. Herman-Giddens, PA, DrPH

CONTENTS

INTRODUCTION

REASONS IT IS IMPORTANT TO HAVE CURRENT AND
SECULAR DATA ON SEXUAL MATURITY STAGES

VALIDITY OF THE INDICATORS

HISTORICAL DATA ON PUBERTAL MILESTONES

AND THE SECULAR TREND PATTERN IN THE UNITED
STATES

EVALUATING US PUBERTAL STUDIES FROM THE 1950S
ON TO ASSESS THE ACCURACY OF ANY SECULAR
CHANGES

ADDITIONAL SUPPORTING EVIDENCE FOR EARLIER
PUBERTY IN THE UNITED STATES

ARGUMENTS AGAINST A DECREASING SECULAR TREND
IN AGE OF PUBERTY IN US CHILDREN

CONCLUSIONS

REFERENCES

Summary

Determining trend directions in ages of pubertal characteristics with certainty in U. S. studies is not possible because studies using similar methods and measurement techniques do not exist, with the exception of the menarcheal data from the government surveys. Taking the methodological differences into account, the data for girls since the 1940s support a continued decline, though less steep than that during the later 1800s and early 1900s as based on menarcheal data. Modern girls appear to start development 5 months to a year earlier than those of 30 to 50 years ago with a longer tempo from onset to menses. The data from NHES and NHANES on menarche are exactly comparable and show a statistically significant drop of over a month per decade in the last 30 years to 12.34 years of age for white girls and 12.06 for black girls. Fewer studies exist for boys and the data are only cautiously suggestive of lessening trend. Testicular measurement has not been used in U. S. studies. Data since the 1940s show a very slight downward trend in onset of pubic hair growth for white boys. The most recent NHANES data on genital growth would indicate a younger age as compared with several decades ago (onset of G2 now 10.1 and 9.5 years of age for white and black boys respectively from the 1988–1992 data set) however; the reliability of

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the assessments has been questioned. The American Academy of Pediatrics Pediatric Research in Office Settings study in the field since 2006 will provide much needed data.

Supporting evidence for earlier puberty in both boys and girls includes limited growth and dental studies showing earlier achievement of final height and mature dentition as well as the obesity epidemic, a condition likely associated with an earlier onset of puberty, at least in girls. Changes in ages of onset of pubertal characteristics have profound public health and medical care implications. Just as rising ages may indicate lack of adequate nutrition or other harmful social or environmental conditions, lowering ages may reflect different kinds of harmful conditions such as overweight or certain environmental contaminants. Reliable trend data are needed for the care of our children and for appropriate public health interventions.

Key Words: Puberty; Age of onset of puberty; Menarche; Age of menarche; Earlier puberty; Tanner staging; United States puberty studies.

INTRODUCTION

The ages of onset and attainment of pubertal milestones are important public health indicators and are required for appropriate clinical care as well. Because the ages for growth and pubertal milestones are not static, ongoing studies are needed to be able to examine secular trends and to keep abreast of timely findings. Unfortunately, the public health system in the United States (US) has never conducted adequate studies to establish average ages for onset and attainment of sexual maturity milestones, except for limited studies on menarche, or to allow for tracking of ages over time. Because solid data do not exist, recently controversy has developed over the question of whether or not these ages are dropping as it had been assumed that the ages had leveled off by the mid-1900s. This assumption has been challenged by several recent publications (1–4). This chapter will examine the available US studies on pubertal milestones and supporting evidence and argue that despite comparability issues (with the exception of three comparable studies on menarche) there is evidence, albeit with varying levels of certainty, that the age of pubertal onset may be dropping in boys and is dropping in girls along with the age of menarche.

REASONS IT IS IMPORTANT TO HAVE CURRENT AND SECULAR DATA ON SEXUAL MATURITY STAGES

Examination of growth velocities and average heights and weights of children at varying ages has been used to demonstrate an important part of the overall condition of a population. For example, in Britain in 1833, a large-scale measurement of children occurred to provide the Parliament with evidence of the effects of having children labor in factories. At that time, working boys 10 years of age averaged 121 cm in height compared with 140 cm in the late 1970s (5). Similar information can be provided from the examination of pubertal milestones. Today, there is an increased interest in pubertal indicators, in particular due to the obesity epidemic and the ubiquity of endocrine disruptor chemicals, many of which have been shown to affect pubertal and reproductive processes. In addition, other social and environmental factors are known to influence puberty (*Table 1*). Because cultural changes in United States are contributing to an increase in some of the factors (such as endocrine disruptors, overweight, or children in households without fathers), it could be expected that there would be a subsequent effect on pubertal milestones. Understanding the relationship and public health implications of these factors is essential to the health of children.

Table 1
Possible causes of earlier puberty: theories and speculations

Genetic differences (5)
Overweight and obesity, decreased physical activity (6–12)
Prenatal and postnatal exposure to endocrine disruptor chemicals (13–15)
Infant soy-based formulas (16)
Girls born small for gestational age (17–19)
Stress, absent fathers, unrelated males in the household (20)
Effects of different types of diet (21,22)
Exogenous hormones (1,23)
Hypersexualization of culture (5,24)

It should be kept in mind that many children have multiple and cumulative exposures.

VALIDITY OF THE INDICATORS

Assessing puberty by the growth of the secondary sexual characteristics lacks precision in that the stages may be difficult to assess accurately and because by the time physical changes are taking place, the hormonal output from the hypothalamic–pituitary–gonadal axis is well under way. Nonetheless, because of the lack of accurate hormonal assessments (25) and the practicality of Tanner staging, most studies of pubertal development have not used invasive procedures and bodily fluids. Studies in the United States examining the stages of sexual maturity have generally used the five points of development commonly known at the Tanner stages (26). Average ages are usually presented for each characteristic separately, such as stage 2 breast development or stage 5 pubic hair growth. The characteristics most likely to represent gonadal functioning are breast development in girls and genital development in boys, in particular testicular growth being the earliest pubertal change. For this reason, more importance may be attributed to the growth of the breast or testes as an indicator of “true” puberty beginning than that of pubic hair growth. When proportions of girls at a given age are examined for breast and pubic hair growth, there is no evidence of a large lag between the two. See, for example, *Fig. 1* or the proportional data from the Pediatric Research in Office Settings (PROS) or National Health and Nutrition Examination Survey III (NHANES III) studies.

Cross-sectional and longitudinal designs are used for puberty studies to provide information on distribution in age of onset (entering a stage) or being in a stage for a given characteristic. Cross-sectional data (point in time) can be used to calculate mean or median ages for onset of a characteristic or “being in a stage” by probit or logit distributions and can give estimates from data from which only a portion of subjects have achieved the characteristics being studied. Means and medians are similar if the distribution is Gaussian as is assumed with the US studies. Better data are obtained from longitudinal studies where a number of observations for each child are made over time, which allows for information on individual variation and differences in progression between stages. The quality of longitudinal data increases with the frequency of examinations and shorter times between examinations. Ages studied should ideally span the time period where all children are still prepubertal to final sexual maturity. Censored data provide less precise estimates.

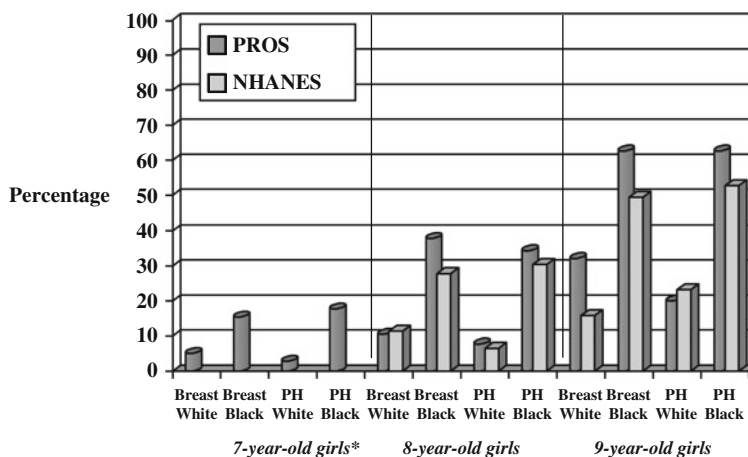


Fig. 1. Percent of 7-year-old, 8-year-old, and 9-year-old girls (2nd, 3rd, and 4th grades) with breast development and pubic hair growth Tanner stage 2 or greater, Pediatric Research in Office Settings (PROS) and National Health and Nutrition Examination Survey (NHANES) III data. *NHANES did not start collecting Tanner stage data until age 8.

Many of the US pubertal studies have published average ages for reaching each of Tanner stages 2 through 5. Questions can be asked using these data, such as the age of onset, the duration of puberty, or whether the age of sexual maturity (stage 5) has changed over time. When evaluating data based on the later stages, the difficulty of accurate assessment must be considered. This is especially true for boys, where changes in genitalia are mostly size related, and there is a normal near two-fold difference in testicular size among individual males. For example, stage 5 genitalia data on White boys from the two 1940s studies show a wide difference—17.3 years of age for the Fels Institute subjects (27) and 15.2 for the Guidance Study (28). The age was 14.9 years in Marshall and Tanner's 1970 study (29) and approximately 16.0 depending on author for NHANES boys in the early 1990s (30–32). For girls, the average age of entering breast stage 5 was a little over 13.5 years in the 1940s studies (28,33), 15.3 in Marshall and Tanner's 1969 study (34), and 15.5 for NHANES White girls in the early 1990s (32). It seems likely that either assessing stage 5 is more subject to error or there is naturally a much greater variation in achievement of final maturity. Whatever the reasons, interpretation of later stage data (35) needs special caution.

The distinction between the age of *onset* of a characteristic and age of attainment or being *in* a stage is important when reviewing Tanner stage data. For girls, many studies that have looked at factors influencing the “age of puberty” have used the age of menarche as their pubertal marker rather than the onset of breast development, no doubt because the former is a far easier and more accurate marker to ascertain. Because influences on menarche may differ from those on the beginning of breast development, it is important to make this distinction. Boys' pubertal studies in the United States usually just present the Tanner stages for genital and pubic hair growth. Very few have examined the age of ejaculation or spermarche for reasons of accuracy and, probably, prudery for the former indicator.

HISTORICAL DATA ON PUBERTAL MILESTONES
AND THE SECULAR TREND PATTERN IN THE UNITED STATES

It is well-known that since the mid-1800s in industrialized countries, children have been getting bigger and reaching maturity more rapidly although periods of economic setback were associated with the leveling off or even a reversal of the secular trend (5,12). In Europe, United States, and Japan, and more recently in some developing countries, the magnitude of the increase in size and age of achievement of maturity has attenuated the formerly well-known differences according to social and occupational class when children in higher socioeconomic conditions matured earlier and were taller than poor children. As a result, in the United States, economic and social class differences with regard to ages of pubertal milestones have apparently disappeared (1,31,32,36,37).

Prior to the mid-1900s, the age of menarche was the basis for assessing secular changes in puberty. Tanner’s well-known graph of the secular trend and ages of menarche, 1840–1970, is, itself, not without controversy (*Fig. 2*). The accuracy of the data has been questioned and the argument made that although the age of menarche has definitely dropped, the difference has been less dramatic (12,39). Obviously, such a steep slope could not continue for long without reaching early childhood. Recently, the controversy has reoccurred, and the issue now is whether there continues to be a drop as it has been widely believed that the age stabilized after the 1960s. In the past, better nutrition and a lower incidence of infectious diseases were thought to be largely responsible for the lowering age of menarche (5,12). The “better nutrition” of that time frame meant adequate calories, protein, and other nutrients as many less well-off

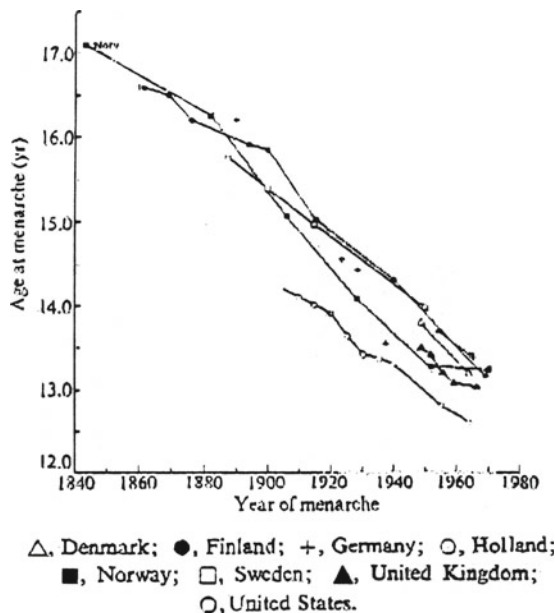


Fig. 2. Trend in menarche in some European countries and the United States in 1840–1970 according to Tanner. Reproduced with permission (38).

children were undernourished and often ill. “Better nutrition” currently in this age of epidemic overweight among US children, especially poorer children, could be defined as the opposite—fewer calories, less protein, and more fruits, vegetables, and fiber. This current state of nutrition, sometimes referred to as “overnutrition,” is likely one of the causes of the current lack of noticeable disparity in pubertal milestones among the various socioeconomic classes. The fact that from the 1950s until the 1970s levels of nutrition were more stable and infectious diseases under reasonable control, at least for all but the very poorest of children, supported the belief that the age of menarche had stabilized.

EVALUATING US PUBERTAL STUDIES FROM THE 1950S ON TO ASSESS THE ACCURACY OF ANY SECULAR CHANGES

Several factors must be kept in mind when considering data for evidence of secular trends:

1. The available studies that recorded stages of secondary sexual characteristics and menarche (*Table 2*).
2. The study populations—are the racial and ethnic characteristics representative of the populations in different areas of the United States, are they weighted correctly if combined for analyses, and are the social classes comparable?
3. Are the studies’ methodologies comparable?
4. Were the proper statistical methods used?

The characteristics of the US puberty studies from the 1940s through the late 1990s on girls and boys are summarized in *Table 2* including the years the data were collected for each study population, the socioeconomic status, the study design, population number for each study, methodologies, and other factors important to consider when examining pubertal data for secular trends.

Only nine studies are available from the 1940s through the 1990s that used professional examiners for Tanner staging and current status from subjects for menarcheal status (rather than age recall, which introduces more error). Many more papers have been published using data from these studies. Pubertal studies that used retrospective recall data for menses or were based on self-assessment by the children such as that by Roche et al. (40) are not included in this discussion, because they are not as precise and would introduce even more comparability problems. Of the nine examiner studies, there are only three longitudinal studies that looked at both girls and boys, not surprising given that although this is the ideal method for puberty studies, it is also the most difficult and expensive. These three were very small, from 20 to under 100 subjects each for girls and boys, and the children were well off economically (27,28,33,41). It is well to keep in mind that these children were likely to have earlier puberty than their less well-off counterparts, especially the two studies from the 1940s, because until fairly recently in the United States well-off children developed earlier than poorer children (12). In addition, the findings are based on very small numbers of children from a few specific communities making applicability to US children more problematic. The strength of the longitudinal design is somewhat weakened by the Fels Institute and the Guidance Study assessing the pubertal stages by using photographs of the nude children (as did Marshall and Tanner). Unfortunately, these are the only data available

Table 2
US puberty studies with examiner assessments since the 1940s: design type, source of data, populations, and age range

Study	Authors and year of publication	Years data collected	Socioeconomic status	Type	Number studied	Source of Tanner stage rating	Source of menarche status	Point estimate	Age increment used in study/ frequency of exam	Age range (years ¹) of population
Study 1: Fels Institute, OH	Reynolds and Wines 1948 and 1951 ²	1940s	High	Longitudinal	49 W girls and 59 W boys	Visual from photographs	"Menstrual record"	Mean	Semiannual	8–18
Study 2: Guidance Study, CA	Nicolson and Hanley 1953 ³	1930s–1940s	High	Longitudinal	95 W girls and 92 W boys	Visual from photographs	NR	Mean	Semiannual	8–17
Study 3: NHES I, II, and III ⁴	1. MacMahace* 1973	NHES, cycles II and III, 1963–1965 and 1966–1970	Mixed	Cross-sectional	1. 5635 W, 1043 B, and 32 O	–	I, recall (self); II, current status; and III, recall (parent)	I, mean; II and III, median/fitte d logistic curve	I, years; II and III, midpoint of the years of age	1. I, 18–79; II and III, 11–14
	2. Anderson et al. 2003 (II and III)				2. 3272					2. 10–15
	3. Harlan et al. 1980 (III)				3. 2688 W and 500 B					3. 12–17
	4. Kaparti et al. 2002 (II and III)				4. II, 3010 and III, 3514					4. II, 8–11 and III, 12–17
Study 4: Lee Study ⁵	Lee 1980	1969–1974	High	Longitudinal	36 boys and 18 girls	Visual by physician	"Menstrual history" (self)	Mean	Semiannual	8.6–17.8
Study 5: Bogalusa Heart Study ⁶	1. Foster et al. 1977	1973–1974	Low to average	Mixed longitudinal/cross-sectional	1. 3524	Visual by "trained examiners"	1. Recall (self)	1. Median	1. Years	1. 5–14
	2. Wattigney et al. 1999	1978–1979 and 1992–1994			2. Cohort 1: 332 W and 238 B and Cohort 2: 348 W and 305 B		2. Recall (self) and postmenarcheal females	2. Mean and analysis of variance between two cohorts	2. Years	2. 8–17
	3. Freedman et al. 2002	1973–1994			3. 9158 C-S, 1253 W L, and 805 B L		3. Current status (self)	3. Median (logistic regression)	3. Midpoint of years	3. 8–17

(Continued)

Table 2
(Continued)

Study	Authors and year of publication	Years data collected	Socioeconomic status	Type	Number studied	Tanner stage rating	Source of menarche status	Point estimate	Age increment used in study/ frequency of exam	Age range (years) ¹ of population
Study 6: Cincinnati school children ⁷	Biro et al. 1995	1984–1987 (boys only)	Mixed	Longitudinal	278 W and 237 B	Visual and orchidometer by physician ⁶	–	Means	Semiannual for 3 years	10–15
Study 7: Pediatric Research in Office Settings (PROS) ⁸	Herman-Giddens et al. 1997	1992–1993 (girls only)	Mixed	Cross-sectional	15,439 W and 1638 B	Tanner staging only for pubic hair Visual by pediatricians and 39% had breasts palpated ⁷	Current status-self	Means/probit analysis and status quo for menarche	Years	3–12
Study 8: HHANES ^{4,9}	Villarreal et al. 1989 Sun et al. 2005	1982–1984	Mixed	Cross-sectional	688 MA girls and 704 MA boys	Visual by physicians	Not done	Median for entry into stage Mean for being in a stage	Years by 3 month intervals	10–17
Study 9: NHANES III ¹⁰	1. Wu et al. 2002 2. Chumlea et al. 2003 3. Sun et al. 2002 4. Anderson et al. 2003 5. Sun et al. 2005 6. Kaparti et al. 2002 7. Herman-Giddens et al. 2001 8. Anderson and Must 2005	1988–1994	Mixed	Cross-sectional	Varied by authors' selection criteria: ~ 500–700 W, ~ 600–800 B, and ~ 400–800 MA	Visual by physicians	1. Current status (self) and recall (self) 2. Current status (self) and recall (self) (or parent for 8–9 year olds)	1. Current status data: mean (probit). Recall data: mean/failure midpoint of years of age 2. Median (probit) 2. Midpoint of a three-month age group	1. Current status: 1. 8–16 years. Postmenarcheal females: 2. 8–19 years of age three-month age group	1. 8–16 3. 8–20 4. 10–15 5. 12–18 B and W and 10–18 MA 6. 8–18 7. 8–19 8. 9–16

B, Black; W, White; O, other; MA, Mexican American; A, Asian, NR, not reported.

¹Years described in whole numbers indicate the range from the beginning of the 1st age to the end of the 2nd age; for example, 3–12 is 3.0–12.99 years old.

²Reynolds and Wines (1948 and 1951) used a five-stage rating system for breast and pubic hair development that had similar definitions to Tanner staging. For genital development, they used two separate scales, a five-stage genital development scale that had some differences compared with Tanner genital development stages: G2 was defined as “enlargement of scrotum, first reddening and texture change.” G3 was defined as “first ‘sculpturing’ and enlargement of penis.” G4 was defined as “pronounced sculpturing and darkening.” G5 was defined as “essentially adult; reddish brown color, loose penile skin, loss of sharp sculpturing.” The second had to do with individual differences GD: genital development in pencil size.

³Nicolson and Hanley (1953) used a four-stage system for breast development. They defined stage 2 as onset (similar to Tanner stage 2), stage 3 as similar to Tanner stage 3, and stage 4 as similar to Tanner stage 5. A five-stage system similar to Tanner staging was used for pubic hair development. For boys, they used a five-stage “sex stage” system of Greulich et al. (1942) that combined assessment of pubic hair and genital development.

⁴National Health Examination Surveys (NHES), Hispanic Health and Nutrition Examination Survey (HHANES), and National Health and Nutrition Examination Survey (NHANES) are nationally representative surveys conducted by the federal government. Data from NHES cycles II and III were combined and the age range was limited to 11–14 years. Harlan et al. (1980) for NHES data did not report means nor medians.

⁵Lee used Tanner staging and breast palpation but not testicular measurement. Most children were White.

⁶The Bogalusa Heart Study used an overlapping panel design of multiple cohorts. Foster et al. (1977) analyzed data from one CS cohort. The analysis of Wattigney et al. (1999) grouped ages as 8–11 (“Early”), 12–13 (“Intermediate”), or 14–17 (“Late”) years of age. Data from this study are not presented because of lack of comparisons to other studies.

⁷Cincinnati School Children: Biro et al. (1995) assessed testicular volume using a Prader orchidometer, and therefore, the data are not comparable to these other studies for genital staging.

⁸Pediatric Research in Office Settings (PROS): Used Tanner staging. Data on the 39% where the breasts were palpated were reported by Kaplowitz and Oberfield (1999).

⁹HHANES: Hispanics surveyed included Mexican Americans from the southwest United States, Cuban Americans from Florida, and Puerto Ricans from the greater New York City area. However, Villareal only analyzed the data from HHANES on Mexican Americans.

¹⁰NHANES: W, non-Hispanic White; B, non-Hispanic Black. Wu et al. (2002) calculated mean menarcheal age separately for current status and recalled age. Only the mean ages from current status data are shown in other tables. Sun et al. (2002) calculated means for “age in a stage” and medians for “age at entry” into a stage. Only medians for “age at entry” are reported in this chapter for comparison with other studies. Anderson et al. (2003) combined data from NHES cycles II and III and limited the age range to 10–15 years.

from around the mid-1900s and none are available on African-American youth from that time that included Tanner staging. (Interesting data from two retrospective studies on girls in a “colored orphanage” in New York state show that their mean age of menarche was 14.3 years in the early 1900s and 13.1 in the late 1930s.) Only the Lee study (41) assigned Tanner stages based on actual visual examinations. The Biro et al. (42) study in Cincinnati only looked at boys and introduced a different scale for genital growth based on orchidometer measurements making it not comparable with other studies for genital findings.

Comments on the Cross-Sectional Studies

THE GOVERNMENT SURVEYS

The rest of US pubertal data come from three cross-sectional studies if one considers the federal government studies, National Health Examination Surveys (NHES) I, II, and III, the Hispanic Health and Nutrition Examination Survey (HHANES), and the NHANES as one study with different phases and populations. Unfortunately, NHANES is no longer doing sexual maturation staging in their ongoing surveys.

NHANES I was a modification and expansion of the Health Examination Survey initiated 10 years earlier, which had carried out three separate programs. The second and third of these programs focused on children from 6 through 11 years of age and 12 through 17 years of age, respectively. Data collection began in April 1971. After NHANES I, data for NHANES II were collected from February 1976 to February 1980. NHANES III data were collected from 1988 to 1992. These surveys use probability samples of the US population to provide representative national data. All surveys have been multistage, highly clustered probability samples (43,44). Data collection teams consisted of specially trained interviewers and examiners. The methods used for training physicians in assessing sexual maturity have been used throughout these government surveys and are subject to quality control protocols as well as interrater reliability checks. These comprised a medical consultant who conducted site visits and observed Tanner staging by participating physicians at least three times per year and compared their ratings with his or her own (30). It is helpful in assessing data quality to be aware that the Tanner staging examinations were performed in mobile examination centers by physicians who were not pediatricians. In addition, the children did not receive complete physical examinations so the Tanner staging was sometimes awkward to perform (Brian Dolan, MD, MPH, e-mail communication, February 1, 2000). Selected households received remuneration to help with the problem of participation refusal.

Special comments on the characteristics of the government surveys (NHES, HHANES, and NHANES) and the need for caution in assessing the validity of findings calculated from the Tanner staging data

The only studies that have produced pubertal data over a period of time representative of the US population due to their largely consistent methods (except for the age range for collection of pubertal data) are the NHES, HHANES, and NHANES. Because of this, it is tempting to regard the results as somewhat of a “gold standard.” [For example, some authors have proposed that the NHANES III findings be used as national reference data for sexual maturation (32).] Although this is true for the surveys’ menarcheal data when the status quo method is used for analysis, it is not

true for the Tanner staging data. Some papers that have been published on analyses of these surveys have not fully described the limitations and weaknesses of the surveys. Therefore, when evaluating the quality of the data, it is well to consider a number of factors (30).

Reasons for concluding that the survey data are reasonably accurate

- Methods for collecting and processing the data have been in place for years and are subject to limited quality control measures.
- Proportions of children with a pubertal characteristic show consistency in proportional increases by years of age.
- Assessment errors are likely to have been similar between the surveys (30,32).
- Height and weight data are consistent with expected changes by pubertal stage.

Reasons that the survey data on Tanner staging may not be accurate enough to provide conclusive results

- NHES did not collect pubertal data on children under 12 years (45,46), HHANES under 10 years (47), and NHANES under 8 years of age—all ages were too late to capture the earliest developers—therefore, calculation of average ages for onset data is biased upward from the actual stage 2 onset age.
- The sexual maturity staging was by visual inspection only. In no case was palpation or measurement used.
- Staging for breast development in girls was probably more accurate than for genital development in boys due to differences in physical properties related to the subjective quality of the assessment (48,49). (For example, 25% of African-American NHANES III boys were graded as entering genital stage 2 at 7.5 years of age and, for Whites, 8.9 years of age (32). Although it is possible that these data may represent earlier puberty than in the past surveys, this degree of change calls for caution in evaluating the results.)
- The staging was performed by physicians who were not pediatricians who, despite their training, may not have been as experienced as pediatric examiners.
- No interrater reliability assessments among the examiners were carried out for pubertal staging.
- NHANES protocol allowed for a stage-1 variance between the physician's assessment and the quality control standard.
- Aspects of pubertal growth such as duration or the relationship between duration and timing of stages are more appropriately examined in longitudinal studies.
- The children did not receive a complete physical exam so had to expose their breast and genital areas just for the Tanner staging, which added a certain level of discomfort.
- Although the subjects were selected to be representative, individual cell numbers by age and race are not large, especially when broken down for further analyses. For example, in NHANES III for 10-year-old boys, a total of 64 were representing 1,153,352 US White boys of that age, 97 were representing 296,542 African-American boys, and 88 were representing 139,641 Mexican-American boys (the minorities were oversampled to provide more stable estimates) (30). Cell sizes are similar for girls.

THE BOGALUSA HEART STUDY

The Bogalusa Heart Study is a well-known community-based study of cardiovascular disease risk factors in schoolchildren from a southern Louisiana community, which was conducted between 1973 and 1994. It was the first to provide data on sexual maturity stages on Black and White children starting at a young enough age to capture the beginning of development, and in a more typical population socioeconomically. Only data for stages 2 and 3 were collected so this study does not add to information on pubertal completion (8,50). Tanner stages were assessed by physicians trained with photographs, and menarcheal information was collected similarly to the government surveys (8,50).

THE PROS STUDY

PROS had the advantage of enrolling large numbers of subjects fairly well spread out across the United States and, unlike the other studies, started collecting data at an age early enough (3 years) to capture the earlier developers (1). It is the only study where the participating physicians had to pass a competency test in Tanner staging and where interrater reliability was examined. Unlike NHES, HHANES, and NHANES where visual inspection only was used to assess breast development, PROS physicians palpated breast tissue in 39% of the subjects. These data were analyzed by Kaplowitz et al. (2), who reported that the higher percentages of very young girls with Tanner 2 breast development than reported in earlier studies could not be explained by observer error that is mistaking fatty tissue for true breast tissue, a concern due to the growing prevalence of overweight children in the United States. As with non-government the other US studies, as well as the Marshall and Tanner British studies of approximately 200 institutionalized children [which have been used for pubertal standards for years (29,34)], the results cannot be assumed to be representative of the US population although the size and subject distribution of the PROS study make this less of an issue than with the smaller studies. Only the government surveys are representative of the population as a whole. Unfortunately, owing to PROS funding limitations, girls were studied only up to 13 years of age, which makes the menarcheal data somewhat less reliable than of NHES and NHANES. Girls were enrolled in the study if they presented for reasons requiring a complete physical examination. Ninety-six percent were for well-child exams, the remaining 4% presented for reasons that would require a complete exam (such as chronic abdominal pain or fatigue). After the study's publication, critics stated that some parents may have brought the children for a problem with pubertal development, thus introducing a selection bias. They proposed that parents might have been too embarrassed to tell their pediatrician if they were worried that their daughter was too fast or too slow in development. The data collection form asked for the reason for the visit if it was not well-child care, and in no case was the reason listed as such a concern (unpublished data). It seems unlikely that the number of parents needed parents to create a bias would be reticent to share such a worry with their child's pediatrician; if such occurred, any introduced biases would be expected to lower the proportion of developers in the older girls (along with an increase in younger girls), an effect that was not seen (4).

Assessing the Girls' Studies

BREAST AND PUBIC HAIR DEVELOPMENT

Table 3A summarizes the average ages of entering into a stage for breast and pubic hair development in US studies. *Table 3B* shows an uncluttered version of the data making for easier comparisons of rounded average ages. Only two studies, Lee's and the PROS study (39%), used palpation for assessing early breast development (41,1). For the onset of breast development, that is, Tanner stage 2, one can see, considering the methodological differences and issues discussed above, that there is a drop in the age of approximately 5 months to 1 year from the 1940s studies to the most recent studies for White girls and a slightly larger drop for Black girls over a shorter period of time. Similarly, depending on which studies are compared for pubic hair, the age of onset appears to have dropped 6 months or more for White girls and 8 months to 1 year for Black girls. If one considers that the Fels Institute (33) and Guidance Study (28) results are likely to present a lower age than the overall US population at the time due to these (White) children's higher socioeconomic status, the drop in age of onset would be even larger. Data for 10-year-old to 11-year-old Mexican-American girls from HHANES and NHANES show a marked increase in the proportions in stage 2 or higher during the approximately 14 years between the studies for breast development (40% increasing to 70%) and pubic hair (23–62%) indicating a drop in pubertal age for this population as well (35).

Data are less convincing for any changes in stages 3 through 5 (*Table 3A*). In particular, it would appear that attainment of stage 5 might be even occurring later now than in the past. However, one has to consider the fact that attainment of adult sexual maturity in the Fels Institute and the Guidance Study in the 1940s at age 13 seems extraordinarily early even for higher socioeconomic status (SES) girls, another indication, if the assessment is accurate, that these well-off girls matured earlier than the US population as a whole. In addition, for reasons discussed above, the later sexual maturity stages may show more observer errors. It is possible that the onset of puberty and the progression are subject to different influences; therefore, it is useful to consider them separately. Of interest are observations of data that find the later the onset of puberty the faster the progression (4,51). It may be that current environmental influences, such as the epidemic of overweight and obesity among children and the prevalence of endocrine disruptors, are triggering an earlier onset, but that progression continues at a slower pace.

MENARCHE

The data on menarcheal age provide the strongest indicator of earlier puberty among girls in the United States in recent decades, rather than a leveling off after the mid-1900s as some have proposed (37). *Table 2* summarizes the design type and statistical information for US publications on menarcheal studies. Reviewing *Table 3A* or *B* and *Fig. 3*, one can see that the average age has dropped for both Black and White girls, though less steeply than what we know for the late 1800s and the first half of the 20th century.

Population-Based Studies on Menarche. Because the methodologies for the NHES and NHANES for menarche are exactly the same, these surveys offer the soundest

Table 3A

Ages of entering into pubertal stages and menarche in US White and Black girls from the 1940s on [SD or CI or standard error (SE) where noted]

Country or study	Years data collected	N type	Mean or median age in years								
			B2	B3	B4	B5	PH2	PH3	PH4	PH5	Menarche
England (Marshall and Tanner 1969)	1969	192 M	11.15 (1.10)	12.15 (1.09)	13.11 (1.15)	15.33 (1.74)	11.69 (1.21)	12.36 (1.10)	12.95 (1.06)	14.41 (1.12)	13.47 W (1.02)
Fels Institute, OH (Reynolds and Wines 1948)	1948	49 L	10.8 (1.1)	11.2 (ND)	ND	13.7 (ND)	11.0 (1.1)	11.9 (ND)	12.5 (ND)	13.9 (ND)	12.9 (1.4) W
Guidance Study, CA (Nicholson and Hanley 1953)	1936–late 1940s	70–97 L	10.6 (1.2)	11.2 (1.1)	ND	13.9 (0.9)	11.6 (0.9)	12.5 (1.0)	13.2 (0.9)	ND	12.8 (1.1)
NHES (MacMahon 1974)	1963– 1970	6710 C-S	ND	ND	ND	ND	ND	ND	ND	ND	12.8 W and 12.5 B
Baltimore (Lee 1980)	1969– 1974	18 L	11.2 (1.6)	12.4 (1.2)	13.1 (0.7)	14.5 (1.6)	11.9 (1.5)	12.7 (0.5)	13.4 (1.2)	14.6 (1.1)	13.3 (1.3)
Bogalusa Heart Study (Foster et al. 1977)	1973– 1974	1059 W and 621 B	10.4 (0.11 SE) W and 10.2 (0.13 SE) B	11.6 (0.9 SE) W and 10.9 (0.1 SE) B	ND	ND	10.9 (0.1 SE) W and 10.1 (0.1 SE) B	11.7 (0.1 SE) W and 10.9 (0.1 SE) B	ND	ND	12.7 (0.1 SE) W and 12.8 (0.1 SE) B

PROS (Herman- Giddens et al. 1997)	1992– 1993	17,077 C-S	10.0 (1.8) W and 8.9 (1.9) B	11.3 (1.4) W and 10.2 (1.4) B	ND	ND	10.5 (1.7) W and 8.8 (2.0) B	11.5 (1.2) W and 10.4 (1.6) B	ND	ND	12.9 (1.2) W and 12.2 (1.2) B
NHANES III (Chumlea et al. 2003, ¹ Sun et al. 2002, and Anderson and must ² 2005)	1988– 1994	~2000 C-S	10.4 (10.1– 10.7) W and 9.5 (9.1– 9.8) B	11.8 (11.5– 12.0) W and 10.8 (10.5– 11.1) B	13.3 (13.0– 13.6) W and 12.2 (11.9– 12.6) B	15.5 (15.0– 15.9) W and 13.9 (13.6– 14.3) B	10.6 (10.3– 10.9) W and 9.4 (9.1–9.7) B	11.8 (11.5– 12.1) W and 10.6 (10.3– 10.8) B	13.0 (12.7– 13.3) W and 11.9 (11.4– 12.2) B	16.3 (15.9– 16.9) W and 14.7 (14.3– 15.1) B	12.55 W ¹ (12.31– 12.79), 12.06 B ¹ (11.84– 12.28), 12.25 MA ¹ (12.04– 12.46), 12.43 All ¹ (12.33– 12.53), 12.57 W ² (12.45– 12.69), 12.09 B ² (11.82– 12.36), 12.24 MA ² (11.88– 12.59), and 12.53 All ² (12.43–12.63)

(Continued)

Table 3A
(Continued)

Country or study	Years data collected	N type	Mean or median age in years									
			B2	B3	B4	B5	PH2	PH3	PH4	PH5	Menarche	
NHANES 1999–2002 (Anderson and Must 2005)												12.52 W (12.38, 12.67), 12.06 B (11.81, 12.32), 12.09 MA (11.81, 12.37), and 12.34 All (12.24, 12.45)

L, longitudinal; C-S, cross-sectional; M, mixed; W, White; B, Black; ND, not done.
Marshall and Tanner: Data included as a point of reference. English institutionalized girls, means, from photos, and onset of a stage. Reynolds and Wines:
Means, from photos once every 6 months, onset of a stage for genital, first appearance of pubic hair, ages 9–21, and upper socioeconomic status (SES) White
girls. NHES, MacMahon: Median, status quo, and representative population. Lee: Mean, age of attainment, from direct examination once every 6 months,
ages 8.6–17.8, 94% W, upper SES, and 94% White. Foster: 50% transition, from direct visual (Tanner) staging, menses from asking girls, ages 5–14, and low
to average SES. PROS, Herman-Giddens: Median age of transition, from direct visual (Tanner) staging, menses by status quo, ages 3–12, mixed SES [39%
of subjects had breast palpation performed with substantial agreement with visual staging (Kaplowitz et al. 1999)]. NHANES, Chumlea, Sun: median age of
transition, from direct visual (Tanner) staging, results of menses analyses vary slightly by author, and ages 8–17, representative.

Table 3B
 Pubertal data for White and Black girls simplified, age of onset

Country or study and reference	Years data collected	N type	Age in years				
			B2	B5	PH2	PH5	Menarche
England (Marshall and Tanner 1969)	1950s–1970s	192 Mixed	11.2	15.3	11.7	14.4	13.5
Fels Institute, OH (Reynolds and Wines 1948)	1930s–1940s	49 L	10.8	13.7	11.0	13.9	12.9 W
Guidance Study, CA (Nicholson and Hanley 1953)	1930s–40s	95 L	10.6	–	11.6	–	12.8
NHES (MacMahon 1974)	1969–1974	C-S	–	–	–	–	12.8 W and 12.5 B
Baltimore (Lee 1980)	1973–1974	18 L	11.2	14.5	11.9	14.6	13.3
Bogalusa Heart Study (Foster et al. 1977 ¹ and Freedman et al. 2002 ²)	1988–1994	1059 W, ¹ 621 B, ¹ 9158 C- S, ² 1253 W L, ² and 805 B L ²	10.4 W and 10.2 B	–	10.9 W and 10.1 B	–	12.7 W 12.8 B
PROS (Herman-Giddens et al. 1997)	1992–1993	15,439 W and 1638 B	10.0 W and 8.9 B	–	10.5 W and 8.8 B	–	12.9 W and 12.2 B
NHANES III (Chumlea et al 2003, Sun et al. 2002, and Anderson and Must 2005)	1988–1992		10.4 W and 9.5 B	15.5 W and 13.9 B	10.6 W and 9.4 B	16.3 W and 14.7B	12.6 W, 12.1 B, and 12.5 All
NHANES 1999–2002 (Anderson and Must 2005)	1999–2002		–	–	–	–	12.5 W, 12.1 B, and 12.3 All

L, longitudinal; C-S, cross-sectional; M, mixed; W, White; B, Black; ND, not done.
 Marshall and Tanner: Data included as a point of reference. English institutionalized girls, means, and from photos.

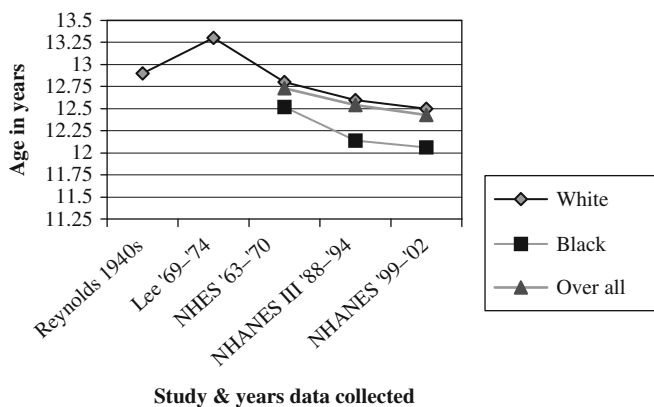


Fig. 3. Mean menarcheal age in US girls, 1940s–2000..

data for comparison, especially if the racial/ethnic populations are compared separately as they should be. In the 25 years between these two surveys, the average age of menarche dropped by about 2.5 months for White girls and 5 months for Black girls (6,36,37,52–54). These differences are statistically significant (6,53,55).

Several authors analyzing NHANES data have compared “overall” menarcheal ages, a term that includes all racial and ethnic population groups in the surveys (6,37,53,54). As a general principle, because we now know there are significant differences among racial and ethnic groups in the achievement of pubertal milestones, and changing proportions of racial and ethnic groups in the overall population over time, the validity of presenting “overall” data is questionable. In addition, racial and ethnic groups have been sampled differently among the various government surveys. Keeping these caveats in mind, comparing the “overall” age from NHES to the latest survey analysis (53), in the 30-year period, we found that the mean age has dropped from 12.76 to 12.34 years of age, a statistically significant difference of 5 months or 1.6 months per decade. This latest analysis of the most recent NHANES data as compared with NHANES III data of 10 years prior found nonsignificant drops in age for White girls from 12.57 to 12.52 years, for Black girls from 12.09 to 12.06 years, and for Mexican-American girls from 12.12 to 12.09 years (53). The authors noted that the statistically significant drop in overall age (from 12.53 to 12.43) in the 10 years was due to a larger change in the racial/ethnic category of “other” in the NHANES population sample. These data indicate that the trend toward a lower age for menarche has continued. Although some authors (35,37) have argued that this drop is not meaningful, many consider this an important difference that has biological, public health, social, and psychological significance for girls, their families, and society in general (6,53,55). It is particularly concerning that the latest NHANES analysis shows that for the decade ending in 2002, the drop in age has continued for the population as a whole (53). Because the drop in age for Whites, African Americans, and Hispanics was not statistically significant, it could be that these groups are stabilizing, although 10 years is probably too short a time period for assessment.

Menarcheal Studies on Specific Populations. The Bogalusa Heart Study findings are almost identical to those from the NHES/NHANES. The age of menarche decreased

by 9.5 months among the Black girls and 2 months among the White girls in the study between 1973 and 1994 (8), and twice as many girls in a later cohort had reached menarche before their 12th birthday than in the cohort 14 years earlier (56). The Fels Longitudinal Study found that girls born in the 1980s reached menarche 3–6 months earlier than those born in the 1930s. The correlation between birth year and age of menarche was small but significant ($r = -.14$; $P = .008$) (57). The PROS study found an average age of 12.88 years for White girls and 12.16 for Black girls (1). Although this shows a drop in age for Black girls, it does not for White girls as compared with previous studies (though the confidence limits include the NHANES average age, data not shown). Because the PROS population included girls only to 13 years of age, just 35.2% of the White girls had reached menarche as compared with 62.1% of the Black girls. Given the requirements of probit analysis, estimates from the data for White girls would be more biased than those for the PROS Black girls or the NHES/NHANES girls from whom data were collected from 8 through 18 years of age. Therefore, the NHES/NHANES menarcheal data are more reliable than those from the White subjects in the PROS study. Of note, the average menarcheal age for Black girls from NHANES III and that from PROS closely agree. (The PROS data on the onset of breast and pubic hair development are likely to be more accurate than data from NHANES, because some of the latter girls had already reached Tanner stage 2 or more by age 8 when their data collection began.) Recently, Biro et al. (58) found among 615 White girls and 541 Black girls recruited at age 9 and followed for 10 years that the mean age of menarche was 12.6 and 12.0 years, respectively, agreeing with NHANES data although theirs was not a representative population. Interestingly, the correlation between onset of puberty and menarche in their study was only .37 (Pearson's correlation coefficient) suggesting that factors that impact onset and menarche may have some differences. In summary, a number of recent studies on menarche have found statistically significant drops in age of menarche for White, Black, and Hispanic girls over the last 30–40 years.

Assessing Boys' Studies

GENITAL AND PUBIC HAIR DEVELOPMENT

Boys' studies of pubertal events in the United States, where professionals rated the boys, are limited to Tanner staging of genitalia and pubic hair growth. Fewer studies exist than for girls, perhaps because boys' puberty is less visible to the public, is harder to measure, and is of less interest than girls' as they are not the child-bearers. No papers have been published on the age of first ejaculation or spermarche with the exception of a study in the 1940s on 37 Black boys and 286 White boys. By interview, it was determined that the mean age of first ejaculation was 13.8 years for both groups (59).

Table 4A summarizes the average ages of entering into a stage for genital and pubic hair development in US studies. *Table 4B* presents summary data for easier comparison. As with girls, the earlier US studies are on small groups of well-off boys. NHES did not do Tanner staging on boys younger than 12 years of age; even then age 12 was too late to use to calculate Tanner 2 data (45). Boys' data are weaker than those from the girls' studies mainly because assessing genital growth visually is even more subjective than for breast development and because there is no counterpart of menses for boys.

Table 4A
Ages of entering into genital and pubic hair pubertal stages in US boys from the 1940s on

<i>Country or study and Reference</i>	<i>Mean/median¹ age of attainment of a stage in years (SD or 95% CI)²</i>									
	<i>G2</i>	<i>G3</i>	<i>G4</i>	<i>G5</i>	<i>PH2</i>	<i>PH3</i>	<i>PH4</i>	<i>PH5</i>		
England (Marshall and Tanner 1970)	11.64 (1.07)	12.85 (1.04)	13.77 (1.02)	14.92 (0.09)	Not reliable	13.90 (1.04)	14.36 (1.08)	15.18 (1.07)		
Fels Institute, OH (Reynolds and Wines 1951)	11.5 (0.9)	NS ³	13.4 (0.7)	17.3 (0.8)	12.2 (1.1)	13.3 (0.8)	13.9 (0.7)	16.1 (1.2)		
Guidance Study, CA (Nicolson and Hanley 1953 ⁴)	11.8 (1.0)	13.1 (1.0)	13.8 (1.0)	15.2 (1.0)	ND	ND	ND	ND		
NHES III (Karpati et al. 2002)	NR ⁵	13.0 (13.0–13.1) All	13.9 (13.8–14.0) All	15.1 (14.8–15.3) All	NR ⁵	13.3 (13.2–13.4) All	14.0 (13.9–14.1) All	15.3 (15.1–15.5) All		
NHES III (Sun et al. 2005)	ND	13.05 (12.91–13.17) W and 12.74 (12.38–13.02) B	13.88 (13.72–14.04) W and 13.72 (13.47–13.94) B	15.07 (14.96–15.18) W and 14.76 (14.42–15.11) B	ND	13.29 (13.20–13.38) W and 13.28 (13.01–13.51) B	14.04 (13.96–14.11) W and 14.03 (13.80–14.24) B	15.39 (15.30–15.49) W and 15.20 (14.95–15.46) B		
Baltimore (Lee 1980)	11.9 (1.1)	13.2 (0.8)	14.3 (0.8)	15.1 (1.1)	12.3 (0.8)	13.9 (0.9)	14.7 (0.9)	15.3 (0.8)		

Bogalusa Heart Study (Foster et al. 1977 ⁶)	11.82 (0.11 ⁷) W and 11.16 (0.185 ⁷) B	NR	NR	NR	12.52 (0.085 ⁷) W and 11.72 (0.125 ⁷) B	NR	NR	NR
HHANES (Villarreal et al. 1989 ⁸)	12.39 (12.14–12.58) H	13.50 (13.27–13.73) H	14.59 (14.35–14.89) H	16.26 (15.94–16.54) H	12.81 (12.43–12.83) H	13.62 (13.36–13.84) H	14.64 (14.32–14.94) H	16.06 (15.71–16.33) H
HHANES (Sun et al. 2005)	ND	13.29 (12.90–13.70) MA	14.38 (14.19–14.59) MA	16.08 (15.76–16.46) MA	ND	13.41 (13.10–13.72) MA	14.42 (14.17–14.69) MA	15.86 (15.61–16.14) MA
Cincinnati School Children (Biro et al. 1995 ⁹)	ND	ND	ND	ND	12.79 (NR) All	13.74 (NR) All	14.63 (NR) All	15.19 (NR) All
NHANES III (Herman-Giddens et al. 2001)	10.1 (9.6–10.6) W, 9.5 (8.9–10.0) B, and 10.4 (9.6–11.1) MA	12.4 (12.0–12.7) W, 11.8 (11.3–12.3) B, and 12.5 (12.2–12.8) MA	13.5 (13.2–13.8) W, 13.4 (13.1–13.6) B, and 13.7 (13.4–14.1) MA	15.9 (15.3–16.4) W, 14.9 (14.4–15.5) B, and 15.7 (15.3–16.2) MA	12.0 (11.7–12.3) W, 11.2 (10.9–11.4) B, and 12.3 (12.1–12.5) MA	12.6 (12.3–13.0) W, 12.5 (12.3–12.8) B, and 13.1 (12.9–13.3) MA	13.5 (13.2–13.8) W, 13.7 (13.5–13.9) B, and 14.1 (13.8–14.4) MA	15.7 (15.3–16.0) W, 15.4 (14.9–15.9) B, and 15.8 (15.5–16.2) MA

(Continued)

Table 4A
(Continued)

Country or study and Reference	Mean/median ¹ age of attainment of a stage in years (SD or 95% CI) ²							
	G2	G3	G4	G5	PH2	PH3	PH4	PH5
NHANES III (Karpati et al. 2002)	9.9 (9.5– 10.4) All,	12.2 (11.9– 12.5) All, 12.4	13.6 (13.4–13.8) All, 13.5	15.8 (15.3– 16.2) All, 16.0	11.9 (11.7– 12.2) All, 12.0	12.6 (12.4– 12.9) All, 12.7	13.6 (13.4– 13.8) All, 13.6	15.7 (15.4– 16.0) All, 15.6
	10.1	(12.1–	(13.2–13.8)	(15.4–	(11.7–	(12.3–	(13.3–	(15.2–
	(9.6–	12.7) W,	W, 13.4	16.6) W,	12.3) W,	13.0) W,	13.9) W,	16.0) W,
	10.6)	11.8	(13.2–13.7)	14.9	11.1	12.6	13.7	15.2
	W, 9.3	(11.3–	B, and 13.8	(14.3–	(10.8–	(12.3–	(13.6–	(14.8–
	(8.7–	12.3) B,	(13.4–14.1)	15.5) B,	11.5) B,	12.8) B,	13.9) B,	15.7) B,
	9.9) B,	and 12.5	MA	and 15.8	and 12.3	and 13.1	and 14.0	and 15.7
	and	(12.2–		(15.4–	(12.1–	(12.9–	(13.8–	(15.4–
	10.4	12.8)		16.3)	12.6)	13.3)	14.3)	16.1)
	(9.6–	MA		MA	MA	MA	MA	MA
NHANES III (Sun et al. 2002 ¹⁰)	10.03	12.32	13.52 (13.22– 13.83) W, 13.40	16.01 (15.57– 16.50)	11.98 (11.69– 12.29)	12.65 (12.37– 12.95)	13.56 (13.27– 13.86)	15.67 (15.30– 16.05)
	(9.61– 10.40)	(12.00– 12.67)						
	W, 9.20	W, 11.78	(13.15–	W, 15.00	W, 11.16	W, 12.51	W, 13.73	W, 15.32
	(8.62–	(11.50–	13.66) B,	(14.70–	(10.89–	(12.26–	(13.49–	(14.99–
	9.64) B,	12.08) B,	and 13.77	15.32) B,	11.43) B,	12.77) B,	13.99) B,	15.67) B,
	and	and	(13.51–	and	and	and	and	and
	10.29	12.53	14.03)	15.76	12.30	13.06	14.08	15.75
	(9.94–	(12.29–	MA	(15.39–	(12.06–	(12.79–	(13.83–	(15.46–
	10.60)	12.79)		16.14)	12.56)	13.36)	14.32)	16.03)
	MA	MA		MA	MA	MA	MA	MA

NHANES III (Sun et al. 2005)	ND	12.44 (11.73– 12.84) W, 12.37 (11.82– 12.70) B, and 12.56 (12.34– 12.80) MA	13.49 (13.12– 13.81) W, 13.46 (13.10– 13.75) B, and 13.76 (13.52– 14.01) MA	15.83 (15.38– 16.36) W, 14.86 (14.38– 15.31) B, and 15.60 (15.23– 16.02) MA	ND	12.55 (11.95– 12.93) W, 12.69 (12.27– 12.99) B, and 13.08 (12.84– 13.33) MA	13.48 (13.12– 13.79) W, 13.79 (13.45– 14.09) B, and 14.08 (13.85– 14.31) MA	15.59 (15.23– 15.96) W, 15.21 (14.71– 15.71) B, and 15.79 (15.49– 16.11) MA
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CI, confidence interval; N, study population number; L, longitudinal; CS, cross-sectional; G, genital; PH, pubic hair; NR, not reported by the authors; ND, not determined; NS, not shown in this table.

Race/ethnicity: M, Mixed; B, Black; W, White; MA, Mexican American; A, Asian. Marshall and Tanner: Data included as a point of reference. English institutionalized White boys, means, from photos, and onset of a stage.

¹Mean and median are considered similar values because a normal distribution is assumed.

²Explained in references.

³Reynolds and Wines (1953) definitions of GD are somewhat different from Tanner GD stages (see *Table 2* legend). Their G3 mean age is not shown here for purposes of comparisons with other studies.

⁴Nicolson and Hanley (1953) used a five-stage “sex stage” system with assessment of pubic hair and genital development; genital stage descriptions were similar to those of Tanner and are reported here as G2–G5. This discrepancy in stage definition affects comparability with other studies that used the Tanner-stage definitions.

⁵Not reported because the youngest age examined was 12 years.

⁶Foster et al. (1977) only presented median ages for transition into G2 and PH2 because of the “limited age range,” 5–14 years, of their population.

⁷Standard errors calculated.

⁸Villarreal et al. (1989) calculated percentages of boys in a given stage by age, means for being in a stage, means (from probit analysis) and medians (from Spearman-Kärber analysis) for entry into a stage. Means for entry into a stage are summarized in this table.

⁹Biro et al. (1995) defined a PS1: “PS1 was defined as the absence of pubic hair and a testicular volume of the larger testis less than 3 cc.” Their PS2 was similar to Tanner’s PH2. Data for comparable Tanner PH stages are presented here. PS1 data are not presented here. Data were not presented by race.

¹⁰Sun et al. (2002) calculated means for “age in a stage” and medians for “age at entry” into a stage. Medians are reported here for comparison with other studies that looked at age of attainment.

Table 4B
Pubertal data for White and All boys simplified

<i>Country</i>	<i>Years data collected</i>	<i>N type</i>	<i>Age in years</i>		
			<i>G2</i>	<i>G5</i>	<i>PH2</i>
Age of onset					
England (Marshall and Tanner 1970)	1950s–1970s	228 Mixed	11.6	14.9	–
Fels Institute (Reynolds and Wines 1951)	1930s–1940s	59 L	11.5	17.3	12.2
Guidance Study (Nicholson and Hanley 1953)	1930s–1940s	92 L	11.8	15.2	ND
Baltimore (Lee 1980)	1969–1974	36 L	11.9	15.1	12.3
Bogalusa Heart Study (Foster et al. 1977)	1973–1974	1153 W	11.8	–	12.5
NHANES III (Herman-Giddens et al. 2001)	1988–1994	536 C-S	10.1	15.9	12.0
Ages of pubertal events in “All” ¹ males					
NHES II/III (Kaparti et al. 2002)	1963–1970	3047 C-S	–	15.1	–
NHANES (Kaparti et al. 2002)	1988–1994	2481 C-S	9.9	15.8	11.9

L, longitudinal; C-S, cross-sectional.
Marshall and Tanner: Data included as a point of reference. English institutionalized boys, means, from photos, and onset of a stage, Reynolds and Wines: Mean, from photos once every 6 months, onset of a stage for genital, first appearance of pubic hair, ages 9–21, and upper socioeconomic status (SES). NHES: Karpati median, onset of a stage, from direct visual (Tanner) staging, ages 12–17, representative population. Lee mean: Age of attainment, from direct examination once every 6 months, ages 9–17, upper SES. Foster mean: 50% transition, from direct visual (Tanner) staging, ages 5–14, low to average SES. NHANES: Herman-Giddens median age of transition, from direct visual (Tanner) staging, ages 8–19, representative population.

¹ All—Black and White for NHES and Black, White, and Mexican American for NHANES.

As with the studies on girls' pubertal markers, similar caveats apply with comparing studies. Data on the onset of genital growth from any of these studies may not be reliable as the assessment was based on visual inspection only. Presumably, sexual maturity ratings of pubic hair are less subjective and, thus, more reliable, except, perhaps, in the early studies that used photographs to assign sexual maturity stages, especially stage 2 (27–29). Early US studies on White boys (*Table 4A*) found the ages of onset of genital growth to be about 11.5–11.9 years and about 12.2–12.5 years for pubic hair growth. Data from NHANES 30–40 years later on boys show the average age of onset of genital growth was 10.1 and 9.5 years of age, respectively, for Whites and African Americans and 12.0 and 11.2 for pubic hair growth (30–32). Mexican-American boys fell in between White and African-American values. When age of onset of pubic hair in the studies on White males is compared with that in earlier studies, there is a drop of about 3 months (*Tables 4A and 4B*), with the exception of Biro et al.'s 1990s study of 515 boys in Cincinnati, Ohio (not shown in table) (42). Data for 10-year-old to 11-year-old Mexican-American boys from HHANES and NHANES show a marked increase in the proportions in stage 2 or higher during the approximately 14 years between the studies for genital development (11% increasing to 46%) but no increase for pubic hair development (7% to 4%) (35) suggesting a drop in pubertal age for this population if the data are accurate.

The average ages of onset of genital stages need to be viewed with caution due to the marked drop from earlier studies (about 1.5 years for White boys) and the lack of testicular measurement as discussed in detail in Herman-Giddens et al.'s study (30). As with girls, the tempo of progress through the stages may be slower than in the past and ages of completion may be later, though these data are not reliable. Lack of adequate, methodologically sound data does not allow for a reasonably definitive assessment of secular changes for boys though the existing data together with growth data (see discussion below) suggest an earlier age of onset of puberty now as compared with several decades ago. The American Academy of Pediatrics' PROS is currently conducting a study of puberty in US boys which includes testicular measurement in early puberty. The study will provide much needed data, and the physical assessments will offer greater reliability than those from NHANES III. However, the problem of assessing secular trends will still exist as there are no comparable data from the past.

ADDITIONAL SUPPORTING EVIDENCE FOR EARLIER PUBERTY IN THE UNITED STATES

Growth Studies

Data are available that could be used to model timing of children's growth spurts and their achievement of final heights. If children are experiencing these milestones earlier now than in the past as data from the Bogalusa Heart Study suggest, this would provide additional evidence of earlier onset of puberty over the last several decades. Freedman et al. examined trends in height among the 5-year-old to 17-year-old children between 1973 and 1992 using over 24,000 examinations. During the study period, the mean height of the children increased by 0.7 cm per decade. For Black boys 9–12 years old, the height increase was 1.8 cm per decade. Because no increase in mature height was found in the 15-year-old to 17-year-old children, the authors suggested their findings "most likely reflect an acceleration of maturity" (60). In an analysis of NHANES III

data on boys as compared with NHES data (about 25 years earlier), final heights had not changed; however, NHANES boys 8–14 years of age were 2.0 cm taller than their earlier counterparts (31).

Dentition

It would stand to reason that if children are experiencing earlier growth spurts, sexual maturation, and final adult height, their dental age of maturation would also be earlier because there is a relationship between dental and sexual maturity (61). The adolescent growth spurt has been found to be highly correlated with the calcification pattern of teeth, especially the lower canines (62). Data on dental maturity based on the lower right canine from 150 White children randomly selected in 1972–1974 were compared with those from 150 White children in 1992–1994, all 8.5–14.5 years of age. Females were 1.5 years earlier in their dental maturation and males were 1.4 years earlier (63).

The Obesity Epidemic

Numerous studies, including observations going back to the middle ages (12), have found an association between higher adiposity and earlier maturation in girls, though the mechanism or direction of causation has not been defined (1,6,9,10–12,26,36,46,50,56,64–67). Only two have reported no association (35,56). The increase in overweight and obesity in US children has become a public health concern. For girls, the percentage above the 85th percentile for body mass index increased from 16 to 27% in the 25 years between NHES and NHANES III (6). This alone, especially because the prevalence of overweight among children is continuing to increase, would predict earlier puberty in girls unless the many studies showing this relationship are discounted. NHANES 1999–2000 data show that over 15% of children over 6 years old are at or above the 95th percentile of sex-specific body mass index for age growth charts as compared with 7 to 11% just 10 years earlier (68). Interestingly, the opposite association with overweight has been found for boys with the thinner boys maturing earlier than their heavier peers, although more research needs to be done on this association (67). Therefore, despite the dramatic increase in adiposity in US children, this may not be an influencing factor when considering changes in timing of boys' maturation.

Findings From the Role of Environmental Factors on the Onset and Progression of Puberty—Expert Panel Workshop, Chicago 2003

To further study US pubertal data and address controversial aspects as well as data reliability, experts gathered in November 2003 at a meeting, “The Role of Environmental Factors on the Onset and Progression of Puberty”—Expert Panel Workshop, held by Serono Symposia International in Chicago. The major conclusion of the workshop, that data for girls are sufficient to suggest a secular trend toward earlier onset of breast development and menarche but insufficient for boys' pubertal markers, has been presented (69). All members agreed that data are not sufficient to conclude that there is *not* a decreasing secular trend. A very detailed paper, “Examination of U.S. puberty timing data from 1940 to 1994 for secular trends: Panel Findings” by Euling et al., on methodological issues in puberty studies, findings, and the content and conclusions from this meeting is expected to be published in 2007 (70).

ARGUMENTS AGAINST A DECREASING SECULAR TREND IN AGE OF PUBERTY IN US CHILDREN

None of the discussed studies, except Biro et al.'s for onset of boys' pubic hair growth (42), show no drop in age of onset of secondary sexual characteristics for boys and girls or age of menarche in the United States as compared with studies conducted several decades ago. Only a few papers, which by necessity must use the same data from the studies presented above, have argued that the data do not support a secular trend toward earlier puberty in US children, even in girls. One argument is that the large variations in sampling, sample sizes, and methods preclude the ability to reach any reasonably reliable conclusions. Another approach is to look for different patterns that would support no change or present new analyses of existing data that may inadvertently introduce errors (71). Breaking down the data by too many criteria (such as age, race, and stage of development) may create a problem with stability of results because of the creation of very small sample sizes (35). A new analysis of government survey data compared proportions by race and ethnicity in stage 2 or higher and mean ages of entry into stages 3 through 5. Although the authors found statistically significant differences for some of the groups and stages with lower ages for NHANES children as compared with NHES children, the authors declared that there was no persuasive evidence for a lower age of puberty (35). A focus on whether the age of complete sexual maturity rather than onset is lower does not help solve the question of whether onset is earlier and those data on completion may be less reliable as discussed above. Of course, stating conclusions from these assessments assumes the same rigor and reliability among the studies being compared, which can be questioned regardless of whether the argument is for or against earlier puberty. Another argument is to state that although there are lower ages now (e.g., the .34-year drop in age of menses over approximately 25 years from the government survey data), the changes are not 'significantly' different, meaning not important (37), a problem discussed at length in a 2004 commentary in *Pediatrics* (55).

Other than for menses, there are no exactly comparable studies, leaving the final call subject to the influence of personal opinion and judgment layered over the data. A careful and cautious review of methods and data (72) states that conclusions regarding a lower age of onset can only be inferred and that evidence for a decrease in the age of menarche is still unsubstantiated (however, when this chapter was written, data from the last two NHANES for menses had not been published). Although it is correct that inference is the only way to assess the onset data, we now have sound data on menses showing that the age continues to fall (6,53). Hopefully, by the time the next NHANES analysis is presented on menses, the age will have stabilized.

CONCLUSIONS

US studies for pubertal milestones with methodologies that not only used similar methods but always included techniques that are now recognized as necessary, such as breast palpation and testicular measurement, do not exist, except for menarcheal data from the government surveys. (Breast palpation for the onset of development was done by Lee for the 18 girls in his study and for 39% of the 17,077 girls in the PROS study.) These differences must be taken into account when assessing data from the available

studies. Even given these concerns, the data for girls support a continued, though less steep, decline in age of puberty and menarche:

1. All the reviewed data for the onset of breast development and pubic hair growth show a consistent downward trend with modern girls starting development 5 months to a year earlier than those of 30–50 years ago, with somewhat larger differences for Black girls (but less data for comparisons). Hispanic girls also show a drop in age.
2. Tanner stage data from NHANES and PROS are in substantial agreement, allaying concerns that the PROS population was different from the US population as a whole.
3. The data do not show achievement of earlier final maturity, but the visual assessment of the “adult” sexual maturity stage may be less reliable and attainment more variable. The data suggest that the time for progression from onset to menses and beyond may be longer than in the past.
4. The soundest data, for age of menarche from NHES (54) and NHANES (53), over an approximately 30-year period show a statistically significant drop of 5 months, with the most recent data from 1999 to 2002. For White girls, this drop was 3.4 months and for Black girls 5.5 months.
5. The experts at the November 2003 meeting, “The Role of Environmental Factors on the Onset and Progression of Puberty”—Expert Panel Workshop, held by Serono Symposia International in Chicago, concluded that data are “sufficient to suggest” a secular trend toward earlier onset of breast development and menarche.
6. Supporting evidence includes the following:
 - I. Growth studies that show mean height increases at an earlier age, but no increase at final maturity, indicative of earlier maturity.
 - II. Earlier dental maturation.
 - III. The continuing increase in overweight and obesity for girls, associated with an earlier onset of puberty. (Interestingly, limited studies have shown an opposite effect for boys, so this is not supportive evidence for them.)

Data for boys are less reliable because of lack of testicular measurement, other differences in methodology, and fewer studies. However, the data suggest that boys may also be experiencing puberty earlier:

1. The studies show a consistent downward trend, though less steep than that for girls (with the questionable exception of the early genitalia stages in NHANES) in the onset of genital growth and pubic hair growth for White, Black, and Hispanics boys.
2. Supporting evidence includes limited growth and dental studies.

Because there is no convincing evidence that onset, progression, or final maturity is earlier (or later) in boys, the PROS study now in the field will provide much needed data.

The preponderance of evidence supports the assessment that puberty (based on the onset of breast and pubic hair growth and age of menses) is occurring earlier now than several decades ago for girls. The evidence for boys is weaker but cautiously suggestive. Changes in ages of onset of pubertal characteristics have profound public health and medical care implications. Just as rising ages may indicate lack of adequate nutrition or other harmful social or environmental conditions, lowering ages may not reflect ideal health or conditions either. Instead, they may indicate detrimental factors such as overweight or environmental contaminants. Reliable trend data, which cannot

wait for longitudinal studies, are important for the care of our children. The Centers for Disease Control should require that NHANES reinstitute this facet of data collection with improved methodology. Questions about whether there is an optimal age for puberty, any negative effects of too early an age of sexual development (such as psychological stress or an increased risk of reproductive cancers), and the causes for the decrease in the age of onset of puberty and menarche in girls need to be answered.

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6

BMI and the Onset of Puberty

Paul Kaplowitz, MD, PhD

CONTENTS

THE INCREASING PREVALENCE OF OBESITY IN US CHILDREN
EPIDEMIOLOGICAL EVIDENCE FROM US LINKING OBESITY AND EARLIER PUBERTY
STUDIES FROM OTHER COUNTRIES LINKING EARLIER PUBERTY AND INCREASED BMI
IS INCREASED BMI A RISK FACTOR FOR EARLY ADRENARCHE?
IS OBESITY RELATED TO EARLIER PUBERTY IN BOYS?
IS INCREASED BODY FAT THE CAUSE OF EARLY PUBERTY IN GIRLS OR THE RESULT OF IT?
THE POSTULATED ROLES OF LEPTIN AND INSULIN RESISTANCE IN THE RELATIONSHIP BETWEEN BMI AND THE TIMING OF PUBERTY
CONCLUSIONS
REFERENCES

Summary

Although there is mounting evidence for a secular trend for earlier puberty in girls over the past 30–40 years, there is disagreement as to what factor(s) might explain this trend. This chapter summarizes the available information that supports a relationship between earlier puberty and the well-documented increase in obesity over the same time period. Although there are few studies that directly measured body fat as a predictor of earlier puberty in girls, there are many that examine the relationship between earlier puberty and increased body mass index (BMI) in girls, which correlates well with increased body fat in young girls. Using the data from the Pediatric Research in Office Settings (PROS) study of pubertal development in US girls between ages 3 and 12, a clear relationship was found in 6-year-old to 9-year-old girls between higher BMI and the presence of breast development and pubic hair. This relationship was stronger for White than for African-American girls. Data from other US studies [the National Health and Examination Survey (NHANES) III, the National Heart, Lung, and Blood Institute Growth and Health Study (NGHS), and the Bogalusa Heart Study] support a role for increased BMI, as well as a few studies from other parts of the world. Whether increased obesity is a cause of or a consequence of earlier puberty in girls is still the subject of debate. There is so far no clear evidence linking obesity to earlier puberty in boys, and a few studies even suggest that obesity in boys may be associated with later puberty. Reasons

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why the effect of obesity may be different in girls than in boys will be discussed. In addition, the possible role of leptin and insulin resistance as factors linking obesity and earlier puberty in girls will be reviewed.

Key Words: Early puberty; BMI; Obesity; Premature adrenarche; Leptin.

THE INCREASING PREVALENCE OF OBESITY IN US CHILDREN

There is ample evidence for the increasing prevalence of obesity in all segments of our population, including children, over the last 30 years. *Table 1* compares the prevalence of obesity, defined as a body mass index (BMI) above the 95th percentile for age and sex, for Black and White boys and girls between the National Health and Examination Survey (NHANES) study from 1963 to 1965, NHANES II from 1976 to 1980, NHANES III from 1988 to 1991, and the recently reported results of NHANES 1999–2000 (1). In the 1963–1965 study, about 5% of the children had a BMI greater than the 95th percentile, but since then there have been striking increases in all groups, with the greatest increase being found in Black girls. In the most recent survey, 22% were classified as obese before they even reached adolescence. In another analysis of the longitudinal changes in BMI, Jolliffe (2) reported that not only was the incidence of obesity in children increased greatly (by 182%) over a 30-year period, but the extent of obesity (the amount by which children’s average BMI exceeded their age- and gender-specific BMI obesity threshold) increased even more, by 247%.

EPIDEMIOLOGICAL EVIDENCE FROM US LINKING OBESITY AND EARLIER PUBERTY

Ideally, one would like to be able to directly assess the influence of body fat mass on indices of puberty. However, in most large-scale epidemiological studies, fat mass has not been directly measured. BMI is a suitable surrogate for body fat, as demonstrated by a study in which both were measured in 100 boys and 92 girls between the ages of 7 and 17 years (3). For girls, the correlation between BMI and fat mass (measured by dual-energy x-ray absorptiometry or DEXA) was 0.94 and 0.96 for Black and White girls, respectively, and for BMI versus percent body fat, the correlations were 0.83 for both races. The correlations were lower for boys (0.85 and 0.86 for BMI versus fat mass and 0.54 and 0.50 for BMI versus percent body fat). Another shortcoming of many of the studies to be reviewed is that the timing of puberty is defined by the age of menarche rather than the age at which breast development appeared (thelarche).

Table 1
Percentage of 6-year-old to 11-year-old children with a BMI greater than the 95th percentile based on four national surveys spanning five decades

<i>Years of study</i>	<i>White boys</i>	<i>Black boys</i>	<i>White girls</i>	<i>Black girls</i>
1963–1965	5.6	2.0	5.1	5.3
1976–1980	7.9	7.9	6.4	11.3
1988–1991	10.4	13.4	10.2	16.2
1999–2000	12.0	17.1	11.6	22.2

Adapted with permission (1).

It is much easier to collect data on when girls reached menarche, which can be ascertained by history and does not require a physical exam. Although the interval between thelarche and menarche is quite variable, it can be assumed that most girls who reached menarche a year or more earlier than the average likely had earlier than average thelarche as well.

In 1997, the results of the cross-sectional Pediatric Research in Office Settings (PROS) study of female puberty were published as discussed in detail elsewhere in this volume (4). Because height and weight were recorded along with staging of breast and pubic hair development by trained pediatricians, it was possible to use this database to test the hypothesis that increased BMI was associated with earlier puberty in Black and White girls. Each female child between ages of 6 and 12 years had her BMI calculated and then converted to a BMI standard deviation or Z-score using the tables derived from the NHANES III study (5). For example, for 7-year-olds, the mean BMI was 16.2 ± 2.2 , and thus a girl whose BMI was 17.3 would have a BMI Z-score of $[17.3 - 16.2] \div 2.2 = 0.5$. For White 6-year-old to 9-year-old girls, the mean BMI Z-scores of girls with breast development were significantly greater than for age-matched girls who were prepubertal (*Fig. 1*); the same trend was noted for Black girls but the differences were smaller (6). For 7-year-old White girls, the mean BMI Z-score of 0.75 in girls with Tanner 2 breast development represented approximately a 10% increase in weight over ideal body weight. In 7-year-old to 12-year-old girls, there was a continuous relationship at each age between greater Tanner breast stage and higher mean BMI Z-scores (*Fig. 2*). The same trends for increased BMI Z-score were found for 6-year-old to 8-year-old White and Black girls who had the appearance of pubic hair but not breast development. White premenarcheal 11-year-old to 12-year-old girls had a mean BMI Z-score of -0.25 versus $+0.29$ for postmenarcheal girls of the same

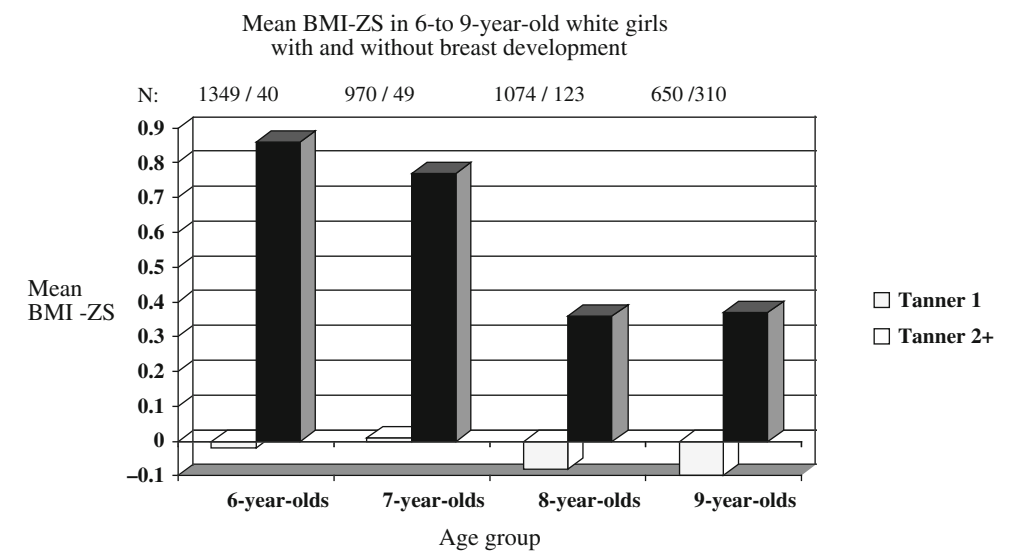


Fig. 1. Mean BMI Z-scores in 6-year-old to 9-year-old White girls with and without breast development, based on the Pediatric Research in Office Settings (PROS) study (6). The number of subjects represented by each bar is indicated at the top of the graph.

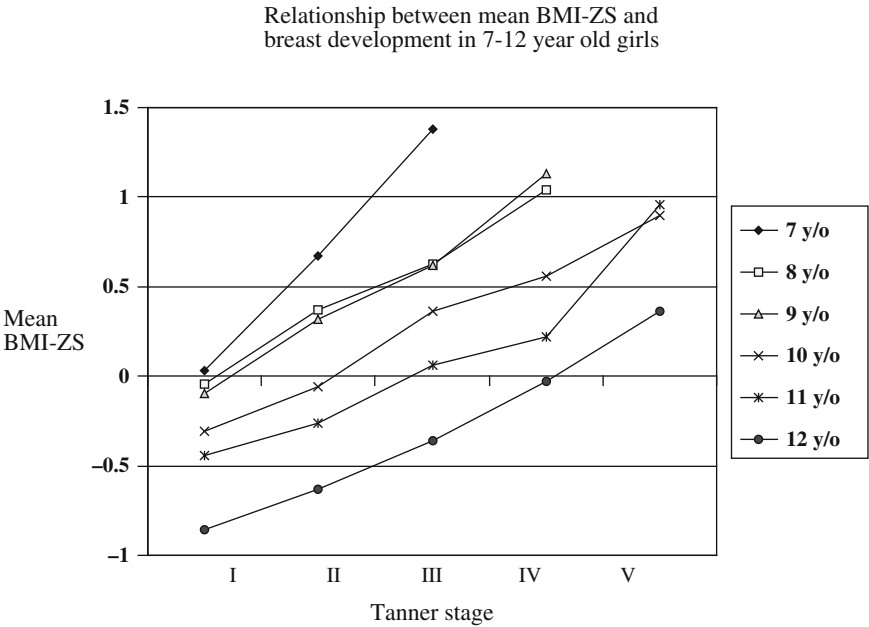


Fig. 2. The relationship between mean BMI Z-scores and Tanner staging of breast development in 7-year-old to 12-year-old girls, based on the Pediatric Research in Office Settings (PROS) study (6). The data include both White girls (90%) and Black girls (10%). y/o, years old.

age; for Black girls, the means were -0.09 versus 0.70 . One potential problem with the PROS study is that breast development was assessed by inspection and not palpation, which can cause confusion with regard to overweight girls, where fat can mimic breast tissue. However, an analysis of the subset of girls (39%) in which Tanner staging by palpation was recorded in addition to staging by inspection showed that the results were very similar to those published for the entire group (7).

The National Heart, Lung, and Blood Institute Growth and Health Study (NGHS) is a cohort study of 2379 girls divided about equally between White and Black girls who were recruited in 1987 at age 9. Each subject was examined yearly for height, weight, skin-fold thickness, and stage of pubertal maturation. A recent analysis of this cohort showed that the mean age at menarche was 12.7 years in White girls and 12.0 years in Black girls. The Black and White girls were separated into three groups according to the age of onset of menarche. Across the entire 9- to 18-year age range studied, the earlier maturing White and Black girls had consistently higher BMIs than the mid-onset girls, who had higher BMIs than the late-onset girls (8). Similar findings were reported when the sum of skin-fold thickness was examined in relation to the timing of menses. However, there was no association between age at menarche and body fat distribution.

Another recent study that examined the interrelationship between menarche and the trend for increasing obesity is the Bogalusa Heart Study, in which seven cross-sectional studies of 5-year-old to 17-year-old girls were conducted in a semirural Louisiana community between 1973–1974 and 1992–1993. During the 20-year study period, the median menarcheal age decreased by approximately 9.5 months among Black

girls versus approximately 2 months among White girls (9). Earlier age at menarche correlated negatively with BMI in both White and Black girls ($r = -.23$ and $-.24$). In addition, the incidence of early menarche (before age 11) was 1.79-fold greater for girls at the 75th percentile for BMI versus girls at the 25th percentile and was 1.4-fold to 1.5-fold greater for girls with skin-fold thickness at the 75th versus the 25th percentile.

As mentioned elsewhere, recent comparison of data from the NHES study (data collected between 1963 and 1970) and NHANES III (collected between 1988 and 1994) show that, using probit analysis, the average age at menarche dropped from 12.75 to 12.54 years over the 25-year period (10). At the same time, the percentage of girls 10–15 years of age who had a BMI above the 85th percentile increased from 16 to 27%. Further analyses were needed to see whether these two findings were related. Similar to what was found in the PROS and Bogalusa studies, premenarcheal girls at each age between 10 and 15 had a significantly lower BMI Z-score than postmenarcheal girls in both NHES and NHANES III studies, the main difference between the studies being the higher BMI Z-scores in NHANES III versus NHES. Table 2 summarizes data for ages 11–13, where there were sufficient numbers of subjects to provide good statistical comparisons; all differences were significant at $P < .01$ or greater. In a logistic regression model, an increased BMI Z-score was associated with a significantly greater likelihood of a girl having reached menarche, after adjusting for age and race. Based on the relationship between BMI Z-score and age of menarche derived from the NHES data, the authors predicted, based on the higher BMIs in NHANES III, that the average age at menarche in NHANES III would be 12.56 years; this was quite close to the actual figure of 12.54 years. This analysis suggests the testable hypothesis that there is a causal relationship between increasing obesity and the modest decline in average age at menarche over a 30-year period in US girls.

A different analysis of the puberty/obesity relationship using the NHANES III data was reported by Wang (11). Both boys and girls were categorized as early maturers if they reached a certain stage of genital (boys) or breast (girls) development earlier than the median age for that stage. Children (ages 8–11) and adolescents (ages 12–14) were analyzed separately. Table 3 summarizes the prevalence of obesity (BMI > 95th

Table 2
Relationship between age at menarche and mean BMI Z-score in 11-year-old to 13-year-old girls

Age	NHES				NHANES III			
	Premenarcheal		Postmenarcheal		Premenarcheal		Postmenarcheal	
	N	BMI Z	N	BMI Z	N	BMI Z	N	BMI Z
11	441	−0.22	65	0.75	204	0.17	57	0.83
12	295	−0.28	245	0.52	96	−0.15	127	0.63
13	153	−0.56	422	0.34	29	−0.33	181	0.71

From the NHES and NHANES III studies. All differences between premenarcheal and postmenarcheal girls were significant at $P < 0.01$. Adapted with permission (10).

Table 3
The prevalence (%) of obesity (BMI > 95th percentile) among girls and boys in the NHANES III study who were early maturers versus average and late maturers (others)

	<i>Girls</i>		<i>Boys</i>	
	<i>Early maturers¹</i>	<i>Others</i>	<i>Early maturers¹</i>	<i>Others</i>
Children	14	8	8	14
Adolescents	17	8	8	17
White	13	5	6	13
Black	20	12	8	19

Children, ages 8–11 and adolescents, ages 12–14. ¹A boy or girl was classified as an early maturer if they reached a certain stage of sexual maturation earlier than the median age for that stage in the population. For example, a girl with Tanner 2 breast development was early if her chronological age was less than the median for breast stage 2. Adapted with permission (11).

percentile) in children who were early maturers versus the prevalence of obesity in those who were either average or later than average maturers (others). Similar differences were found between early maturers and the others in the percent of subjects who had a BMI above the 85th percentile. For example, in 8-year-old to 11-year-old girls, 32% of early maturers and 20% of the others met the definition of overweight. It was concluded that fatness (measured as both BMI and skin-fold thickness) was related to sexual maturation in both boys and girls, with early-maturing boys being thinner and early-maturing girls being fatter than their later-maturing counterparts.

A recent study that involved only Black girls also found a strong relationship between increased BMI and earlier maturation. In a group of 147 Black girls between ages 8 and 10, it was found that girls who had Tanner stage 2 or greater breast development had a 6.1-fold higher risk of being overweight (BMI > 85th percentile) and a 8.2-fold higher risk of being obese (BMI > 95th percentile) compared with prepubertal girls (12). It was of note that, in contrast to breast development, increasing stage of pubic hair development was unrelated to either increased BMI or increased percentage of body fat.

STUDIES FROM OTHER COUNTRIES LINKING EARLIER PUBERTY AND INCREASED BMI

Although the association is not as striking in other countries as in the United States, there is limited data supporting a relationship between BMI and the timing of puberty. A study from the Netherlands compared pubertal maturation in both boys and girls from three cross-sectional national surveys conducted in 1965, 1980, and 1997 (13). There was a decrease of 0.25–0.3 years in the age of onset of breast development and the age of menarche during this period. At a given age, the probability of a girl having had menarche increased with increasing BMI up to a BMI of 20 (+1 SD), with no further increase in the probability of menarche with higher BMIs. No data on the impact of BMI on the timing of puberty in boys was provided.

In a random sample of over 3500 children between the ages of 6 and 13 from northern Italy, it was reported that different stages of puberty in both boys and girls

were reached about 1 year earlier than in Tanner's study on White British girls (14). The mean BMI Z-scores were significantly ($P = .006$) higher in girls at breast stage 2 than in girls at stage 1, but the magnitude of the difference was not large (+0.09 vs. -0.18). However, it is striking that the mean BMI Z-scores in this population are significantly lower than those found in the pubertal girls in the PROS study (Fig. 1), indicating a far lower prevalence of obesity in northern Italy compared with the US.

A study of breast and pubic hair development in 1231 Lithuanian girls of ages 7–11.6 found that the pubertal girls and those with adrenarche only had higher mean BMI (SDS) standard deviation scores (0.39 and 0.45) than did prepubertal girls (0.03) (15). However, the pubertal and adrenarcheal girls were 1–2 years older on average than the prepubertal girls, and again, the level of obesity in this population appears to be much lower than in US girls.

IS INCREASED BMI A RISK FACTOR FOR EARLY ADRENARCHE?

The mechanisms by which adrenal androgen secretion is activated in 6-year-old to 10-year-old girls and boys, leading to the appearance of pubic hair, are incompletely understood and likely involve intra-adrenal changes in the activity of certain adrenal steroidogenic enzymes. They are distinct from the activation of estrogen secretion by the ovarian, which is under the control of hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion. A number of studies point to a relationship between obesity and premature adrenarche, including an investigation of 216 consecutive patients seen at a single clinic in Paris. Although the 21 girls with appearance of pubic hair before age 2 had normal BMIs, of the 160 girls with appearance of pubic hair between the ages of 4 and 8, 52 (32.5%) had BMI Z-scores that were greater than or equal to +2 SD (16). In this age group, there was a significant correlation ($r = .27$, $P = .004$) between BMI Z-score and dehydroepiandrosterone sulfate (DHEA-S). Similarly, in the PROS study, it was found that 6-year-old to 8-year-old girls with pubic hair but no breast development had greater BMI Z-scores than age-matched girls without pubic hair or breasts (6).

Additional support for an effect of obesity on adrenarche came from a longitudinal study in Germany in which 42 White children had yearly 24-hour urine samples analyzed for the main adrenal androgen, DHEA-S (17). BMI was tracked by yearly measurements and was related to levels of urinary DHEA-S production. Although there was no association in the cross-sectional analysis between BMI and urinary DHEA-S, it was observed that there was a significantly higher increase in DHEA-S during the year when a child had the highest rise in BMI compared with the year when the BMI rise was lower. This suggests that an increase in body fat may play a critical role in turning on adrenal androgen secretion.

IS OBESITY RELATED TO EARLIER PUBERTY IN BOYS?

In contrast to the large number of studies pointing to a relationship between obesity and early puberty and menses in girls, there is little data pointing to a similar relationship in boys. The previously cited study based on the NHANES III data (11) points to the possibility that obesity may actually result later rather than earlier puberty

in boys. However, these results should be interpreted with caution, because of problems with the ascertainment of genital stage in the boys in NHANES III. This study appears to show that puberty is starting earlier in boys as well as girls, based on the observation that 25% of 8-year-old boys were already at genital stage 2 (18). However, clinicians have noted no increase in the number of boys being referred for precocious puberty, which is defined as the onset of testicular enlargement at less than 9 years of age, and which is still uncommon. Thus, it seems possible that criteria for attainment of stage 2 genital development in boys were not adequately defined in NHANES III, resulting in an over classification of prepubertal boys as early pubertal. In one of the few other studies that looked at the relationship between BMI and puberty in boys, the results were similar to those reported for NHANES III (11). Northern Italian boys with pubertal testicular size had a lower mean BMI Z-score (-0.26) versus prepubertal boys (-0.15) ($P = .003$) (14).

In contrast, a Spanish study that focused on boys between the ages of 11 and 14 years and girls between ages 10 and 13 found a positive relationship between age of pubertal onset and BMI in boys, but not in girls. However, the sum of skin-folds and the percent body fat did not differ according to the age of pubertal onset in either boys or girls (19). Laron (20) compared obese children referred to their clinic and age-matched non-obese Israeli boys and girls between the ages of 10 and 16 years. He found that obese children were taller up to age 14, but there was no difference in the age of appearance of pubic hair or facial hair or the age at testicular or genital enlargement in obese boys, or in the age of menarche or the appearance of breasts or pubic hair in obese girls. The last two studies suggest that the relationship between obesity, BMI, and the timing of puberty may differ depending on the population studied in both boys and girls.

One reason that data on increased BMI and the timing of puberty in males may be limited is that because there is no single easily defined pubertal event such as menarche in boys, it is simply more difficult to study this relationship. One needs a large sample of healthy boys with recorded heights and weights and physical exam with Tanner staging done by experienced personnel, or longitudinal growth records detailed enough to allow one to accurately determine the time of the pubertal growth spurt, and very few growth studies are suitable for obtaining this information. Furthermore, data based only on BMI in boys as a measure of body fat may be misleading, because the correlation between BMI and body fat is much lower in boys than in girls (3). This may be because during male puberty, the increasing muscle mass related to the anabolic effect of rising testosterone levels will cause an increase in weight and BMI independent of any increase in body fat.

IS INCREASED BODY FAT THE CAUSE OF EARLY PUBERTY IN GIRLS OR THE RESULT OF IT?

Studies that show a relationship between early puberty and increased BMI in girls do not answer the question of whether increased body fat predisposes girls to earlier puberty or whether earlier puberty in some girls leads to an estrogen-mediated increase in body fat. A review of the effects of gonadal steroids on body composition in adults concluded that estrogens and possibly progesterone largely account for the greater degree of body fatness in women as opposed to men, because these hormones appear

to work together to favor the storage of excess calories as fat, with estrogens promoting deposition of fat in peripheral adipose tissue depots (21). It is possible that the early pubertal female produces enough gonadal steroids to cause a significant increase in body fat. However, as noted above, the PROS data indicate that the relationship between mean BMI Z-score and pubertal stage in 7-year-old to 12-year-old girls is continuous and clearly apparent at Tanner stage 2 (*Fig. 2*). Estradiol levels are modestly elevated compared with prepubertal girls at Tanner stage 2 breast development (22), but the increase in estradiol between stages 2 and 3 is much greater. It is not clear if the brief and modest increase in estradiol production found at stage 2 of puberty could cause the clear increase in BMI observed in early pubertal 6-year-old to 9-year-old White girls shown in *Fig. 1*.

There is evidence from three longitudinal studies that the increase in obesity precedes the onset of early puberty in girls. In a large population-based study conducted in Sweden, growth data collected using physician records until age 6 and school records from ages 7 to 18 allowed determination of the age at peak height velocity as a measure of the timing of puberty. There was a negative correlation between the change in BMI between the ages of 2 and 8 years and the age of the peak height velocity (23). An increase of one BMI unit between ages 2 and 8 was associated with an average of 0.11 years earlier for peak height velocity, and for children with higher changes in BMI, the effect on the timing of puberty was as great as 0.6 years in boys and 0.7 years in girls. This study suggests that over-nutrition in early childhood can result in an earlier onset of puberty; in this study, the effect seemed to apply to both females and males.

A group of 197 5-year-old girls was enrolled in a longitudinal study at age 5 and then reexamined at ages 7 and 9 (24). Fat mass and body fat percentage were calculated using a formula employing height, weight, subscapular and the triceps skin-fold thickness, and bioelectrical impedance. At age 9, girls were classified as earlier ($n = 44$) or later ($n = 136$) maturers based on breast development stage examined by inspection, using serum estradiol levels, and using a parent-assessed pubertal development scale. It was found that girls with a higher percent of body fat at age 5 and girls with higher body fat or higher BMI percentiles at age 7 were significantly more likely than their peers to be classified as having earlier pubertal development by age 9. The strongest Spearman rank correlation (0.53) was between percent body fat at age 7 and breast development at age 9. Similar findings were reported in a Bogalusa Heart Study report comparing anthropometric data in early to mid childhood in girls who reached menarche before age 12 versus those who reached it age 12 or later. It was found that girls who reached menarche early already had higher BMIs and triceps skin-fold thickness between the ages of 5 and 7 years (25).

The Fels Longitudinal Study of girls, which began enrolling patients born in 1929 who had heights and weights measured every 6 months throughout childhood, may provide some insight into the relationship between earlier puberty and increased BMI. A recent report on 211 White girls who were born between 1929 and 1983 examined their BMI trends as a function of the age of the child relative to menarche and whether puberty was early (menarche ≤ 11.9 years), average (12–13.1 years), or late (≥ 13.2 years) (26). The key finding was that mean BMI in the three groups was similar prior to and at the time of menarche in early, average, and late menarche girls but

that 4–6 years after menarche, the early-maturing girls had clearly higher BMIs. This could be interpreted as evidence that increased body fat develops as a consequence of earlier puberty, rather than that increased body fat leads to earlier puberty. However, one should be cautious in interpreting a study in which only 211 girls represent a time span of 54 years, and only 62 of these girls were born between 1965 and 1983, a period during which the prevalence of obesity has increased greatly. It may be proposed that the relationship between earlier puberty and increased BMI in US girls is a relatively recent phenomenon.

THE POSTULATED ROLES OF LEPTIN AND INSULIN RESISTANCE IN THE RELATIONSHIP BETWEEN BMI AND THE TIMING OF PUBERTY

Leptin, the fat-derived hormone that is critical in regulating energy balance, also appears to be necessary for normal reproductive function. In parallel with studies in the leptin-deficient ob/ob mouse, humans with rare mutations of the leptin gene have been discovered who are very obese and remain prepubertal (27) unless given recombinant leptin, which restores pulsatile gonadotropin secretion (28). This makes sense from an evolutionary perspective, as it does not make sense for a female to be able to become pregnant during periods of caloric deprivation, as there would not be sufficient fat stores to carry a pregnancy successfully to term.

Several studies, both cross-sectional and longitudinal, have shown a marked rise in serum leptin concentrations in young girls starting as early as age 7 and continuing as they progress through puberty at least until age 15 (22,29,30). In contrast, in boys, leptin levels seem to rise transiently and then decrease after Tanner stage 2 to prepubertal levels, which are about one-third of those seen in late-pubertal girls. These changes in leptin levels are paralleled by increasing body fat during female puberty and decreasing body fat during male puberty. In at least one cross-sectional study, the rise in serum leptin was well established 2 years before clear increases in serum LH and estradiol levels were observed (22). This would be consistent with the hypothesis that higher leptin levels are one of the factors which are critical in allowing puberty to progress, rather than a result of the hormonal increases of puberty.

If the relationship between body fat and earlier menarche in humans is mediated by leptin, one would predict that leptin levels would be related to age at menarche. This was examined in a study of 343 healthy, White girls from central Ohio who were recruited at Tanner stage 2 of puberty between the ages of 8.3 and 13.1 (31). Menstrual history, height and weight, body composition by DEXA, and leptin were measured every 6–12 months over a 4-year period. As expected, leptin was highly correlated with body fat mass ($r = .81$). Higher leptin levels up to a level of 12 ng/ml were associated with a decline in the age of menarche by about 1 month per 1 ng/ml increase in leptin. In addition, the group of girls who remained premenarcheal for the entire 4 years of the study had significantly lower leptin levels than the groups who reached menarche during the study. The authors concluded that a threshold blood level of leptin in girls may be needed for the establishment of normal menses.

A possible relationship between leptin and precocious puberty in girls has been examined in two studies. Palmert et al. reported that whereas mean leptin levels in girls with central precocious puberty are slightly elevated compared with BMI-matched

controls, leptin levels in most of these girls fall within the normal range (32). A study from Germany reported that girls with central precocious puberty (CPP) showed no significant difference in leptin levels at pretreatment and after 1 and 2 years of treatment compared with healthy girls with the same BMI (33). These two studies would again suggest that an increase in leptin is not the trigger for the onset of puberty in most precocious girls, which is not surprising in that many girls with true precocious puberty are not overweight and may have a genetic basis for their earlier puberty. However, these findings do not rule out the possibility that higher leptin levels in obese girls may be one factor in their earlier, though not precocious, onset of puberty compared with girls of normal weight.

The role of leptin in the regulation of the onset of adrenal androgen secretion has also received recent attention. A preliminary study in seven US girls with premature adrenarche and eight age-matched controls showed a higher BMI and a greater than two-fold increase in leptin in the girls who were in adrenarche (34). In contrast, a Berlin study compared 26 obese prepubertal girls with 26 normal weight prepubertal girls and 30 girls with premature adrenarche with 30 age-matched controls. Before the onset of the appearance of pubic hair, adrenal androgen levels were to some extent related to higher BMI and leptin. However, in children with premature adrenarche, no clear correlation was found between increased adrenal androgen secretion and leptin or BMI (35).

Several studies also suggest a role for both insulin resistance and obesity in the pathogenesis of premature adrenarche, which has been associated with an increased risk of developing polycystic ovary syndrome in adolescence (36,37). Ibanez found hyperinsulinemia as well as other cardiovascular risk factors in girls with a history of premature adrenarche compared with girls without premature adrenarche matched for stage of puberty and bone age (37).

The relative roles of increased BMI and insulin resistance in the timing of puberty in girls cannot be easily separated, because increased body fat is known to cause insulin resistance. It is possible, however, that insulin resistance, not obesity, is the primary cause of earlier puberty, though direct evidence is limited. Indirect evidence favoring insulin resistance as the culprit comes from two observations: (i) the finding that earlier puberty and menarche in Black girls versus White girls cannot be entirely explained by the tendency of Black girls to have higher BMIs than age-matched White girls (6,10), and (ii) a report that prepubertal Black girls compared with age- and BMI-matched White girls had higher fasting insulin levels and higher first-phase insulin concentrations during a hyperglycemic clamp study (38). Thus, it may be proposed that the same genetic differences that result in relative insulin resistance early in life in Black girls are responsible for their earlier onset of both puberty and adrenarche. There are few data directly assessing insulin levels in relation to the timing of puberty. However, the Bogalusa Heart Study found slightly but significantly higher fasting insulin levels at ages 5–7 in girls who reached menarche before age 12 compared with girls who had later menarche (25). Although this could be attributed to the higher BMIs in the early-maturing girls at ages 5–7, it was found that insulin levels showed an independent positive association with early menarche.

CONCLUSIONS

Many of the studies reviewed in this chapter demonstrate convincingly that girls (but not boys) with an increased BMI (and therefore increased body fat) start puberty and reach menarche earlier than girls with a normal BMI. This observation, combined with the increasing prevalence of obesity in the United States and many other countries, provides a plausible explanation for why breast development and menarche have been found in some studies to be occurring earlier now than 30–40 years ago. The same considerations also may apply to earlier development of pubic hair, although the hormonal basis for this is quite distinct from the hormonal basis for thelarche and menarche. However, there are some cautions which should be stated regarding this hypothesis.

First, not all studies reviewed have found the same relationship between obesity and earlier puberty in girls, particularly some from other countries. It may be that those countries (e.g., Israel and Spain) have not experienced an increase in obesity to the same degree as the United States and that the same trends may either appear later or not at all in other parts of the world. It is also possible that the different genetic background in other countries may mask the association between puberty and obesity. It is worth noting, however, that recent reports from the Netherlands and northern Italy do show a correlation between BMI and earlier menarche, though not as large as is seen in US studies. Second, there is still controversy regarding the question of whether the increase in BMI precedes the earlier onset of puberty or is a consequence of it. This issue may not be resolved until larger longitudinal studies are able to follow a group of girls with a wide range of BMIs from the age of 5 years through at least the age of 13 years, with careful anthropomorphic measurements and pubertal staging at regular intervals. Third, the relative roles of higher leptin levels and differences in insulin sensitivity in mediating the effect of BMI on earlier puberty are still not resolved, but a role for insulin sensitivity is suggested by its ability to explain why Black girls mature earlier than White girls, a consistent finding in US studies that cannot be explained by their greater obesity. Finally, although there is much less data on boys, there is no solid evidence to date that increased BMI is associated with earlier puberty in boys, and in fact the opposite may prove to be the case.

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7

Puberty Timing Remains Unchanged

*Peter A. Lee, MD, PhD
and Christopher P. Houk, MD*

CONTENTS

BACKGROUND
DESCRIPTION OF LARGE SURVEYS
EVIDENCE SUPPORTING A TREND TOWARD AN EARLIER
PUBERTY
MAJOR TOPICS THAT CONFOUND CLAIMS OF A TREND
OF EARLIER PUBERTY
CONCLUSIONS: *EVIDENCE OF A CONTINUING SECULAR TREND
OF PUBERTY*
REFERENCES

Summary

It has been presumed by the media and the public that puberty is occurring earlier now than in past decades. This is the result of reports of earlier breast development in girls (Tanner stage 2, Br2) and earlier genital development (Tanner genital stage 2, G2) in boys. Nonetheless, owing to the lack of representative data, the intra- and inter-observer variability in Tanner staging, and the fact that initial sex hormone-driven physical changes do not always indicate the onset of puberty, bona fide evidence of an earlier pubertal onset is not available. The gold standard verifying pubertal onset involves documentation of hormonal responsiveness of the hypothalamic–pituitary–gonadal (HPG) axis. The evidence cited for an earlier onset of puberty involves the age of the first physical changes of puberty. In girls, the appearance of breast may simply be due to fatty tissue deposition, particularly in overweight children; alternatively, breast tissue may be a consequence of non-HPG-stimulated estrogen production. In males, genital staging is poorly defined and therefore subjective and prone to significance within and between observer disagreements. Inexperienced observers, unacquainted with the normal variation in prepubertal genital size and appearance, may erroneously assign G2 based on size alone. This is a possible explanation of recent studies showing a high percentage of 9-year-old boys in G2 in recent surveys in contrast to findings in the last 1970s. Data remain insufficient because of problems with sample size and selection, race, socioeconomic status (SES), assessment, and statistical methods to conclude that there is a significant continuation of the secular trend toward an earlier pubertal onset in boys or girls.

Key Words: Secular change; Puberty; Menarche; Tanner stages.

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BACKGROUND

More than 5 years ago (October 30, 2000), *Time* magazine ran a cover story that suggested that a trend toward earlier puberty in American children 30 years ago was certain. This type of media coverage has led the perception by most, both within and outside the medical community, that this trend has been unquestionably confirmed. Therefore, most would probably find it surprising that this claim of a trend toward earlier puberty in America has not been, and perhaps cannot be, substantiated. To explore this issue further, we present both the supporting and contradictory evidence regarding this issue.

It is clear that a secular trend toward earlier puberty took place over the time interval from the early 1800s to 1960s based on the progressive decline in age of menarche in European countries (1–3). This shift has been attributed to the effects of improved nutrition, control of infectious disease, and a progressive improvement in socioeconomic and public health conditions. In contrast to the convincing trend of earlier puberty that appears to have plateaued in the 1960s, contemporary controversy about pubertal timing revolves around whether this trend has continued.

One complexity of the modern information age is that the effects of isolated events and individual cases tend to be amplified, influencing the public, clinicians, and researchers. Furthermore, given that the current research climate discouraging visual examination of pubertal development has prompted the use of survey tools such as adolescent self-reporting of pubertal development [as planned for the next National Health and Nutrition Examination Survey (NHANES)], the ability to determine the epidemiological trends in pubertal development in the American population may never be answered with certainty. This switch to self-reporting will introduce a new set of uncertainties given what is already known about the behavior of adolescent girls involved in longitudinal studies to falsify age of menarche.

The data used to assess whether there have been changes in the age of puberty from the 1960s to the present come from large surveys, primarily from US government-sponsored programs. To help give the reader a better understanding of the available data sources, and the limitations inherent to them, we have described the three largest population studies from the United States since the 1960s.

DESCRIPTION OF LARGE SURVEYS

Large national surveys of pubertal staging in the United States include: (i) National Health Examination Survey (NHES) (1966–1970), (ii) the Hispanic Health and Nutrition Examination Survey (HHANES) (1982–1984), and (iii) the NHANES III (1988–1994). Pubertal stages were included for ages 12–17 years for NHES, 10–18 for HHANES, and 8–18 years for NHANES III. HHANES included Mexican Americans, Cuban Americans, and Puerto Ricans and can be compared with NHANES III Mexican Americans.

NHES cycles II–III were conducted from 1963 to 1970 (4–6) by the Centers for Disease Control and Prevention (CDC) and provide the first pubertal timing data of US children from these national surveys. Although Tanner stage data were not collected in NHES II, information on Tanner staging was collected in NHES III (1966–1970) for children 12 years of age and older.

NHANES I (1971–1973) and NHANES II (1976–1980) did not collect pubertal staging data. In contrast, the other studies from this enterprise labeled HHANES and NHANES III did collect pubertal data. Data from these studies were recorded as having reached a given stage of puberty at the age of examination.

Three recent surveys (NHES, HHANES, and NHANES III) provide sufficient data to estimate the timing of sexual maturity for US White, Black, and Mexican-American children. The sampling strategies used in these studies provide for over-sampling to assure adequate sampling of minority populations (Blacks and Hispanics). Appropriate statistical analyses must be chosen to account for this weighting. In these three large national data sets, comparisons between racial groups for pubertal stages are possible.

Additional important studies include the longitudinal and cross-sectional Bogalusa Heart Studies (1973–1994) (7,8) and the practice-based research network study [Pediatric Research in Office Settings (PROS), 1992–1993] that examined a large cohort of girls aged 3–12 years. This cross-sectional, racially diverse PROS study included 17,077 subjects. Participants were enrolled at well-child visits (95.74%) or visits involving complete physical examinations (4.26%). These studies documented physical exam findings to include heights, weights, and Tanner staging. Tanner staging was done by observation only with no palpation of breast or testicular volume. Because of the cutoff age of 12 years, 64.8% of Whites and 37.9% of Blacks had not yet reached menarche by study end.

EVIDENCE SUPPORTING A TREND TOWARD AN EARLIER PUBERTY

The basis for the position that puberty is continuing to occur at younger ages has come from these large cross-sectional studies mentioned in the previous section (NHES I–III, HHANES, NHANES III, and PROS). Based on the data from these studies showing a declining mean and/or median age of pubertal stage transition, the conclusion has been made that the secular trend toward an earlier age of puberty in American girls is continuing.

Because breast development is a reliable biomarker of estrogen, it has historically been considered a more reliable marker of puberty than the isolated development of pubic hair. With this background, statistical analysis of the data from the PROS or NHANES III suggests a trend toward an earlier onset of breast development in Whites than the accepted ages from earlier small US and British studies. Additional evidence has come from comparisons between the onset of breast development in Black and White girls from the PROS study and the Bogalusa Heart Study (9). The data from the NHANES III study showing that children are now taller and heavier during late childhood and at onset of Tanner stage 2 development have been postulated as an explanation for the earlier onset of pubertal changes in the United States.

Additional support for an earlier pubertal onset comes from a statistical trend toward an earlier menarche of 2.5–4 months (10) seen from the NHANES III data in 1994 when compared with the data from the NHES II and III compiled in 1966 and 1970.

In boys, the evidence for an earlier pubertal onset comes primarily from the age of Tanner 2 genital development documented in the NHANES III study compared with smaller previous studies and accepted standards for lower age limits of puberty. The other available earlier large-scale study data, NHES III, were not helpful for corroborating these data as they were not collected on boys less than 12 years of age.

Other pubertal markers in boys, such as attainment of later pubic hair or greater genital stages, did not show consistent significant difference on comparisons of NHES III and NHANES III data.

MAJOR TOPICS THAT CONFOUND CLAIMS OF A TREND OF EARLIER PUBERTY

Data that fail to support or refute the claims toward earlier puberty, as discussed below, include the following:

1. The inexactness and subjective nature of Tanner staging.
2. A lack of racially diverse historical comparison data.
3. The socioeconomic differences between study groups from the United States.
4. That the initial physical changes associated with pubertal onset do not always indicate pubertal onset [classically defined as an activation of the hypothalamic–pituitary–gonadal (HPG) axis].
5. Despite the statistically significant, albeit marginal, decline in the onset of breast development identified in the United States, there is no evidence that sexual maturity is being reached earlier.

Although most of the media focus has been on the trend toward earlier puberty in girls, some suggestions have been made that males are also showing an earlier puberty. We complete our discussion of this topic with a brief review of the available data from US boys.

The Inexactness and Subjective Nature of Tanner Staging

Tanner staging is by its very nature arbitrary in that it attempts to characterize a continuous developmental process into one of the five distinct stages. This fact provides for a degree of subjective interpretation in Tanner staging and must be taken into account when applying and interpreting the results of statistical analyses from data collected from a multitude of observers. One problem in ascertaining Tanner breast stage 2 is further confounded by the increasing incidence of obesity in US children. Overweight peripubertal girls commonly manifest a degree of disproportionate adipose deposition in the chest accompanied by slight areolar maturation that to some may be classified as the onset of true breast development. In addition, in overweight girls, a small amount of breast tissue may be palpable because of an adipose-related increase in serum estrone and/or decreased metabolic clearance of sex steroids rather than a pubertal HPG axis. This subjective nature of Tanner staging and inter-observer variability that stem from it add additional uncertainty about the conclusions of large-scale studies that employ multiple observers.

A Lack of Representative, Racially Diverse Historical Comparison Data

Until recently, the data used in the United States for age of puberty were extrapolated from studies in Great Britain (11,12) involving middle class Whites. In addition, small-scale studies of racially homogenous populations from the United States from the 1940s through the 1970s have been used to estimate the age of pubertal milestones (1,13,14). These small non-representative samples from the United States showed that for girls breast development occurred between 10.6 and 11.2 years, pubic hair

developed between 11.0 and 11.8 years, and menarche was reached between 12.8 and 13.3 years (15–17). In boys, the same studies showed a mean age of onset of genital growth from 11.5 to 11.9 years (11,12,18). Largely because of the paucity of racial sampling, these studies should not be considered representative of the US population and are therefore inadequate as a reference population for newer studies. The larger scale studies, NHES III, PROS, and NHANES, report data for the ages sampled but do not include all ages in question. Although these data suggest a trend, because of limitations cited, comparisons with earlier studies cannot be used to provide conclusive evidence of the trend toward earlier puberty.

AGE OF PHYSICAL PUBERTAL CHANGES

Onset in Females. The PROS study was the first to suggest the question of earlier pubertal development (19). The mean age of onset of breast and pubic hair stage 2 for Black girls in the study was 8.9 and 8.8 years, respectively, and the data for White girls was 10.0 and 10.5 years, respectively. In this study, 6.7% of White girls and 27.2% of Black girls had some breast development while 7 years of age—that is, by their eighth birthday. At the time, this was considered lower than the prevailing definition of precocious puberty (i.e., Tanner stage 2 breasts before 8.0 years). It is important to point out that the traditional definition of precocious puberty was developed from studies of British girls of mid-socioeconomic status (SES) and small US studies (1), data nearly entirely constructed from a White population. Appropriately, the PROS study, together with data from the 1966–1970 NHES, prompted the assessment of pubertal age from racially diverse populations that would better represent children from the United States.

Because historical data primarily represent the middle-class White population, a key consideration is whether the traditional age of precocious female puberty of 8.0 years (approximately -2 SD or 2.3%) is different than the 6.7% of 7 year old for White girls assigned Tanner stage 2 breast in the PROS study, especially when the discernment of Tanner stage 2 is often subjective. Furthermore, it is unclear whether the traditional mean age of onset of Tanner stage 2 breasts of 10.4–10.6 years, based on earlier small cross-sectional or longitudinal data from 1940 through 1970, is later than the 10.0–10.5 years noted in the PROS study.

The large national surveys have confirmed the racial difference identified by the PROS study (20). The NHES data found a significant difference between Black and White girls with respect to pubertal development. This racial difference was also seen in the NHES and NHANES studies, although these studies did not collect pubic hair and breast development data on children younger than 12 and 10 years of age, respectively. The NHANES III data have also confirmed earlier puberty in Black girls compared with White or Mexican-American girls (21).

Based on NHANES data, the median age of entry into pubertal stages and the approximate early limits are listed in *Table 1* (22). The ages for White girls are similar to historical data.

The youngest age when NHES and NHANES III inter-survey comparisons could be made was 12–13 years, an age when the majority of girls in both groups had passed Tanner breast stage 2. Assessment based on the percentage of 12-year-old Black or White girls in stage 2 or more breast and pubic hair development from NHES and

Table 1
National Health and Nutrition Examination Survey (NHANES) III data: median age of entry
(years)

	<i>White</i>	<i>Black</i>	<i>Mexican American</i>
Breast stage			
2	10.4	9.5	9.8
3	11.8	10.8	11.4
4	13.3	12.2	13.1
5	15.5	13.9	14.7
Pubic hair stage			
2	10.6	9.4	10.4
3	11.8	10.6	11.7
4	13.0	11.9	13.2
5	16.3	14.7	16.3
Approximate early limit of onset (2.5 percentile)			
Breast stage 2	8.0	6.6	8.8
Pubic hair stage 2	8.0	6.7	7.4

NHANES III was not significantly different (23). For Mexican-American girls, the youngest age sampled in both HHANES (1982–1984) and NHANES III (1988–1994) was 10 years. In HHANES, 40% of 10-year-old girls had Br2 compared with 70% for NHANES, a significantly larger proportion. However, this difference over slightly more than a decade raises questions of whether the stage assignment, SES, or population sampled was the same. Hence, data are insufficient to determine differences within the White and Black groups and questions concerning the Mexican-American group.

Is Menarche Continuing to Occur Earlier? Because menarche is the pubertal milestone least prone to subjective interpretation, it has been commonly cited as evidence for the secular trend toward earlier puberty. By the middle of the 20th century, the mean age of menarche was 12.5–13.0 years. Analyses from more contemporary studies have shown a continued trend both toward and against earlier menarche.

The application of different statistical analyses to the same database has led to different conclusions. One study reports that the average age of menarche dropped from 12.75 to 12.54 years, a drop of about 2.5 months, felt by the authors to be a statistical difference (10). Another report (23), also using probit analysis of NHANES III data, assessed ages at which 10, 25, 50, 75, and 90% of girls reached menarche, the median age being 12.43 years. When the median value was compared with NHES data (11), a decline of about 4 months was found, a difference “not more significant than that reported for US girls in 1973.” Thus, application and interpretation of pubertal milestones can lead to different conclusions, resulting in contradictory conclusions.

Given the identified differences in pubertal patterns between ethnicities, it is paramount that any analysis of the age of menarche data accounts for ethnicity with individual sub-analyses. From NHES cycles II and III, the mean age at menarche was reported at 12.80 years for Whites and 12.48 years for Blacks (20). Using data from NHANES III (1988–1994), we calculated median ages (11) at menarche of White,

Table 2
National Health and Nutrition Examination Survey (NHANES) data:
age of menarche (years)

	Median age	−2.5 percentile
Entire cohort	12.43y	10.45y
White	12.55	10.65
Black	12.06	9.70
Mexican American	12.25	10.05

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12.55 years; Black, 12.06 years; and Mexican-American, 12.25 years (*Table 2*). When comparing percentiles, the age of menarche was significantly earlier for Black girls than for White girls at the 10th, 25th, and 50th percentiles; however, these differences were not present at the 75th and 90th percentiles. Mexican-American girls were significantly earlier than White girls only for the 25th percentile.

Onset in Boys. The issue of whether male puberty is occurring at younger ages rests on whether data are comparable and accurate. Comparison of the mean, median, or percentage at age 12 for Tanner stage 2 pubic hair for White boys between the early studies (5,13,18), NHES, and NHANES (23) do not show differences. If Tanner stage 2 genital data are reliable, the comparative data suggest that Tanner stage 2 genital has declined by 1–2 years over that last three decades (13,14,22,24). However, ascertainment of genital stage 2 seems particularly difficult based on results of the NHANES III study in which multiple examiners assigned Tanner stage 2. Consistency of assignment of Tanner stage 2 must be demonstrated together with verification that the onset of puberty is reflected in increased testicular volume, the physical finding that suggests HPG pubertal activity (25).

The Socioeconomic Differences Between Study Groups From the United States

In addition to racial makeup of the study populations used, the analysis of secular trends in puberty must also account for differences in SES in study participants. Although it is clear that racial differences occur for ages of pubertal development, comparisons may not be appropriate unless study design includes representative sampling concerning SES status. For example, although the Bogalusa Heart Study found that Black girls reached menarche earlier than White girls, over a 20-year period this longitudinal study found that the age of menarche for Black girls decreased by 9.5 months whereas that of White girls decreased by about 2 months (9). Because socioeconomic conditions may have changed more for Black girls in Mississippi, the site of the Bogalusa study, than for Black girls in the United States during these two decades, these data may not be representative of any overall secular trend of Black girls. Although some portions of the US population may show a secular trend toward earlier puberty, this cannot be established for the US population as a whole without comparable data.

The value of SES is further highlighted by the differences in pubertal development between underdeveloped countries or regions that show a later puberty than industrialized areas. For example, girls in Senegal are shorter and thinner than similar-aged girls from developed countries; Senegal girls have a median age of breast development of 12.6 years and menarche of 15.9 years (26). Pubertal delays in both sexes can be ascribed to malnutrition, greater energy expenditure, and stressful living conditions. Intra-country differences between urban and rural environments have also been demonstrated (27), which emphasize the impact of poor socioeconomic conditions (28). These SES differences may even override racial differences in pubertal development. For example, median ages for Tanner stage 2 breasts (11.5 years) and Tanner stage 5 breasts (15.7 years) were similar for Black and White girls in Johannesburg (29), whereas Black girls had later Tanner stage 2 pubic hair (11.3 vs. 12.1 years) and completion of pubic hair development (Tanner stage 5 pubic hair, 14.8 vs. 16.2 years) compared with White girls. The median age of menarche was 10 months later in Black girls than for the White girls (13.9 years vs. 13.1 years), in contrast to earlier median menarche in Black girls in better SES conditions. This lack of difference between Whites and Blacks in Johannesburg has been attributed to the overall better nutrition and social conditions of White girls in this region.

In addition, recent investigations from developed countries have also shown significant differences in the mean age of menarche within similar races. For example, Scandinavian countries have reported a mean age of menarche for Whites of 13.0–13.2 years (30,31) compared with the 12.6–12.9 years for White girls in the United States (10,11,19,32). This difference may be related to inherent differences between races suggesting that once a threshold of SES and nutrition has been reached in a population that pubertal onset and tempo may remain stable. Additional examples of this phenomenon come from Hong Kong, a country that has enjoyed a stable SES status where pubertal milestone data in girls have shown no change over the last four decades after comparing studies from 1962, 1979, and 1993 (33). Likewise, lack of shift is reported in an Italian study for the onset of breast and pubic hair development and menarche.

***That the Initial Physical Changes Associated With Pubertal Onset
do not Always Indicate Pubertal Onset (Classically Defined as an Activation
of the HPG axis)***

DESCRIPTION OF PHYSIOLOGIC PUBERTY

It has been known since the 1970s that low levels of gonadotropins are episodically secreted during childhood well before pubertal onset. The onset of puberty occurs when this low level of basal activity transitions to a more robust and amplified gonadotropin release pattern. Ideal studies to verify the onset of puberty and evaluate the secular trend toward earlier pubertal onset would include hormonal measurements a pubertal HPG axis. Therefore, to answer the question “Is puberty occurring earlier?” hormonal documentation of a pubertal gonadotropin release pattern is necessary. Such studies involve measurement of basal and/or gonadotropin-releasing hormone (GnRH)-stimulated gonadotropins, primarily luteinizing hormone (LH), together with sex steroids and other markers of gonadal function. If hormone measurements are consistent with the physical changes of puberty and growth, this is consistent with the onset of puberty. On the contrary, physical changes of puberty unaccompanied by pubertal hormonal measurements indicate that puberty has not yet begun.

SIGNIFICANCE OF EARLY PHYSICAL PUBERTAL CHANGES

Without hormonal verification, the Tanner stage 2 physical development cannot be said to reflect the onset of puberty—particularly in those children with minimal pubertal progression. Because puberty is a progressive process, timely progression of changes is of key importance. If there is progression of breast development from Tanner 2 to Tanner 3, HPG axis activation can be assumed. However, the finding of Tanner stage 2 pubic hair suggests adrenarche and by itself does not support pubertal onset. In some cases, these initial physical changes may be a consequence of obesity or the ubiquitous hormone-like substances (aka: endocrine disruptors) found in our modern environment (34).

POTENTIAL CAUSES OF EARLY PHYSICAL DEVELOPMENT OTHER THAN HPG AXIS

Further difficulties in judging a secular trend of puberty include possible physiologic relationships with body weight. In fact, this relationship was identified from studies in the early 1970s that showed that secondary sexual characteristics were reported earlier in heavier girls. However, no clear relationship between overnutrition (overweight and obesity) and demonstration of onset of pubertal HPG activity was shown. Because it is clear that the prevalence of obesity has increased in children aged 2–19 years and that the degree of obesity has also increased (35) between the years between the surveys (1971 and 2002), this finding suggests that for girls, overnutrition may be tightly connected to the initial physical changes in pubertal development. Evaluation from the Bogalusa Heart Study that compared skinfold thickness, height and weight, and menarche in White and Black girls (8,9) showed that Black girls aged 5–9 years were taller and heavier than similarly aged White girls. This was predictive of earlier menarche but did not account for all of the variation in age of menarche between Whites and Blacks.

The relationship between weight and initial pubertal changes has not been observed for boys (9). Over a 25 to 30 years period, both the national surveys, NHES and NHANES (36), and the Bogalusa Heart Study (7,9) data show that boys have become taller during childhood and heavier at the end of puberty but not taller. How weight or body composition influences the onset of puberty remains unclear.

Pertinent correlations have been made between the onset of Tanner stage 2 and body composition. However, the relationship between body size and the attainment of pubertal events may be indirectly related to a critical mass not being obligatory. For example, the onset of puberty in children with chronic childhood malnutrition is not related to body size or body fat (37). A recent longitudinal study found that girls beginning with breast development had earlier menarche and greater skinfold thickness, body fat, and percent body fat, both a year before and throughout puberty. This has been interpreted as evidence of a risk of adult obesity (38).

Despite the Statistically Significant, Albeit Marginal, Decline in the Onset of Breast Identified in the United States, There Is no Evidence That Sexual Maturity Is Progressing or Being Reached Earlier

Data as outlined below show in *Table 3* that puberty is being completed at the same ages as previously. Hence, if puberty is actually starting earlier, the duration of pubertal development is longer, an unlikely situation in an enhanced physiological condition.

A better interpretation may be that the changes that are being interpreted as onset of puberty are unrelated to the actual onset of puberty as driven by the HPG axis.

PROGRESSION AND COMPLETION OF PUBERTY IN FEMALES

Sun et al. (19) re-analyzed the NHES III, HHANES, and NHANES III Tanner stage data in girls by race (Table 3). Because NHES III included children aged 12 or older, age of attainment of percentiles, including the median, of breast and pubic hair stages 3, 4, and 5 were calculated. No significant differences were found between Black and White girls for ages of entry into breast and pubic hair stages 3, 4, or 5, except stage 5 pubic hair in the NHANES III White girls. The 50th and 75th percentiles of Tanner stage 5 pubic hair were significantly different, but later.

Table 3
Ages of entry (years) by percentile to Tanner Stages from National Health Examination Survey (NHES) (1966–1970), Hispanic Health and Nutrition Examination Survey (HHANES) (1982–1984), and National Health and Nutrition Examination Survey III (NHANES III) (1988–1994) for girls

	Stage 3		Stage 4		Stage 5	
	NHES	NHANES	NHES	NHANES	NHES	NHANES
White						
Breasts						
10 percenile	9.63	10.39	10.22	10.29	11.61	11.29
25 percenile	10.52	11.07	11.43	11.53	13.44	13.12
50 percenile	11.52	11.82	12.78	12.90	15.47	15.16
75 percenile	12.52	12.58	14.13	14.28	17.50	17.19
90 percenile	13.42	13.25	15.35	15.52	19.33	19.02
Pubic Hair						
10 percenile	7.94	8.00	10.69	10.63	10.47	12.50
25 percenile	9.08	9.08	11.66	11.62	12.15	14.26
50 percenile	10.34	10.28	12.74	12.73	14.02	16.13
75 percenile	11.60	11.48	13.83	13.83	15.89	17.99
90 percenile	12.73	12.55	14.80	14.83	17.58	19.67
Black						
Breasts						
10 percenile	7.89	6.82	8.03	8.07	10.13	9.60
25 percenile	9.03	7.77	9.65	9.67	12.10	11.47
50 percenile	10.29	9.39	11.46	11.46	14.29	13.54
75 percenile	11.55	11.01	13.26	13.24	16.48	15.62
90 percenile	12.69	12.47	14.89	14.85	18.45	17.49
Pubic Hair						
10 percenile	7.94	8.00	9.03	7.61	10.47	10.19
25 percenile	9.08	9.08	10.36	9.15	12.15	12.13
50 percenile	10.34	11.48	11.85	10.87	14.02	14.29
75 percenile	11.60	12.55	13.33	12.59	15.89	16.45
90 percenile	12.73	X	14.66	14.28	17.58	18.37

Mexican American						
Breasts						
10 percenile	10.14	9.53	11.22	10.74	12.49	11.92
25 percenile	11.02	10.40	12.40	11.83	13.66	13.26
50 percenile	11.98	11.37	13.72	13.04	14.97	14.74
75 percenile	12.95	12.33	15.04	14.25	16.27	16.23
90 percenile	13.83	13.20	16.22	15.33	17.45	17.57
Pubic Hair						
10 percenile	10.58	9.69	11.69	10.62	13.02	13.50
25 percenile	11.37	10.62	12.74	11.82	14.09	14.64
50 percenile	12.24	11.65	13.92	13.15	15.27	16.24
75 percenile	13.11	12.68	15.09	14.48	16.45	17.84
90 percenile	13.89	13.60	16.14	15.79	17.51	19.27

Bolded pairs are significantly earlier for NHANES, whereas bolded and italic pairs are significantly later. Data reproduced with permission (23).

Comparisons of HHANES/NHANES for Mexican-American girls included significantly earlier and later changes. Earlier attainment was present for Br4 at the 50th and 75th percentiles and for PH4 at the 10th, 25th, and 50th percentiles. Later age at entry into stage PH5 in the NHANES than the HHANES girls was found for the 50th, 75th, and 90th percentiles.

Table 4
Ages of entry (years) by percentile to Tanner Stages from National Health Examination survey (NHES) (1966–1970), Hispanic Health and Nutrition Examination Survey (HHANES) (1982–1984), and National Health and Nutrition Examination Survey III (NHANES III) (1988–1994) for boys

	Stage 3		Stage 4		Stage 5	
	NHES	NHANES	NHES	NHANES	NHES	NHANES
White						
Genital						
10 percentile	11.45	10.77	12.45	11.91	13.29	13.00
25 percentile	12.21	11.56	13.13	12.66	14.13	14.34
50 percentile	13.05	12.44	13.88	13.49	15.07	15.83
75 percentile	13.89	13.31	14.64	14.32	16.00	17.32
90 percentile	14.65	14.10	15.32	15.07	16.84	18.66
Pubic hair						
10 percentile	11.88	11.00	12.67	11.95	13.68	13.54
25 percentile	12.55	11.74	13.32	12.67	14.49	14.51
50 percentile	13.29	12.55	14.04	13.48	15.39	15.57
75 percentile	14.04	13.37	14.76	14.27	16.30	16.67
90 percentile	14.71	14.11	15.41	15.01	17.11	17.64

(Continued)

	<i>Stage 3</i>		<i>Stage 4</i>		<i>Stage 5</i>	
	<i>NHES</i>	<i>NHANES</i>	<i>NHES</i>	<i>NHANES</i>	<i>NHES</i>	<i>NHANES</i>
Black						
Genital						
10 percentile	10.96	10.86	11.96	11.83	12.67	11.95
25 percentile	11.81	11.58	12.79	12.60	13.66	13.33
50 percentile	12.74	12.37	13.72	13.46	14.76	14.86
75 percentile	13.68	13.16	14.64	14.31	185.86	16.39
90 percentile	14.53	13.87	15.47	15.08	16.85	17.77
Pubic hair						
10 percentile	11.58	11.14	12.35	12.38	13.20	12.90
25 percentile	12.39	11.87	13.14	13.05	14.15	13.99
50 percentile	13.28	12.69	14.03	13.79	15.20	15.21
75 percentile	14.17	13.51	14.91	14.53	16.25	16.42
90 percentile	14.97	14.25	15.71	15.20	17.19	17.52
Mexican American						
Genital						
10 percentile	11.90	10.96	12.73	12.06	13.95	13.26
25 percentile	12.56	11.72	13.51	12.87	14.96	14.37
50 percentile	13.29	12.56	14.38	13.76	16.08	15.60
75 percentile	14.03	13.41	15.25	14.66	17.20	16.84
90 percentile	14.69	14.17	16.03	15.46	18.21	17.96
Pubic hair						
10 percentile	11.98	11.63	12.79	12.64	13.97	13.82
25 percentile	12.66	12.32	13.56	13.32	14.86	14.75
50 percentile	13.41	13.08	14.42	14.08	15.86	15.79
75 percentile	14.16	13.84	15.27	14.83	16.85	16.83
90 percentile	14.84	14.52	16.04	15.51	17.74	17.76

Bolded pairs are significantly earlier for NHANES, whereas bolded and italic pairs are significantly later. Data reproduced with permission (23).

These studies suggest that there is no evidence of earlier puberty development for the intermediate stages of puberty in girls.

PROGRESSION AND COMPLETION OF PUBERTY IN MALES

The NHANES III data reported earlier puberty in Black boys compared with White and Mexican-American boys (19). Although an early assessment of the NHANES found more advanced Tanner staging for ages (20) compared with the NHES data suggesting that puberty might be occurring earlier in boys, it is impossible to substantiate these data. Age 12 comparisons of Tanner stage 2 pubic hair sexual maturity from NHES and NHANES III were not significant for Black or White boys (19). When comparisons are made between the NHES, HHANES, and NHANES data (Table 4), there are data suggesting that stages are being attained at earlier, the same, and later ages (19). More data are needed before this can be considered as evidence that puberty is beginning

earlier in White boys, particularly as the data concerning the later stages of puberty fail to show a difference or suggest attainment at older ages.

CONCLUSIONS: EVIDENCE OF A CONTINUING SECULAR TREND OF PUBERTY

In summary, it cannot be concluded that puberty is starting or occurring earlier over recent decades because (i) of the inexactness and subjective nature of Tanner staging, (ii) of the lack of racially diverse historical comparison data, (iii) of the socioeconomic differences between study groups from the United States, (iv) pubertal onset is indicated by an activation of the HPG axis that may not be associated with the initial physical changes, and (v) there is no evidence that sexual maturity is being reached earlier.

Differences in study size and group selection methods, race, ethnicity, SES, longitudinal versus cross-sectional studies, marker assessment methods, and statistical techniques do not allow the conclusion that the secular trend in the age of onset of puberty in boys or girls is continuing. The primary data come from the NHES (1966–1970), HHANES (1982–1984), and NHANES III (1988–1994) of White, Black, and Mexican-American children. Evidence for lack of change is strongest for Black and White girls and Black boys, and only if one accepts that criteria are consistent (e.g. Tanner stage 2) and sampling controls for SES status, could one suggest earlier puberty in White boys and Mexican-American boys and girls.

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III

ISOLATED BREAST DEVELOPMENT

8

Premature Thelarche

*Linda A. DiMeglio, MD, MPH
and Adda Grimberg, MD*

CONTENTS

INTRODUCTION
PHYSIOLOGY OF BREAST DEVELOPMENT
EARLY BREAST DEVELOPMENT
GONADOTROPIN-INDEPENDENT PRECOCIOUS PUBERTY
GONADOTROPIN-DEPENDENT PROCESSES
EXPOSURE TO EXOGENOUS ESTROGENS
NONHORMONAL CAUSES OF INCREASED BREAST TISSUE
DIAGNOSTIC APPROACH
CONCLUSION
REFERENCES

Summary

Premature thelarche is the development of breast tissue prior to the normal age of pubertal onset. it can arise as a result of a number of different etiologies. This chapter provides an overview both of the physiology of normal breast development and of the causes of early thelarche, including benign self-limited premature thelarche, gonadotropin-independent precocious puberty, gonadotropin-dependent precocious puberty, exposure to exogenous estrogens, and non-hormonal mechanisms. A careful history and physical examination is essential in order to determine the proper diagnostic approach. it is important for the clinician to differentiate benign premature thelarche from other causes that can have deleterious consequences, such as true precocious puberty.

Key Words: Breast morphogenesis; Growth hormone; Neonatal Gynecomastia; Ovarian Cysts; Ductal ectasia.

INTRODUCTION

Thelarche is defined as the “beginning of breast development.” Therefore, premature thelarche (PT) simply is breast tissue development at an inappropriately young age. Premature thelarche can be categorized and conceptualized based on its timing and ultimate attributed etiology, with benign PT (BPT) and gonadotropin-dependent precocious puberty (GDPP) lying at opposite ends of a clinical spectrum. This chapter will

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begin by discussing the physiology of normal breast development, explore various forms of PT, and conclude with a diagnostic approach.

PHYSIOLOGY OF BREAST DEVELOPMENT

Normal Morphogenesis

Mammary glands are evolutionarily modified apocrine sweat glands containing two major components: epithelial networks and surrounding mesenchymal or stromal connective tissue (1,2). The epithelium forms a branched network of lumened alveoli and ducts that ultimately reach the thoracic surface and open through the nipple. The epithelial compartment consists of two cell types: luminal secretory cells that produce milk postpartum and basal myoepithelial cells that encase the secretory cells and contribute to milk delivery through their contractile properties (2). Although the stromal compartment primarily consists of adipocytes forming the breast fat pad, it also contains fibroblasts, hematopoietic cells, blood vessels, and neurons (2). Cellular interactions and signaling between the epithelial and stromal compartments are important for breast development and function.

Breast development begins embryologically with the formation of two mammary ridges or milk lines of ventral ectoderm at 4 weeks' gestation (1). Initially extending from the axillae to the inguinal regions, the mammary ridges atrophy everywhere except the pectoral region, where they persist as mammary disks (1,3). The mammary disks then thicken, and the epidermis continues to grow into the underlying mesenchyme, branching as it elongates and penetrates throughout the remainder of gestation (1,3) (*Fig. 1A*). Fifteen to 25 secondary buds in each breast ultimately develop into the alveoli, lobules, and ducts, whereas the mesenchyme develops into the fat pad. The nipples begin as depressed mammary pits of epidermis at the sites of the primary buds and do not elevate above skin level until birth; this elevation stems from proliferation of surrounding areolar mesenchyme, which first appears at 5 months' gestation (1,3).

Postnatally, the breasts, containing only the major ducts, remain quiescent until puberty. In females, at puberty, club-shaped terminal end buds appear at the tips of the ducts. The terminal end buds contain highly proliferating cells that push the ductal progression into the fat pad, which also grows during puberty. The inner body cells of the terminal end buds develop into luminal cells, whereas the outer cap cells become the myoepithelial cells of the new ducts (2). The terminal end buds disappear once the ducts have branched and proliferated throughout the entire fat pad (2). With each menstrual cycle, side branches form and disappear (*Fig. 1B*).

The female breast has two additional major developmental stages (*Fig. 1C and D*). During pregnancy, the alveolar epithelium expands, forming alveoli from epithelial precursor cells (2). The fully differentiated alveolar cells then synthesize and secrete milk during lactation. Cessation of suckling leads to involution, the stage of luminal cell apoptosis and alveolar remodeling. Persistence of stem cells allows the cycle of alveolar regeneration and apoptosis to occur with each and every pregnancy (2).

Hormonal Control

Estrogen and progesterone are the principal hormones stimulating breast development during puberty. Estrogen receptors, especially the alpha type, in both the stroma and epithelium stimulate the ductal outgrowth of puberty and the estrous

cycle (2,3). In contrast, progesterone receptors in the epithelium, especially the B isoform, lead to proliferation of the alveolar cells and their lobular development (2,3). Progesterone receptor expression itself is also induced by estrogen (2). Expression of both estrogen and progesterone receptors by the epithelial cells is evident from birth, but it is not until the hormone levels rise with pubertal ovarian activation that breast development occurs. However, should the hormone levels rise prematurely because of pathologic conditions, or the receptors exhibit enhanced sensitivity to the normal low prepubertal hormone levels, then PT ensues.

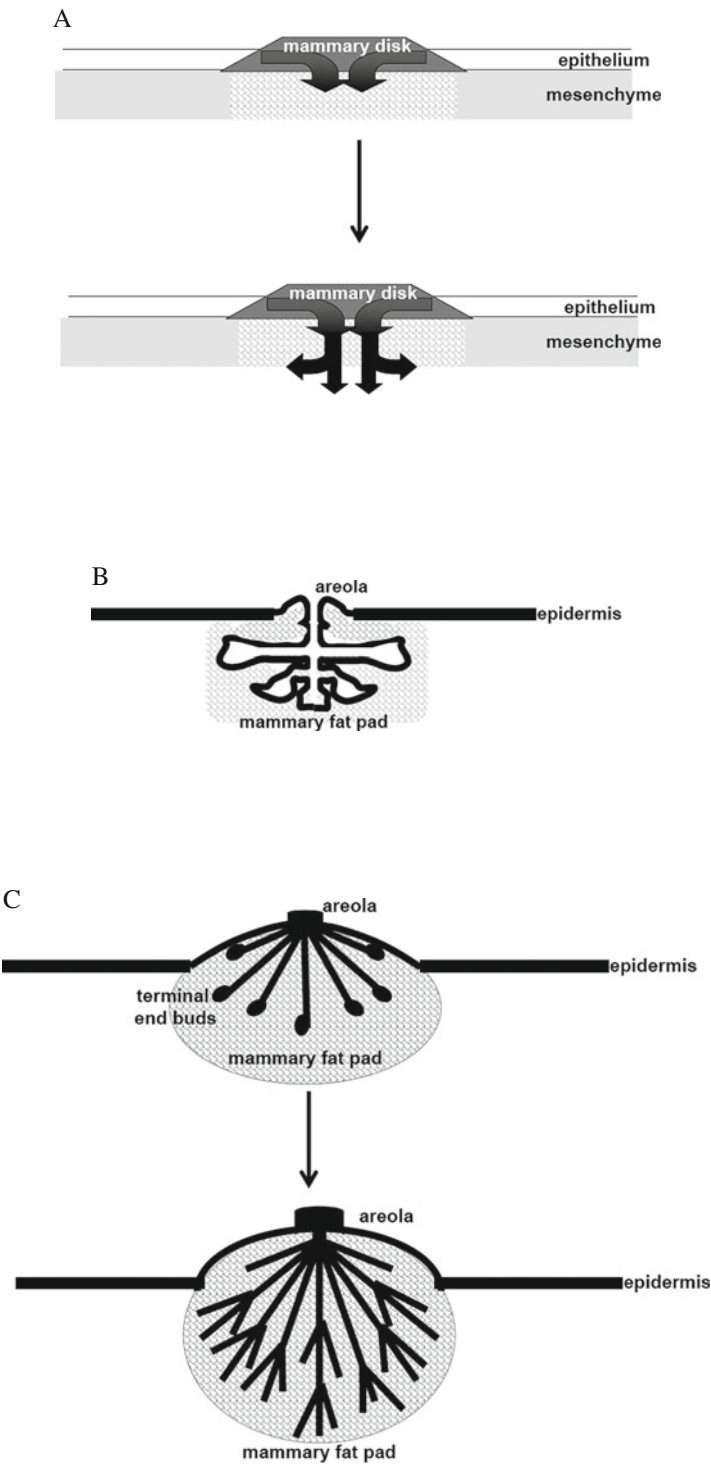
The further increase in estrogen and progesterone during pregnancy stimulates the additional expansion of the epithelial ducts and alveoli, respectively. Estrogen and progesterone production by the corpus luteum during early pregnancy is supported by prolactin and by placental lactogens after mid-pregnancy (2). However, prolactin is the main hormone that regulates lactation. Although the majority of prolactin comes from pulsatile secretion by pituitary lactotrophs, local prolactin secretion by mammary epithelium leads to paracrine effects (2,7,8). Oxytocin stimulates the milk let-down (7).

Growth Hormone/Insulin-Like Growth Factor Axis

Breast cancer research has led to an appreciation for a role of the growth hormone (GH)/insulin-like growth factor (IGF) axis in normal breast development. GH receptor (GHR) expression has been found in normal, benign hyperplastic and malignant lesions of the breast and, to a lesser degree, breast stroma. Despite wide interindividual variability, GHR expression levels do not correlate with lesion histology (9). In contrast, GH expression, detected in the ductal luminal epithelial and myoepithelial cells and the scattered stromal fibroblasts of normal human mammary glands, progressively increases in both the epithelia and supporting stroma of benign fibroadenomas, preinvasive intraductal carcinomas, and invasive ductal carcinomas with lymph node metastases (10). Experiments with GHR knock-out mice demonstrate the importance of stromal GH signaling for normal ductal development (11).

Ductal development is also impaired in both IGF-I and IGF-I receptor (IGF-IR) knock-out mice (12). Reduced proliferation in the terminal end buds of these mice supports the role of IGF-I as a mitogen for mammary epithelial cells. The role of IGF-I as a survival factor for epithelial cells is supported by the reduced apoptosis and hence the delayed involution of the breasts of transgenic mice overexpressing human IGF-I or IGF-II (12). Evidence for IGF-I proliferative and survival effects on mammary cells stemming from research on breast cancer is reviewed elsewhere (13,14). IGF-binding protein (IGFBP)-5 is the most abundant of the six IGFBPs expressed in murine mammary glands, whereas IGFBP-2 is the most abundant in human milk (15). Mouse studies found IGFBP-5 and IGFBP-3 expression to be the most prominent in pubertal terminal end buds, in the stroma, and in the pregnant epithelium (12).

There are numerous interactions between the GH/IGF axis and estrogen. For example, normal GH/IGF axis function is important for ovarian steroidogenesis and ovulation (16), whereas estrogen can affect both GH secretion and GH signaling (17). IGF-I and estrogen are synergistic in stimulating breast epithelial cell proliferation. This was seen in both terminal end bud formation (18) and Michigan Cancer Foundation MCF-7 breast cancer cells (19,20) and has been attributed to enhanced progression through the G₁ phase of the cell cycle (19).



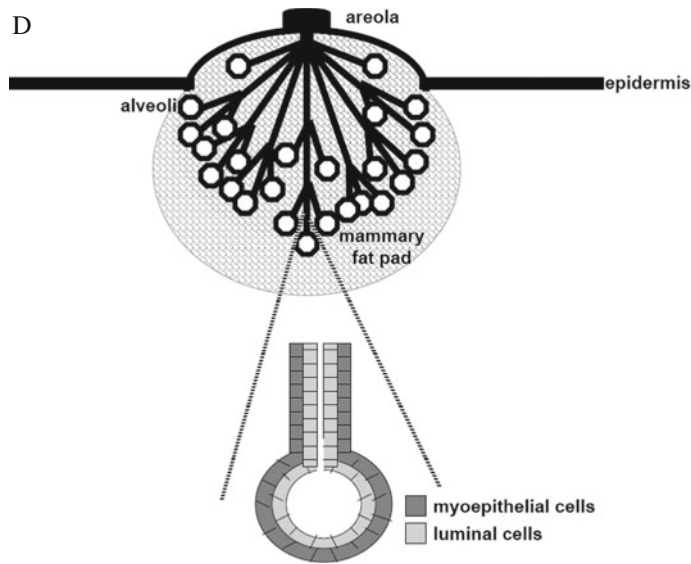


Fig. 1. Stages of breast development. (A) Embryogenesis. Epithelial cells from the mammary disk invade the underlying mesenchyme, forming a series of branches. (B) Birth. The lumens of the primary branches of the epithelial network open to the thoracic surface through the nipple, which first elevates above skin level at birth due to proliferation of the underlying areolar mesenchyme. The breasts remain quiescent in this stage until puberty. (C) Puberty. In females, cellular proliferation in the terminal end buds gives rise to further branching of the epithelial duct system until it has proliferated throughout the entire mammary fat pad. The mammary fat pad itself also grows during puberty and is composed of primarily of adipocytes, but also fibroblasts, hematopoietic cells, blood vessels, and neurons. (D) Pregnancy. Epithelial precursor cells proliferate to form alveoli, which fully differentiate to synthesize and secrete milk during lactation. The inner layer of luminal secretory cells produce milk, whereas the surrounding myoepithelial cells are contractile and lead to milk delivery. When suckling ceases, involution occurs to return the breasts to their pre-pregnancy state; this involves luminal cell apoptosis and alveolar remodeling. Adapted from (1,2,4–6).

Other Growth Factors

In addition to the aforementioned hormone systems, local growth factors contribute to breast development. The best studied of these is the epidermal growth factor (EGF), which acts through four related erythroblastosis oncogene B (ERBB) tyrosine kinase receptors. Like estrogen, EGF is synergistic with IGF-I in stimulating G_1 phase progression and, hence, proliferation of breast epithelial cells (12). Signal transducer and activator of transcription 5 (STAT5), a downstream mediator of hormone receptor signaling, is activated by prolactin only in breast epithelium but by GH and EGF preferentially in the stroma (21). Alveolar development in pregnant mice requires stromal ERBB1 and, for complete functional differentiation, ERBB4 (2). Later alveolar development during pregnancy requires receptor activator for nuclear factor B ligand (RANK-L), an osteoclast differentiation factor in the tumor necrosis factor (TNF) family that is induced by prolactin (2). Thus, as we consider possible causes of PT, we must recognize that they may involve alterations of hormone action as well as known or as of yet unknown local growth factors.

EARLY BREAST DEVELOPMENT

Neonatal Gynecomastia

The earliest class of premature breast development is neonatal gynecomastia. Neonatal gynecomastia is a normal variant of infant somatic development, seen in as many as 70% of infants in the first months of life. Most studies hold that neonatal gynecomastia occurs equally in males and females, although Schmidt et al. (22) found that palpable breast tissue was present more frequently and that breast size was larger in 3-month-old girls compared with boys. Neonatal gynecomastia is accompanied by galactorrhea in about 5% of cases (23). The fluid, also known as “witch’s milk,” is cloudy and similar in fat and electrolyte composition to maternal milk (24).

The etiology of neonatal gynecomastia is perhaps best explained by the following hypothesis. Maternal estrogens are transferred to the fetus transplacentally prior to birth. After parturition, maternal estrogen levels in the newborn bloodstream fall, stimulating secondary prolactin secretion by the neonatal anterior pituitary. Hyperprolactinemia, coupled with a prolonged effect of maternal estrogens and stimulation from ovarian estrogens secreted during the minipuberty of infancy in female infants, results in unilateral or bilateral breast enlargement through breast ductal system hypertrophy. Acini appear and the breast becomes vascular (25).

Neonatal gynecomastia resolves spontaneously within several months with a decline in neonatal prolactin production and quiescence of the breast tissue. However, repeatedly expressing milk from the breast, as advocated by some superstitions and cultural practices (26), can lead to a hyperplastic secretory state that persists for many months (*Fig. 2*).

Benign Premature Thelarche

BPT is defined as the onset of isolated breast development without any other physical or hormonal signs of pubertal maturation before the age of 6 years in African-American girls and before the age of 7 years in Caucasian girls (28,29). BPT may be either unilateral or bilateral (*Fig. 3*). Despite breast enlargement, nipple and areolar development and maturation are absent. Vaginal mucosa estrogenization (thickening and dulling) is uncommon, and uterine enlargement is rare. There are no adrenarcheal changes, linear growth spurt, or advancement in skeletal maturation.

Most girls with BPT present in the first 2 years of life (30–32). BPT is rare over age 4. Mills et al. (33) reported that BPT was detected within the first 13 months of life in 87.5% of cases. Van Winter et al. (34) reported that girls aged 6 months to 2 years accounted for 60% of all BPT patients, with a peak onset of 18 months. BPT is felt to be a normal childhood variant due to its nonprogressive course. However, differentiating BPT from other forms of precocious puberty can be difficult.

Longitudinal follow-up studies and clinical trials involving girls with BPT provide some epidemiologic data about BPT, including an overall incidence of 20.8 per 100,000 patient-years (34). In most cases, PT does not progress with time, and sometimes, the gynecomastia even disappears completely. Mills et al. (33) observed 46 girls with BPT over 3–5 years. Fifty-seven percent had no change in breast development, 11 percent had progressive breast enlargement without other signs of precocious puberty, and 32 percent resolved completely. Girls over 2 years of age at the onset of thelarche (30–32)



Fig. 2. Neonatal gynecomastia with galactorrhea because of cultural nipple stimulation. Reprinted with permission (27).

and those with breast development beyond Tanner stage II (35) are less likely to later have breast tissue regression. BPT may be in part genetically determined, as cases in sisters have been reported (36).

Despite multiple investigations, the etiology of BPT remains poorly understood. Various hypotheses have been proposed, and BPT most likely arises from any one or a combination of these mechanisms. First, some young girls may have an increased intrinsic sensitivity of breast tissue to small physiologic amounts of estrogens (estradiol and/or estrone) (30,37). Estradiol levels in girls slowly rise from infancy through childhood until puberty (38). If some girls have increased sensitivity to low levels of estradiol, this could explain early thelarche at age 6 or 7. Second, some children may have a prolonged “minipuberty of infancy,” with delayed inactivation of the hypothalamic–pituitary–gonadal axis, leading to higher than usual levels of estradiol later in infancy.

Third, a temporary activation of the hypothalamic–pituitary–ovarian axis with secretion of luteinizing hormone (LH), follicle-stimulating hormone (FSH), or both can stimulate ovarian follicle maturation, cyst formation, and estradiol production (30,39,40). Some studies have reported LH secretion during sleep in children with BPT

to be similar to that seen in GDPP and normal puberty (41), whereas other studies have not found increases in LH secretion (42). When compared with normal children, girls with BPT have higher FSH levels, both at baseline and after gonadotropin-releasing hormone (GnRH) stimulation (43,44). Measurements of inhibin B, a hormone produced by ovarian granulosa cells after FSH stimulation, are also higher in girls with PT than in controls (44).

BPT could also result from elevated circulating levels of estradiol or estrone, as has been shown by ultrasensitive, recombinant cell bioassay (31,45). There are multiple potential sources of the hormone excess. Ovarian overproduction of estradiol from intrinsic follicular maturation can be minimal, intermittent, or sporadic. Lyon et al. (46) reported four girls with unsustained sexual maturation attributable to autonomously



Fig. 3. Two year old African-American girl with benign premature thelarche. Breast development had been first noted by the family prior to 12 months of age. .

functioning, estrogen-producing ovarian cysts. Of note, all these girls came to attention as a result of breast development accompanied by vaginal bleeding.

Estradiol may also come from adrenal estrone. Delay in fetal adrenal gland regression with an increase in adrenal androgen precursors may result in increased circulating estradiol. Although one study has shown elevation of dehydroepiandrosterone (DHEA) in girls with BPT (47), others have not seen this increase (42). Increased peripheral aromatase activity can be another source of estrogens. In overweight children, increased total body fat leads to greater aromatization, increased conversion of androgens to estrogens, and earlier breast development (48). Some cases of prepubertal gynecomastia in boys and isosexual precocious puberty (presenting with thelarche) in girls are due to a familial aromatase excess syndrome resulting from aberrant P450 aromatase gene expression (49). Environmental estrogens, discussed in the section “exposure to Exogenous Estrogens” below may also be a factor in familial cases.

Occasionally, BPT is associated with other medical issues, which may be simply coincidental, reflect cases of incomplete GDPP, or provide insight into the etiology of BPT. In a series of 83 Brazilian girls treated with GH, PT developed in four girls after 2–60 months of treatment (50). Two of these girls had isolated growth hormone deficiency GHD and two had mosaic Turner’s syndrome; they were aged 5.6–7.8 years at thelarche. The authors speculated that GH might produce breast enlargement by acting directly on GHRs located in the breast tissue or indirectly through generation of IGF-1, through stimulation of other lactogenic receptors, or through a “refeeding mechanism.”

GONADOTROPIN-INDEPENDENT PRECOCIOUS PUBERTY

Exaggerated Thelarche

In 1990, Stanhope and Brook (51) described a group of girls with a “thelarche variant,” who had cyclic breast development that appeared to be intermediate between BPT and GDPP. Subsequently, Garibaldi (52) coined the term “exaggerated thelarche” for this phenomenon. Exaggerated thelarche is defined as early breast development to Tanner stage III or greater with mildly accelerated skeletal maturation and/or increased growth velocity but no adrenarcheal hair development or biochemical signs of precocious puberty. Ovaries are enlarged for age, and peak stimulated estradiol levels fall midway between BPT and GDPP but with an FSH-predominant response. Some cases of exaggerated thelarche have been attributed to ovarian cysts (42).

Girls with “chronic fluctuating” or “stuttering” thelarche have repeated self-limited breast budding episodes, occasionally with bone age advancement and/or height velocity acceleration. FSH-predominant gonadotropin pulses may induce formation of small estrogen-secreting cysts, resulting in fluctuating PT (39). It is also possible that these cysts represent a form of partial autonomous ovarian function (53).

McCune–Albright Syndrome

McCune–Albright syndrome (MAS) is characterized by a clinical triad of gonadotropin-independent precocious puberty, café au lait skin macules, and polyostotic fibrous bone dysplasia. MAS is caused by postzygotic activating mutations of the *GNAS1* gene in a mosaic tissue distribution; girls with MAS can sometimes present

with PT, before bone and skin manifestations are apparent (53,54). Recent evidence suggests that although it is unlikely that many patients with BPT have *GNAS1* gene mutations (55), some girls with exaggerated and/or chronic fluctuating thelarche do have activating mutations of the *GNAS1* gene (53). MAS is discussed in further detail in Chapter 17.

Ovarian Cysts and Tumors

Other types of ovarian cysts and lesions, besides those associated with *GNAS1* gene mutations, can also result in PT. PT is a frequent initial presentation of juvenile ovarian granulosa cell tumors (56,57), although these children go on to develop pubarche and vaginal bleeding. Gonadal and adrenal tumors are reviewed in detail in Chapter 19.

GONADOTROPIN-DEPENDENT PROCESSES

Central Precocious Puberty

PT may serve as a harbinger of true GDPP, with the first sign of pathology being breast enlargement. In a series of 55 Japanese girls aged 0 to 5 years referred for PT, 21.8% were immediately classified as precocious puberty, 20% had findings suspicious for precocious puberty, and 58.2% had isolated abnormal breast development. Later age of onset and rapid pace of breast development can raise suspicion of true precocious puberty, and following cases with these features is essential to monitor for subsequent late complications. It has been estimated that 14–18% of girls with BPT develop GDPP (31).

Unfortunately, there are no clear criteria to differentiate girls with BPT from girls with early GDPP, though girls with onset of thelarche after age 2 years or with “exaggerated thelarche” seem to be at greater risk of GDPP. Children with neurofibromatosis are also at risk of developing GDPP, in association with optic gliomas, and may initially present with PT (58).

Thelarche as First Sign of Normal Puberty

Breast development is the first sign of normal puberty in more than 80% of girls (58). In 1999, the Lawson Wilkins Pediatric Endocrine Society issued a statement redefining the normal lower limit of age of pubertal onset for thelarche (and/or adrenarche) in African-American girls (6 years) and Caucasian girls (7 years) (28). These recommendations were based on a study by Herman-Giddens et al. (60) utilizing data from 17,077 American girls of mixed ethnic background presenting to primary care physicians for ambulatory care. Although some pediatric endocrinologists have expressed reservations about these new guidelines (61), it seems that over the past several decades, there has indeed been a downward secular trend in the age of thelarche (60) without a concomitant change in the age of menarche (62). It is possible that obesity simply promotes earlier onset of breast development but not progression through puberty.

EXPOSURE TO EXOGENOUS ESTROGENS

Medications

Oral ingestion of estrogen-containing medications, such as oral contraceptives and postmenopausal preparations, can cause PT. Ingestion of cimetidine, a selective H₂

blocker, has also been implicated in gynecomastia in males and may be a cause of PT in infancy (63). Unwitting ingestion of sex steroids can come from adrenal extracts, sold by nutritional supplement stores to enhance energy or immunity, though they usually cause signs of virilization. Now that estrogen preparations are also available transdermally, this constitutes another potential mechanism for unintentional transmission.

Cosmetics

Estrogen exposure can also occur transdermally through cosmetics, including topical anti-aging creams and ointments. The Food and Drug Administration does not regulate cosmetics containing less than 10,000 IU of estrogen per ounce. Adult hormone-replacement therapy with oral ethinyl estradiol uses 4000–10,000 IU of estrogen per day. Cosmetics incorporating estrogens include hormone-containing hair care products commonly applied to African-American children's hair (64,65). Topical exposure to tea tree oil and lavender oils found in shampoos, soaps, and body lotions has been reported to cause premature breast development in prepubertal boys (REF). It is also possible that these compounds are a cause of benign premature thelarche in girls (98).

Isoflavones

Another potential source of exogenous estrogen action comes from dietary phytoestrogens, plant compounds containing an estrogen-like phenolic group. Isoflavones, the most prevalent class of phytoestrogens in human diets, are nonsteroidal compounds with structural homology to estrogen and the ability to bind the estrogen receptor (66,67). Soybeans and all soy-protein products are particularly rich sources of isoflavones (66). High Performance liquid chromatography (HPLC) analysis of commercially available soy-based infant formulas revealed similar qualitative isoflavone compositions with quantitative variability related to interbatch differences of the soybean isoflavone content and to processing effects from heat exposure and changes in pH (68). In all the soy-based formulas tested, about two-thirds of the total isoflavone content consisted of conjugates of genistein (68). Structurally homologous to 17- β -estradiol, genistein binds both α -type and β -type estrogen receptors, as well as sex hormone-binding globulin. Genistein can act as an estrogen agonist, estrogen antagonist, anti-androgen, and inhibitor of tyrosine kinase and topoisomerase (69).

Concern for the potential effects of soy-based infant formulas is based mostly on indirect evidence. In utero and postnatal ingestion of higher doses of genistein by rats leads to mammary gland hyperplasia and abnormalities in vaginal cellular maturation, ovarian antral follicles, and spermatogenesis (70). Four-month-old infants consuming 800–1000 ml of soy-based formula are exposed to 35–50 mg isoflavones/day. This corresponds to 6–9 mg isoflavones/kg body weight/day, an order of magnitude greater than the 0.7 mg/kg body weight/day dose shown to affect the hormonal regulation of women's menstrual cycle (68). The ready absorption of isoflavones from infant soy formulas, the lower renal clearance than in adults, and the lower affinity of isoflavones relative to estradiol for serum-binding proteins all suggest a high bioavailability of these compounds in infants consuming soy-based formula diets (68).

Despite this potential for potent estrogenic effects and its wide-spread use [estimated at 25% of all formula sold in the United States (71)], soy-based formula use has not led to rampant disturbances of sexual development (69). The only long-term safety study

was a retrospective cohort evaluation of 20 or 30 year olds who had been fed soy-based ($n = 248$) or cow-milk ($n = 563$) formulas as infants in a clinical trial. In the telephone interview, women who had been soy fed reported 8-hour longer duration of menstrual bleeding, greater menstrual discomfort, increased use of asthma or allergy drugs, and a greater tendency for sedentary activities (72). Human data on soy formulas and PT remain scarce and inconsistent (69).

The Puerto Rican Experience

A tripling of the reported incidence of PT in Puerto Rico from 1978 to 1981 prompted study of possible environmental triggers (73). A matched-pair, case-control study using standardized home interviews to assess exposures did not find any associations in girls over age 2 years, but for those under age 2, positive correlations were found between PT and maternal history of ovarian cysts, soy-based formula diet, and consumption of various meat products (73). Cytosol receptor assays of homogenated meat products from Puerto Rico and the continental United States revealed high estrogen-like content in samples of poultry from Puerto Rico and the United States as well as in a US beef sample (74). Fifty-eight percent of the girls had involution of their breast tissue within 2–6 months of withdrawing local chicken, milk, and beef from their diets (74). The offending agents were attributed to animal husbandry practices used to tenderize the meat. A smaller case-control study found higher serum levels of phthalate plasticizers, but not pesticides, in the serum of Puerto Rican girls with PT (75). None of the studies to date have provided conclusive answers to the etiology of the Puerto Rican epidemic.

NONHORMONAL CAUSES OF INCREASED BREAST TISSUE

Benign Masses

Other conditions can mimic PT. The most common is fatty breast tissue, which is often related to overall obesity. Although, the breast may resemble real thelarche, by contour palpation reveals absence of glandular tissue. Clinicians should also be cognizant of the variety of benign breast tumors. Fibroadenomas are the most common breast mass found in older childhood, adolescents, and young women (76–78). Hemangiomas occur in younger children, are benign, and tend to spontaneously regress by age of 6–7 years (79–81). Lymphangiomas can also occur rarely in the prepubertal breast, but these require surgery as they do not resolve spontaneously; if possible, surgical resection should be deferred until after pubertal development (81,82). Although more common in adulthood, pediatric myxoid breast lesions can be seen as part of Carney Complex, an autosomal dominant, familial lentiginosis and multiple neoplasia syndrome. Inactivating mutations in the gene encoding the type 1 α regulatory subunit of protein kinase A (PRKARIA) are found in 45–65% of familial cases (49,83).

Malignant Tumors

The overwhelming majority of pediatric breast tumors are benign. Primary breast carcinomas are exceedingly rare in childhood. Most malignancies of the breast are metastatic lesions (77,78,84). Rhabdomyosarcoma is the most frequent cancer, followed by leukemia or lymphoma and then neuroblastoma (3,84,85).

Abscess

A mass that is tender and fluctuant is most likely an abscess, usually caused by *Staphylococcus aureus*, *Escherichia coli*, or *Pseudomonas* infection (3). Nipple piercing is an increasingly prevalent cause of breast infections among adolescents, and cultured organisms are commonly *S. aureus*, *Pseudomonas*, and β -hemolytic streptococci (86). Spread of local cutaneous infection, foreign body, epidermal cyst, trauma, and shaving periareolar hair are other causes of inflammatory breast lesions in children (3,77). Treatment is antibiotic therapy, with aspiration, or incision and drainage reserved for refractory cases only.

Ductal Ectasia

Rarely, infants may present with a breast mass and bloody nipple discharge. This is generally due to mammary ductal ectasia (87), although it can also occur with chronic mastitis. In ductal ectasia, maternal estrogen, progesterone, and prolactin, as well as infantile prolactin, produce duct hyperplasia and secretion (88). There are limited reports of malignant tumors in children with palpable masses and bloody discharge (89).

DIAGNOSTIC APPROACH

Although isolated PT is benign, GDPP and GIPP are not, making differentiation essential. Correct diagnosis of the nature of sexual precocity is critical, because evaluation and treatment of children for each condition varies. In children with PT, the history and physical examination will aid greatly in guiding the diagnostic approach (Fig. 4).

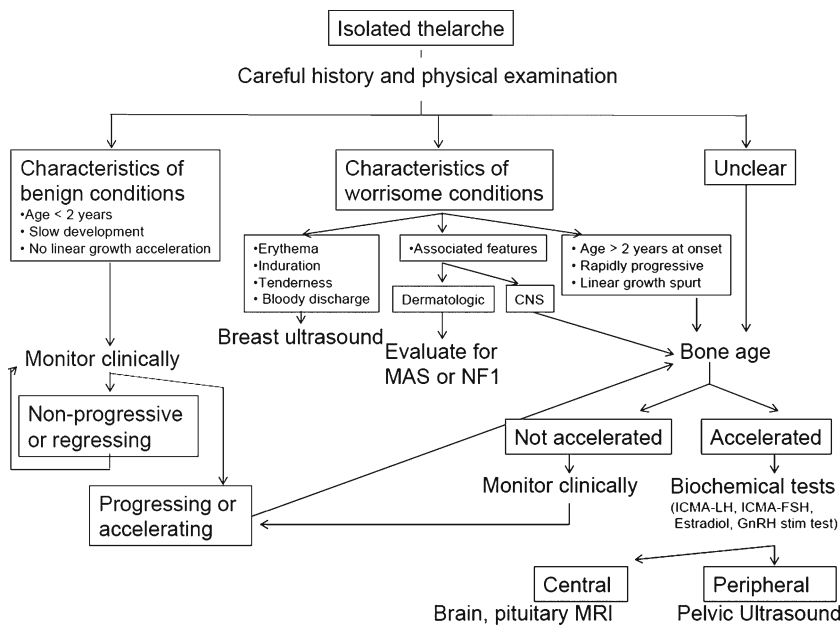


Fig. 4. An algorithm for the work-up of premature thelarche.

History and Physical Examination

Many cases of PT do not require any evaluation beyond an initial assessment. History should focus on the timing of the breast development and any noted changes in breast size. A general medical history should also be obtained, noting that children with a history of central nervous system lesions, pathology, or trauma are at greater risk of developing GDPP than other children their age. Previous growth records should be reviewed to see whether children have been growing in length/height along an established percentile or crossing percentiles upward or downward (especially after age 2). Medication history should include any possible ingestion or applications of topical estrogens. Of note, a history that includes waxing–waning of breast development, vaginal spotting, and/or bone pain should make the clinician suspicious of MAS.

Physical examination must include both inspection and palpation of breast tissue. Children with BPT will not have thickening and pigmentation of the nipples and areolae, as seen in other forms of progressive precocious puberty. Palpation will distinguish between fatty deposition and the presence of true glandular tissue. Erythema, tenderness, and induration, especially in unilateral gynecomastia, should alert concern for local infectious or inflammatory processes. Careful inspection should also seek other signs of sex hormone action, such as virilization (clitoromegaly, pubic and axillary hair, hirsutism, and acne) and vaginal estrogenization. Skin changes may indicate syndromes like MAS or neurofibromatosis. If history, growth patterns, and physical exam are not suspicious for a pathologic process, many children can just be followed without further evaluation. The amount of breast tissue present will increase only minimally over time or may even decrease.

Diagnostic Tests

If questions remain, a bone age evaluation (x-ray of the left hand and wrist) is often helpful. In cases involving prolonged sex-steroid exposure, estrogen will result in a maturation of the epiphyses and consequent bone age advancement relative to the chronologic age.

Some cases of PT will present at a classic age without growth acceleration, other signs of estrogenization, pubic hair, or bone age advancement, and some cases of true precocious puberty will present with all these features. However, because BPT and GDPP are points along a spectrum, about one-third of the children with premature breast enlargement will not present with classic features of either entity (42). In these cases, further laboratory assessments may be beneficial. Children with an advanced bone age or a predicted adult height that is more than 2 SD below their mid-parental height deserve a more thorough endocrine evaluation.

Biochemistry

In general, estradiol measurements are not useful, unless elevated to greater than 20 pg/ml. Although highly sensitive estrogen assays may demonstrate high estradiol levels for ages below this threshold, these ultrasensitive assays are not clinically available and vary with time. Similarly, although children with BPT will have lower levels of sex hormone-binding globulins or thyroxine-binding globulin than those with GDPP, as they do not have circulating estrogens that will increase these plasma proteins, these are not routinely measured.

Random LH and FSH levels have a limited value in the work-up of precocious puberty, although ultrasensitive chemiluminescent LH measures now available have utility in differentiating pubertal from prepubertal children, with some pediatric endocrinologists using values greater than 0.3 IU/ml as diagnostic of GDPP. Distinguishing BPT from GDPP can involve GnRH stimulation testing, as described more fully in Chapters 15 and 16. Responses to GnRH stimulation tests of children with BPT are either normal (43,90) or FSH-predominant with a baseline elevation of FSH (30). Prepubertal LH concentration at baseline and after GnRH stimulation (30,91) is in contrast to children with GDPP, who demonstrate an LH predominant response (42). However, even this testing is not absolutely determinative. Because FSH secretion and ovarian activity are not completely quiescent during childhood, an FSH predominant response can also be seen in very early GDPP, making the line between prepuberty and puberty difficult to ascertain. Also, some patients may have heterophile antibodies resulting in erroneously high LH levels at baseline and after GnRH stimulation, which can lead to an erroneous diagnosis of GDPP (92).

Imaging Studies

Pelvic ultrasonography, with high-frequency transducers and real-time scanners, offers the ability to rapidly and noninvasively assess uterine and ovarian size and morphology. Normal standards from birth through puberty are readily available (93,94). Pelvic ultrasound may help differentiate BPT from pathologic precocious puberty (39); however, given the overlap between normal and abnormal ranges for all observed parameters, it is impossible to use ultrasound alone to make a concrete diagnosis. A study comparing 48 patients with BPT aged 1.1–6.7 years with age-matched normal control subjects found no significant differences in uterine parameters or ovarian volumes between the groups (95). Ovarian morphology, however, differed with more BPT girls having varying degrees of ovarian cyst development than controls.

If there is a need to image the breast, ultrasonography is the preferred imaging modality (96,97). Benign masses are readily discriminated from cysts, malignant masses, or ductal ectasia. Surgical interventions such as biopsies may produce later severe breast deformities, which are disabling both cosmetically and functionally.

CONCLUSION

Premature thelarche is often a benign, self-limited condition although it can be a harbinger of early true precocious puberty or other, rarer conditions. A suggestion as to which patients should be discharged from clinic, which should be observed periodically without medical intervention, and which children should receive long-acting GnRH analogs can be gleaned from the data above. It is important to remember that BPT does not require treatment, just careful observation. Treatments for other forms of precocious puberty are covered elsewhere in this text.

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9

Gynecomastia

Epidemiology, Pathophysiology, and Management

Samar N. Rahhal, MD
and John S. Fuqua, MD

CONTENTS

INTRODUCTION
EPIDEMIOLOGY
DEVELOPMENTAL ANATOMY AND PATHOLOGY
ANDROGEN AND ESTROGEN PHYSIOLOGY IN MALES
ENDOCRINE CHANGES DURING GYNECOMASTIA
DIFFERENTIAL DIAGNOSIS OF GYNECOMASTIA
EVALUATION OF GYNECOMASTIA
TREATMENT
CONCLUSIONS
REFERENCES

Summary

Gynecomastia, defined as a palpable disk of breast tissue in males, is a relatively common condition. It is usually benign and self-resolving, especially in neonates and pubertal boys. An imbalance in the estrogen-to-androgen ratio is believed to be the culprit in the pathophysiology of gynecomastia. An increase in estrogen or a decrease in androgen is found in most pathologic entities associated with gynecomastia, such as hypogonadism, tumors, or enzymatic defects. A thorough history and physical examination is essential in distinguishing between benign and pathologic gynecomastia, as well as in directing further workup. Treatment of gynecomastia is usually for social and cosmetic reasons, and it is unnecessary in cases where it is mild or transient. However, in persistent or severe cases, treatment may be advisable. Androgens, antiestrogens, and P450 aromatase inhibitors have all been studied, with mixed results. Surgical treatment, while invasive and likely to leave scars in severe cases, is a definitive and more effective treatment option.

Key Words: Florid phase; Fibrous phase; Estrogen-to-androgen ratio; Klinefelter syndrome; Testicular tumors; Adrenocortical tumors; Aromatase excess syndrome; Danazol; Tamoxifen.

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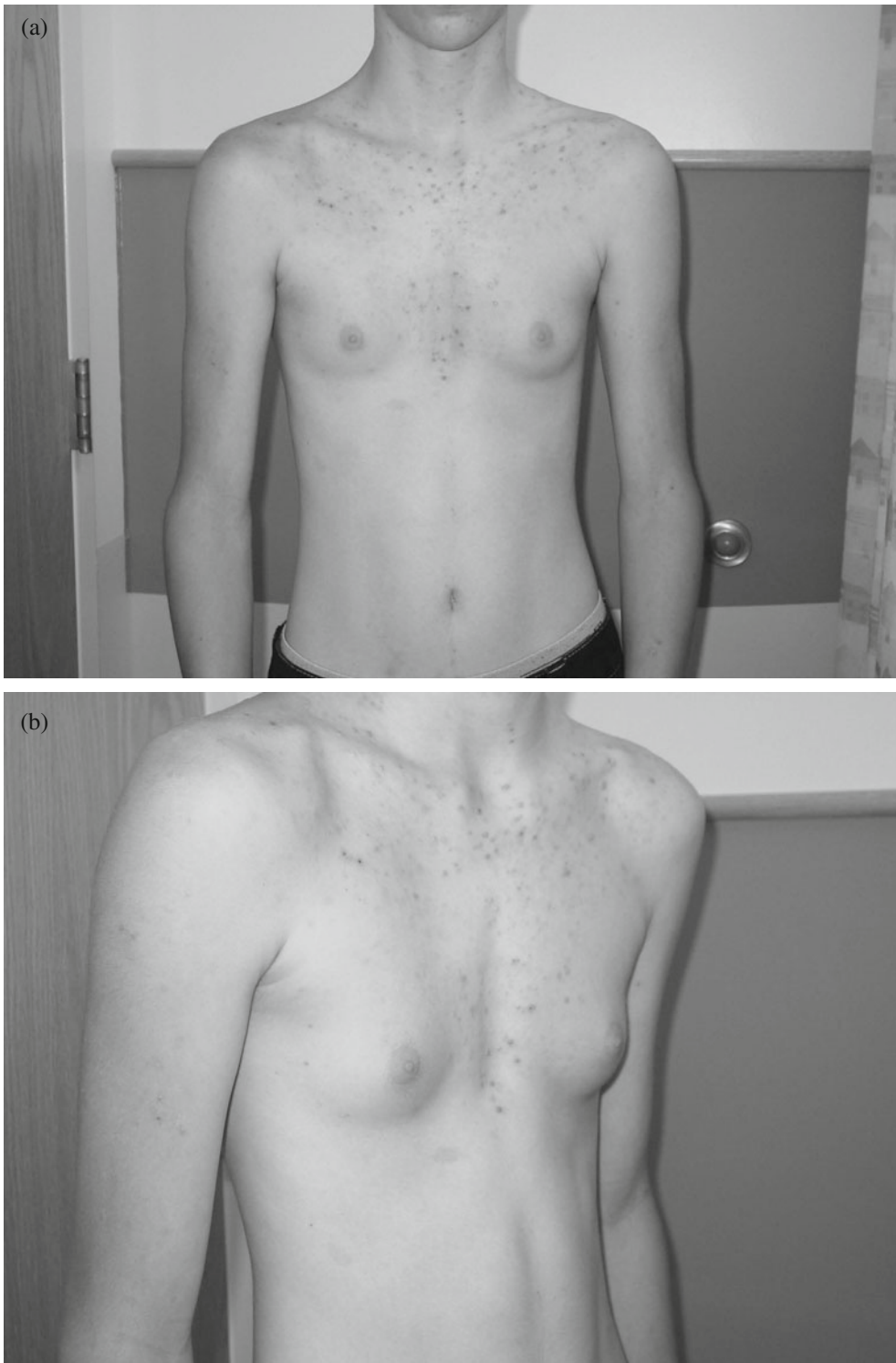


Fig. 1. Gynecomastia in an adolescent boy with Klinefelter syndrome (A) Frontal view (B) Right oblique view.

INTRODUCTION

Gynecomastia is a common condition in which one or both breasts are enlarged in a male (*Fig. 1*). Although it is most commonly physiologic and of little consequence, it may also be caused by one of a host of pathologic conditions. Distinguishing physiologic gynecomastia from pathologic gynecomastia is an important part of its evaluation in the pediatric patient.

Criteria used for the diagnosis of gynecomastia vary between studies, but most series of pediatric cases have considered it to be a palpable disk of breast tissue greater than 0.5 cm in diameter (*1–3*). The tissue is usually centrally located beneath the nipple, and it is usually less than 4 cm in diameter. Most often, the individual is unaware of its presence, although it may be painful, particularly early in its development (*4*).

EPIDEMIOLOGY

Gynecomastia has three characteristic peaks in incidence in the general population. In the newborn and in the infant up to 2 years of age, it is a very frequent finding, and it is thought to represent the effects of placentally derived estrogens and sex steroids produced during the “mini-puberty of infancy.” Its incidence distinctly decreases in mid-childhood but shows a sharp increase as adolescence ensues. Finally, gynecomastia is a frequent finding in elderly men.

A number of studies, most conducted in the 1960s and 1970s, have investigated the prevalence of gynecomastia in teenage males (*Table 1*). The most widely quoted study evaluated 1890 boys between the age of 10 and 16 years attending a Boy Scout camp (*1*). In this cross-sectional study, gynecomastia was present in 38.7% of boys, with a peak of 64.6% of those between the age of 14 and 14.5 years (*Fig. 2*). Several longitudinal studies have confirmed these data. The largest of these (*5*) followed 536 males for 3 years. Bilateral or unilateral breast development was found to occur in 48.5%, most commonly at Tanner stage 4. Gynecomastia occurred more commonly in those with earlier pubertal development, a finding that has been confirmed by another study (*6*). The remaining longitudinal studies of the prevalence of gynecomastia (*2,7*) have documented prevalences of 69 and 22%. The largest cross-sectional study used

Table 1
Data on the prevalence and duration of gynecomastia in adolescent males

<i>Study</i>	<i>Number of subjects</i>	<i>Overall prevalence (%)</i>	<i>Age at peak prevalence</i>	<i>Pubertal stage at peak prevalence</i>	<i>Duration</i>
Nydic et al. (<i>1</i>)	1890	38.7	14–14.5	NR	< 2 years
Lee (<i>7</i>)	29	69	13	III	1–1.5 years
Fara et al. (<i>3</i>)	61	34.6	NR	NR	NR
Harlan et al. (<i>6</i>)	3522	4.2	13	III–IV	NR
Moore et al. (<i>2</i>)	135	22	13.6	III	0.5–2.5 years
Nizzoli et al. (<i>10</i>)	790	29.4	NR	NR	NR
Biro et al. (<i>5</i>)	536	48.5	NR	IV	0.5–1 year

NR, not reported.

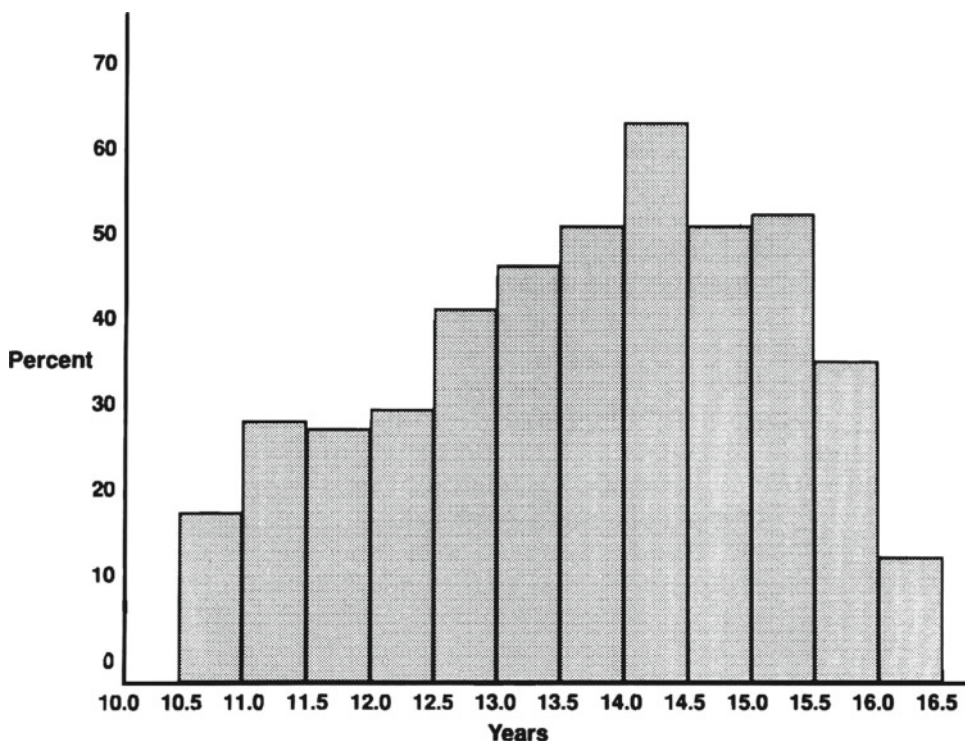


Fig. 2. Prevalence of gynecomastia at different ages, derived from the data of Nydick et al. (1). Reproduced with permission (8).

the United States Health Examination Survey to assess the prevalence of secondary sexual characteristics in 3522 adolescent boys (6). Although the prevalence was only 4.2%, this study was not designed specifically to assess gynecomastia, and the study procedures appear to have been less sensitive for its detection (8). The duration of physiologic gynecomastia in adolescence appears to be relatively short, typically less than 2 years (1,2,5,7) (Table 1).

Gynecomastia occurring in the prepubertal population is distinctly uncommon. Although data on the incidence and prevalence in this population are generally lacking (9), an investigation into an epidemic of gynecomastia occurring in a single primary school in Milan, Italy, in 1977 led to surveys of other schools in the city (3) and in surrounding towns (10). These investigations revealed that 2.9–3.2% of over 250 preschool and school-aged boys had at least 0.5 cm of palpable breast tissue. However, it is unclear whether these data are applicable to the general population, as environmental or food contamination was suspected as the cause of the epidemic, and this may have also increased the prevalence in surrounding areas. Clinical experience suggests that the incidence in the US population is lower.

Although gynecomastia has been assumed to be uncommon in young and middle-aged adults, several studies have indicated that it may be a frequent finding. Nuttall (11) examined 306 unselected military reservists between the age of 17 and 58 years and found that 36% had gynecomastia more than 2 cm in diameter that was generally not recognized by the subject. Similar findings have been reported in an autopsy study

(12). The prevalence of gynecomastia clearly increases with increasing age, particularly as the population examined reaches senescence.

DEVELOPMENTAL ANATOMY AND PATHOLOGY

The mature female breast consists of 15–20 lobes, each with its own duct structure and independent opening onto the nipple. Each of these lobes functions independently from the others, and they are separated by dense collagenous tissue and fat. The individual lobes consist of many smaller interconnected lobules, also separated by collagenous tissue. Milk secretion occurs in the acini that are connected together by intralobular ducts. The intralobular ducts empty into larger interlobular ducts, eventually connecting into the main lactiferous duct that opens at the nipple.

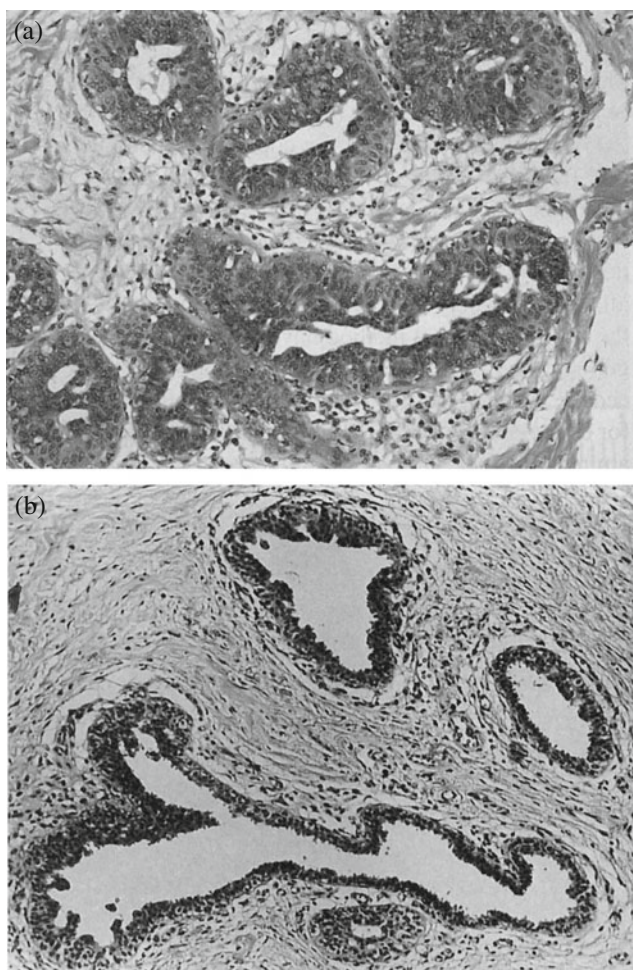


Fig. 3. (A) Florid phase of gynecomastia, showing epithelial proliferation with an abundance of loose connective tissue, edema, and a slight lymphocytic infiltrate. (B) Fibrous phase of gynecomastia, showing decreased proliferation of the ductal epithelium and the development of denser collagenous tissue. Reproduced with permission (18).

In both male and female fetuses, the formation of the breasts begins early in gestation, with the development of a cluster of 15–25 invaginations in the thoracic portion of the mammary lines (13). During the second trimester, these invaginations penetrate the subcutaneous mesenchyme and begin to branch into successive generations of cords. By the time of delivery, these have become canalized and further branched, becoming the lactiferous ducts and small interlobular ducts. Occasionally, these reach the stage of lobule development with acinar structures. This results in the formation of neonatal breast buds, an almost universal occurrence. Neonates with larger breast buds may have secretory function, known as “witch’s milk.” Madlon-Kay (14) noted this phenomenon in 5.9% of 640 neonates followed serially over 2 months. Regression of breast tissue begins by early infancy in both males and females, coming to consist of lactiferous ducts and larger interlobular ducts. During the remaining prepubertal years, there is gradual growth and branching of the duct system (15).

With the onset of gynecomastia, characteristic histologic changes occur over a typical time course. This pattern has been separated into three phases: florid, intermediate, and fibrous (Fig. 3) (16,17). Florid gynecomastia refers to the proliferation of small ducts with hyperplasia of the ductular epithelium, increased loose connective tissue stroma with edema, and vascular congestion. This phase lasts less than 12 months and often merges into the intermediate stage by 6 months. The intermediate stage is a transition between the florid phase and the fibrous phase and usually lasts 6–12 months. The fibrous phase is thought to be an end stage, in which the ductular epithelium regresses and the connective tissue stroma becomes densely collagenous and hyalinized (18) (Fig. 4). Typically, lobular development with the formation of acini does not occur in gynecomastia, although it has been reported in rare cases (19,20). The progression of this histopathology proceeds regardless of the etiology, but it is important to understand when dealing with the treatment of gynecomastia.

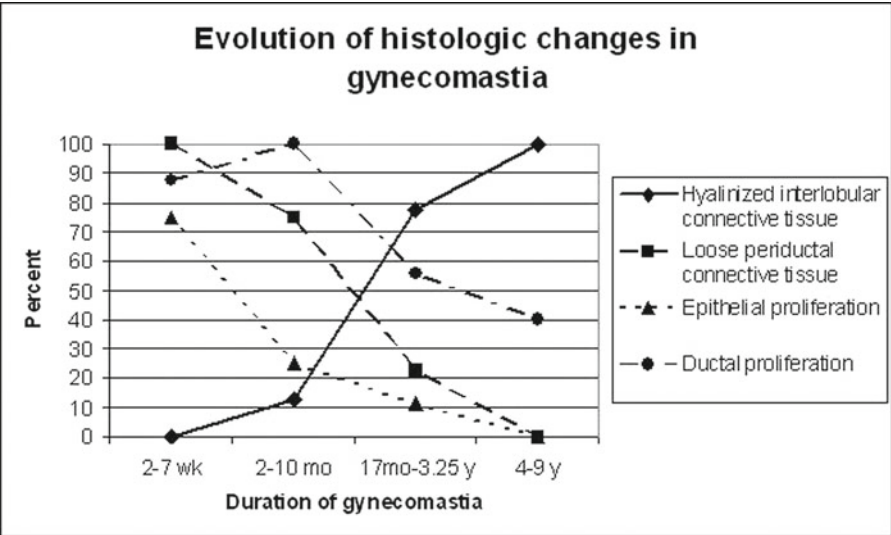


Fig. 4. Time course of the evolution of the histopathologic changes seen in gynecomastia. Redrawn from data with permission (16).

ANDROGEN AND ESTROGEN PHYSIOLOGY IN MALES

Androgens are the primary sex hormones in the normal human male, and in adulthood, they are present in concentrations several times those of estrogens. Testosterone and androstenedione are the two major androgens, with testosterone being present in higher concentrations. In normal adult men, 17–35 μmol of testosterone is produced per day versus 7–14 μmol of androstenedione (21). Testosterone is principally produced by the Leydig cells of the testes under the influence of the gonadotropin luteinizing hormone (LH) produced in the pituitary, and the main site of production of androstenedione is the adrenals. Follicle-stimulating hormone (FSH), the other pituitary gonadotropin, enhances Sertoli cell function in men, and it may also act to augment the effect of LH on the Leydig cells (22). Testosterone exerts a negative feedback effect on the pituitary gonadotropes, rendering them less sensitive to gonadotropin-releasing hormone (GnRH).

The testes also produce the estrogens estradiol and estrone, albeit in far less quantities than androgens (23). Direct production from the testes accounts for only 20% of estrogens in males. The peripheral conversion of testosterone and androstenedione is responsible for around 80% of the estrogens in males. The enzyme responsible for this conversion is the cytochrome P450 aromatase, found in several tissues of the body, including adipose tissue, muscle, testis, and bone (24). There is also significant interconversion of estrogens from estradiol to estrone and vice versa that is regulated by a wide variety of enzymes (21) (*Fig. 5*).

Concentrations of gonadotropins, as well as sex steroids, vary in males through infancy, childhood, adolescence, and adulthood. At birth, the concentration of estrogens in cord blood is high, reflecting maternal as well as placental estrogens, although the levels decline by 1 week of age (25). At this time, there is a gonadotropin surge that stimulates the Leydig cells of the testis to produce testosterone as well as estradiol. This is the period known as the “mini-puberty of infancy” in newborn males. It lasts approximately 6 months, with a peak in serum hormone levels at 2–3 months of age (26). After this period, sex hormone concentrations decline to levels seen in childhood, when there is relative pituitary quiescence and low secretion of FSH, LH, and sex steroids (26).

The hallmark of pubertal onset is the activation of the hypothalamic–pituitary–gonadal axis. Although the triggers of this activation are poorly understood in males as well as in females, they result in an increase in the amplitude and frequency of GnRH pulses and subsequent LH and FSH secretion. In response to LH and FSH, the gonadal production of sex steroid increases several fold (27). In males, the testosterone level increases after pubertal onset and continues to steadily increase until late in puberty, when it plateaus (28). Estrogen also increases in the pubertal male, and its role in bone maturation and epiphyseal closure is well known. Klein et al. (28), using ultrasensitive assays, showed that estrogen levels first increase significantly in males in the year immediately preceding the peak growth velocity, or approximately 3 years after pubertal onset. Estrogen levels were shown to continue to increase and remain at a relatively higher level at the end of puberty as compared with prepuberty levels. Thus, both androgens and estrogens are found at high concentrations during male puberty. Furthermore, it has been shown that at various stages of puberty and at different times of the day, there may exist relatively higher concentrations of estrogens than androgens (29). Based on these data, gynecomastia is hypothesized to result from an increase in the estrogen-to-androgen ratio.

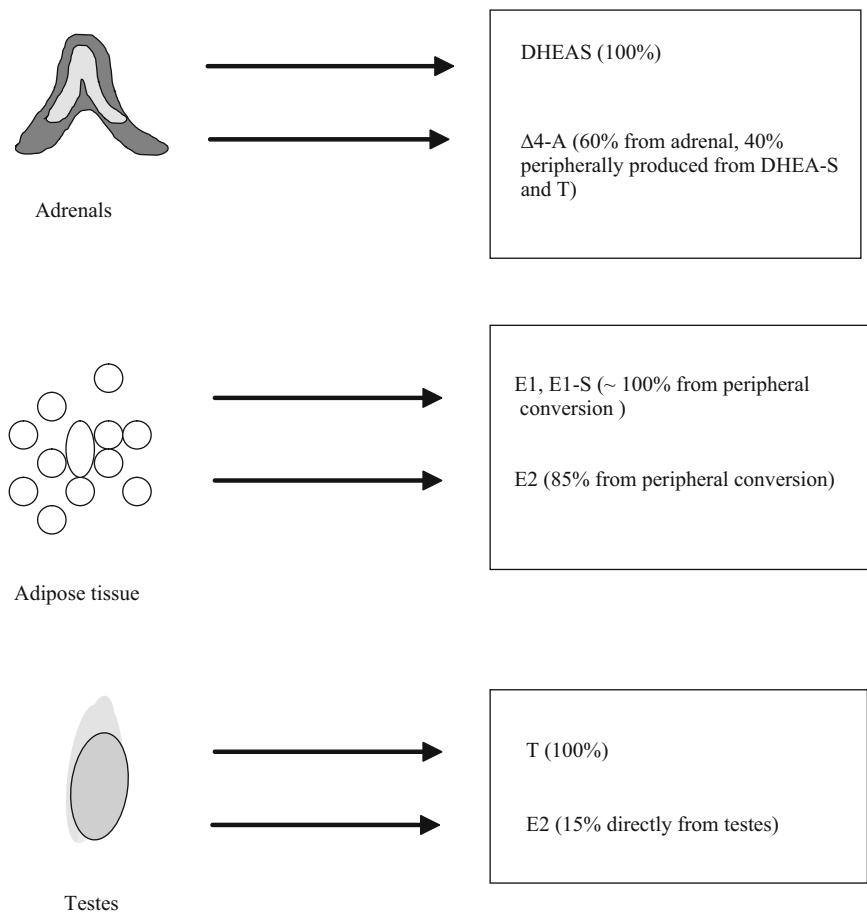


Fig. 5. Production and interconversion of sex steroids. Modified with permission (21). DHEA-S, dehydroepiandrosterone sulfate; T, testosterone; E1, estrone; E2, estradiol; $\Delta 4$ -A, $\Delta 4$ -androstenedione; and E1-S, estrone sulfate.

ENDOCRINE CHANGES DURING GYNECOMASTIA

The male breast is similar histologically to the female breast at birth, and its subsequent growth and development is dependent on the hormonal milieu (21,30), with estrogen being the primary hormone responsible for breast development. Gill et al. (31) showed that male transgenic mice with overexpression of aromatase develop histopathological changes similar to gynecomastia. Androgens, on the contrary, are antagonistic hormones to breast development. In the mouse breast, it has been shown that the administration of estradiol results in doubling of the dry weight and DNA content of the breast (32). This effect, however, is inhibited by dihydrotestosterone (DHT). Casey et al. (32) studied a genetically engineered mouse that lacks a functional androgen receptor and concluded that DHT effects were not exerted through the androgen receptor but may have involved estrogen receptors. In humans, a study evaluating aromatase activity in pubic skin fibroblasts found increased aromatase activity in eight men with gynecomastia when compared with five normal subjects

(33). It stands to reason, therefore, that the development of gynecomastia might occur in a high-estrogen and/or a low-androgen state, and indeed, most of the pathological causes of gynecomastia would fit into either or both of these categories. An increase in the estrogen-to-testosterone ratio is believed to be the cause of gynecomastia, and clinical studies have sought to prove this (Table 2). However, the evidence for this remains controversial, with part of the problem being that serum levels of different hormones do not adequately reflect breast tissue hormone concentrations.

Biro et al. (5) prospectively followed 536 boys aged 10–15 years at 6-month intervals to assess for the development of gynecomastia during puberty and to document hormonal changes at that time. At the onset of gynecomastia, they did not find a statistically significant difference in estrogen-to-testosterone ratios when compared with those of the control group. They did, however, find lower serum free testosterone levels

Table 2
Studies of androgen-to-estrogen ratios in gynecomastia

Study	Subjects	Hormones studied	Results
Biro et al. (5)	536 boys aged 10–15, 48.5% developed gynecomastia	E2 T Free T DHEA-S SHBG	No difference in E2/T ratio at onset of gynecomastia ↓ Free T levels in boys who developed gynecomastia
Moore et al. (2)	54 pubertal boys, 30 developed gynecomastia	T E2 E1 DHEA-S Δ4-A	No difference in T/E2 ratio
Large and Anderson (29)	11 boys with pubertal gynecomastia 8 with simple delayed puberty 2 normal adult men	24-h profiles of T Δ4-A E2 E1	↑ E1 in boys with gynecomastia
Lee et al. (7)	29 pubertal boys, 20 developed gynecomastia	T E2	↓ T/E2 ratio with onset of gynecomastia

Δ4-A, Δ4-androstenedione; DHEA-S, dehydroepiandrosterone sulfate; E1, estrone; E2, estradiol; T, testosterone; ↑, increase; ↓, decrease.

as well as higher sex hormone-binding globulin (SHBG) levels in those boys who developed gynecomastia.

Moore et al. (2) studied plasma levels of various hormones throughout puberty in 30 boys who developed gynecomastia and 24 who did not. They found that the ratio of $\Delta 4$ -androstenedione and dehydroepiandrosterone sulfate to estrone and estradiol was significantly lower in the gynecomastia group. However, they did not find a significant difference in the testosterone-to-estradiol ratio between the groups.

Large and Anderson (29) studied 24-h profiles of circulating hormones in boys with and without gynecomastia. Although they did not find any difference in testosterone or androstenedione levels over 24 h, they found that estradiol and estrone were high relative to testosterone in the afternoon and evening in all subjects. Those boys with gynecomastia had higher levels of estrone.

Lee et al. (7) followed 29 boys with genital Tanner stages 2–4 for a period of 24–48 months, measuring serum hormones at 6-month intervals. Of the 29 males, 20 developed some degree of gynecomastia and 9 did not. There was no significant difference between the two groups in serum levels of each hormone at each stage. However, there was a significant increase in serum estradiol levels with the onset of breast hyperplasia. This increase in estradiol was not paralleled by an increase in testosterone levels, thus decreasing the testosterone-to-estradiol ratio from 154:1 to 120:1.

The abovementioned studies highlight the fact that no conclusive evidence shows consistent hormonal imbalance or abnormalities in patients with physiologic pubertal gynecomastia compared with controls. Nevertheless, the different pathologic situations in which gynecomastia manifests can usually be identified as states of increased estrogen, decreased androgen, or both (*Table 3*).

DIFFERENTIAL DIAGNOSIS OF GYNECOMASTIA

Physiologic Gynecomastia

The most common cause of gynecomastia is benign physiologic gynecomastia. Here, gynecomastia is not a sign of an underlying disorder but rather a common finding occurring in three distinct peaks in the human male’s lifetime.

Table 3
Differential diagnosis of gynecomastia according to hormonal state

<i>Increased estrogen</i>	<i>Decreased androgen</i>	<i>Both</i>
Physiologic neonatal gynecomastia	Secondary hypogonadism	Primary hypogonadism
Testicular tumors	Androgen receptor defects	
Adrenocortical tumors	Antiandrogen drugs	
hCG-secreting tumors	Hyperprolactinemia	
Aromatase excess syndrome		
Estrogen exposure or drugs		
Hyperthyroidism		
11- β -Hydroxylase deficiency		

NEONATAL GYNECOMASTIA

Sixty to ninety percent of all newborns have some degree of palpable breast tissue at birth (30). This is most likely secondary to the transfer of maternal and placental estrogens. Neonatal gynecomastia in the male infant is usually bilateral and more likely to be palpated than visualized. It is transient, usually lasting several weeks but sometimes longer (27). Controversy exists as to whether the hormonal surge during the mini-puberty of infancy in male babies contributes to neonatal gynecomastia, especially when it persists longer than usual. Schmidt et al. (34) examined 1126 healthy infants at 3 months of age and found that 72.6% of boys have gynecomastia greater than 3 mm. They did not find a correlation between estradiol levels and breast size in the male infants. This could be attributed to the limited sensitivity of the assays. However, if real, this lack of correlation suggests that neonatal gynecomastia persists without a subsequent estrogen surge in the male infant.

PUBERTAL GYNECOMASTIA

Breast tissue enlargement is an extremely common finding in pubertal boys. Some studies estimate the incidence to be around 40% of pubertal boys (1,5,35). However, given the transient nature of pubertal gynecomastia, this is most likely an underestimation of the true incidence (22). Pubertal gynecomastia, by definition, occurs in the setting of male sexual development, typically in mid-puberty to late puberty (30). Pubertal gynecomastia is often asymmetric and frequently tender (22). As discussed earlier, the exact etiology of pubertal gynecomastia has not been proven. However, it is attributed to imbalances in androgen and estrogen concentrations in the highly variable hormonal milieu of puberty.

ADULT MALE

Gynecomastia is found to occur commonly in elderly men. In this age group, numerous pathologic factors may lead to gynecomastia, including certain medications and pathologies such as liver disease. However, it has also been described as a common finding in otherwise healthy older men. Men older than 70 years show decreases in the mean levels of total and free testosterone, as well as an increased rate of peripheral aromatization. These changes can all contribute to imbalances in the estrogen-to-androgen ratio (27).

Hypogonadism

PRIMARY HYPOGONADISM

In patients with primary testicular failure, testosterone levels are typically very low. This results in the loss of negative feedback on the gonadotropes and increase in LH production. LH increases testicular estrogen production, and an imbalance in the estrogen-to-androgen ratio results. Primary testicular failure can be a result of several disorders, including viral orchitis, testicular trauma or torsion, uncorrected cryptorchidism or anorchism (vanishing testes syndrome), and chromosomal abnormalities, as in Klinefelter syndrome. Klinefelter syndrome is a chromosomal disorder whereby the affected individual has at least two X chromosomes and at least one Y chromosome, with the XXY karyotype being most common. Almost all patients have dysgenetic testes that are small and firm. There is a substantial variation in clinical

presentation. However, other findings typically include eunuchoid body proportions, sparse hair growth, and gynecomastia. Patients with Klinefelter syndrome may have low normal testosterone levels, and their estrogen levels are found to be elevated (36,37). The risk of breast cancer is 20 times higher in patients with Klinefelter syndrome when compared with males in the general population. Nearly all of those with breast cancer have gynecomastia, suggesting that it may be a predisposing factor. However, the overall incidence of breast cancer remains extremely low and thus does not justify screening or prophylactic resection (38).

SECONDARY HYPOGONADISM

Hypothalamic or pituitary dysfunction may lead to abnormalities in gonadotropin release or synthesis. Causes of secondary dysfunction are varied. Central nervous system (CNS) disorders such as craniopharyngiomas, germinomas, radiation, and trauma often result in pituitary insufficiency. Genetic forms of pituitary hormone deficiencies, such as the PROP-1 mutation, can also result in secondary hypogonadism. Kallmann syndrome is an example of isolated hypogonadotropic hypogonadism that is caused by abnormalities in the *KALX* gene. It is associated with other midline defects, including anosmia and possibly cleft lip and palate. Gynecomastia has rarely been reported in patients with Kallmann syndrome (39).

Tumors

TESTICULAR TUMORS

Testicular tumors are very rare causes of gynecomastia, accounting for only 3% of all cases (40). However, they are potentially life threatening and therefore must always be ruled out. Although extremely rare in children, tumors of the gonadal stroma, including Leydig cell and Sertoli cell tumors, can be associated with gynecomastia. In some cases, gynecomastia can be the presenting sign, even before the tumors are detected on physical examination (41). These tumors, especially Leydig cell tumors, secrete large amounts of estrogens, and peripheral aromatization of androgens further increases the estrogen-to-androgen ratio. The typical biochemical profile is low LH, FSH, and testosterone concentrations with elevated estradiol and estrone levels. Germ cell tumors of the testes, especially choriocarcinomas, have also been associated with gynecomastia (40). These tumors produce human chorionic gonadotropin (hCG) that acts to stimulate Leydig cells to produce estrogens and testosterone. Because estrogens are usually produced in larger quantities, this results in disproportion between the two hormones. Other biochemical abnormalities include elevated levels of hCG and α -feto-protein that serve as tumor markers.

ADRENOCORTICAL TUMORS

These are extremely rare in children, accounting for 0.2% of all childhood malignancies (42). There is a bimodal occurrence, with one peak occurring at age less than 5 years and the other in adulthood. In contrast with adults, approximately 80–100% of these tumors in children are hormonally active (42). Depending on the hormones that are secreted, there is significant variability in clinical presentation. The incidence of purely feminizing adrenocortical tumors in children is extremely low (43). More commonly, the clinical presentation is a combination of virilization and feminization,

including gynecomastia. These tumors secrete huge quantities of adrenal sex steroids, both androgens and estrogens. The tumor cells have been shown to have increased intrinsic aromatase activity, so that the estrogen produced is not solely derived from peripheral aromatization (44). There is usually a greater fold increase in the concentration of estrogens when compared with androgens. This is illustrated by a case of a 6-year-old boy with an adrenal tumor who presented with gynecomastia as well as pubic and facial hair (45). His testosterone concentration was increased four to five times the upper limit of normal for his age. However, his estrone levels were increased to 30–40 times the upper limit of normal, resulting in an increase in the estrogen-to-androgen ratio.

OTHER HORMONE-PRODUCING TUMORS

Bronchogenic carcinomas and retroperitoneal tumors have been associated with gynecomastia. These tumors produce hCG, and this leads to increased production of sex hormones. However, these tumors are extremely rare in children.

TUMORS MIMICKING GYNECOMASTIA

Chest wall tumors may appear similar to gynecomastia. However, on palpation, they differ from the breast glandular tissue seen in true gynecomastia, and the mass may be eccentrically located beneath the nipple. Such masses are usually unilateral. Examples of masses reported in the literature include lipomas, neurofibromas (46), and giant cell fibroblastoma (47).

Enzyme Defects

AROMATASE EXCESS SYNDROME

Mutations in the aromatase gene leading to its overexpression have been reported in the literature. The disorder is usually familial, with an autosomal dominant pattern of inheritance (48). Families with this disorder have a pattern of heterosexual precocity in males and isosexual precocity in females. Gynecomastia is a common feature, typically presenting around puberty. Biochemically, these patients have high levels of estradiol and estrone. The increased concentrations of estrogens exert a negative feedback effect on the pituitary, resulting in suppressed gonadotropin secretion (49). Treatment with an aromatase inhibitor lowers estrogen concentrations and reverses this negative feedback, causing normalization of gonadotropin as well as testosterone levels (50).

OTHER ENZYMATIC DEFECTS

Defects in the androgen receptor in XY infants have been described. The defects can be either complete, resulting in purely female external genitalia, or partial, resulting in ambiguous, under-virilized genitalia. Unlike patients with 5- α -reductase deficiency, these patients exhibit features of hypogonadism at puberty, including sparse hair growth and gynecomastia. As expected, these patients have normal to high androgen levels as well as high-normal estrogen levels (21). However, the effective estrogen-to-androgen ratio is increased.

Exogenous Sources

MEDICATIONS

A large number of medications have been implicated in the pathogenesis of gynecomastia. These drugs interfere with estrogen and/or androgen metabolism (*Table 4*). Spironolactone, a diuretic, blocks the binding of androgen to its receptor. Ketoconazole, an antifungal, inhibits steroid synthesis, including testosterone synthesis.

Table 4
Drugs associated with gynecomastia by class

<i>Class</i>	<i>Drugs</i>
Antimicrobials	Ethionamide Isoniazide Ketoconazole Metronidazole
Antineoplastic drugs	Alkylating agents Combination chemotherapies Methotrexate Vinca alkaloids
Cardiovascular drugs	ACE inhibitors Amiodarone Calcium channel blockers Digitalis preparations Methyldopa Reserpine Spironolactone
Drugs affecting the nervous system	Diazepam Haloperidol Phenytoin Phenothiazines Tricyclic antidepressants
Hormones/hormone-related drugs	Anabolic steroids Aromatizable androgens Estrogens Finasteride Flutamide Growth hormone Human chorionic gonadotropin
Gastrointestinal drugs	Histamine-2 receptor blockers, particularly cimetidine Omeprazole
Drugs of abuse	Alcohol Amphetamines Cannabis Heroin Methadone

Both drugs displace both androgens and estrogens from SHBGs (30) but have a greater effect on androgen displacement. All of these effects result in an increase in the estrogen-to-androgen ratio, causing gynecomastia. Cimetidine, a histamine-2 receptor blocker, has also been shown to cause gynecomastia. Cimetidine blocks the androgen receptor and is also believed to decrease hydroxylation of estradiol, resulting in increased concentration of estrogen (51). Antiandrogens, such as flutamide and bicalutamide, are also known to cause gynecomastia (52). These drugs, used most commonly in the treatment of prostate cancer, bind to the testosterone receptor and thus effectively increase the estrogen-to-androgen ratio. Antineoplastics, especially alkylating agents can also cause gynecomastia. These drugs are used in the treatment of testicular cancer and cause testicular injury. The resultant rise in pituitary gonadotropins leads to increases in estrogen secretion, increasing the estrogen-to-androgen ratio (53). Other drugs have been associated in the literature with gynecomastia; however, their mechanisms of action are poorly understood. These include growth hormone (9), calcium channel blockers (54), enalapril, and diazepam (55).

ESTROGEN EXPOSURE

Inadvertent exposure to creams containing estrogen has been reported to cause gynecomastia in children, as well as rapid increase in height percentiles and advancement of bone age (56). These authors reported three boys whose mothers were postmenopausal and used estrogen vaginal creams. After eliminating the boys' exposure, their gynecomastia regressed. Other cases have been shown to result from enteral exposure to estrogen: the ingestion of the meat of cows treated with estrogen (3).

Other Endocrine Systems

HYPERTHYROIDISM

Hyperthyroidism results in an increase in the concentration of SHBG. Because androgens bind SHBG with more affinity than estrogens, the elevated SHBG level results in an increase in the free estrogen-to-androgen ratio and can manifest as gynecomastia. There is also evidence to suggest increased peripheral conversion of androgens to estrogens in hyperthyroidism, further increasing the ratio (57). The gynecomastia resolves with the normalization of thyroid function (58).

CONGENITAL ADRENAL HYPERPLASIA (CAH)

Gynecomastia secondary to CAH is more commonly seen in 11- β -hydroxylase deficiency than in 21-hydroxylase deficiency (59,60). Gynecomastia is thought to be the result of peripheral aromatization of accumulated androgens, especially androstenedione (61).

PROLACTIN

The role of prolactin in gynecomastia is uncertain. Although elevated serum prolactin results in galactorrhea, it has not been associated with breast enlargement (62). However, hyperprolactinemia inhibits LH secretion and causes hypogonadotropic hypogonadism. The resultant imbalance in the estrogen-to-androgen ratio is likely to be the cause of the gynecomastia seen in patients with prolactinomas or who are on psychotropic medications (63).

Idiopathic

Prepubertal gynecomastia is generally thought to be pathological. However, there are several case series in the literature in which no cause could be elucidated in the majority of the boys (64,65). It is essential to realize that idiopathic gynecomastia is a diagnosis of exclusion. A careful history, physical examination, and pertinent laboratory studies are warranted to exclude all the possible pathological causes of gynecomastia.

EVALUATION OF GYNECOMASTIA

Because gynecomastia is a very common occurrence in pubertal boys (1), a major goal of its evaluation is to distinguish physiologic cases from pathologic cases. Although an in-depth evaluation of most cases of gynecomastia is not necessary, an endocrine evaluation is required in cases of significant breast development in a pubertal boy (66) and in all cases of breast enlargement in prepubertal boys. Confirming the presence of breast tissue is an important step, as a number of conditions may mimic gynecomastia, such as lipomas, lymphangiomas, and cavernous hemangiomas. These lesions usually are different from typical gynecomastia upon palpation, and they are often eccentrically located beneath the nipple or areola.

History

Perhaps one of the most important historical features that should be elicited is the duration of the breast enlargement, as it has important prognostic and therapeutic implications. Gynecomastia that has been present for less than 1 year has a high likelihood of being in the florid stage as opposed to more long-standing gynecomastia, which may have transitioned into the fibrous stage and hence may be less likely to spontaneously resolve. Tenderness is often a symptom early in the course of gynecomastia because of the high degree of edema and vascular congestion seen histologically. Tenderness may be more severe than one would expect, often to the point that simply the pressure of a shirt or bed sheets may be uncomfortable to the boy. Tenderness usually resolves within several months after the onset. The interviewer should also elicit other features of pubertal development and attempt to determine the time course of puberty. Physiologic gynecomastia is most often seen in the middle stages of puberty and may occur more often in boys whose pubertal development has been relatively rapid (5). As there is a wide variety of medications and other substances that may cause gynecomastia, a careful history of medications or drugs of abuse should be obtained (*Table 4*). In some situations, waxing and waning gynecomastia may be related to intermittent non-compliance with prescribed medications (67).

Physical Examination

A good general physical examination should be performed, with special attention to funduscopy, the abdominal examination to detect hepatosplenomegaly or masses, and pubertal status. Several examination techniques of the breast are helpful. As it is important to differentiate between simple adipose tissue and true gynecomastia, the detection of a mass of breast tissue beneath the nipple and areola is critical. The examiner may gently pinch the skin between thumb and forefinger to detect a firm mass. Alternatively, one may push down on the nipple to compress any breast tissue between

the nipple and the chest wall. Gynecomastia will be evident as a tissue mass beneath the compressing finger. If the breast fullness is due to adipose tissue alone, the compressing finger will pass down directly to the chest wall through a ring of adipose tissue, sometimes called the “doughnut” sign, as it is similar to putting a finger through the hole of a doughnut. It is often very helpful to measure the size of the disk of breast tissue with a tape measure, as this will allow for easier detection of changes over time and help determine the need for laboratory evaluation (66). The disk of breast tissue may be firm or relatively soft, depending on its size and duration. It is not adherent to deep tissues.

Examination of the genitalia will allow for assessment of the pubertal stage as well as the testicular size, symmetry, consistency, and the presence of testicular masses. The size of the testes should be appropriate for the stage of puberty. Testes that are unusually small for the boy’s pubertal development may indicate primary testicular disease or central hypogonadism. Testes that are abnormally soft or firm are suggestive of gonadal dysgenesis or Klinefelter’s syndrome, respectively. Significant asymmetry or the presence of a palpable testicular mass is concerning for tumor.

Laboratory Evaluation

Laboratory studies are not necessary in the vast majority of boys presenting with gynecomastia, particularly in those presenting with a small amount of gynecomastia of relatively short duration and who are in the middle stages of puberty. These patients almost always have physiologic gynecomastia. However, boys who are prepubertal, those with large amounts of gynecomastia, those presenting with gynecomastia at an unusual pubertal stage, and those with concerning historical or physical findings should have additional studies performed beyond a history and physical examination.

A wide variety of laboratory studies have been proposed to evaluate gynecomastia (30,66,68). Core studies that should be performed include LH, FSH, total testosterone, estradiol, and β -hCG. Secondary studies that should be obtained depending on the clinical situation include assessment of hepatic function, thyroid function, prolactin, and estrone (50). One algorithm for the laboratory approach to the patient with pathologic gynecomastia is presented in *Fig. 6*.

Imaging Studies

Imaging studies of the breast are rarely necessary in the evaluation of gynecomastia. If required, however, both ultrasound and mammography are potentially useful modalities, particularly if the subareolar tissue does not appear to be breast tissue (69). Gynecomastia appears on ultrasound as either a subareolar triangular sonolucency or a hyperechoic fibroglandular tissue (69). The mammographic appearance of gynecomastia is either nodular or dendritic, correlating with either the florid stage or the fibrous stage of gynecomastia, respectively. Gynecomastia can be clearly distinguished from the carcinoma of the breast on mammography (70).

Additional imaging modalities may be required, depending on the results of earlier evaluation. These might include computed tomography or magnetic resonance imaging of the adrenal glands or ultrasonography of the testes. Ultrasound examination of the testes may detect small tumors that are not evident on physical examination (71). Biopsy of gynecomastia is rarely indicated unless there remains a concern for malignancy after mammography (72).

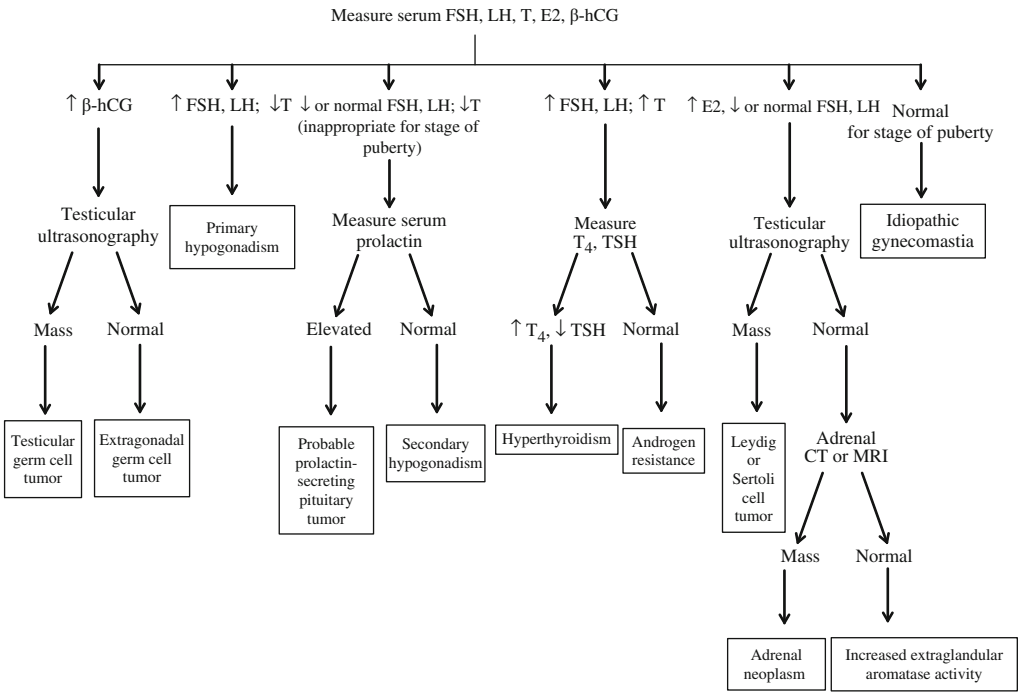


Fig. 6. Algorithm for the evaluation of gynecomastia. CT, computed tomography; E2, estradiol; T, testosterone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MRI, magnetic resonance imaging; β-hCG, beta subunit of human chorionic gonadotropin; T4, thyroxine; TSH, thyroid-stimulating hormone; ↑, high; and ↓, low. Modified with permission (30).

TREATMENT

Indications

Because the vast majority of boys with gynecomastia have physiologic, transient breast development that usually lasts less than 2 years, treatment of gynecomastia is not usually required. Indeed, many boys who have small amounts of gynecomastia are not even aware of its presence. For some patients, however, gynecomastia is a major psychological and emotional burden, especially in cases in which there is a large amount of breast development, resembling the breasts of a girl in mid-puberty. These adolescents are often maliciously teased or physically assaulted because of their gynecomastia. This may lead to social withdrawal and limitation of physical activity, particularly swimming or other activities involving the removal of clothing. Boys with significant gynecomastia often will insist on wearing multiple layers of clothing, may sit or stand in a hunched posture, and frequently cross their arms over their chests, all in an attempt to hide the gynecomastia. In some cases, particularly early in the course of gynecomastia, the patient may experience very bothersome breast tenderness. Although this often resolves within a few months, it may persist for much longer. Patients who have had long-standing gynecomastia, who are experiencing psychological or emotional distress, or who have significant breast pain are potential candidates for treatment.

Treatment Options

OBSERVATION

The average boy with gynecomastia has a small amount of breast tissue, usually less than 4 cm in diameter (8) and typically 2 cm or less. The duration is generally less than 2 years (2,5,7) and usually less than 1 year. For this reason, observation is an acceptable option for most general adolescent patients. For those with more severe breast development, observation may also be appropriate, particularly if they are not bothered by it. Many patients, however, insist on treatment, especially those with the indications discussed in the previous section.

MEDICAL TREATMENT

Between 1950–2000, a large number of medications were proposed as treatment options for gynecomastia. In the medical literature, case reports and uncontrolled retrospective studies of different agents abound. Unfortunately, there is a paucity of well-designed, scientifically rigorous, randomized, controlled trials. Given the high spontaneous resolution rate, this is a difficult condition to study (30), and prospective, blinded, and controlled studies of large numbers of subjects are required to obtain an accurate estimate of the efficacy of a drug. In addition, most of the recently published series report the treatment of adults, often in a very specific population such as prostate cancer patients.

Older studies evaluated the effects of a number of agents. Testosterone may be aromatized to estradiol and may lead to gynecomastia. Hence, it is not recommended for the treatment of gynecomastia (73), although it is often indicated in cases of hypogonadism. DHT, a non-aromatizable androgen, has been studied in 40 adolescents and adults after transdermal administration (74). Full or partial resolution was noted in the majority of patients in this uncontrolled trial, and there appeared to be a strong correlation between efficacy and the systemic DHT concentrations achieved. However, this has not been studied further in a systematic fashion.

Danazol is a weak androgen that reduces gonadotropin secretion. Its use in gynecomastia was evaluated in a randomized, placebo-controlled study of 55 subjects (75). Although there was a statistically significant decrease in the size of the breast tissue, the change was unlikely to have been clinically important. Danazol was partially effective in a retrospective review of adults in a surgery clinic (76). However, adverse effects are common and include acne, weight gain, muscle cramps, and diaphoresis (77).

Clomiphene citrate is a triarylethylene compound with antiestrogenic effects (78). In males, it increases gonadotropin secretion and estradiol and testosterone concentrations (79). It has been studied in several older uncontrolled series of adolescent males with pubertal gynecomastia (78–80) with variable results. A dose of 50 mg daily showed variable efficacy, whereas 100 mg/day resulted in complete or partial resolution in 64% of subjects (79). Effects on the relative ratios of androgens to estrogens were also variable. Adverse effects included rash and gastrointestinal upset. Unfortunately, the efficacy data have not been confirmed in randomized, placebo-controlled trials.

Tamoxifen, an estrogen receptor antagonist, has been evaluated for the treatment of gynecomastia both in adults and in pubertal boys. There have been a large number

of uncontrolled trials suggesting efficacy, in terms of both pain reduction and breast size reduction (76,81–85). The largest of these treating pubertal gynecomastia (85) was a retrospective uncontrolled review of 38 patients treated with either tamoxifen or raloxifene, another estrogen receptor inhibitor. The mean reduction in breast size was only 2.1–2.5 cm, and a large number of the patients went on to have surgical breast reduction. Two randomized, placebo-controlled crossover studies of the efficacy of tamoxifen in the treatment of gynecomastia in adults have been published (86,87). These studies showed successful pain relief but limited efficacy in clinically important reduction of breast size. Both studies were limited by small sample sizes and relatively short treatment durations. Thus, although suggestive data exist, tamoxifen remains unproven as a treatment for gynecomastia in the absence of large-scale, well-designed trial.

Inhibition of the P450 aromatase enzyme offers the ability to limit estrogen production while potentially increasing testosterone secretion by stimulating gonadotropin secretion. In a transgenic mouse model expressing the human aromatase gene, administration of an aromatase inhibitor led to the reversal of a variety of defects, including gynecomastia (88). There has been a limited amount of published experience in humans. In a small, uncontrolled series, boys with pubertal gynecomastia appeared to respond to treatment with anastrozole (89). However, a well-designed, prospective, double-blind, placebo-controlled study of 80 adolescents with gynecomastia failed to show any significant difference between anastrozole and placebo in the percentage of subjects achieving a 50% or more reduction in breast tissue (38.5 vs. 31.4%, respectively) (90). Hence, in the absence of any additional data, this agent is not recommended for the treatment of gynecomastia.

SURGICAL TREATMENT

Surgical therapy for gynecomastia has been a long-standing treatment option. Despite the reported successes of medical therapies in some publications, many of the study subjects went on to have surgical mammoplasty, even after partial regression. This remains the treatment of choice for many patients, given the limited efficacy of medical therapy. The surgical procedure is generally performed through an areolar incision, thus minimizing scarring. The breast tissue is removed, taking care to preserve a layer of subcutaneous fat beneath the nipple–areolar complex to avoid adhesion to the chest wall and to create a normal contour (*Figs. 7A and 7B*). Liposuction may be required to reduce the surrounding fatty tissue. Patients typically wear a compressive bandage for 2 weeks after surgery to minimize the risk of hematoma or seroma formation. Larger degrees of gynecomastia, approaching the appearance of the female breast, may require additional steps to reduce redundant skin and to reposition the nipple and areola to their normal location on the chest wall.

CONCLUSIONS

Gynecomastia is a relatively common condition with characteristic peaks in incidence, specifically in the neonatal period, adolescence, and senescence. It is believed to be the result of an increase in the estrogen-to-androgen ratio. In most cases, it is a benign finding; however, it can be associated with several pathological processes. These include hypogonadism, tumors, enzyme and receptor defects, and

some medications. Most of these disorders can be excluded with a thorough history, physical examination, and a brief laboratory evaluation when necessary. In pediatric patients, treatment is frequently not required, because in most neonatal and adolescent cases, gynecomastia is transient. However, for persistent and severe cases, several medications have been studied, including androgens as well as antiestrogens. Unfortunately, there are strikingly few randomized, controlled trials that are essential to document benefit in this often transient condition, and evidence for a medical treatment with clinically important results remains inconclusive. Surgical treatment is also an option, and in many cases, it is the treatment of choice.



Fig. 7A. Preoperative view of an adolescent male with gynecomastia.

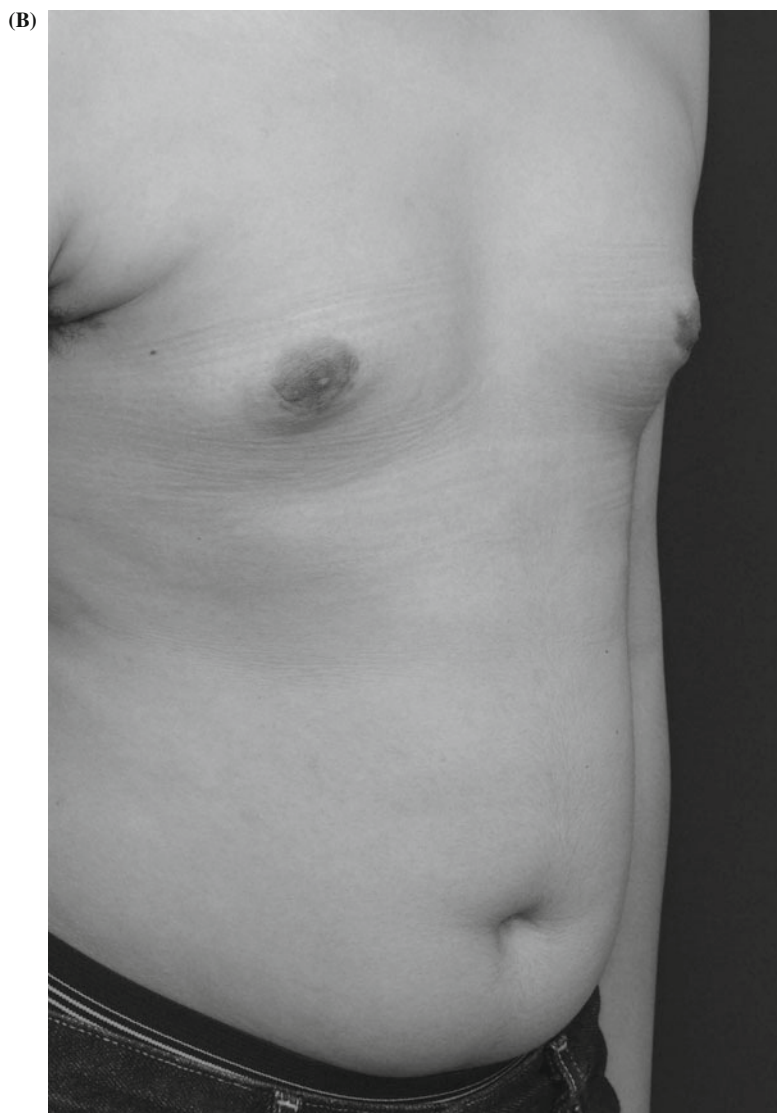


Fig. 7B. Postoperative view of the same patient. Note the normalization of the breast contour with minimal scarring of the areola. Photographs courtesy of Robert Havlik, MD, Indiana University School of Medicine.

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IV

ADRENAL ABNORMALITIES AND PUBERTY

10

Premature Adrenarche

Harbingers and Consequences

Alicia Belgorosky, MD, PhD, María Sonia Baquedano, PhD, Gabriela Guercio, MD and Marco A. Rivarola, MD

CONTENTS

INTRODUCTION
NORMAL ADRENARCHE
PREMATURE ADRENARCHE
CONCLUSIONS
REFERENCES

Summary

Adrenarche occurs only in higher primates, typically at 6–8 years of age in humans, when the innermost layer of the adrenal cortex, the zona reticularis (ZR), develops. This is an event of postnatal sexual maturation in which there is an increase in the secretion of adrenal androgens, mainly dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), not accompanied by an increase in cortisol secretion. Although adrenarche might be a multifactorial event, the main process regulating the production of adrenal androgens continues to represent one of the most intriguing mysteries in adrenal functional differentiation. Premature adrenarche, the early increase in adrenal androgen production, might be associated with ovarian hyperandrogenism and polycystic ovarian syndrome (PCOS) in females, as well as with insulin resistance in the two sexes, during puberty development and adult life, and even in childhood. However, many questions remain with incomplete answers, such as (i) the mechanisms of adrenarche and premature adrenarche, (ii) the physiological actions of adrenal androgens, acting as either direct ligands or pro-hormones, and (iii) the relationship between the activation of adrenal androgen secretion, growth restriction during fetal life, and chronic diseases in adulthood, transforming adrenal androgens in markers of diseases important for human health. Future research might contribute to provide responses for a better understanding of these questions.

Key Words: Adrenarche; Premature adrenarche; Adrenal cortex–medulla interaction; GH–IGF system; Adrenal androgens.

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INTRODUCTION

Adrenarche occurs only in higher primates, typically at 6–8 years of age in humans, when the innermost layer of adrenal cortex, the zona reticularis (ZR), develops. This is an event of postnatal sexual maturation in which there is an increase in the secretion of adrenal androgens, mainly dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), not accompanied by an increase in cortisol secretion (*1*). The ZR is in theory the morphological equivalent of the fetal zone of adrenal cortex. The primate adrenal produces large amounts of DHEA and DHEAS during fetal development, which decrease rapidly after birth, because the fetal zone virtually disappears during the first few months of postnatal life (*2–6*). Thereafter, longitudinal studies have shown a progressive increase in serum concentration of DHEA and DHEAS in healthy boys and girls, beginning at 6–8 years of age, roughly in parallel with an increase in skeletal age (*7–15*). However, in contrast to the latter proposal, adrenarche might begin earlier in childhood, as suggested by studies performed in healthy children measuring DHEA as well as the DHEA metabolites in 24-h urine samples (*16*).

Although adrenarche might be a multifactorial event, the main process regulating the production of adrenal androgens continues to represent one of the most intriguing mysteries in adrenal functional differentiation. Although a non-adrenocorticotrophin (ACTH) pituitary factor stimulating adrenal androgen production has been proposed to work in concert with ACTH (*17*), no circulating factor has been found that might initiate this process. Moreover, according to recent studies, it has been suggested that the increment of adrenal androgen production is associated with the enlargement of ZR (*18–21*). In addition, the regulation of adrenal steroidogenesis in ZR is a complex process, because adrenarche requires both the acquisition of 17,20-lyase activity of P450c17 and a decrease of 3- β -hydroxysteroid dehydrogenase (3 β -HSD) expression (*18–20,22–27*). Several factors, such as the insulin-like growth factor (IGF) system, insulin, the immune system, and also a possible modulatory role of the adrenal medulla, have been proposed, among others, to be involved in the regulation of the functional differentiation of adrenal ZR cells (*18–28*). However, the mechanism of adult adrenal cortex remodeling and zonation has to be elucidated.

The fact that adrenarche is confined to higher-order primates indicates that this phenomenon is a relatively recent evolutionary development. Although the importance of the fetal production of DHEA and DHEAS is related to their role as substrates for the massive rise in estrogen biosynthesis during gestation (*29*), the significance of adrenarche in human physiology remains unknown. In addition, although no DHEA receptor has been genetically cloned, there are strong data to support the possibility of the existence of a plasma membrane receptor for DHEA (*30,31*).

Adrenarche can be considered as the puberty of the adrenal gland, but the two processes (adrenal and gonadal puberty) are independent. However, androgens of adrenal origin had been postulated to be able to initiate activation of the hypothalamic–pituitary–gonadal axis, because central precocious puberty often occurs in children untreated or poorly treated for congenital adrenal hyperplasia (*1,15,32*), suggesting a possible relationship between adrenarche and gonadarche. On the contrary, the adrenal insufficiency does not represent a limiting factor for the development of gonadarche (*33*).

Pubarche has often been taken as the clinical sign of adrenarche; therefore, the terms adrenarche and pubarche have been usually used as synonyms (15,33). However, dissociation between pubarche and biochemical markers of adrenarche can occur in girls with precocious puberty, who have pubarche long before adrenarche (34,35). The opposite situation has been reported in some girls with Turner's syndrome, mainly in those with premature ovarian failure (35).

Premature adrenarche, the early increase in adrenal androgen production, might be associated with ovarian hyperandrogenism and polycystic ovarian syndrome (PCOS) in females as well as with insulin resistance in the two sexes, during puberty development and adult life, and even in childhood (15,18,36–42). Then, improving our knowledge on the mechanism involved in the regulation of adrenarche represents an important challenge to medical science.

NORMAL ADRENARCHE

Development and Zonation of the Human Adrenal Gland

The developmental program that gives rise to the adrenal gland begins early during embryogenesis and continues into adult life. There are undeniable species-specific differences in the structural and functional organization of the human and great primate adrenal cortex compared with non-primate species (43).

The fetal adrenal cortex derives from a common adrenogonadal precursor lineage that also gives rise to the steroid-secreting cells of the gonads. In human embryos, these adrenogonadal progenitors first appear in the fourth week of gestation. Cells destined to generate the adrenal cortex migrate from the coelomic epithelium forming the primitive adrenal gland by the eighth week of gestation. This rudimentary adrenal gland contains an inner cluster of large, eosinophil cells, termed the fetal zone. Shortly thereafter, a second group of cells develops to form a densely packed outer zone of cells, the definitive zone. At the same time, the adrenal cortex becomes encapsulated, and chromaffin cells originating from the neural crest migrate through the fetal cortex to progressively colonize the center of the gland to form the future medulla (43,44). However, it is not until 12–18 months of age that the medulla becomes adult-like in appearance (45).

Genes encoding many transcription factors have been linked to adrenocortical cell development and to modulation of steroidogenic function (44,46). The large inner fetal zone expressing cholesterol side-chain cleavage enzyme (CYP11A) and 17 α -hydroxylase (CYP17), but not 3 β -HSD, is the site of synthesis of large amounts of DHEA and DHEAS since early in development (47). Definitive zone cells have a proliferation phenotype that persists throughout gestation, and they acquire mineralocorticoid synthesis capacity only late in gestation. A third zone, the transitional zone, develops between the definitive zone and fetal zone around mid-gestation, and it expresses enzymes required for the synthesis of cortisol (47).

After birth, a strong remodeling of the adrenal gland occurs; the medullary islands coalesce to form a rudimentary medulla, the fetal zone regresses by the third postnatal month, primarily by apoptosis (3,48), and the definitive zone develops into the adult adrenal. These morphologic changes are accompanied by a rapid drop in DHEA and DHEAS production due to the involution of the fetal zone. In preadrenarche children, the zona glomerulosa (ZG) and the zona fasciculata (ZF) are clearly present, but

only focal islands of ZR cells, insufficient to influence serum DHEAS levels, can be identified at the age of 3–5 years (4,19,20,49). After adrenarche, there is a development and thickening of a continuous ZR associated with detectable increases in circulating DHEA and DHEAS (4,11,50).

Peak levels of DHEA and DHEAS occur at the age of 20–25 years and decline thereafter (51). This decrease in adrenal androgens with aging is often called adrenopause. There appears to be a reduction in the width of the ZR with aging, without overall changes in the width of the adrenal cortex. This suggests that this phenomenon is specific to the ZR and not to global atrophy of the adrenal gland with aging (51,52).

The origin of the adrenocortical zones and the regulation of their proliferation are incompletely understood. At present, there are three theories for the zonation of the adrenal cortex (53). First, the transformational field theory involves the replacement of zonal tissue by ZF (54–59). Second, the zonal theory involves the equal proliferation of all three zones (55). Third, the progenitor cell proliferation/migration theory, and the most accepted one, proposes that proliferation of cortical cells takes place in the outermost layers of the adrenal cortex. The theory would be valid for differentiation of ZG and ZF during fetal life, as well as for ZR during postnatal life. Hence, all cells of the adrenal cortex would have a common origin, which becomes functionally differentiated in the appropriate zone environment (Fig. 1).

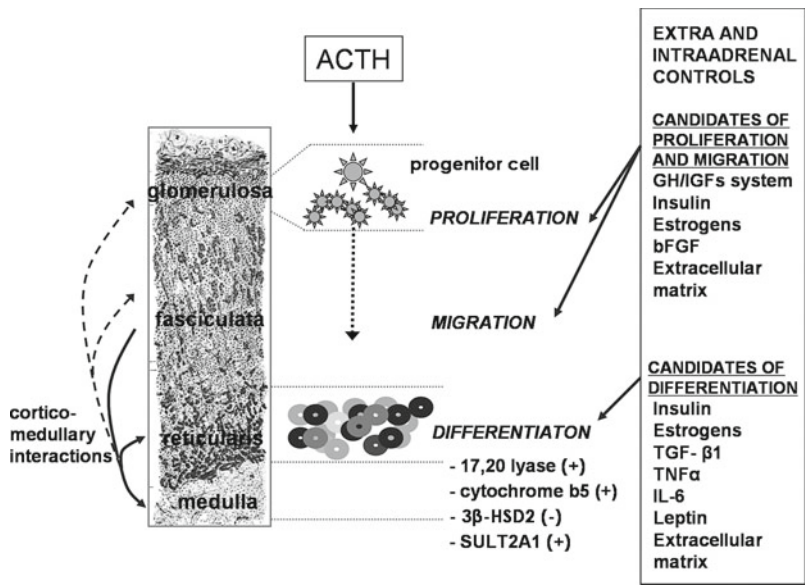


Fig. 1. Growth and differentiation (zonation) of adult adrenal gland. The scheme depicts the progenitor cell proliferation/migration theory and its putative regulation for zona reticularis (ZR) formation. Under the permissive action of adrenocorticotrophin (ACTH), directly essential for cell proliferation and for cortisol secretion in zona fasciculata (ZF), and indirectly for adrenal medulla function, progenitor cell proliferation in the periphery, as well as subsequent migration toward the center of the gland, might be stimulated by several factors, as shown. Upon arrival to the ZR area, other factors might stimulate (and inhibit) transcription of specific genes necessary for the differentiated function of ZR cells. In turn, factors secreted by the adrenal medulla might contribute to adrenal cortex zonation, particularly in ZR.

Although the accumulated data point strongly to the progenitor cell proliferation/migration theory in adrenal gland differentiation, the evidence is not direct. On the contrary, there are few data concerning the mechanisms responsible for the apparent shrinkage of ZR with aging.

Functional Zonation, Ontogeny of Steroidogenic Enzyme Expression in Postnatal Life, and Implications for Adrenarche

In the adult adrenal, the outer cortex has differentiated through the process of zonation into three steroidogenically and morphologically distinct zones: ZG, ZF, and ZR. These zones are responsible for the production of aldosterone, cortisol, and DHEA/DHEAS, respectively. Although some enzymes and cofactor proteins are common to all adrenal zones, the specific classes of steroids produced are determined predominantly by zone-specific expression of the characteristic steroidogenic enzymes for each zone (22). The coexpression of 3β -HSD/ Δ^{4-5} isomerase, 3β -HSD (HSD3B2), and aldosterone synthase (CYP11B2) in the ZG leads to aldosterone production, whereas the coexpression of 3β -HSD and cytochrome P450 17α -hydroxylase/17,20-lyase, P450c17 (CYP17), along with 11β -hydroxylase, P450c11 (CYP11B1), in the ZF results in the production of cortisol. The expression of P450c17 in the ZR along with low levels of 3β -HSD expression leads to the synthesis of DHEA and DHEAS (22).

The cleavage of cholesterol to pregnenolone is the first, rate-limiting, and hormonally regulated step (23) in the biosynthesis of steroid hormones common to all steroidogenic cells (*Fig. 2*). The cholesterol side-chain cleavage enzyme, P450scc (CYP11A), catalyzes three sequential reactions: 20α -hydroxylation, 22 -hydroxylation, and cleavage of the 20 – 22 carbon bond of cholesterol to yield pregnenolone. Because P450scc resides on the inner mitochondrial membrane (60), the movement of cholesterol from the outer to the inner membrane is regulated by the steroidogenic acute regulatory protein (StAR) (61). The expression of CYP11A is similar in all three zones of the adrenal cortex, and there are no changes in adrenal reticularis expression of CYP11A at adrenarche (49). However, as CYP11A (with StAR) is the quantitative regulator of steroidogenesis, alterations in its activity may contribute to the genesis of premature and/or exaggerated adrenarche.

The specific repertoire of enzymes distal to CYP11A in a cell determines the fate of pregnenolone metabolism and defines the function of that cell. The conversion of pregnenolone to DHEA requires only the P450c17 enzyme. CYP17 is the gene encoding this single microsomal enzyme responsible for the metabolism of pregnenolone to 17α -hydroxypregnenolone (17α -hydroxylase activity) and 17α -hydroxypregnenolone to DHEA ($17,20$ -lyase activity) (62,63). The $17,20$ -lyase activity of the P450c17 enzyme is necessary for androgen production, whereas the 17α -hydroxylase activity of the same enzyme is necessary for both androgen and glucocorticoid production. The efficiency of the 17α -hydroxylation reaction in the human is comparable using either Δ^5 steroid substrate (pregnenolone) or Δ^4 steroid substrate (progesterone), whereas the efficiency of the $17,20$ -lyase reaction predominantly occurs using the Δ^5 steroid substrate (17α -hydroxypregnenolone) for the reaction (64). Although, qualitatively, P450c17 expression has been shown to increase with adrenarche, it is similar in ZF and ZR of the human adrenal. However, cortisol production does not appreciably

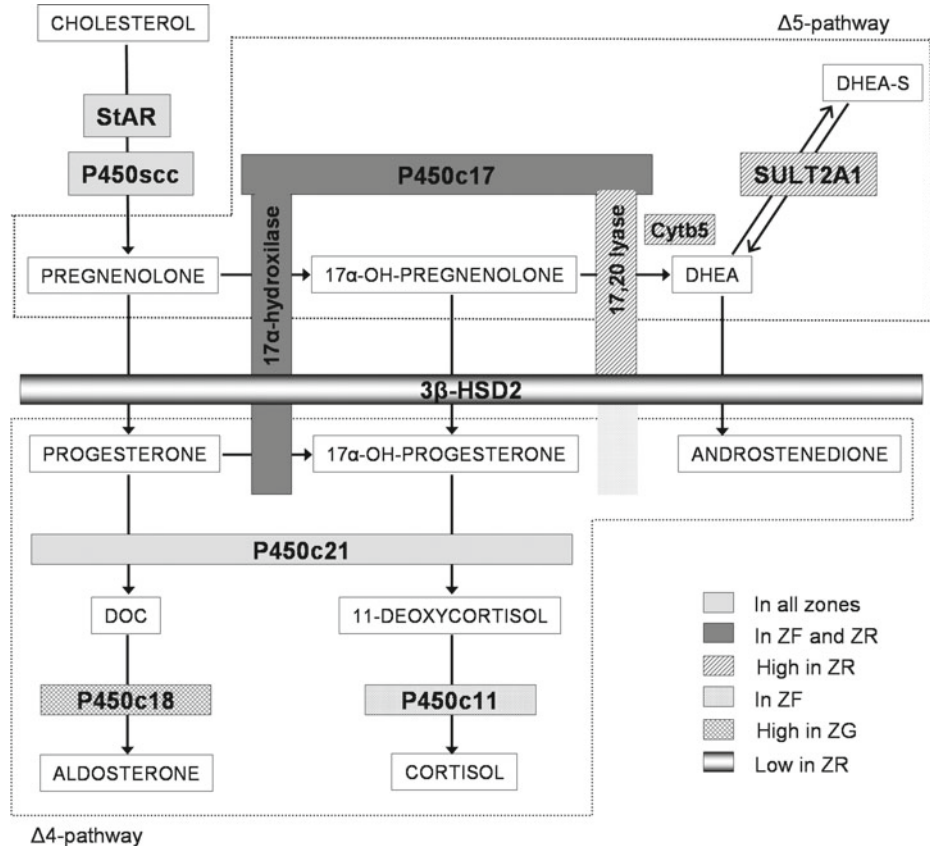


Fig. 2. Steroidogenic pathways in the adrenal cortex. The Δ^5 pathway, predominant in zona reticularis (ZR), is depicted in the upper section of the scheme. The Δ^4 pathway, leading to aldosterone, cortisol, and androstenedione, requires the action of type 2 3-beta-hydroxysteroid dehydrogenase (3β -HSD2) enzyme (lower section).

change with adrenarche. Thus, it has been suggested that a specific induction of 17,20-lyase activity of CYP17 is responsible for the greater C_{19} steroid production by the adrenal reticularis as adrenarche progresses (23). The ratio of 17,20-lyase to 17 α -hydroxylase activity of P450c17 is regulated posttranslationally by at least three factors: the abundance of the electron-donating protein P450 oxidoreductase (POR) (64,65), the presence of cytochrome b5 (23,66), and the serine phosphorylation of P450c17 (24)—all of which could be influenced at adrenarche.

P450c17, like all microsomal cytochrome P450, receives electrons from NADPH through the intermediacy of POR. As the 17 α -hydroxylase and 17,20-lyase require the reception of a separate pair of electrons, P450c17 must sequentially interact with two molecules of POR in order to catalyze both reactions. On this line, it has been showed that increasing the molar ratio of POR to P450c17 increased the ratio of 17,20-lyase to 17 α -hydroxylase activity (65). POR is expressed in all three adrenal zones, with highest expression in the ZR. At adrenarche, the expression of POR increases in all zones of the adrenal (49), which should increase both activities of P450c17, particularly

17,20-lyase activity, thus boosting the overall capacity to produce DHEA. However, this is not sufficient to explain the biochemical changes in the ZR that occur during adrenarche.

Human cytochrome b5 acts principally as an allosteric effector that interacts primarily with P450c17 POR complex to further stimulate 17,20-lyase activity (25). Immunohistochemical studies have shown that cytochrome b5 colocalizes along with P450c17 to the adrenal ZR (26), with minimal expression in the preadrenarche adrenal gland. It increases during adrenarche (49), supporting an important role for cytochrome b5 in DHEAS biosynthesis. Lower expression of cytochrome b5 in the ZF, at least partially, explains the discrepancy between high glucocorticoid production and relatively low DHEA production by these cells, despite the dual activities of CYP17. Moreover, it has been found that the area of the adrenal cortex that expresses cytochrome b5 also declines with aging (67), which would contribute to the reduction of the Δ^5 , C₁₉ synthetic pathway (68).

Phosphorylation of P450c17 on serine/threonine residues increases the 17,20-lyase activity, whereas dephosphorylation inhibits this activity (27). Alterations of P450c17 phosphorylation status is an attractive mechanism for driving the increased DHEAS production during adrenarche. However, altered phosphorylation of P450c17 has yet to be demonstrated in children at adrenarche or, for that matter, in children with premature adrenarche.

Another key regulatory process in DHEA biosynthesis is the flux of steroids from the Δ^5 pathway to the Δ^4 pathway. Enzymes such as 3 β -HSD and 21-hydroxylase, P450c21 (CYP21), normally act to decrease DHEA production through competition with P450c17 in the case of 3 β -HSD and through the removal of steroid precursors in the case of P450c21.

In humans, there are two 3 β -HSDs: type 1 3 β -HSD is found in liver, skin, placenta, and other peripheral tissues and type 2 3 β -HSD (3 β -HSD2) is expressed in adrenals and gonads (69). Pregnenolone, 17 α -hydroxypregnenolone, and DHEA are all substrates for the 3 β -HSD, which irreversibly transforms these Δ^5 steroids into their Δ^4 congeners. Therefore, 3 β -HSD2 activity is essential to aldosterone and cortisol production in ZG and ZF. On the contrary, 3 β -HSD2 expression in the ZR would drain steroid flux away from the Δ^5 pathway leading to DHEA and would produce instead androstenedione. Reverse transcriptase-polymerase chain reaction experiments show that adrenarche is associated with a decline in 3 β -HSD2 expression (20), and immunohistochemistry studies localize this deficiency to the ZR (19,49). However, the mechanisms regulating the poor or absent expression of 3 β -HSD2 in ZR cells are not defined as yet.

P450c21 metabolizes steroid products of 3 β -HSD2 toward mineralocorticoids and glucocorticoids. Although deficient P450c21 activity leads to increased C₁₉ steroid production and the clinical manifestations of androgen excess (70), the increase in C₁₉ steroids seen at adrenarche does not result from diminished P450c21 expression within the ZR (19).

It is worth mentioning that most DHEA synthesized by the fetal adrenal cortex and the ZR of the adult adrenal is first sulfated by the DHEA sulfotransferase enzyme (SULT2A1) and secreted as DHEAS. Expression of SULT2A1 remains low through early childhood, increases after adrenarche in the ZR, and continues into adulthood (2,49). Once Δ^5 steroids are sulfonated, they are no longer available as substrates

for 3 β -HSD2 or P450c17, thereby attenuating glucocorticoid and mineralocorticoid production and supporting adrenal androgen production.

Consequently, adrenarche would result from an increase in 17,20-lyase activity of P450c17 that is related with high levels of cytochrome b5 expression and an increase in the expression of SULT2A1 along with a decrease in 3 β -HSD2 expression in the adrenal ZR to maximize DHEAS production by these cells. This clearly shows that the regulation of adrenarche is a complex event at multiple levels of regulation of adrenal steroidogenesis.

Regulators of Adrenal Androgen Production

The pituitary hormone ACTH is the primary regulator of both fetal adrenal development and adult adrenal cortex homeostasis and steroidogenic function (61,71–73). Given various biological events triggered by ACTH in the adrenal cortex, it appears that most of these effects are induced by various related proteins synthesized and secreted by the different zones of the cortex and /or that locally produced factors may synergize or antagonize the direct biological effects of ACTH to generate combinatorial responses in the different cell subpopulations.

Several experiments and clinical observations have shown that ACTH is necessary but not sufficient to induce adrenarche. Patients with ACTH resistance fail to undergo adrenarche (74,75), and patients with ACTH deficiency have undetectable DHEAS levels (76). However, ACTH levels do not change at times when circulating DHEA levels change drastically, such as during adrenarche or aging (77), suggesting that other factors must be involved in the specific regulation of DHEA and DHEAS synthesis in the adrenal gland. These factors should be involved either in the regulation of adrenal enzyme expression and action or in the growth and trophic maintenance of the ZR itself.

The roles of several growth factors have been examined. IGF-I and IGF-II, acting through type 1 IGF receptor (IGF-R1), affect growth and differentiation of a wide variety of cell types and can act as autocrine, paracrine, or endocrine factors (78). Although IGF-I mediates many of the postnatal somatotrophic actions of growth hormone (GH), IGF-II has been involved in the regulation of fetal development. Both IGF-I and IGF-II enhance steroidogenic enzyme activity of P450c17 and 3 β -HSD (79). In this regard, a recent study of IGF-I, IGF-II, and IGF-R1 mRNA expression and immunolocalization in human adrenals from early infancy to late puberty shows a very low IGF-R1 expression in the ZR, suggesting that the IGF system is not directly involved in the regulation of adrenal androgen through ZR cells (21). However, it has been proposed that IGF-I and perhaps IGF-II, by autocrine, paracrine, or endocrine stimulation, could be a factor involved in the postnatal mechanisms of progenitor adrenal cell proliferation and migration (21). Although IGF-II circulating and tissue levels are highest during fetal life and decrease postnatally (21,78), a postnatal role of IGF-II in adrenal gland could not be discarded (21,80).

Basic fibroblast growth factor (bFGF) is a potent mitogen in primary cultures of bovine adult adrenal cortical cells (81). It also stimulates proliferation of cultured fetal and definitive zone cells (82). As bFGF is also a potent angiogenic and neurotrophic factor, its trophic effects could be also due to effects on the growth and maintenance of the vascularization and innervation of the adrenal cortex. Nevertheless, little is known about the expression and role of bFGF in postnatal human adrenal gland function, including adrenarche.

The possible role of the transforming growth factor $\beta 1$ (TGF- $\beta 1$) in adrenarche is not clear. TGF- $\beta 1$ stimulates 3β -HSD2 activity in adult human adrenal cells. A local decrease of TGF- $\beta 1$ production might be involved in the steroid hormone changes observed at adrenarche (83).

Interleukin-6 (IL-6) also appears to be a local factor that stimulates DHEA secretion. Interestingly, IL-6 receptor is expressed with high density in the ZR (84). Cytokines produced by the inner zones of the human adrenal cortex, such as tumor necrosis factor α (TNF α) (85), would participate in the differentiation and apoptosis of the ZR (86,87). Recently, an inhibitory effect of TNF α on the HSD3B2 promoter has been shown, which is in agreement with the low expression of 3β -HSD2 in the ZR at adrenarche (88).

A potential role for steroids, particularly estradiol, in promoting adrenal androgen production has been suggested. High concentrations of estradiol enhance basal and ACTH-stimulated DHEA and DHEAS production by human fetal adrenal cells in culture (89,90). The mechanism of action seems to be a direct inhibition of 3β -HSD2 enzyme activity by high estrogen levels (91). In addition, it has been shown that estradiol increases cell proliferation in the human cell line H295R (92). However, children with gonadal dysgenesis have a normal rise of DHEAS with chronological age (CA) (33). Furthermore, there are no significant sex differences in the age of adrenarche, which supports that estrogen is not a major factor. However, estrogen is not solely an endocrine factor but instead is produced in many extragonadal sites and acts locally in a paracrine and intracrine fashion. Within these sites, aromatase action can generate high levels of estradiol locally without significantly affecting circulating levels (93). Taking this into account, it is of interest that a preliminary study described the presence of aromatase expression in prepubertal and pubertal human adrenal glands as well as the immunolocalization of estrogen receptor β (ER β) in the ZR (94).

More recently, other candidate hormones, related to control of body mass, such as insulin and leptin, have been suggested as the triggers of adrenal growth and adrenarche. In vitro, leptin has been shown to increase the 17,20-lyase activity of the P450c17 enzyme in human adult adrenal cells, presumably through P450c17 phosphorylation (95).

It is worth mentioning that components of extracellular matrix can induce intracellular cell signals or interact with hormonal or growth factor transduction pathways, leading to specific adrenocortical cell behavior, such as proliferation, migration, apoptosis, and gene expression (96–98).

Finally, the integrated control of adrenocortical function involves cortico-medullary interactions, the gland's vascular supply, its neural input, the immune system, growth factors, as well as signals provided by the extracellular microenvironment (28,99). Although the physiological triggers of adrenarche remain speculative, it is reasonable to speculate that adrenarche is probably the result of the interplay of several factors.

Interaction Between Adrenal Medulla and Adrenal Cortex

The adrenal gland consists of two endocrine tissues of different embryological origin. During embryogenesis, the adrenal primordium consists of mesodermally derived fetal adrenal cells that later become steroid-producing cells. Adrenomedullary chromaffin cells originate from neural crest precursor cells that migrate into the adrenal anlagen in

the sixth week of gestation, in the human, and later differentiate into chromaffin cells in the adrenal medulla, under the influence of adrenocortical steroids (28,100,101). In addition, during human intrauterine life, it has been reported that a well-formed medulla is not present until after birth (43–45,102–104).

The main secretory products are the catecholamine epinephrine (E) and norepinephrine (NE). E is synthesized from NE methylation by phenylethanolamine *N*-methyltransferase (PNMT). However, E secretion in adrenal medulla is in a 5:1 ratio with NE. Thus, plasma E is mainly exclusively derived from the adrenal medulla in which it is synthesized from its precursor NE, whereas plasma NE is predominantly derived from sympathetic nerve endings, acting as a neurotransmitter. In addition to catecholamines, chromaffin cells produce and secrete a wide variety of neurotransmitters, neuropeptides, and proteins (105–107).

Historically, the two cell populations within the adrenal gland, the steroid-producing adrenocortical cells and the catecholamine-producing chromaffin cells, were considered as two independent endocrine systems. However, human adrenal cortex and medulla appear to be interwoven and show multiple contact zones without separation by connective tissue or interstitium (103,108–111). Evidences obtained from in vitro studies and animal models, as well as the analysis of human adrenal pathophysiology, have demonstrated the critical importance of cortical–chromaffin crosstalk for the functioning of the two endocrine systems in the gland (112–124). The signaling between the two compartments is considered to be reciprocal, and adrenocortical glucocorticoids regulate the expression of catecholaminergic enzymes including tyrosine hydroxylase (114), dopamine- β -hydroxylase (115), PNMT (116,117), and neuropeptides (28,118) produced by chromaffin cells. In humans, there are also strong evidences suggesting that high intra-adrenal glucocorticoid concentrations are necessary for the maintenance of adrenal E synthesis. For instance, patients with acquired secondary adrenal insufficiency have diminished basal E secretion (125), and patients with classic 21-hydroxylase deficiency have adrenomedullary dysfunction and major structural changes in the adrenal medulla, such as dysplasia, reduced expression of the key catalytic enzyme tyrosine hydroxylase, and depletion of E-containing secretory vesicles (126,127). Moreover, an impaired stress-induced adrenomedullary capacity, leading to defective glucose elevation and possibly reduced heart rate during exercise, has also been described in patients with classic 21-hydroxylase deficiency (128).

Conversely, catecholamines and neuropeptides are required to regulate adrenocortical steroidogenesis (28,119–121). In vitro studies have shown that E stimulates the expression of adrenocortical cP450 enzymes in bovine adrenocortical cells in culture (123,124). Moreover, although there was no atrophy of the adrenal cortex in catecholamine-deficient mice lacking tyrosine hydroxylase, ultrastructurally, the organelles of cortical cells displayed features of a hypofunctional state (112). In addition, the lack of adrenarche has been previously described in treated congenital adrenal hyperplasia patients (129,130), suggesting an impairment of adrenal maturation of ZR. Thus, chromaffin cells might be involved in the functional differentiation of the ZR, that is, in the mechanism of adrenarche development. On the contrary, Weise et al. (131) described that plasma concentrations of E, and of its metabolite metanephrine, decreased significantly with advancing age, whereas in puberty, in healthy boys and girls, a close inverse relationship between E and DHEAS levels was observed.

However, in the latter study, a causal relationship was not proved and an age-related change in the clearance of catecholamines has to be ruled out. In addition, a recent study provides in vitro evidences that DHEAS stimulates the production (synthesis and secretion) of catecholamines in PC12 cells, suggesting that ZR cells exert a continuous direct tonic effect on adrenal chromaffin cell production of catecholamines (132). Moreover, Yoshida-Hiroi et al. (133) have shown that corticotropin-releasing receptor-1 (CRHR-1)-null mice exhibited a marked depletion in the storing of E in secretory granules that could not be completely normalized by ACTH treatment, suggesting that CRHR-1 is required for a normal chromaffin cell structure and function. In this line, Ibáñez et al. (134) have proposed that CRH might have the capacity to act as an adrenal androgen secretagogue. Therefore, it can be speculated that CRH might modulate the communication between chromaffin and ZR cells.

Glasow et al. (135) have detected full-length human leptin receptor (Ob-R) mRNA expression in human adrenal cortex and adrenal medulla, but a weak staining of the Ob-R protein in adrenal medulla was observed. In this line, no significant direct effect on adrenomedullary catecholamine release was found. These data suggest that leptin might not be directly involved in the regulation of catecholamine release. However, an inverse correlation between serum leptin and plasma E levels in healthy individuals has been described (136–138). Moreover, because obese individuals have been shown to have elevated leptin concentrations and resistance to the lipolytic effect of E, a role of the adrenomedullary system on adipose tissue might be suggested (139–141).

In conclusion, the future progress in characterizing the mechanism of chromaffin–cortical interaction might contribute to our knowledge on the mechanism of adrenarche development and its consequences, particularly in patients with disorders of adrenal androgen production.

The Immune System and Adrenal Androgen Production

Adrenal ZR cells are the only adrenocortical cells that constitutively express major histocompatibility complex (MHC) class II molecules (86). It has been proposed that, perhaps, the expression of these MHC class II molecules facilitates interactions between cells in ZR and immune cells, such as lymphocytes and others, suggesting that these cells are predestined for direct interaction with the immune system (83,142).

In clinical conditions of chronic inflammatory disease, and similar to aging, adrenal androgen production clearly decreases (143–146). However, several studies, in the two clinical states, described the existence of a dissociation between adrenal androgens and cortisol secretion (147–155). The mechanism of ZR functional decline and involution, similar to adrenarche, is still poorly understood.

A very challenging study by Wolkersdörfer et al. (156) has shown that normal T-cell function may be critical for the production of DHEA, because the incubation of human adrenocortical cells with lymphocyte-conditioned medium did not increase DHEA secretion. Thus, the immune–endocrine interaction might not be mediated by cytokines but requires direct cell interaction between lymphocytes and ZR cells. In addition, specific immunostained CD4+ and CD8+ T lymphocytes in direct cellular contact with ZR cells have been described in normal human adrenal tissues.

Finally, that an interaction between the immune system and ZR cells might play a role in the development of adrenarche is an interesting and testable hypothesis. Future research efforts will help to delineate the relevance of the immune system, not only in the development and functional differentiation of ZR cells but also in the clinical features of hyperandrogenism.

The IGF System and Insulin Sensitivity

There are some evidences that the GH-IGF system and insulin might be regulating factors of adrenal androgen production at adrenarche. For instance, serum IGF-I levels rise and fall in a pattern similar to that of serum DHEAS levels, and normal puberty is characterized by a state of transient insulin resistance associated with an increase of not only gonadal sex steroid production but also adrenal androgens. Thereby, a role has been proposed for the GH-IGF system and insulin in the developmental changes taking place at adrenarche (157–163). Indeed above, several in vitro studies showed that the IGF system and insulin might modulate adrenal steroidogenesis, not only cortisol but also DHEAS secretion.

The GH-IGF system and adiposity have been considered the major contributors of insulin resistance at puberty (157,161,164–168). Several studies have shown pubertal female–male differences in insulin sensitivity, normal girls being less insulin sensitive than normal boys (161,167–171). However, we found that these sex differences were clearly evident in late prepuberty, when girls became more insulin resistant than boys (169,171). In addition, a similar finding was described by Hoffman et al. (168) in a small sample of subjects.

In vivo studies of the implications of insulin resistance and the GH-IGF system on the regulation of adrenal androgen secretion in normal children at adrenarche are scarce. Bloch et al. (172) have found that healthy children at adrenarche were more insulin resistant than younger ones, and an inverse relationship between insulin sensitivity and DHEAS levels was also found. On the contrary, no relationship between DHEAS levels and insulin sensitivity was observed in normal prepubertal and adolescent subjects of both sexes, by Caprio et al. (173). Finally, although Smith et al. (174) described a positive correlation between DHEAS levels and basal or stimulated insulin responses when prepubertal and pubertal children were analyzed together, they were unable to detect a significant correlation in the prepubertal group alone.

In adult men, in contrast to adult women, DHEAS concentrations correlate positively with insulin sensitivity (175), suggesting a physiological sexual dimorphism. Guercio et al. (169,171) have studied the relationships between the GH-IGF-I, insulin sensitivity, and adrenal androgens in normal prepubertal and pubertal boys and girls. In this study, it was found that, as previously described (176,177), serum DHEAS levels increased during prepuberty in both sexes. However, as it is shown in Fig. 3, and in contrast to Denburg et al. (42), in prepubertal boys, no correlation was found between serum DHEAS levels and insulin sensitivity, or serum DHEAS and serum IGF-I levels, suggesting that neither the GH-IGF-I axis nor the insulin sensitivity is involved in adrenarche. Insulin sensitivity decreased in the transition from early to late puberty in boys following changes in body mass index (BMI) and correlating with DHEAS levels, suggesting that peripheral insulin could be involved in adrenal androgen steroidogenesis, particularly during early puberty in boys.

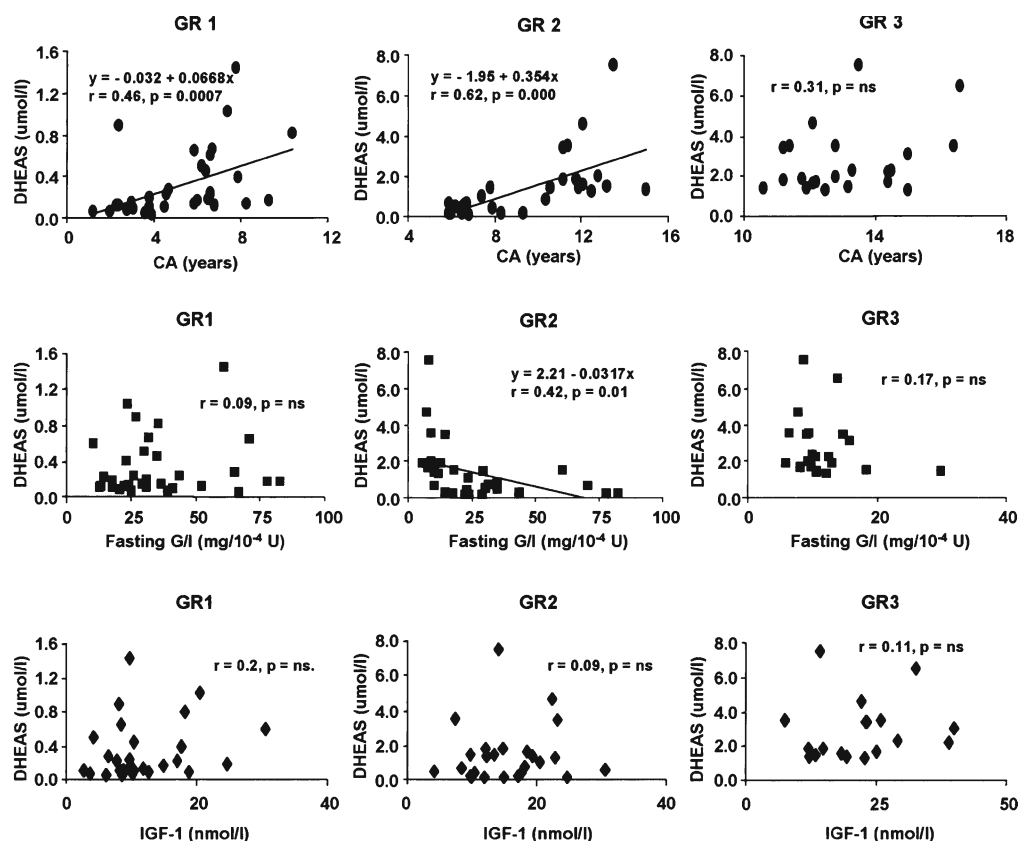


Fig. 3. Correlations between serum dehydroepiandrosterone sulfate (DHEAS) and chronological age (CA), serum DHEAS and a marker of insulin sensitivity (fasting serum glucose/insulin ratio), and serum DHEAS and serum insulin-like growth factor-1 (IGF-I), in normal boys of three different age groups. Group 1 (GR1): prepubertal boys with testicular volumes greater than 4 ml. GR3: pubertal boys with testicular volumes 4–25 ml. GR2: transition between prepuberty and puberty was conformed by the oldest half of GR1 (5.9 years old or older, in late prepubertal boys) and those boys of GR3 with the smallest testicular volumes (4–8 ml testes, in early pubertal boys). In GR1, age at adrenarche, serum DHEAS increased with age, but no consistent correlation with insulin sensitivity or serum IGF-I was observed. Reprinted with permission (169) and The Endocrine Society.

Contrarily to boys, Guercio et al. (171) found a significant decrease of insulin sensitivity in normal prepubertal girls as well during pubertal development. Although they did not determine serum estradiol in this study, they proposed that the sex differences in insulin sensitivity might be secondary to differences in the estrogen milieu, because sex steroids, androgens, as well as estrogens can regulate insulin sensitivity (178–180) and adipogenesis (181,182) in opposite ways. In girls, Guercio et al. (171) also found a significant negative correlation between serum DHEAS levels and insulin sensitivity during prepubertal and pubertal development, and a positive one between serum DHEAS levels and serum IGF-I, but limited to the prepubertal period (Fig. 4). These data suggested that in normal girls insulin is involved in the regulation of adrenal androgen steroidogenesis during the lifespan and might be one of the peripheral regulators involved in the mechanism of adrenarche in girls. The study

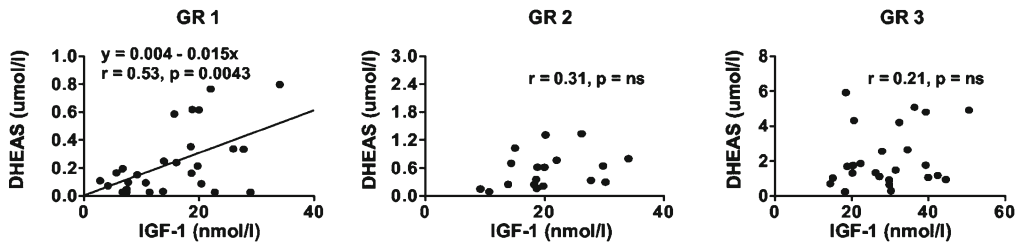


Fig. 4. Correlations between serum dehydroepiandrosterone sulfate (DHEAS) and serum insulin-like growth factor I (IGF-I) in normal girls of three different age groups. GR1: Tanner stage 1 of breast development. GR3: Tanner stages 2–5 of breast development. GR2: transition between prepuberty and puberty was conformed by the oldest half of GR1 (5.3 years old or older, in late prepubertal girls) and those girls of GR3 with Tanner stage 2 of breast development. In GR1, age at adrenarche, serum DHEAS levels correlated positively with serum IGF-I and negatively with insulin sensitivity (not shown). Reprinted with permission (171) and The Endocrine Society.

of Guercio et al. (171) also suggested that the GH–IGF axis might be an important metabolic signal involved in the maturational changes of human adrenal at the time of adrenarche.

Information regarding serum adrenal androgen levels in individuals with GH deficiency has been conflicting, and limited to a small number of reports, mainly derived from studies performed in children (183–189). It has been proposed that the presence of an intact ACTH reserve is a necessary feature of the GH-dependent action (190). However, in the few studies available in children, this point was not carefully considered.

Although the available data are not conclusive, as it is shown in Fig. 5, we propose that GH might stimulate adrenal androgen production directly or throughout the modification of peripheral or intra-adrenal IGF system. However, an inhibitory effect of GH

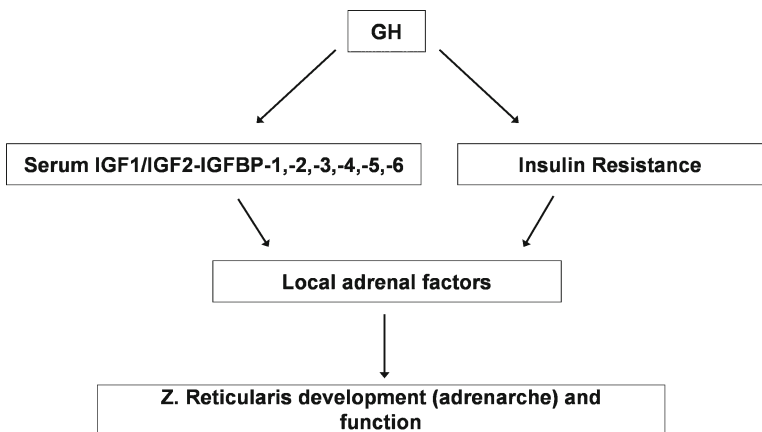


Fig. 5. Putative modulatory role of the growth hormone–insulin-like growth factor I (GH–IGF-I) system on adrenarche. GH, either by directly affecting peripheral insulin sensitivity or through the systemic and local IGF–IGFBP insulin like growth factor binding protein system, might interact with local adrenal factors to modulate zona reticularis (ZR) development and function.

on 11 β -HSD type 1 (191) activity leading to decreased peripheral cortisol production, and subsequent activation of hypothalamic–pituitary axis, has to be ruled out.

PREMATURE ADRENARCHE

Definition—Clinical and Laboratory Characteristics

Premature pubarche is a clinical term used to describe the appearance of pubic hair before the age of 8/9 years in girls/boys, respectively, in the absence of other clinical signs of puberty. Premature or exaggerated adrenarche is defined as the elevation of adrenal androgens above prepubertal levels in children with premature pubarche, provided that the elevation is not due to defined disorders of the gonads or adrenals, such as gonadal precocious puberty, tumors, or enzymatic defects of steroidogenesis (192). As published by Guercio et al. (171), in prepubertal girls, the normal mean \pm SD serum DHEAS level, the main marker of ZR function, was $0.24 \pm 0.22 \mu\text{mol/l}$ (mean \pm SD age = 5.0 ± 3.08 years). However, there was an age difference during prepuberty. In early prepubertal girls (2.2 ± 1.45 years), serum DHEAS level was 0.08 ± 0.09 , whereas in late prepuberty (7.5 ± 1.5 years), the level was $0.39 \pm 0.22 \mu\text{mol/l}$. The corresponding level in prepubertal boys (169), with a mean \pm SD age of 5.22 ± 2.29 years, was similar to that in girls: $0.31 \pm 0.33 \mu\text{mol/l}$. An age difference within prepuberty was also found in boys: early prepuberty (3.19 ± 1.0 years) 0.16 ± 0.20 and late prepuberty (7.13 ± 1.25 years) $0.46 \pm 0.22 \mu\text{mol/l}$.

It should be stressed that the increase of serum DHEAS detected in late prepuberty is the initiation of a long-term biological process of a gradual increment in the serum levels of the steroid, peaking in the early twenties, and followed by a slower and gradual decrement from the third decade to reach very low levels at senescence.

Differential Diagnosis

The differential diagnosis of premature pubarche has important clinical implications. It is more common in girls than in boys (193), and it might be associated with other signs of mild androgen action, such as acne, body hair, or advanced bone age. It is mandatory to discard several conditions leading to excessive androgen production. Congenital defects of steroidogenesis are classically present before birth, and therefore, female external genitalia are masculinized to varying degrees. Mild defects, however, might induce minimal masculinization and should be discarded. Most commonly, premature pubarche is the first sign of non-classical 21-hydroxylase deficiency (194). Other congenital defects of steroidogenesis are also possible. Virilizing ovarian or adrenal tumors are extremely rare, but they should be included in the differential diagnosis. One diagnosis to discard is idiopathic precocious puberty. Usually, this condition starts with breast development (thelarche), and therefore, it is easy to diagnose. However, as it occasionally happens with normal puberty, the onset of pubarche might precede breast development. Occasionally, medications for central nervous system (CNS) disorders might induce the development of premature pubic hair.

In boys also, the first condition to differentiate from is simple virilizing congenital adrenal hyperplasia due to 21-hydroxylase deficiency. These boys do not lose salt, and premature pubarche might be the first sign of androgen excess. Clinically, there is growth acceleration and increased bone age. The external genitalia are usually

stimulated, contrasting with small testes, in the first years of life. Later on, chronic androgen excess leads to early maturation of the hypothalamic GnRH pulse generator and development of true precocious puberty. As in girls, virilizing adrenal tumors should be discarded. Testicular disorders are rare. Testicular tumors are usually palpable and might be single (usually Leydig cell tumor) or multiple (Sertoli–Leydig cell tumors). Finally, other conditions, such as familial male precocious puberty produced by gain-of-function mutations of the luteinizing hormone receptor (testotoxicosis), or stimulation of the testes by a human chorionic gonadotropin (hCG)-secreting choriocarcinoma, should be considered.

Imaging studies and laboratory hormonal tests are helpful to establish a correct diagnosis in the two sexes. Adrenal and ovarian tumors can be detected by appropriate image diagnosis. Because the etiology of idiopathic premature pubarche is unknown, the diagnosis is supported by the detection of a moderate elevation of serum adrenal androgens (usually DHEAS) along with the exclusion of the abovementioned disorders. For the detection of 21-hydroxylase deficiency, it is important to determine the basal serum concentration of 17-hydroxyprogesterone along with serum androgens. If they are not elevated, the 17-hydroxyprogesterone response to an iv ACTH test is useful for detecting non-classical 21-hydroxylase deficiency (195). Finally, analysis of the *CYP21* gene will confirm the diagnosis.

Association of Premature Adrenarche With the Risk of Chronic Disease as Adult

Mounting evidence, arisen in epidemiological studies, indicates that events occurring in the earliest stages of human development, such as fetal growth restriction, may influence the development of several disorders in adulthood, such as central distribution of body fat, insulin resistance, the metabolic syndrome, type 2 diabetes, hypertension, and ischemic cardiovascular disease (196). It has been suggested that lower birth weight could result in re-programming many metabolic pathways, which might have long-term unfavorable consequences on body health. In 1998, Ibañez et al. (40) reported that premature pubarche (and exaggerated adrenarche), hyperinsulinism, and ovarian hyperandrogenism were associated with low birth weight in girls. This finding linked exaggerated adrenarche with the risk of developing central obesity, hyperinsulinism, and PCOS. More recent studies found that the relationship between lower birth weight and higher childhood adrenal androgen levels was similar in boys and girls. Furthermore, adrenal androgen levels were highest in small-for-gestational-age infants who gained weight rapidly during childhood (197). Charkaluk et al. (198) studied a large population of children with premature pubarche ($n = 216$) classified according to their sex and the age at the onset of pubarche. They confirmed that premature pubarche was rare in boys (13.4% of the cohort). They suggested that premature pubarche occurring in children aged less than 2 years is probably different from that occurring in older ones. In agreement with previous reports, 4-year-old to 7.9-year-old girls with premature pubarche tended to be obese and had a higher incidence of intrauterine growth retardation.

Indeed, insulin resistance and hyperinsulinemia are common features seen in prepubertal girls with premature adrenarche. In many of these girls, significantly high ACTH-stimulated Δ^5 steroid levels (17 α -hydroxypregnenolone and DHEA), associated

with low sex hormone binding globulin, low insulin-like growth binding protein-1, high IGF-I levels, and an altered lipid profile, have been reported. It has been shown that ACTH-stimulated hormones correlated inversely with insulin sensitivity and directly with IGF-I levels, suggesting that hyperandrogenism might be linked to insulin resistance and the IGF system (36–39,199–201). Administration of metformin to girls with premature pubarche appears to prevent the increase in DHEAS levels (202).

Obesity was more frequently reported in girls with precocious pubarche, and the correlations between DHEAS levels and adiposity indexes suggest that overweight might influence the onset of adrenarche (38,39,201–204). Moreover, in normal children, a greater increase in urinary DHEAS during the period of the greatest rise in BMI was found (205). These effects might be related to increased insulin and leptin levels, associated with an increased adiposity (205,206). Leptin has been implicated in adrenarche by modulating CYP17 phosphorylation (95). However, in girls with precocious adrenarche, serum leptin levels have been found to be similar (207) or higher (203) than in controls.

On the contrary, the GH-IGF-I system might modulate adrenal androgens (207,208). Indeed, a report by Silfen et al. (39) found that insulin levels in Hispanic girls with premature adrenarche did not differ from those of control girls, but IGF-I was higher and IGFBP-1 was lower in premature adrenarche.

It is then evident that premature adrenarche shares many characteristics with PCOS, suggesting that they might be different expression of similar underlying disorders. Therefore, the risk of developing PCOS at adolescence or soon thereafter in girls with premature adrenarche should alert primary care physicians to follow the evolution of sexual development, age of menarche, and menstrual cycles in these girls.

On the basis of the discussed above, premature pubarche has to be included among conditions prone to develop central obesity, insulin resistance, and its complications for adult life. It is advisable, then, in clinical practice, to study these children in terms of BMI, insulin sensitivity, and lipid profile to assess the feasibility of implementing preventing measures involving quality and quantity of food intake, recreational activities, and exercise.

CONCLUSIONS

The mechanism behind precocious adrenarche is as intriguing as that behind normal adrenarche. From a cellular perspective, adrenarche depends on the mechanisms regulating postnatal zonation of ZR. The migration theory of zonal formation assumes that, starting at the age of 6 years, proliferation of progenitor cells would take place in the periphery of the cortex, followed by migration of these cells toward the center of the gland. Upon arrival, these cells would place themselves adjacent to focal areas of ZR cells, already present, and they would acquire expression of genes specific for ZR cells. This process of cell accumulation would result in the formation of a new zone. In premature pubarche, ZR formation would start earlier and/or it is exaggerated. The trigger for this earlier activation of ZR differentiation could act at the level of progenitor cell proliferation, migration, or cell differentiation, or, perhaps, at several levels.

It is interesting that adrenarche requires the permissive presence of basal levels of ACTH but that ACTH is not the primary stimulator. On the contrary, under an acute iv ACTH stimulation (postadrenarche), there is no response of serum DHEAS during

the first hours, but there is a response after several days, suggesting that the response might need proliferation of ZR cells. Activation of IGF-R1 by high levels of insulin in patients with insulin resistance, or by IGF-I in other subjects (39), might induce proliferation of adrenal progenitor cells in some girls with premature or exaggerated adrenarche.

Estrogens might participate in the mechanism of premature adrenarche at the level of ZR cell differentiation through activation of ER β . To explain the higher incidence of premature pubarche in girls compared with boys, it might be proposed that peripheral estrogens could be added to local estrogens synthesized by adrenal medulla aromatase to act on ER β (94). Furthermore, DHEA itself, but not DHEAS, can be an agonistic ligand for ER β (208), suggesting the existence of a positive feedback system within ZR. Other potential candidate factors playing a role in normal differentiation of ZR cells, such as cytokines (IL-6 and TNF α), leptin, and components of the extracellular matrix or of adrenal medulla, might be responsible for premature adrenarche.

Until recently, the secretion of adrenal androgens, as well as the growth of pubic hair in children, was considered as a trivial physiological event, and premature pubarche a minor deviation of normality. However, the numerous recent studies discussed in this review have changed our concept of these events. However, many questions remain with incomplete answers, such as (i) the mechanisms of adrenarche and premature adrenarche, (ii) the physiological actions of adrenal androgens, acting as either direct ligands or pro-hormones, and (iii) the relationship between the activation of adrenal androgen secretion, growth restriction during fetal life, and chronic diseases in adulthood, transforming adrenal androgens in markers of diseases important for human health. Future research might contribute to provide responses for a better understanding of these questions.

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11

Pubertal Consequences of Being Born Small for Gestational Age

*Dyanne A. Wilson, FRACP,
Wayne S. Cutfield, FRACP
and Paul L. Hofman, FRACP*

CONTENTS

INTRODUCTION

PUBERTY IN INDIVIDUALS BORN SGA

ADRENARCHE IN INDIVIDUALS BORN SGA

CONCLUSIONS

REFERENCES

Summary

There is increasing evidence that the in utero environment has a profound effect on later health. Fetal nutritional restriction, usually caused by uteroplacental insufficiency and that manifests with low birth weight for gestational age, is associated with neurocognitive problems, reduced growth potential, and an increased risk of the adult diseases comprising “the metabolic syndrome.” There are also well-characterized metabolic abnormalities, the best described being reduced insulin sensitivity in childhood and pathological insulin resistance in later life. A growing body of evidence indicates that early life events can also modify aspects of puberty. Puberty occurs slightly earlier and at a different tempo, with an earlier but attenuated growth spurt resulting in reduced final height. There is also an increased incidence of premature and exaggerated adrenarche, ovarian hyperandrogenism, and polycystic ovary syndrome (PCOS). These conditions are discussed in more detail as are possible underlying etiologies especially insulin resistance and the amplifying effect of obesity.

Key Words: Small for gestational age; Puberty; Premature adrenarche; Polycystic ovary syndrome; Insulin resistance.

INTRODUCTION

With the technological advances in obstetric and neonatal care over the past 50 years there are increasing numbers of individuals being born small for gestational age (SGA). It has become apparent that the growth and development of these individuals through childhood and adolescence is unique, and as adults they exhibit distinct metabolic and

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endocrine differences. Although research has focused primarily on either early life complications of being born SGA or the effects of SGA on later adult disease, there is a growing body of evidence that pubertal growth and development is altered. This review will outline our current knowledge on the effects of being born SGA on puberty and growth.

To understand the effect of SGA on puberty, it is important to both define what is meant by SGA and outline known SGA abnormalities that could affect puberty, namely, metabolic and growth alterations. The definition of SGA has varied internationally both in clinical and in research settings. In 2001, the International SGA Advisory Board released a consensus development statement for the management of short children born SGA (1). They established a definition for SGA to be used internationally. SGA represents the group of infants with birth weight and/or birth length at least 2 SD below the mean for gestational age for the given population. This approximates the third percentile on growth charts. Infants can be further classified as being born SGA with respect to weight, length, or both weight and length (SGA_W , SGA_L , and SGA_{WL} , respectively). Although both birth measurements should be performed routinely (at least in western society), birth length is inherently less accurate even when measured diligently (e.g., in the setting of research). Birth weight, however, is accurate and therefore is often the key measurement used to classify an individual as being born SGA.

This definition of SGA (≤ -2 standard deviation score (SDS)) is useful in terms of growth surveillance and management; however, research has shown that metabolic and endocrine abnormalities are present in individuals in the lowest 10th of birth parameters (i.e., $P \leq 10$ th percentile or $SDS \leq -1.7$ standard deviation score (SDS)) (2). As such, this cutoff (which represents a birth weight < 2500 g at term gestation) is commonly used when investigating consequences of being born SGA. Despite international improvements in nutrition, sanitation, societal infrastructure, and health including antenatal care, the prevalence of SGA is increasing. A large Swedish cohort showed that the incidence of SGA in the early 1970s was 5.4% of the population (3). In 2003, 7.9% of live infant births in the United States were of low birth weight (LBW – defined as < 2500 g), however this includes premature as well as SGA. (4).

Intrauterine growth retardation (IUGR) is a term often incorrectly interchanged with SGA. IUGR is defined as diminished growth velocity (weight or length or both) of the fetus, implying growth failure (1,5). This can only be determined with serial intrauterine growth assessments (at least two). Intrauterine growth assessments are inherently inaccurate however, and therefore, caution should be exercised when labeling a fetus as having IUGR. IUGR can be further subclassified as symmetrical (reduced growth of head, length, and weight) and asymmetrical IUGR (reduced weight with sparing of length and head circumference). Symmetrical IUGR is usually caused by a fetal abnormality or insult early in pregnancy, whereas asymmetrical IUGR is usually associated with an insult later in pregnancy (i.e., third trimester).

SGA is the consequence of many etiologies (1,6). By far, the largest number and commonest cause of SGA is uteroplacental insufficiency. There are multiple reasons for uteroplacental insufficiency including maternal factors during pregnancy such as hypertension of pregnancy, gestational diabetes, smoking, alcohol or drug use, and congenital infections; demographic factors such as maternal age, maternal height, prepregnancy

weight, race, and parity; maternal co-morbidities such as cardiovascular, renal, and connective tissue diseases (e.g., systemic lupus erythematosus); and uterine/placental factors including single uterine artery, placenta hemorrhage or abruption, and placenta previa. Other causes of SGA include multiple gestation and history of previous SGA, and a large group of infants born SGA are of unknown cause or idiopathic. A minority of individuals born SGA will be the consequence of chromosomal, genetic, and congenital abnormalities of the fetus. Often infants who are SGA have a history of IUGR; however, this is not universal.

The metabolic and endocrine consequences of being born SGA have been the focus of research for many years. Barker et al. (7–9) pioneered this research with their observations that individuals born with an LBW were at higher risk of cardiovascular disease in adult years. It has been subsequently well established that SGA individuals have a higher risk of an assortment of diseases in adulthood, including hypertension, cardiovascular disease, insulin resistance, type 2 diabetes, dyslipidemia, and obesity (together which encompass the metabolic syndrome) (10–14). This association is independent of environmental factors or lifestyle choices that individuals make postnatally, although these potentiate the effects of being born SGA, influencing the rate and degree of disease manifestation. Metabolic abnormalities in individuals born SGA are present even in childhood (2,15). Insulin resistance (the earliest marker of the metabolic syndrome) is evident in otherwise healthy, non-obese, prepubertal SGA children (2), as evaluated by modified minimal model (16–18). Using a short intravenous glucose tolerance test, Soto et al. (19) documented that 1-year-old infants born SGA have insulin resistance, further implying that this sequela to an adverse intrauterine environment occurs very early in life.

The association of SGA with later development of diseases of the metabolic syndrome led to the theories of “fetal origins of adult disease” or “fetal programming.” This concept postulates that fetal exposure to an adverse intrauterine environment leads to fetal adaptation by alteration of endocrine pathways, causing permanent metabolic changes, including reduced insulin sensitivity. Further theories such as the thrifty phenotype hypothesis have helped to further explain this concept (20). This suggests the fetus perceives the unfavorable intrauterine environment as comparable with its future (postnatal) environment and adapts through permanent metabolic and endocrinological alterations to ensure survival. If the postnatal environment is unfavorable, the individual is appropriately adapted. However, when there is mismatch between the in utero and postnatal environment (as is most often the case), the adaptations are not suited to the postnatal environment and lead to early development of disease. In contrast to the thrifty phenotype hypothesis, the fetal salvage hypothesis suggests that the initial adaptations to an adverse, nutrient constrained in utero environment are made to ensure fetal survival (2). These changes will be beneficial in an adverse postnatal environment but maladaptive in a typical nutrient-rich western society.

In addition to the risk of the metabolic syndrome, growth failure is also prevalent in many SGA individuals. Most infants born SGA exhibit catch-up growth (with growth velocity greater than the median for chronological age and gender) and establish a normal growth pattern (defined as a height SD score above -2.0) by 2 years of age at the latest (1). However, 10–15% of SGA infants fail to exhibit catch-up growth and continue to grow parallel to but below -2 SDS (5,21,22). These

individuals have a seven times higher risk of short adult stature (final height < -2 SD) compared with the normal population (5). SGA is now accepted worldwide as a cause of short stature, and GH administration has become registered as a treatment for these individuals (1).

PUBERTY IN INDIVIDUALS BORN SGA

Puberty is the dynamic period that encompasses development of secondary sexual characteristics, change in body composition, acquisition of bone mass, and maturation of cognitive functioning. It is also the time of rapid longitudinal growth, with the second highest growth velocity (surpassed only by growth during infancy).

The commencement of puberty is hallmarked by reawakening of the hypothalamic–pituitary–gonadal (HPG) axis, which remains relatively quiescent during childhood because of inhibition of the gonadotrophin-releasing hormone (GnRH) neurons in the hypothalamus. Many neural connections and neurotransmitters including gamma-aminobutyric acid GABA (23) and neuropeptide Y (NPY) (24,25) are believed to be responsible for inhibition of this axis from about 6 months of age until late childhood/early teenage years, when the removal of inhibitory factors, together with stimulatory and permissive factors, such as glutamate, transforming growth factor- α (TGF- α), insulin-like growth factor-I (IGF-I), insulin, kisspeptin and leptin, allows gradual and rhythmical hypothalamic release of GnRH into the hypophyseal portal system (23–25). GnRH stimulation of the pituitary leads to release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The first biochemical sign of puberty is nighttime secretion of LH and FSH, with eventual release throughout the day in a rhythmical pattern. LH and FSH stimulate and promote maturation of the gonads (ovaries and testicles), allowing development of follicles and sperm, and hormone production (estrogens, testosterone, and inhibin B). Hormonal feedback ensures continued maturation of the HPG axis and sustained hormone release. Hormone production by the gonads leads to the development of secondary sexual characteristics and an adult-like body habitus. The onset of puberty is classified as breast development (breast budding) in females and testicular enlargement (≥ 4 ml volume) in males and occurs between 8 and 13 years and 9 and 14 years, respectively (26).

The specific triggers for puberty and timing in each individual are unknown; however, many believe that they are determined in the large majority during fetal and early postnatal life, when the HPG axis is active (27). Genetic factors do play a role and likely contribute to at least 50% of the variation of pubertal timing between individuals (26,28); however, environmental factors such as nutrition, co-morbidities, foreign adoption, and climate/geography are all believed to contribute to some degree (28,29). In addition, there is growing evidence of the contribution of the intrauterine environment on the timing of puberty.

Timing of Puberty in Individuals Born SGA

Many studies have evaluated the timing of pubertal development in individuals with a history of SGA. Determining the timing of pubertal onset in a large cohort of individuals is not straightforward however. Ideally, children would require at least four to six monthly examinations during peripubertal years to document initial (Tanner stage 2) development (breast budding and testicular enlargement). This is a time-consuming

exercise, without taking into account likely errors including interexaminer variability. As a surrogate, in females at least, menarchal age has served as a marker of pubertal timing. Recollection of age of menarche has been shown to be reliable, especially after a short interval (30). Studies also show that menarchal age is highly correlated with age at the appearance of breast buds (Tanner stage 2) and is considered to be an indicator of the timing of onset of puberty, except in extreme circumstance such as illness or late onset of puberty, when there is an altered tempo between Tanner stage 2 and menarchal age (28,31). The situation is more difficult in males and must rely on examination. The fewer studies evaluating male development reflect this difficulty, and often data are present only in conjunction with other clinical situations or studies (e.g., GH treatment and monitoring). Such interventions may affect the timing of puberty with potentially misleading results.

Although there are some discrepancies among researchers, larger studies suggest that the onset of puberty although well within the normal range occurs earlier in individuals born SGA.

A British cohort of 1818 females born in 1946 were examined 2 yearly throughout childhood and further assessed at a mean age of 14.5 years (27). At that time, during 1960–1961, the females were questioned for the age of menarche. Menarchal age was positively related to birth weight, with those individuals born in the lowest fifth of birth weight having menarche 4 months earlier than those born in the highest fifth. This was not statistically significant however. In contrast, weight at 7 years of age showed a strong negative correlation with menarchal age. Those individuals of highest weight at 7 years had menarche 7 months earlier than the lighter females. Combining the two factors, the earliest menarchal age was seen in females with LBW who grew rapidly during childhood (highest weight at 7 years of age), with childhood weight contributing the greatest. A Swedish cohort of 1231 children (males and females) born during 1973–1977 were examined annually until the age of 16 years (32). There was no difference in the age of pubertal onset in relation to birth weight in males. In contrast, females born SGA had an onset of puberty 5 months earlier and menarchal age 4 months earlier than normal controls. A further Swedish study (3) followed a cohort of 3650 children born at full term. About 5.4% of the individuals were born SGA and had the onset of puberty at an earlier age (although within normal age range) compared with controls [individuals born appropriate for gestational age (AGA)].

Koziel and Jankowska (33) documented the age of menarche in relation to birth weight in 883 Polish females, when routinely examined at 14 years of age. A positive correlation was found, between birth weight and the timing of menarche, with SGA (defined as birth weight < 10th percentile in this study) individuals being 2.5 times more likely to have experienced menarche by the age of 14 years compared with individuals born AGA.

Adair (34) published data on a cohort of females born in the Philippines between 1983 and 1984, whose growth and development was followed during childhood and teenage years. There was no significant relationship between the age of menarche and birth weight, unless birth length was taken into account. Menarche occurred the earliest in females born “long and light” (suggesting asymmetrical IUGR), whereas menarche occurred latest in those infants born “short and light” (suggesting symmetrical IUGR).

Like Cooper et al. (27) (suggesting symmetrical IUGR), They documented that postnatal growth was a factor strongly associated with menarchal age, with the females with higher body mass index (BMI) and highest skinfolds in peripubertal years (8–9) having earlier puberty. Combining these two factors, females born SGA, who had accelerated growth during childhood, had the earliest menarchal age, whereas if the females born SGA remained lean, menarchal age was not affected.

In contrast to the above studies, smaller studies have shown no change in the timing or in fact delayed onset of puberty in individuals with SGA or IUGR. Leger et al. (35) compared the age of menarche in 133 females born SGA with 152 females born AGA. There was no difference in menarchal age between the groups. Lienhardt et al. (22) suggested that puberty is delayed by 1 year in females and 2 years in males with a history of IUGR compared with controls. Boonstra et al. (36), in follow-up of 75 short children with a history of SGA being treated with GH, demonstrated no effect of SGA on the timing of puberty in comparison with local population reference range.

Overall, the evidence suggests that the timing of puberty is earlier (but still within normal range for population) in females who were born SGA in comparison with AGA controls and population means. This association is not as clear in males (which may be in part due to the difficulty assessing pubertal onset in a cohort of males in comparison with ascertaining menarchal age in females). However, it may actually indicate that there is sexual dimorphism with regard to the effect of being born SGA on the timing of puberty (i.e., SGA affects pubertal timing in females, with no effect in males). Precocious puberty (the onset of puberty at < 8 years in females and < 9 years in males) is known to be much more common in females compared with males and is increasingly more likely to be due to an idiopathic or unknown cause (28). It may ensue therefore that females have an underlying predisposition to early puberty. Changes in the intrauterine environment, which lead to being born SGA, may therefore only affect individuals (females) who have an underlying risk of early puberty.

Pubertal Tempo and Characteristics in Individuals With SGA

Owing to the association of SGA and short adult stature, research has focused on the magnitude and timing of the pubertal growth spurt. The pubertal growth spurt contributes to 15–18% (22,37) of final adult height. There is some suggestion that the pubertal growth spurt is attenuated both in magnitude and in duration in individuals born SGA, which significantly contributes to reduced final height.

Research looking at this association is confounded by many factors. First, over the past 100 years, there has been a secular trend of increasing final adult height, of 1–3 cm per decade (38). Although some of this gain in final height (at least in the first half of the 20th century) may have been attributable to the younger onset of puberty, over the past forty years, there has been less variability in the timing of puberty; despite this, final adult height continues to rise (28,38). Other factors especially nutrition and body composition are likely to be the fundamental reason for this serial population height gain. Therefore, when evaluating growth, it is important to take this factor into account; anticipated adult height should be 1–3 cm greater than mid-parental height. Second, it is well documented that individuals with later onset of puberty (such as individuals with constitutional delay of growth and development) have an attenuated adolescent growth spurt (22,28,31). When comparing groups, it may be wise therefore to exclude

individuals with markedly delayed puberty, as this may skew results. Finally, the use of GH in the treatment of short stature in individuals with SGA will influence the adolescent growth spurt. The growth attained when treating individuals with a history of SGA with GH is highly variable compared with the results that are achieved in conditions such as GH deficiency. There is a large interindividual variability, and therefore, any comments about pubertal growth spurt when on GH should be made with caution.

A French cross-sectional study of 517 fully grown individuals (approximately half of whom had a history of SGA) showed that individuals born SGA had a final height (adjusted for target height) significantly less when compared with individuals born AGA (39). The individuals with AGA had gained 3–4 cm over target height (consistent with secular trend in increasing population height gain), whereas the individuals with SGA failed to meet/or just met their respective target heights.

Lazar et al. (21), followed 128 children with persistent short stature obtaining growth data and pubertal evaluation throughout childhood, until final height (defined by growth velocity < 2 cm per year and bone age > 15 years in girls and > 17 years in boys). The onset of puberty was earlier in the SGA children, and menarchal age was also earlier compared with the AGA group (although both occurred within the normal range for population). Duration of puberty, total pubertal growth, and peak height velocity were similar between the SGA and AGA groups, suggesting a normal pubertal tempo in the SGA group. The pattern of growth during puberty, however, was significantly different in the SGA group. In contrast to the normal timing of peak height velocity occurring at Tanner stages 4 and 5 in boys and Tanner stages 3 and 4 in girls, the SGA group reached peak height velocity at Tanner stage 3 in boys and Tanner stage 2 in girls. Consistent with the altered growth pattern, bone age maturation occurred earlier in the SGA group, in conjunction with the peak height velocities. Despite this alteration in growth pattern, pubertal height gain was not different between the groups. Earlier puberty is normally associated with greater pubertal height gain to compensate for earlier epiphyseal closure (31). Therefore, the SGA group who entered puberty earlier should have had greater height gain during puberty compared with the AGA group. This suggests that the abnormal growth pattern of earlier peak height velocity in SGA individuals actually attenuates height gain and is likely to contribute in part to the reduced adult final height of SGA individuals.

In contrast, Boonstra et al. (36) obtained three monthly growth and pubertal data on 75 SGA individuals receiving GH treatment. In this cohort, there was no effect of birth measurements on the timing or tempo of puberty. The interval between Tanner stage 2 and menarche was shorter in individuals with later onset of puberty (consistent with the finding in children with constitutional delay of growth and development), higher prepubertal BMI, and less bone age delay and resulted in reduced pubertal height gain.

In addition, this study exemplified the unreliability of bone age evaluation in the assessment of bone maturation in individuals with SGA compared with a normal population (36). Bone age evaluation in children with a history of SGA gives a false reassurance of growth potential. Biologically, children with SGA develop more quickly than their bone age assessment suggests. Other studies concur, showing that pubertal onset occurs at an “inappropriately” young bone age in SGA children (22). This discrepancy between bone age assessment and development may reflect that SGA

children do have a biologically different developmental and growth pattern through childhood and adolescence. The unreliability of bone age is not a unique problem to children born SGA. Bone age evaluation is subjective, with interindividual and intraindividual variabilities, and therefore, this diagnostic tool should be used with caution in all clinical and research settings.

Is the Association of SGA and Altered Timing and Tempo of Puberty Biologically Plausible?

Despite extensive research into human and animal puberty, the exact triggers for normal pubertal initiation and development remain elusive. As highlighted above, many neurotransmitters and neuropeptides (GABA, NPY, TGF- α , insulin, IGF-I, leptin, and sex hormones) either directly or indirectly inhibit or stimulate GnRH neurons in the hypothalamus. The contribution of each substrate during different stages of development is not known.

Given an association between the timing of pubertal onset and being born SGA, many postulate that the timing of puberty is set during fetal or early postnatal life, when the HPG axis is active (27). In this sense, the reproductive path of the individual is set early in life, in anticipation of the demands postnatally.

Insulin, one of the most important hormones for fetal growth and development, has been proposed as having a role in the regulation of puberty prenatally (as well as postnatally as discussed later in this section) (5). Insulin receptors are present throughout the reproductive system, both in the central nervous system and in gonads; therefore, any changes in insulin regulation (such as insulin resistance, which is known to occur in SGA individuals) may directly affect the HPG axis and therefore the timing and regulation of puberty (40–43).

A possible explanation for a direct association between SGA, insulin resistance, and the timing of puberty can be explained by the “fetal salvage hypothesis” (see “introduction”) as discussed earlier (2). Exposed to an adverse, nutrient-constrained intrauterine environment, fetal growth is compromised, resulting in IUGR and eventual SGA at birth. To ensure adequate fuel supply to vital organs, the fetus adapts by developing specific insulin resistance to glucose regulation, diverting glucose away from less essential tissues such as fat and muscle. Postnatally, when there is nutritional abundance, the insulin-resistant individual develops compensatory hyperinsulinemia to maintain euglycemia. Insulin resistance, however, has been shown to be tissue specific, and the elevated insulin levels may alter physiology and development in other tissues such as brain and gonads (44). The change in metabolic regulation of insulin and glucose, as suggested by this model, could directly affect the HPG axis in utero and continue to do so postnatally. The hyperinsulinemia of the SGA individual during infancy and childhood (in the face of an abundant fuel supply) will influence the HPG axis through direct binding to the multiple insulin receptors along its axis and may influence the timing, duration, and magnitude of puberty. We have indirect evidence supporting an effect of insulin sensitivity on pubertal timing (unpublished results). In children who have evidence of constitutional delay of growth and development (as defined by a family history and delayed bone age), insulin sensitivity is increased when compared with healthy, age-matched controls.

An alternative theory for the association of SGA and insulin resistance is reviewed by Kanaka-Gantenbein et al. (6). This suggests that SGA is a direct consequence of insulin resistance in utero. Rare genetic causes of severe insulin deficiency in utero (e.g., as a result of secretory defect or severe insulin resistance as seen in leprechaunism) result in significant intrauterine growth development and therefore an individual born SGA (6). In the vast majority of cases, SGA and insulin resistance are not due to genetic causes however, instead due to uteroplacental insufficiency, and this explanation for the association of SGA and insulin resistance should not be inferred without evidence.

It is possible that an adverse intrauterine environment influences the development and consequent regulation of the HPG axis independently but in unison with the development of insulin resistance in individuals born SGA. Other metabolic pathways are known to be permanently affected or “programmed,” when exposed to an adverse intrauterine environment, including the hypothalamic pituitary axis (HPA) (6). It is therefore feasible that fetal programming of the HPG axis leads to permanent changes in an SGA individual, leading to alterations in pubertal timing, duration, and character.

There are teleological benefits for early puberty in individuals with IUGR and SGA. The fetus exposed to an unfavorable intrauterine environment predicts that the extrauterine environment will be similarly adverse. By pooling all their resources into early sexual maturation, at a sacrifice of linear and physical growth, the individual maximizes its chances of successful reproduction and gene propagation (before the early onset of disease and death).

IGF-I is another hormone that potentially has a role in HPG axis control and regulation prenatally and postnatally. This hormone is also essential for the growth and development of the fetus. IGF-I deficiency is characterized by prenatal growth retardation. Fetal IGF-I levels are influenced by placental factors, fetal nutrition, and insulin (45–47). In a nutrient-restricted in utero environment, the lower insulin concentration will result in lower IGF-I levels, and the low levels of Insulin and IGF-I may affect the development of the HPG axis in utero and influence pubertal timing postnatally.

Regardless of the effects of insulin and IGF-I on the HPG axis prenatally, these hormones are thought to have a role postnatally. As hypothesized, the SGA individual having developed glucose-specific insulin resistance in utero will develop hyperinsulinemia and in addition high serum IGF-I levels in childhood (when corrected for height and BMI), in the nutritionally abundant postnatal environment (48). These hormones, binding to their receptors along the HPG axis, will directly signal and stimulate the HPG axis and influence puberty in the SGA individual.

The importance of insulin and IGF-I on the pubertal axis is supported by associations in non-SGA children. Serum insulin, IGF-I, and leptin levels are positively correlated with body mass and increase with increasing fat percentile (49,50). Studies show that the timing of puberty is negatively correlated with prepubertal body weight (51–53). The higher serum insulin, IGF-I, and leptin levels in children with a higher BMI are thought to directly stimulate the HPG axis and trigger or permit puberty to proceed. BMI generally increases in peripubertal years (especially in females where there is also an increase in body fat : lean muscle); therefore, critical body fat percentage (and hence hormone concentrations) is likely to be pivotal for puberty to proceed. In contrast, anorectic children who have low levels of serum insulin, IGF-I, and leptin have markedly delayed puberty (29). These hormones may also act indirectly to stimulate

puberty, by promoting bone maturation, which acts as a signal of biological readiness for puberty. Consequently, an individual born SGA has a higher risk of early onset puberty if there is excessive weight gain in childhood. Excessive weight gain will lead to further increases in serum insulin, IGF-I, and leptin levels, signaling the readiness for puberty.

Estrogens and androgens are likely to influence the timing of puberty. As will be discussed later in section “Adrenarche in individuals born SGA”, individuals with SGA are at risk of premature and exaggerated production of androgens and estrogens. This is due in part to direct stimulation of high insulin levels in the gonads and due to earlier onset of sex hormone production by the adrenal gland. Not only do androgens and estrogens promote bone maturation, further signaling biological readiness for reproduction, but estrogens will have direct positive feedback on the HPG axis promoting activation.

Animal Studies

Nutritional restriction in female rats during pregnancy causes IUGR of the fetal pups, with subsequent birth of LBW pups, producing an animal model of IUGR and SGA. Using this model, Engelbregt et al. (54) evaluated the timing of puberty in IUGR pups compared with controls and postnatally food-restricted rats. Both female and male IUGR rats had delayed onset of puberty (assessed by vaginal opening and balano-preputial separation, respectively).

In the rat model, in contrast to the finding in humans, SGA is associated with delayed puberty. An attempt to simulate human development in animal models is, however, fraught with problems. Genetically and biologically rats and humans are very different. Although this study provided a model of IUGR, postnatally rats had no food regulation. Rats are inherently lean animals and will not overfeed. In order to mimic the human model of nutritional abundance, a high-fat diet should have been enforced on the rats, which may have led to different results.

Summary

Although the biological triggers and regulation of puberty remain unknown, the association of SGA and early onset of puberty is plausible. There are many hormonal and biological candidates for signaling, maturing, and triggering the HPG axis, leading to early puberty. It is likely that both prenatal and postnatal factors influence the HPG axis, although the precise mechanism remains unknown.

ADRENARCHE IN INDIVIDUALS BORN SGA

Adrenarche refers to the stage of growth and development in mid-childhood, when there is increased production of androgens from the zona reticularis of the adrenal cortex. Clinically, this results in increased sweat production, sebum production with associated odor, oily skin and acne, and axillary and pubic hair development. This process is independent of the HPG axis and puberty, usually occurring 2 years prior to puberty (onset about 6 years of age) (50). The zona reticularis is active during fetal and early postnatal life, producing large amounts of the androgens, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenedione. These are converted peripherally to testosterone and estrogens. During the first year of life, the fetal adrenal cortex is replaced by an adult-like adrenal. In conjunction with this, involution of the

zona reticularis causes a marked decline of androgen concentrations over the first few months of life. In contrast to well-developed and functioning zona fasciculata and zona glomerulosa, the adrenal reticularis remains poorly developed until mid-childhood. Androgen concentrations therefore remain low from infancy until mid-childhood, when the zona reticularis is eventually formed, and an unknown trigger leads to increased production of androgens and adrenarche ensues.

The cells of the zona reticularis are responsible for the production of androgens, in addition to the production of cortisol. Androgen and cortisol production have a common early pathway and share the precursors, 17-hydroxypregnenolone and 17-hydroxyprogesterone. Under regulation of adrenocorticotrophic hormone (ACTH), the enzyme cytochrome P450_{scc} and the [steroidogenic acute regulatory protein (StAR)] uptakes and converts cholesterol to pregnenolone and progesterone. Pregnenolone and progesterone undergo 17 α -hydroxylation by the enzyme cytochrome P450c17 to produce 17-hydroxypregnenolone and 17-hydroxyprogesterone. These precursors predominantly undergo further hydroxylation to produce cortisol. To produce DHEA and androstenedione, cleavage of the C17–C20 carbon bond (17,20 lysis) is required, which is also catalyzed by cytochrome P450c17 (*Fig. 1*).

The enzyme cytochrome P450c17 is pivotal to the production of androgens, and increasing data suggest that posttranslational regulation of this enzyme is the key to initiation and control of androgen production and adrenarche (55). Cytochrome P450c17 is a single enzyme with two different actions: 17 α -hydroxylation and 17,20 lysis (C17–C20 carbon bond cleavage). Prior to adrenarche, there is virtually no 17,20-lyase activity, and therefore minimal androgen production. At adrenarche, 17,20-lyase activity increases, leading to androgen production. Three processes are believed to regulate 17,20-lyase activity: electron transfer, cofactor interaction, and phosphorylation (55–57). First, electron transfer is essential for cytochrome P450 function. Cytochrome P450 oxidoreductase (POR) is the flavoprotein responsible

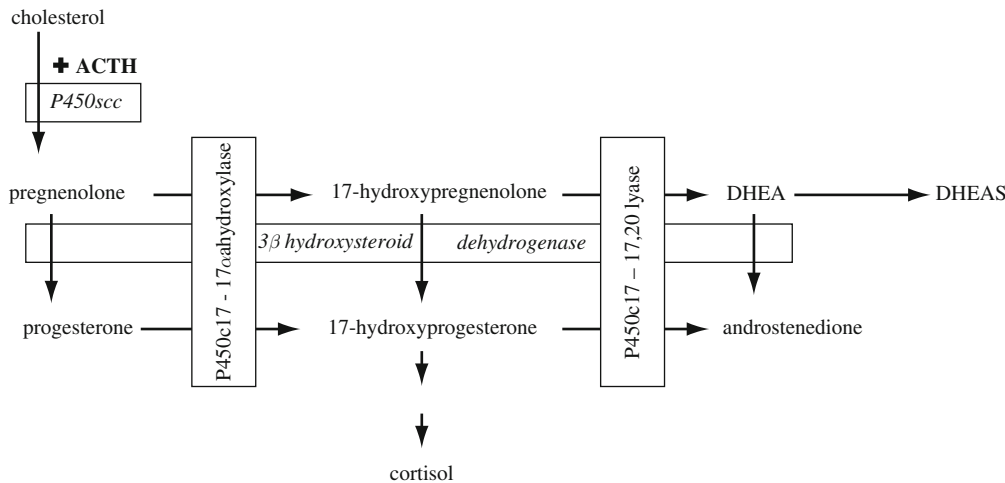


Fig. 1. Schematic representation of adrenal steroid biosynthetic pathways in zona reticularis. In early childhood, the 17,20-lyase activity of cytochrome P450c17 is negligible. Adrenarche is heralded by the activation of this 17,20-lyase activity and increased androgen production.

for electron transfer. POR is expressed in the adrenal, predominantly in the reticularis, with increased expression coinciding with adrenarche. This will lead to increased cytochrome P450 activity (both 17 α -hydroxylase and 17,20 lyase). Second, interaction of the cofactor cytochrome b_5 with cytochrome P450c17 dramatically increases 17,20-lyase activity. The adult adrenal reticularis expresses high levels of cytochrome b_5 (in comparison with low levels in the preadrenarchal gland). Finally, serine/threonine phosphorylation of cytochrome P450c17 enhances electron transfer, leading to markedly increased 17,20-lyase activity (57). In contrast, the dephosphorylation of cytochrome P450c17 reduces 17,20-lyase activity, and the kinase, protein phosphatase 2A, has been identified as causing the dephosphorylation of cytochrome P450c17 (58). Although all three processes are essential for androgen production, it has been proposed that adrenarche is hallmarked by serine phosphorylation of the cytochrome P450c17 enzyme. The mechanism for this increase in serine phosphorylation remains unknown.

Insulin and IGF-I are important hormones for growth and development (both prenatally and postnatally). Both of these hormones have been proposed as possible biological triggers for induction and control of adrenarche. Serum IGF-I concentrations and insulin resistance correlate with DHEA secretion and DHEAS concentrations (50,59–61). The adrenal cortex expresses both insulin and IGF-I receptors. Insulin and IGF-I enhance adrenal androgen production in vitro, by augmenting both adrenal androgen production and expression of adrenal steroidogenic enzymes (50). Further evidence to link serum insulin and IGF-I levels to adrenarche is the observed relationship between high prepubertal body weight (high BMI) and higher androgen levels, earlier adrenarche, and risk of premature adrenarche (50,61–64). BMI is associated with elevated serum insulin and IGF-I levels and therefore may act as nutritional signals in the modulation of adrenal androgen production (50).

Timing of Adrenarche in Individuals Born SGA

There are an increasing number of studies illustrating an association between SGA and premature adrenarche (onset <6 years) or exaggerated adrenarche (which occurs at the appropriate time but progresses with higher androgen production and more abundant axillary and pubic hair growth than is typical) (55).

On retrospective review of 102 age-matched and Tanner stage-matched females with premature adrenarche, Ibanez et al. (65,66) showed a strong association of premature adrenarche with SGA. Consistent with this finding, an Australian group reviewed the birth data and current nutritional status of 79 females with premature adrenarche (63). About 35% of the females had a history of being born SGA or had a low ponderal index at birth (ponderal index = weight/length³ and indicates degree of thinness). In addition, 65.1% of the females with premature adrenarche were overweight or obese (compared with a national overweight/obesity incidence of 20% in the same-aged population). They suggested that prepubertal weight gain potentiated the risk of premature adrenarche in females born SGA.

Ghirri et al. (67) evaluated a small group of girls with a history of SGA for clinical and biochemical markers of early adrenarche/exaggerated adrenarche between the ages of 6 and 7.5 years. They showed that compared with AGA controls, the girls had elevated levels of DHEAS despite no clinical signs of adrenarche. Although a small

cohort, this prospective study suggests that differences in adrenal function in relation to androgen production are present earlier in individuals with a history of SGA.

Following this small study, the large prospective study, Avon longitudinal study of parents and children (ALSPAC), is following approximately 14,500 pregnancies from the early 1990s. A subcohort of children ($n = 851$) were recruited at 8 years of age for anthropometric data and blood screen (having had anthropometric data also obtained at 3 years) (62). DHEAS and androstenedione levels were significantly higher in children born SGA and were also significantly higher in children with highest weight at 8 years. This effect was continuous with androgen levels inversely proportional to birth weight and positively related to current body size. Further analysis suggested that individuals born SGA who showed rapid postnatal weight gain by 3 years of age had higher adrenal androgens. This again highlights a compounding effect of a postnatal environmental on a prenatal risk. Individuals with a history of SGA at birth are more likely to have exaggerated androgen production in childhood if there is rapid weight gain during childhood.

In contrast to the association of SGA and high androgen levels in mid-childhood, newborns with LBW have lower plasma and urine DHEAS levels together with hypoplasia of the fetal zone of the adrenal glands compared with newborns with a normal birth weight (68–70). The reason for this is not known and may indicate a stress response. It does, however, suggest that the adrenal cortex is susceptible to the effects of an adverse intrauterine environment and that such an insult results in changes to the structure and function of the fetal adrenal gland. It is possible therefore that in addition to causing changes in adrenal structure and function, an adverse intrauterine environment leads to alteration in the regulation and timing of adrenal function, resulting in premature adrenarche in children who were born SGA.

One of the concerns for individuals born SGA having premature adrenarche or exaggerated adrenarche is that it may increase the risk of earlier puberty and loss of final height. Regulation of adrenarche and puberty is considered largely independent, and premature adrenarche does not necessarily lead to early puberty. Adrenarche is dependent on a functioning HPA axis, and although not triggered or regulated by ACTH, ACTH is believed to be an important permissive factor for adrenarche to proceed. The growth factors, insulin and IGF-I, are proposed as key hormones in triggering and regulation of adrenarche. In contrast, puberty is dependent on an intact and functional HPG axis. The association of SGA and altered timing and tempo of puberty biologically plausible. IGF-I and insulin are again implicated as important hormones in the triggering and/or regulation of this axis. An abnormality or excess concentration of these hormones could theoretically trigger both adrenarche and puberty. In addition, premature or exaggerated adrenarche promotes bone growth and accelerates bone maturation (seen as an advanced bone age). It is possible that physiological maturation (as assessed by bone age) could then signal the HPG axis of “biological readiness” for puberty. In addition, premature or exaggerated adrenarche will accelerate bone maturation, leading to early bone fusion and further reduction in height potential.

Individuals born SGA have an increased risk of premature adrenarche. Both SGA and premature adrenarche are associated with insulin resistance. Zhang et al. (57), observing the relationship of these three factors, suggested a common mechanism to explain the association. They postulated that individuals born SGA have abnormal

serine phosphorylation possibly due to in utero programming. 17,20-Lyase activity, and therefore androgen production, is subsequently increased. As discussed earlier, the hallmark of adrenarche is increased 17,20-lyase activity, which is likely to be dependent on the phosphorylation of cytochrome P450c17. In contrast, serine phosphorylation of insulin receptors interferes with insulin action, resulting in insulin resistance. Insulin binding to its receptors triggers autophosphorylation of tyrosine residues, leading to the phosphorylation of intracellular substrates such as insulin receptor substrate 1 (IRS-1) and IRS-2 initiating signal transduction and various cellular functions (71). Serine phosphorylation of the insulin receptor still allows binding of insulin but prevents autophosphorylation of tyrosine, and hence post-receptor insulin resistance. Therefore, abnormal serine phosphorylation of cytochrome P450c17 and insulin receptors, by fetal programming, could be the common event that links SGA, premature adrenarche, and insulin resistance.

SGA and GONADAL FUNCTION

SGA is associated with polycystic ovarian syndrome (PCOS) in females. PCOS is a disorder of ovarian function with heterogeneous signs and symptoms, including oligomenorrhea and/or anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovaries on ultrasound. It is also associated with insulin resistance and hyperinsulinemia, and therefore, females with PCOS are at risk of metabolic syndrome. Insulin resistance and hyperinsulinemia are key factors in the pathogenesis, progression, and clinical spectrum of the disease. Insulin resistance and hyperinsulinemia directly and indirectly (through excessive production of LH from the pituitary) promote excessive production of androgens from the ovaries. In addition, hyperinsulinemia lowers sex hormone-binding globulin, increasing the free androgen index. Excessive free androgen results in hirsutism, acne, oocyte dysfunction, and suppression of FSH from pituitary leading to decreased ovulation and oligo/amenorrhea. As discussed in Introduction, individuals with a history of SGA have insulin resistance present early in childhood, giving a plausible link for the association of SGA and PCOS. In addition, individuals with a history of premature adrenarche have a higher risk of developing PCOS (72). This association with premature adrenarche is not unexpected given that both disorders have insulin resistance as a major underlying pathogenic factor. Interestingly, the use of insulin sensitizers in LBW girls presenting with premature adrenarche prevented the later development of PCOS (73) (Fig. 2).

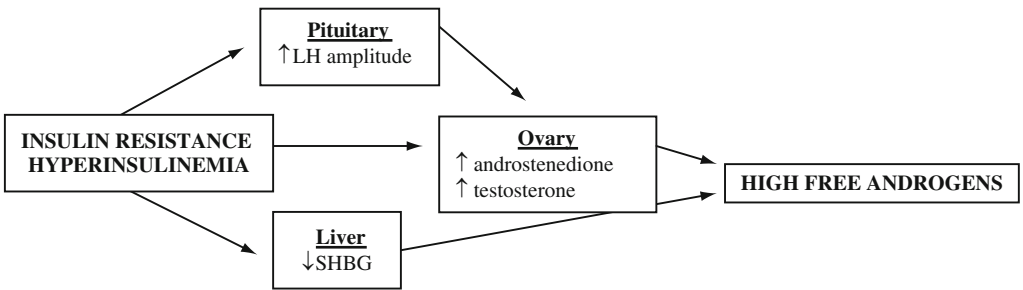


Fig. 2. Diagrammatic representation outlining the consequences of insulin resistance and hyperinsulinemia on increasing ovarian androgen production.

Evidence for ovarian dysfunction is present in LBW females during late childhood and puberty. Ibanez et al. (65) investigated 104 girls with precocious adrenarche for the presence of functional ovarian hyperandrogenism (comprising oligo/amenorrhea, hirsutism, and hyperandrogenism). Functional ovarian hyperandrogenism and PCOS are considered as part of the same spectrum of disease. LBW was associated with one or more of premature adrenarche, functional ovarian hyperandrogenism, and hyperinsulinemia. In fact, with decreasing birth weight, there was an increased risk of having all three pathologies.

Abnormal serum phosphorylation is again implicated as a possible mechanism for functional ovarian hyperandrogenism, PCOS, insulin resistance, and hyperinsulinemia and could explain the association in individuals with SGA. Serine phosphorylation of cytochrome P450c17 and insulin receptors both enhances androgen production from the ovary and adrenal gland and causes insulin resistance that further compounds ovarian hyperandrogenism (Fig. 3).

Functional ovarian hyperandrogenism and PCOS are part of a clinical spectrum ranging from biochemical abnormalities of androgens and ovulation to clinically apparent PCOS with hirsutism, acne, obesity, amenorrhea, and acanthosis nigricans. It is not surprising therefore that both are associated with SGA. Biochemical abnormalities of gonadal function have subsequently been shown to be present in females born SGA, further strengthening the association of SGA and gonadal dysfunction. Ibanez et al. (74) evaluated apparently healthy female adolescents with a history of being born SGA. These non-obese, postmenarchal adolescents, with a history of normal onset of adrenarche and puberty, had no clinical signs of hyperandrogenism and had a history of regular menstruation.

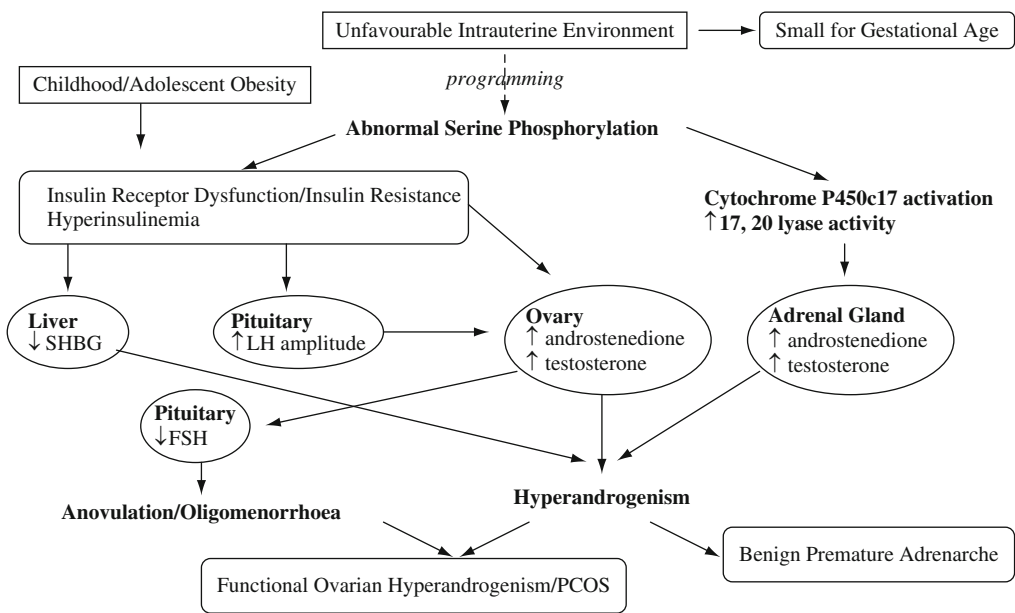


Fig. 3. Cartoon summarizing the proposed mechanisms involving liver, pituitary, adrenal, and ovaries, whereby small for gestational age (SGA) results in premature adrenarche, hyperandrogenism, and polycystic ovary syndrome (PCOS).

Biochemical assessment showed that compared with females born AGA, the SGA adolescents had a 10 times increased incidence of anovulation (assessed over 3 months), hyperandrogenism, and hyperinsulinemia.

Although ovarian hyperandrogenism and PCOS are observed with increased frequency in SGA adolescents, it is likely that the clinical manifestation is worsened by obesity. This association with obesity, particularly increased visceral fat accumulation, probably reflects worsening insulin resistance with greater compensatory hyperinsulinism (75). This is supported by the treatment of adolescents with insulin sensitizers such as metformin that results in the improvement of PCOS symptoms as do weight loss and increased physical activity (76). Obesity and fat mass partitioning, however, may also be altered by the underlying metabolic changes in PCOS. Treatment with combinations of antiandrogens and insulin sensitizers in young women with PCOS caused a reduction in fat mass and an increase in lean mass (77).

There is further evidence of HPG axis dysfunction in individuals born SGA. Functional gonadal abnormalities are suggested by reduction in ovarian and testicular size in individuals with SGA (78), reduced uterine volume in females (78), and hypersecretion of FSH in infancy (suggesting diminished production of inhibin B and estrogen by gonads) (79). de Bruin et al. (80) have shown that there is a lower percentage of primordial follicles in ovaries of IUGR female fetuses. Investigating male infertility, Francois et al. (81) showed that unexplained male subfertility (assessed by semen analysis) was associated with LBW, suggesting abnormality of Sertoli cell structure and/or function. Given the importance of prenatal period for development of the HPG axis and reproductive organs, it is possible that development is also affected by the adverse intrauterine environment of the SGA individual.

CONCLUSIONS

There is growing evidence that the antenatal and early infant environment affect gonadal and adrenal function in puberty. Although this effect may be subtle in terms of advancing pubertal onset, the attenuation in the pubertal growth spurt results in an important and often unanticipated reduction in final height. The dyssynchrony between bone age and pubertal onset in SGA children also should be considered, and predicted final heights using bone age estimation are likely to be overestimated. Adrenal and gonadal hyperandrogenism and PCOS represent important disorders that are more prevalent in SGA women. The associated adult diseases, social problems related to the hyperandrogenism, and issues of fertility make this disorder especially important to identify and treat early. Although programming of puberty and growth in SGA adolescents is likely to involve changes both centrally (hypothalamus and pituitary) and peripherally (gonads and adrenal glands), insulin resistance appears to play an important if not fundamental role in their development. These abnormalities are generally amplified by obesity, and SGA children and adolescents at highest risk of these pubertal alterations are those with the highest BMI.

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12

Congenital Adrenal Hyperplasia

*Lenore S. Levine, MD
and Sharon E. Oberfield, MD, With
editorial assistance of Lauren Antler, BA*

CONTENTS

INTRODUCTION

LIPOID ADRENAL HYPERPLASIA

3 β -HYDROXYSTEROID/ Δ 4,5-ISOMERASE DEFICIENCY

17-HYDROXYLASE/17,20-LYASE DEFICIENCY

21-HYDROXYLASE DEFICIENCY

11 β -HYDROXYLASE DEFICIENCY

COMBINED 21-HYDROXYLASE DEFICIENCY

AND 17 α -HYDROXYLASE/17,20-LYASE DEFICIENCY

(P450-OXIDOREDUCTASE DEFICIENCY)

CONCLUSIONS

REFERENCES

Summary

Congenital adrenal hyperplasia, a family of autosomal recessive diseases of cortisol synthesis, is commonly associated with disordered puberty. Perturbations of pubertal onset and progress are determined by which hormones are overproduced and which are deficient. There may be precocious pubarche or puberty, sexual infantilism and failure to initiate or complete pubertal development, menstrual irregularity, hirsutism and infertility. In addition to glucocorticoid treatment to replace the deficient cortisol and mineralocorticoid if salt-wasting is present, treatment may include sex steroids, LHRH agonists, aromatase inhibitors and genital surgery.

Key Words: 21-Hydroxylase deficiency; Lipoid adrenal hyperplasia; Ambiguous genitalia; Androgen excess.

INTRODUCTION

Congenital adrenal hyperplasia (CAH), a family of autosomal recessive diseases of cortisol synthesis, is commonly associated with disordered puberty (1–4). Perturbations of pubertal onset and progress are determined by which hormones are overproduced and which are deficient. A deficiency in each of the enzymatic activities required

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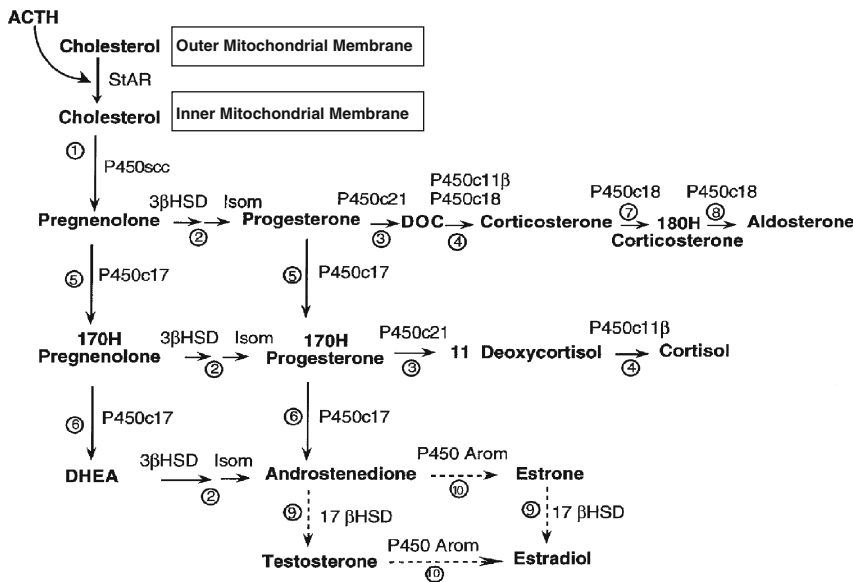


Fig. 1. Simplified scheme of adrenal steroidogenesis. Two reactions (dotted arrows) occur primarily in gonads, not in the adrenal gland. Chemical names for enzymes are shown above or to right of arrows; circled numbers refer to traditional names: (1) 20,22-desmolase; (2) 3β-hydroxysteroid dehydrogenase/isomerase; (3) 21-hydroxylase; (4) 11β-hydroxylase; (5) 17α-hydroxylase; (6) 17,20-lyase; (7) 18-hydroxylase; (8) 18-oxidase; (9) 17β-hydroxysteroid dehydrogenase; (10) aromatase; StAR = steroidogenic acute regulatory protein; DOC = 11-deoxycorticosterone.

for cortisol synthesis has been described (*Fig. 1*). Combined defects, as well, have been recently reported (5). As a result of the disordered enzymatic step, there is decreased cortisol synthesis, increased adrenocorticotrophic hormone (ACTH) through the negative feedback system, overproduction of the hormones prior to the enzymatic step or not requiring the deficient enzyme, and deficiency of the hormones distal to the disordered enzymatic step. Thus, CAH may present with virilization of the affected female infant and subsequent signs of androgen excess in both males and females, throughout childhood, peripuberty, and adulthood. Incomplete virilization of the male and signs of sex hormone deficiency in both males and females also occur. Salt-wasting crisis secondary to aldosterone deficiency, or hormonal hypertension secondary to increased deoxycorticosterone (DOC), a mineralocorticoid, may also occur but will not be discussed in detail in this chapter (1–3,5–11). The affected enzymatic activities of adrenal steroidogenesis, and their genes, chromosomes, and cellular location are presented in *Table 1*. This chapter will present an overview of the pubertal disorders associated with the enzymatic deficiencies resulting in CAH.

LIPOID ADRENAL HYPERPLASIA

Lipoid adrenal hyperplasia, a rare form of CAH, is due to a deficiency of cholesterol desmolase activity. As a result, there is a deficiency in all of the adrenal hormones: glucocorticoids (cortisol), mineralocorticoids (aldosterone), and the sex steroids (*Fig. 1 and Table 2*) (8). In lipoid adrenal hyperplasia, the gene coding for P450_{scc}, a

Table 1
Enzymes and Genes in Congenital Adrenal Hyperplasia

<i>Enzymatic Activity</i>	<i>Enzyme</i>	<i>Cellular Location</i>	<i>Gene</i>	<i>Chromosomal Location</i>
Cholesterol Desmolase (side chain cleavage)	P450 _{scc} (CYP11A1)	Mitochondrion	CYP11A1	15q23–q24
3 β -Hydroxysteroid Dehydrogenase	3 β -HSD (3 β -HSDII)	Endoplasmic Reticulum	HSD3B2	1p13.1
17 α -Hydroxylase/17,20-lyase	P450 _{c17} (CYP17)	Endoplasmic Reticulum	CYP17	10q24.3
21 α -Hydroxylase	P450 _{c21} (CYP21A2)	Endoplasmic Reticulum	CYP21A2	6p21.3
11 β -Hydroxylase	P450 _{c11} (CYP11B1)	Mitochondrion	CYP11B1	8q21–22
Combined 21 α -Hydroxylase and 17 α -Hydroxylase/17,20 lyase	P450-oxidoreductase	Microsome	POR (P450 oxidoreductase)	7q11.2

Adapted from: Levine L.S. Congenital Adrenal Hyperplasia. *Pediatr Rev* 2000;21:159–170.

Table 2
Lipoid CAH

<i>Enzymatic deficiency</i>	<i>Signs and symptoms</i>	<i>Laboratory findings</i>	<i>Therapy specific to puberty (in addition to glucocorticoid or mineralocorticoid therapy)</i>
Lipoid CAH (cholesterol desmolase deficiency)	Salt-wasting crisis Male pseudo-hermaphroditism Incomplete female puberty	Low levels of all steroid hormones, with decreased/absent response to ACTH Decreased/absent response to HCG in males ↑ ACTH ↑ PRA	Gonadectomy of male pseudohermaphrodites Sex hormone replacement consonant with sex of rearing

Adapted from: Levine LS. Congenital Adrenal Hyperplasia. In Manual of Endocrinology and Metabolism, 3rd Edition. Ed. Lavin, N. Lippincott, Williams & Wilkins 2002.

mitochondrial enzyme, has been found to be normal in the majority of cases, albeit rare mutations of the P_{450} scc gene have also been found (8,12,13). Most often, lipoid adrenal hyperplasia is due to a mutation in the gene for steroidogenic acute regulatory protein (StAR) (8,14–16). The StAR gene is located on chromosome 8p11.2 and is expressed in adrenals and gonads (Table 1) (8,14,17). StAR is a mitochondrial protein that promotes the movement of cholesterol from the outer to the inner mitochondrial membrane. Because cholesterol desmolase enzymatic activity is necessary for sex hormone synthesis in the gonad, there is also deficiency of gonadal steroids in this disorder (8,14,17). Affected infants usually present early in life with salt-wasting crisis manifested by cardiovascular collapse, hyponatremia, and hyperkalemia. Males have phenotypically female external genitalia. Females exhibit no genital abnormalities. Increased pigmentation, secondary to increased ACTH, may be of such a degree as to produce “bronzing” in the newborn. Occasionally, infants have been reported to present with salt-wasting crisis beyond the newborn period (1,8,18). Because of the gonadal sex steroid deficiency, males are unable to produce gonadal steroids at the time of puberty. Affected females may have sufficient gonadal function remaining at puberty to begin feminization and progress to menarche, but gonadal failure ultimately ensues (1,8,19). Furthermore, development of bilateral ovarian cysts appears to be a common phenomenon and is speculated to occur because of persistent anovulatory cycles with resultant increase in LH stimulation (20). Laboratory evaluation in patients with lipoid adrenal hyperplasia reveals low levels of all steroid hormones, with no response to ACTH or human chorionic gonadotropin (hCG) administration. ACTH and plasma renin activity (PRA) are very elevated. Imaging studies of the adrenal gland will reveal marked enlargement of the adrenals secondary to the accumulation of lipid droplets. Females with this disorder have normal internal and external genitalia. Males, as noted above, are phenotypically female (1,2,8). Males incorrectly diagnosed as females with adrenal insufficiency have been noted subsequently to have inguinal gonads, which has led to the correct diagnosis (21) (Table 2).

3 β -HYDROXYSTEROID/ Δ 4,5-ISOMERASE DEFICIENCY

3 β -Hydroxysteroid (3 β -HSD)/ Δ 4,5-isomerase deficiency is also a rare form of CAH, occurring in fewer than 5% of patients (1,2). The 3 β -HSD enzyme, located in the endoplasmic reticulum, mediates both 3 β -HSD and isomerase activities. CAH caused by 3 β -HSD/ Δ 4,5-isomerase deficiency results from a mutation in the HSD3 β 2 gene, located on chromosome 1 (Table 1) (9,22–24). 3 β -HSD/ Δ 4,5-isomerase is necessary for the conversion of pregnenolone to progesterone, 17-hydroxypregnenolone to 17-hydroxyprogesterone (17-OHP), and dehydroepiandrosterone (DHEA) to Δ 4-androstenedione (1,23). Decreased ability to convert these Δ 5 steroids to Δ 4 steroids results in decreased synthesis of cortisol, aldosterone, and androstenedione (Fig. 1). In the testes, it results in decreased ability to form testosterone. The deficiency of cortisol results in increased ACTH with overproduction of Δ 5 steroids, including DHEA (Table 3). The increased level of DHEA is sufficient to result in some virilization of the external genitalia in affected females, although the virilization is not as marked as in the other forms of virilizing CAH, 21-hydroxylase deficiency and 11 β -hydroxylase deficiency (1,9). Newborn female infants with 3 β -HSD/ Δ 4,5-isomerase deficiency may have clitoromegaly and partial fusion of the labial folds. Males with this disorder

Table 3
3 β -HSD Deficiency

<i>Enzymatic Deficiency</i>	<i>Signs and Symptoms</i>	<i>Laboratory Findings</i>	<i>Therapy specific to puberty (in addition to glucocorticoid or mineralocorticoid therapy)</i>
3 β -HSD Deficiency Classic form	-Salt-wasting crisis -Male and female pseudohermaphroditism -Precocious pubarche -Disordered puberty	- \uparrow Baseline and ACTH-stimulated Δ 5-steroids (pregnenolone, 17-OH pregnenolone, DHEA and their urinary metabolites - \uparrow Δ 5/ Δ 4 serum and urinary steroids - \uparrow ACTH - \uparrow PRA -Suppression of elevated adrenal steroids after glucocorticoid administration)	-Surgical correction of genitalia and sex hormone replacement as necessary consonant with sex of rearing
3 β -HSD Deficiency Nonclassic form	-Precocious pubarche, disordered puberty, menstrual irregularity, hirsutism, acne, infertility	- \uparrow Baseline and ACTH-stimulated Δ 5-steroids (pregnenolone, 17-OH pregnenolone, DHEA and their urinary metabolites) - \uparrow Δ 5/ Δ 4 serum and urinary steroids -Suppression of elevated adrenal steroids after glucocorticoid administration	

Adapted from: Levine LS. Congenital Adrenal Hyperplasia. In Manual of Endocrinology and Metabolism, 3rd Edition. Ed. Lavin, N. Lippincott, Williams & Wilkins 2002.

manifest a deficiency of prenatal testosterone and are born with varying degrees of ambiguity of the external genitalia ranging from hypospadias to more significant degrees of incomplete virilization with partial fusion of the scrotal folds. Most infants with 3β -HSD/ Δ 4,5-isomerase deficiency have aldosterone deficiency and present in the newborn period with salt-wasting crisis. Postnatally, there is continued excessive DHEA secretion with growth acceleration and the early onset of pubic/axillary hair (1,5,9,25).

At puberty, symptoms of ongoing excessive adrenal androgens include hirsutism, clitoromegaly, acne, menstrual irregularity or amenorrhea, and infertility. Increased pigmentation of skin creases occurs secondary to increased ACTH. There have been reports of males with this disorder undergoing normal male puberty. However, this occurs with marked elevation of the Δ 5 steroids sufficient to produce adequate levels of testosterone (1,6,9). Laboratory evaluation reveals elevation of the Δ 5 steroids, specifically the diagnostic hormone 17-hydroxypregnenolone and DHEA, with a further rise following ACTH stimulation, to levels of 10,000–60,000 ng/dl and 3000–12,000 ng/dl, respectively. ACTH levels are increased, and in those with aldosterone deficiency, PRA is markedly elevated as well. An attenuated form of this disorder (non-classic) with milder signs of androgen excess has been reported, suggestive of a less severe enzyme deficiency. However, genetic studies in these patients have been normal (26,27). Recent studies have linked increased production of Δ 5 steroids with variable degrees of insulin resistance. These findings may become apparent peripubertally (27). Glucocorticoid administration to individuals with 3β -HSD deficiency results in a decrease in ACTH followed by a decrease in the overproduced adrenal androgens (Table 3) (1,6,9).

17-HYDROXYLASE/17,20-LYASE DEFICIENCY

17-Hydroxylase/17,20-lyase deficiency is another relatively rare form of CAH, described in approximately 150 patients (1,2,10,28). P540c17, found in the endoplasmic reticulum, is responsible for catalyzing steroid 17-hydroxylation and 17,20-lyase reactions (Fig. 1). It is coded for by a gene located on chromosome 10 expressed both in the adrenal cortex and in the gonads (Table 1) (10). Approximately 20 different genetic lesions have been documented in these patients (1,2,10,29–31). The molecular basis for isolated 17,20-lyase deficiency, a rare occurrence, has been elucidated (32). In 17-hydroxylase/17,20-lyase deficiency, there is deficient 17-hydroxylation, by which pregnenolone and progesterone are converted to 17-hydroxypregnenolone and 17-OHP, respectively, and deficiency in the 17,20-lyase reaction that is required for conversion of 17-hydroxypregnenolone and 17-OHP to DHEA and Δ 4-androstenedione, respectively (Fig. 1) (1,2,10,31). Similar to other forms of CAH, the deficiency in cortisol results in increased ACTH. Overproduction of DOC, a mineralocorticoid, ensues, producing hypertension and hypokalemia, which may be the presenting symptoms. Because this enzymatic deficiency is present also in the gonad, there is a deficiency of gonadal sex steroids as well, so that affected males are incompletely virilized and are phenotypically female or ambiguous (1,2,10,31).

Males with this disorder are unable to undergo normal male puberty because of testosterone deficiency. Affected females have normal female external genitalia and may present with the failure of sexual development at adolescence. Sex steroid replacement needs to be offered to both males and females, consistent with sex of

Table 4
17 α -OH/17,20 lyase deficiency

<i>Enzymatic Deficiency</i>	<i>Signs and Symptoms</i>	<i>Laboratory Findings</i>	<i>Therapy specific to puberty (in addition to glucocorticoid therapy)</i>
17 α -OH/17,20 lyase deficiency	-Male pseudohermaphroditism -Sexual infantilism -Hypertension	- \uparrow DOC, 18-OH DOC, corticosterone, 18-hydroxycorticosterone - Low 17 α -hydroxylated steroids and poor response to ACTH -Poor response to HCG in male pseudohermaphroditism - \downarrow PRA - \uparrow ACTH -Hypokalemia -Suppression of elevated adrenal steroids after glucocorticoid administration	-Surgical correction of genitalia and sex hormone replacement in male pseudohermaphroditism consonant with sex of rearing -Sex hormone replacement in female

Adapted from: Levine LS. Congenital Adrenal Hyperplasia. In Manual of Endocrinology and Metabolism, 3rd Edition. Ed. Lavin, N. Lippincott, Williams & Wilkins 2002.

rearing. 17-Hydroxylase/17,20-lyase deficiency is diagnosed by the presence of low levels of all 17-hydroxylated steroids, with a poor response to ACTH and HCG administration. Levels of DOC (10–40×), 18-OH DOC (30–60×), corticosterone (B) (30–100×), and 18-OHB (10×) are markedly elevated, and PRA and aldosterone are suppressed. Glucocorticoid administration results in the suppression of the overproduced hormones. As DOC is suppressed, there is a resolution of the volume expansion and PRA increases, thus stimulating aldosterone secretion (*Table 4*) (1,2,10,31).

21-HYDROXYLASE DEFICIENCY

21-Hydroxylase deficiency is the most common form of CAH, affecting approximately 90% of individuals with CAH (1–3,6). It occurs with a worldwide frequency of approximately 1:15,000 newborns, with increased frequency among certain ethnic groups (Yupik Eskimos, La Reunion, France).

Molecular genetic analysis has demonstrated that there are two highly homologous human P450c21 genes: one active (CYP21B, CYP21A2) and one inactive (CYP21P, CYP21A). The two genes are located in tandem with two highly homologous genes for the fourth component of complement (C4A and C4B). A number of other genes of known and unknown function are also located in this cluster (1–3,6,7,33,34).

The genetic mutations in patients with 21-hydroxylase deficiency have been extensively studied. Most patients are compound heterozygotes, having a different mutation on each allele. Approximately 75% of mutations are recombinations between the inactive CYP21A gene and the active CYP21A2 gene, resulting in microconversions. Large gene conversions and gene deletions also occur (1–3,6,7,33–36).

The classic form of the disorder results from the combination of two severe deficiency genes, whereas the non-classic form of the disease results from a combination of a severe CYP21A2 deficiency gene (found in the classic form of the disease) and a mild CYP21A2 deficiency gene or a combination of two mild deficiency genes. Point mutations, gene conversions, and gene duplications have been found in the mild CYP21A2 deficiency genes. A valine-to-leucine substitution in codon 281 is a frequently found point mutation and is highly associated with HLA-B14DR1 (1–3,6,7,33–36).

In this disorder, there is decreased ability to 21-hydroxylate progesterone and 17-OHP to DOC and 11-deoxycortisol (S), respectively (*Fig. 1*). As a result, there is decreased cortisol secretion, increased ACTH, adrenal hyperplasia, and overproduction of the steroids prior to 21-hydroxylation. 17-OHP is the most elevated and is the diagnostic hormone in this disorder. There is overproduction of the adrenal androgens, especially $\Delta 4$ -androstenedione, and by peripheral conversion, testosterone, resulting in virilization, the hallmark of this disorder (1–3,6,7,33,34,36).

In addition, approximately two-thirds of these patients will have aldosterone deficiency presenting with salt-wasting crisis in the newborn period. Because this disorder begins in utero, the female fetus is exposed to excessive adrenal androgens, resulting in virilization of the external genitalia ranging from clitoromegaly, with or without mild degrees of labial fusion, to marked virilization of the external genitalia such that the female infant appears to be a male infant with hypospadias (occasionally with the appearance of a penile urethra) and undescended testes. With severe virilization, there is a urogenital sinus with one outflow track to the perineum. As with

all forms of CAH, a female infant will have normal ovaries, fallopian tubes, uterus, and proximal vagina. The virilization of the external genitalia, however, may require reduction clitoroplasty and vaginoplasty (1–3,6,7,33,34,36).

Postnatally, there is continued virilization with progressive clitoromegaly and penile enlargement and rapid growth. Although rapid growth and tall stature are present early in childhood, bone age advancement is greater than height advancement, resulting in short final height in late or poorly treated patients. Premature development of pubic or axillary hair may occur. Additionally, signs of androgen excess secondary to late or inadequate treatment include acne, delayed menarche or primary amenorrhea, menstrual irregularity, hirsutism, and infertility. True precocious puberty may occur with bone age advancement to 10 years or older, contributing to short final height. Increased ACTH secretion results in increased pigmentation of skin creases, nipples, and genitalia. Unilateral testicular enlargement may occur secondary to stimulation of adrenal rest tissue and formation of adrenal rest tumors (1–3,6,7,33,34,36).

Treatment is aimed to decrease ACTH and overproduction of androgen precursors and androgens. With optimal control, normal progress of puberty and fertility can be achieved. However, peripheral blockade of androgens or use of GnRH agonist may be required in childhood to prevent continued bone age advancement and to further suppress elevated sex steroid hormone levels (1–3,6,33,34,36–41) (Table 5). A milder non-classic form of 21-hydroxylase deficiency is well recognized. It occurs most commonly in Ashkenazi Jews, with an estimated frequency of 0.1%. There is no sign of salt wasting in the non-classic disorder, and female genitalia are normal at birth. Signs of androgen excess may appear in childhood, including premature pubarche, acne, and hirsutism. Menstrual irregularity and infertility may be presenting symptoms. Males with this disorder may also present with unilateral testicular enlargement similar to males with the classic disorder (1–3,6,33,34,36). Similar to other conditions associated with hyperandrogenic states, relative insulin resistance has been reported in this population (42). However, many patients with the non-classic form of 21-hydroxylase deficiency, both males and females, may go undiagnosed because of the lack of symptoms (41). Those requiring treatment usually respond to lower doses of glucocorticoid therapy than is needed in the classic form.

11 β -HYDROXYLASE DEFICIENCY

CAH caused by 11 β -hydroxylase deficiency accounts for 5% of reported cases of CAH. It occurs in approximately 1:100,000 births in a diverse Caucasian population but is more common in Jews of North African origin (1,2,11). P450c11 β , a mitochondrial enzyme, is coded for by CYP11B1. It mediates 11 β -hydroxylation in the zona fasciculata leading to cortisol synthesis. P450c18, also located in the mitochondria and coded for by CYP11B2, mediates 11 β -hydroxylase, 18-hydroxylase, and 18-oxidase activities in the zona glomerulosa leading to aldosterone synthesis. These genes lie on chromosome 8q21–22 (Table 1). CAH caused by 11 β -hydroxylase deficiency results from a mutation in the CYP11B1 gene. A number of mutations in this gene have been reported in patients with 11 β -hydroxylase deficiency. Almost all Moroccan Jewish patients with this disorder have a point mutation in codon 448 in CYP11B1, resulting in an arginine-to-histidine substitution (1,2,11,43,44).

Table 5
21-OH deficiency

<i>Enzymatic Deficiency</i>	<i>Signs and Symptoms</i>	<i>Laboratory Findings</i>	<i>Therapy specific to puberty (in addition to glucocorticoid or mineralocorticoid therapy)</i>
21-OH deficiency Classic form	-Salt-wasting crisis -Female pseudohermaphroditism -Postnatal virilization	-↑↑ Baseline and ACTH-stimulated 17-OH progesterone and pregnanetriol -↑↑ serum androgens and urinary metabolites -↑ ACTH -↑ PRA -Suppression of elevated adrenal steroids after glucocorticoid administration	-Vaginoplasty and clitoral recession in female pseudohermaphroditism -LHRH agonist treatment and ? aromatase inhibitors with precocious puberty
21-OH deficiency Nonclassic form	Precocious pubarche, disordered puberty, menstrual irregularity, hirsutism, acne, infertility	-↑ Baseline and ACTH-stimulated 17-OH progesterone and pregnanetriol -↑ Serum androgens and urinary metabolites -Suppression of elevated steroids after glucocorticoid administration	

Adapted from: Levine LS. Congenital Adrenal Hyperplasia. In Manual of Endocrinology and Metabolism, 3rd Edition. Ed. Lavin, N. Lippincott, Williams & Wilkins 2002.

Table 6
11 β -Hydroxylase Deficiency

<i>Enzymatic Deficiency</i>	<i>Signs and Symptoms</i>	<i>Laboratory Findings</i>	<i>Therapy specific to puberty (in addition to glucocorticoid therapy)</i>
-11 β -Hydroxylase Deficiency	-Female pseudohermaphroditism -Postnatal virilization in males and females -Hypertension	- $\uparrow\uparrow$ Baseline and ACTH stimulated compound S and DOC and their urinary metabolites - \uparrow ACTH - \downarrow PRA -Hypokalemia -Suppression of elevated steroids after glucocorticoid administration	- Vaginoplasty and clitoral recession in female pseudohermaphroditism

Adapted from: Levine LS. Congenital Adrenal Hyperplasia. In Manual of Endocrinology and Metabolism, 3rd Edition. Ed. Lavin, N. Lippincott, Williams & Wilkins 2002.

In this disorder, the enzymatic deficiency results in a block in 11-hydroxylation of 11-deoxycortisol (compound S) to cortisol and 11-DOC to corticosterone (B). Decreased cortisol results in increased ACTH, adrenal hyperplasia, and overproduction of 11-deoxycortisol and DOC. As in 21-hydroxylase deficiency, there is shunting into the androgen pathway with overproduction of adrenal androgens, especially androstenedione, and by peripheral conversion, testosterone (*Fig. 1*). This results in virilization, similar to 21-hydroxylase deficiency, with prenatal virilization of the female fetus, and postnatal virilization of affected males and females. The excessive DOC secretion results in sodium and water retention and plasma volume expansion. Hypertension and hypokalemia may ensue (*1,2,11,45*).

The diagnosis of 11 β -hydroxylase deficiency is based on marked elevation of serum 11-deoxycortisol (1400–4300 ng/dl) and DOC (183–2050 ng/dl). Increased excretion of their metabolites tetrahydro-11-deoxycortisol (THS) and tetrahydro-11-deoxycorticosterone (TH-DOC) in a 24-h urine can confirm the diagnosis. Serum androstenedione and testosterone and urinary ketosteroids are also elevated. PRA and aldosterone are suppressed secondary to the volume expansion mediated by the excessive DOC, and hypokalemia may also be present. Glucocorticoid therapy results in the suppression of the excessive 11-deoxycortisol, DOC, and androgens. As DOC is suppressed, there is remission of the volume expansion, PRA and aldosterone rise and hypokalemia reverses (*Table 6*) (*1,2,11,45*). A milder form of 11 β -hydroxylase deficiency has also been reported, presenting later in childhood, adolescence, or adulthood with signs of androgen excess: premature pubarche, acne, hirsutism, menstrual irregularity, and infertility. As in 21-hydroxylase deficiency, glucocorticoid therapy provides suppression of the excessive adrenal androgens. Reversal of signs of postnatal virilization ensues. Furthermore, decline in excess DOC results in normalization or decline in blood pressure. Pubertal progress and fertility should be normal with optimal therapy. However, with late or suboptimal treatment, there have been reports of premature pubarche, hirsutism, irregular cycles, and amenorrhea. Limited reports are available with respect to fertility. Treatment for these issues is as described above, in the section “21-Hydroxylase Deficiency” (*1,2,11,45*).

COMBINED 21-HYDROXYLASE DEFICIENCY AND 17 α -HYDROXYLASE/17,20-LYASE DEFICIENCY (P450-OXIDOREDUCTASE DEFICIENCY)

Recently, a form of CAH that appears biochemically to be a combined form of 21-hydroxylase deficiency and 17-hydroxylase deficiency has been demonstrated to be due to mutations in the gene for the P450-oxidoreductase, located on chromosome 7. Some of the patients also have skeletal abnormalities associated with Antley–Bixler syndrome, for example, craniosynostosis, radio/humeral synostosis, and proptosis. To date, the mutations of the P450-oxidoreductase include missense and frameshift mutations, resulting in disturbances of the complex electron transfer cascade that is necessary for steroidogenesis to occur (*5,46–48*). In this condition, one may have increased ACTH, 17-OHP, progesterone, and DOC and decreased cortisol and androgens postnatally. Prenatally in this disorder, virilization can occur in the female fetus because of a postulated alternative androgen pathway. This proposed pathway

Table 7
 Combined deficiency of 21 α -hydroxylase and 17 α -hydroxylase/17,20 lyase (P450 oxidoreductase deficiency)

<i>Enzymatic Deficiency</i>	<i>Signs and Symptoms</i>	<i>Laboratory Findings</i>	<i>Therapy specific to puberty (in addition to glucocorticoid or mineralocorticoid therapy)</i>
Combined deficiency of 21 α -hydroxylase and 17 α -hydroxylase/ 17,20 lyase (P450 oxidoreductase deficiency)	Females: -Prenatal virilization of external genitalia - Primary amenorrhea Males: -Feminized or under-masculinized external genitalia Maternal: -Gestational virilization	-Variable combinations of 21 and 17 α -hydroxylase/ 17,20 lyase deficiency (e.g.- $\uparrow\uparrow$ ACTH, $\uparrow\uparrow$ 17OHP, $\uparrow\uparrow$ progesterone, normal or $\downarrow\downarrow$ androgens or \downarrow or normal cortisol.)	-Sex hormone replacement as needed

involves the activity of 5α -reductase-1 and 3β -HSD dehydrogenase. Furthermore, this mechanism may explain the reported virilization of the mother during pregnancy with an affected fetus (Table 7).

In this disorder, the most critical issue remains that of cortisol deficiency, which if unrecognized may result in adrenal crisis and death. Pubertal events in this population have not been well described, but at least one female patient has reached adulthood and was reported to have no breast development, primary amenorrhea, a hypoplastic uterus, and bilaterally enlarged cystic ovaries (48). More information about this condition should be available in the future.

CONCLUSIONS

In all forms of clinical adrenal hyperplasia (excluding some mild non-classic forms), use of glucocorticoid therapy is necessary to prevent adrenal insufficiency and to suppress increased ACTH and overproduced steroid hormones. Use of mineralocorticoids and salt is limited to the salt-wasting disorders. In those conditions with sex steroid deficiency (lipoid adrenal hyperplasia, 17-hydroxylase/17,20-lyase, or 3β -HSD/ $\Delta 4,5$ -isomerase), sex hormone replacement therapy to induce or maintain normal secondary sexual characteristics is often required. Therapy most often is begun at an age appropriate for puberty and the achievement of a satisfactory final height. Estrogen therapy to induce breast development is often begun with Premarin or conjugated estrogen patches. A progestational agent is added to induce menses in the genetic female, and therapy is often subsequently changed to an oral contraceptive agent. Testosterone enanthate is used to induce male pubertal changes. Testosterone patches may also be used. Menstrual irregularity or amenorrhea may occur in females with the virilizing disorders. Oral contraceptives may also be used in those patients. Luteinizing hormone-releasing hormone (LHRH) agonist therapy or the use of both aromatase inhibitors and androgen receptor blockade and even growth hormone may be needed to optimize outcomes (49–54). Females with severe virilizing 21-hydroxylase or 11-hydroxylase deficiency may require revision vaginoplasty (1,2,34,37,49).

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13

Polycystic Ovary Syndrome in the Peripubertal Period

Selma Feldman Witchel, MD

CONTENTS

INTRODUCTION
STEROIDOGENESIS
INSULIN AND STEROIDOGENESIS
PUBERTY
DEFINITION OF PP
DIFFERENTIAL DIAGNOSIS OF PP
RELATIONSHIP OF PP TO PCOS
OVARIAN MORPHOLOGY
INSULIN RESISTANCE
NEUROENDOCRINE FUNCTION
GENETICS OF PP AND PCOS
LOW BIRTH WEIGHT
FETAL PROGRAMMING OF OVARIAN DYSFUNCTION
CONCLUSIONS
REFERENCES

Summary

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by hyperandrogenism and chronic anovulation. The hyperandrogenism is secondary to excessive ovarian and/or adrenal androgen secretion. PCOS is a familial disorder with the phenotype reflecting both genetic factors and environmental influences. Signs and symptoms of PCOS often emerge during the peripubertal years. Indeed, for some girls, early development of pubic hair, known as premature pubarche (PP), is the earliest manifestation of PCOS. Understanding the path by which PP evolves to PCOS will help to identify risk factors and novel therapeutic options.

Key Words: Premature Pubarche; Premature Adrenarche; Polycystic Ovary Syndrome; Hyperandrogenism.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by hyperandrogenism and chronic anovulation. The hyperandrogenism is secondary to excessive ovarian and/or adrenal androgen secretion. This common disorder affects approximately 5% of reproductive-aged women (1). Clinical features include hirsutism, acne, oligo/amenorrhea, infertility, and male-pattern baldness. PCOS is a familial disorder with the phenotype reflecting both genetic factors and environmental influences. Signs and symptoms of PCOS often emerge during the peripubertal years. Indeed, for some girls, early development of pubic hair, known as premature pubarche (PP), is the earliest manifestation of PCOS (2). Understanding the path by which PP evolves to PCOS will identify factors that predict risk and ascertain novel therapeutic options. Lacking a conclusive diagnostic test, the diagnosis of PP involves exclusion of other disorders associated with hyperandrogenism.

Whereas many consider PCOS to be a specific diagnostic entity, physicians and scientists struggle to reach a consensus definition (3,4). In 1990, a consensus conference sponsored by National Institutes of Health (NIH) concluded that the diagnosis of PCOS could be defined as hyperandrogenism and chronic anovulation after exclusion of other disorders associated with androgen excess (5). More recently, the American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) revised the diagnostic criteria for PCOS to include two of three findings: (i) oligo/anovulation; (ii) clinical and/or biochemical signs of hyperandrogenism; and (iii) polycystic ovaries (PCO) along with exclusion of other disorders (6). Because steroidogenesis plays a prominent role in both PP and PCOS, the steroidogenic pathway will be briefly reviewed.

STEROIDOGENESIS

The adrenal cortex, ovary, and testis synthesize and secrete a variety of steroid molecules using similar enzymes. Tissue-specific expression of distinct steroidogenic enzymes endows each steroidogenic tissue with its unique secretory profile. The adrenal cortex is composed of three compartments. The outer compartment is the zona glomerulosa, which is responsible for aldosterone synthesis. The middle compartment is the zona fasciculata, where cortisol is synthesized. The inner compartment is the zona reticularis, where the adrenal androgens are synthesized. The ovary consists of two steroidogenic compartments: theca and granulosa cells. In the testis, Leydig cells synthesize and secrete testosterone.

Adrenal steroidogenesis is primarily driven by adrenocorticotrophic hormone (ACTH) secretion acting through the ACTH receptor. The ACTH receptor is a seven-transmembrane domain G-protein-coupled receptor encoded by the melanocortin 2 receptor (*MC2R*) gene (7). Through a negative feedback system, ACTH regulates cortisol secretion. The adrenal androgens include dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenedione. These adrenal androgen hormones do not bind to the androgen receptor but readily undergo peripheral conversion to more potent androgens, for example, testosterone. Although influenced by ACTH, the regulatory mechanism(s) for adrenal androgen secretion is poorly described. Aldosterone synthesis and secretion are primarily governed by serum potassium concentrations and the renin-angiotensin network.

In the ovary, luteinizing hormone (LH) stimulates theca cells to produce androstenedione, which diffuses to granulosa cells where follicle-stimulating hormone (FSH) induces expression of aromatase. Aromatase, encoded by *CYP19A1*, is the enzyme that converts androgens to estrogens. In the testis, LH stimulates Leydig cells to synthesize and secrete testosterone.

Starting from cholesterol as the basic building block for endogenous steroid hormones, tissue-specific enzymatic activity results in the synthesis of mineralocorticoids, glucocorticoids, and sex steroids. Cholesterol can be acquired from circulating lipoproteins or synthesized de novo. From the cytosol, steroidogenic acute regulatory protein (StAR) promotes transport of cholesterol across the outer mitochondrial membrane. During this rate-limiting step in steroidogenesis, the 37-kDa StAR precursor protein is phosphorylated and converted to a 30-kDa mature form after uptake and processing by mitochondria (8–10).

The majority of steroidogenic enzymes are cytochrome P450 enzymes. In the mitochondria, the cholesterol side-chain cleavage (P450_{scc}) enzyme encoded by *CYP11A1* converts cholesterol to pregnenolone. The enzyme 17 α -hydroxylase/17,20-lyase (P₄₅₀_{C17}) encoded by *CYP17* serves as the “gatekeeper” of steroidogenesis. Through its actions, pregnenolone is sequentially converted to 17 α -hydroxypregnenolone (17-Preg) and dehydroepiandrosterone DHEA. In the absence of P₄₅₀_{C17} activity, the zona glomerulosa synthesizes mineralocorticoids. In the zona fasciculata, the presence of the 17 α -hydroxylase activity results in the synthesis of glucocorticoids. In the zona reticularis and gonads, the presence of both 17 α -hydroxylase and 17,20-lyase activities enables sex steroid biosynthesis. The 17,20-lyase activity is influenced by the presence of cytochrome b₅ and post-translational modifications (11). Cytochrome b₅ is a membrane-bound electron transfer protein. By forming a complex with P₄₅₀_{C17} and cytochrome P₄₅₀ oxidoreductase, cytochrome b₅ appears to act as a “switch” to promote 17,20-lyase activity (12).

Cytochrome P450 oxidoreductase, encoded by *POR*, is a flavoprotein involved in electron transport for all microsomal P450 enzymes. Mutations in the *POR* gene have been identified in the Antley–Bixler syndrome when associated with ambiguous genitalia and in a single young woman with primary amenorrhea; the steroid hormone pattern suggests combined P₄₅₀_{C17} and P450_{C21} deficiencies (13,14). The enzyme 3 β -hydroxysteroid dehydrogenase type 2, encoded by *HSD3B2*, converts the Δ 5 steroids, pregnenolone, 17-Preg, and DHEA, to their respective Δ 4 steroids, progesterone, 17 α -hydroxyprogesterone (17-OHP), and androstenedione.

In the adrenal cortex, 21-hydroxylase encoded by *CYP21* converts progesterone and 17-OHP, respectively, to deoxycorticosterone and 11-deoxycortisol. In the zona glomerulosa, aldosterone synthase, encoded by *CYP11B2*, converts deoxycorticosterone to aldosterone. In the zona fasciculata, 11 β -hydroxylase, encoded by *CYP11B1*, converts 11-deoxycortisol to cortisol. In the zona reticularis, DHEA is converted to DHEAS through the actions of DHEA sulfotransferase encoded by the *SULT2A1* gene. Recent data challenge the concept that DHEAS serves as a circulating storage pool for sex steroids in that DHEA, but not DHEAS, was more readily converted to other sex steroid metabolites (15).

In Leydig cells in the testis, the enzyme 17 β -HSD type 3 encoded by *HSD17B3* converts androstenedione to testosterone. In androgen target cells such as the prostate

and external genitalia, testosterone is converted to dihydrotestosterone (DHT) by 5 α -reductase encoded by the *SRD5A2* gene. Theca cells of the ovary synthesize androstenedione, which diffuses into granulosa cells where aromatase (P₄₅₀c-arom) encoded by *CYP19* converts androstenedione to estrone and subsequently into estradiol by 17 β -HSD type 1.

Androgen, estrogen, and glucocorticoid actions are mediated by specific nuclear hormone receptors. Recent investigations have demonstrated that non-classical cell membrane receptors also mediate hormone actions.

INSULIN AND STEROIDOGENESIS

Insulin has both metabolic and mitogenic actions. The metabolic actions involve regulation of carbohydrate and fat metabolism by influencing insulin-stimulated glucose uptake in target tissues such as skeletal muscle and adipose tissue, insulin-stimulated suppression of hepatic glucose production, and insulin-mediated suppression of lipolysis. These insulin actions are mediated largely through the insulin receptor substrate proteins and downstream mediators. In the case of glucose transport, mediators such as phosphatidylinositol 3-kinase (PI3-K) are essential to induce translocation of the insulin-responsive glucose transporter-4 (GLUT-4) to the cell surface (16).

Tissue culture experiments provided the initial evidence that insulin and insulin-like growth factor (IGF)-1, acting through their cognate receptors, augment steroidogenesis (17). In a clinical study, ACTH-stimulated hormone responses were greater during insulin infusion than during saline infusion (18). More recent studies have probed the molecular mechanisms through which insulin and IGF-1 promote steroidogenesis. Acting through their cognate receptors and the PI3-K pathway, insulin and IGF-1 promote ovarian 17,20-lyase activity (19). However, unlike the metabolic effects of insulin to stimulate glucose uptake, the stimulatory effects of insulin on both P₄₅₀C₁₇ activity and *CYP17* mRNA expression in human theca cells also depend on the co-activation of the cyclic adenosine monophosphate (cAMP)-signaling pathway (20). In cultured mouse Leydig cell tumor cells, IGF-1 promotes transcription of the *StAR* gene predominantly through the protein kinase C pathway (21). As will be discussed below in the section entitled Insulin Resistance, these mitogenic actions of insulin and IGF-1 play important roles in the pathophysiology of PCOS, and perhaps in PP, as well.

PUBERTY

Puberty refers to the process through which reproductive competence develops. In humans, two distinct physiological processes, gonadarche and adrenarche, are associated with puberty. The age at onset of puberty and sequence of pubertal development varies between ethnic groups (22,23). Gonadarche is characterized by the growth and maturation of the gonads and increased secretion of sex steroids. Gonadarche is due to the increased pulsatile gonadotropin-releasing hormone (GnRH) secretion associated with the re-activation of the hypothalamic pulse generator and the ensuing increase in pituitary LH and FSH secretion.

Pubarche, the appearance of sexual hair, represents increased DHEAS secretion associated with adrenarche (24). Adrenarche is a phenomenon that appears to be limited to our own species and to the great apes (25). In humans, the absence of adrenarche does

not prevent gonadarche or lead to infertility (26,27). The physical findings associated with adrenarche include the development of pubic hair, axillary hair, acne, and apocrine body odor. Despite increased adrenal androgen secretion, no apparent changes in cortisol or ACTH concentrations are evident at adrenarche (28). Investigation of human adrenal tissue reveals that at the time of adrenarche, 3 β -HSD expression decreases whereas expression of cytochrome b₅, cytochrome P₄₅₀ oxidoreductase, and DHEA sulfotransferase increases (29). These changes augment the 17,20-lyase activity of P₄₅₀c₁₇ leading to increased conversion of 17-hydroxypregnenolone to DHEA and DHEAS. Although findings have been inconsistent, clinical studies suggest that insulin, IGF-I, and growth hormone (GH) concentrations influence the timing, onset, and progression of adrenarche (30). Higher IGF-I concentrations are found among prepubertal African-American children, a factor that may contribute to the clinical observation that African-American children experience adrenarche/pubarche earlier than other ethnic groups (31).

To date, the molecular mechanism(s) responsible for the regulation of adrenal androgen biosynthesis and the onset of adrenarche remains to be identified. Transcription factors belonging to the GATA binding protein (GATA) family appear to play roles in steroidogenesis (32). Two members of this family, GATA-4 and GATA-6, are expressed in fetal and adult adrenal cortex and gonads. In the adrenal cortex, GATA-6 influences transcription of steroidogenic enzymes including DHEA sulfotransferase (33,34). Recent data suggest a synergistic interaction between GATA factors and other transcription factors to promote transcription of the *HSD3B2* gene, suggesting that these proteins are relevant to both normal regulation and pathologic disorders of steroidogenesis (35). A recent microarray analysis of ovarian theca cells from women with PCOS showed increased expression of *GATA-6* (36).

DEFINITION OF PP

PP is defined as the premature development of pubic hair, axillary hair, or adult-type apocrine odor (Table 1). Traditional 8 years age has been used for girls and 9 years for boys. The differential diagnosis includes premature adrenal pubertal maturation [premature adrenarche (PA)], congenital adrenal hyperplasia (CAH), Cushing’s syndrome, androgen-secreting tumors, and glucocorticoid resistance. Rarely, hyperprolactinemia and exogenous androgen exposure have been associated with PP/precocious puberty (37).

Table 1
Differential diagnosis of premature pubarche

Premature adrenarche
Congenital adrenal hyperplasia
Cushing’s syndrome
Adrenal/ovarian tumors
Glucocorticoid resistance
Exposure to exogenous androgen
Hyperprolactinemia

DIFFERENTIAL DIAGNOSIS OF PP

Premature Adrenarche

Children with PP due to PA typically have androgen concentrations commensurate with the stage of pubic hair development. In other words, serum DHEA, DHEAS, and androstenedione concentrations tend to be elevated for chronological age but are within the range typical for the child's stage of pubic hair development. Generally, clitoromegaly, increased testicular volume, and penile enlargement do not occur. Children with PA may be overweight (38). Review of growth records shows normal to slightly accelerated growth velocity. Skeletal maturation may be slightly advanced but is usually within 2 SD of the average for age. The diagnosis of PA is based on clinical parameters and exclusion of other disorders associated with PP and hyperandrogenism.

The usual natural history of PA is slow progressive development of pubic and axillary hair. Girls with PA have insulin resistance, hyperinsulinemia, dyslipidemia, and decreased sex hormone-binding globulin (SHBG) concentrations (39–41). Prepubertal boys with PA, similar to girls with PA, show higher IGF-1 concentrations and decreased insulin sensitivity, independent of obesity (42). In one study of Spanish and Italian girls, mean age at menarche for girls with documented PP was 6 months earlier compared with healthy controls (43). As discussed below in the section entitled Relationship of PP to PCOS, it has become apparent that some girls with PA develop signs and symptoms of persistent hyperandrogenism and ultimately develop features typical of PCOS (44).

Congenital Adrenal Hyperplasia

The virilizing CAHs comprise a group of autosomal recessive disorders characterized by impaired cortisol biosynthesis due to loss of function mutations in steroidogenic enzymes. Cortisol deficiency leads to a loss of negative feedback inhibition with subsequent increased ACTH and adrenal androgen secretion. The clinical features depend on the specific mutation and its effect on enzyme function. The most frequently encountered type of CAH is 21-hydroxylase deficiency due to mutations in the 21-hydroxylase (*CYP21A2*) gene. Less commonly, CAH is due to 3 β -HSD deficiency associated with mutations in the 3 β -HSD type 2 (*HSD3B2*) gene or 11 β -hydroxylase deficiency associated with mutations in the 11 β -hydroxylase (*CYP11B1*) gene.

The phenotypic spectrum ranges from classical forms with presentation during infancy or early childhood to the non-classical forms. Typical presentations for the milder forms include PP, hirsutism, irregular menses, acne, infertility, and clitoromegaly/phallic enlargement. Linear growth velocity and skeletal maturation, assessed by bone age radiograph, are usually accelerated in affected children. For adolescents and young adults, an ascertainment bias occurs because symptoms are due to the androgen excess. Young adult males with CAH are generally asymptomatic and identified through family studies.

The incidence of 21-hydroxylase deficiency varies from 1 in 15,000 livebirths for the classical forms to approximately 1 in 1000 for the milder forms (45–47). The *CYP21A2* gene is located at chromosome 6p12 in close proximity to a highly homologous pseudogene, *CYP21A1P*. The coding regions of *CYP21A2* and *CYP21A1P* show 98% homology (48,49). To date, over 100 mutations have been identified (50). However,

the vast majority of affected alleles carry one of the more common mutations. These common mutations represent gene conversion events in which the functional gene has acquired deleterious sequences from the pseudogene.

Pharmacologic stimulation with ACTH is often necessary to confirm diagnosis of the milder forms of 21-hydroxylase deficiency. After the blood sample for basal hormone determinations has been obtained, 0.25-mg synthetic ACTH is administered by intramuscular or intravenous bolus injection. Stimulated levels can be obtained at either 30 or 60 minutes. Available data suggest that mutations will be found on both *CYP21A2* alleles if the ACTH-stimulated 17-hydroxyprogesterone value is greater than 1500 ng/dl (51). Although molecular genotype results are necessary for certainty, ACTH-stimulated 17-OHP values between 500 and 1500 ng/dl suggest heterozygosity for *CYP21A2* mutations. However, 50% of heterozygous mutation carriers show ACTH-stimulated 17-OHP values less than 500 ng/dl (52).

CAH due to 3β -HSD deficiency is associated with mutations in the *HSD3B2* gene. Affected infants of both sexes, with severe loss of function mutations, can present with genital ambiguity. In females, the accumulation of DHEA is sufficient to virilize the external genitalia. In males, decreased *HSD3B2* activity impairs testosterone biosynthesis resulting in undervirilization. Correlation of ACTH-stimulated 17-hydroxyprogesterone and DHEA levels with *HSD3B2* genotyping showed that non-classical 3β -HSD deficiency is uncommon. Indeed, similar to 21-hydroxylase deficiency, ACTH-stimulated 17-hydroxypregnenolone concentrations are 9–10 SD above age-matched and pubertal stage-matched values for subjects with *HSD3B2* mutations on both alleles (53–55). Mild forms of 11β -hydroxylase deficiency due to mutations in *CYP11B1* are extremely rare.

Individuals with classical salt-losing CAH require both glucocorticoid and mineralocorticoid replacement therapy. Glucocorticoid therapy without mineralocorticoid treatment is generally sufficient for patients with simple virilizing and late-onset forms of CAH. However, mineralocorticoid therapy may be beneficial in patients with simple virilizing CAH. The goal of treatment is suppression of adrenal androgen concentrations while avoiding excessive glucocorticoid exposure. Both undertreatment and overtreatment can be associated with short stature. Accelerated bone maturation in patients with inadequate glucocorticoid replacement or poor compliance may lead to adult short stature. Excessive glucocorticoid dosage impairs linear growth velocity. For glucocorticoid replacement therapy, hydrocortisone, 7–16 mg/m²/day, may be administered approximately every 8 hours. Adolescents and adults may prefer prednisone and dexamethasone because of the less frequent dosing schedule. Affected individuals need to wear Medic-Alert IDs, know when to increase their glucocorticoid dose for acute physiological stress, and know how to administer a short-acting intramuscular form such as Solu-Cortef for medical emergencies.

Cushing's Syndrome/Adrenal Tumors/Gonadal Tumors

Cushing's syndrome refers to the clinical features associated with excessive exposure to endogenous or exogenous glucocorticoids. Exogenous Cushing's syndrome results from high-dose glucocorticoids used for their anti-inflammatory properties to treat a variety of disorders, that is, inflammatory bowel diseases, connective tissue disorders, asthma, and so on. The endogenous forms of Cushing's syndrome are due to increased

corticotropin (CRH), ACTH, or cortisol secretion. In children under 8 years of age, adrenal tumors are the most common cause of endogenous Cushing's syndrome. Approximately 10–30% of adrenal adenomas and carcinomas secrete other steroid hormones such as androgens in addition to cortisol (56). Ovarian androgen-secreting tumors, Leydig cell tumors, and human chorionic gonadotropin (hCG)-secreting tumors are rare causes of PP.

Steroid-secreting adrenocortical neoplasms can be associated with Beckwith–Wiedemann syndrome, Li–Fraumeni syndrome, and Carney complex. The major features of Beckwith–Wiedemann syndrome include macroglossia, gigantism, abdominal wall defects, and hemihypertrophy; affected children have an increased risk of Wilms' tumor, adrenal tumors, and hepatoblastoma (57). Li–Fraumeni is a heterogenous inherited cancer disorder; mutations in the *p53* and *CHEK2* genes have been identified (58). Carney complex is a heterogenous autosomal dominant disorder associated with spotty skin pigmentation, cardiac myxomas, thyroid tumors, and testicular tumors (59). In approximately 40% of families, Carney syndrome is associated with loss of function mutations in the protein kinase A regulatory subunit 1- α (*PRKARIA*) gene (60). The typical adrenal histology is characterized by primary pigmented nodular adrenocortical disease (PPNAD), in which the adrenal glands contain dark nodules scattered within atrophic adrenal glands. The micronodules are usually visible on imaging studies giving an irregular contour. PPNAD can be associated with “periodic” Cushing's syndrome characterized by intermittent excessive glucocorticoid secretion.

Cushing's disease refers specifically to increased pituitary ACTH secretion often due to a pituitary tumor, but this disorder may also involve excessive hypothalamic CRH secretion. Patients with multiple endocrine neoplasia type I due to loss of function mutations in the *menin* (*MEN-1*) gene and those with McCune–Albright syndrome due to activating mutations in the *GNAS1* gene can develop ACTH-secreting pituitary adenomas. Ectopic tumors secreting ACTH or CRH can also cause excessive glucocorticoid secretion (61–63).

Impaired linear growth velocity is a characteristic feature of excessive glucocorticoid exposure in children. Depression, anxiety, and morbid obesity can be associated with mildly elevated cortisol concentrations. The diagnosis of Cushing's syndrome requires confirmation of inappropriately elevated cortisol secretion with loss of both physiological negative feedback inhibition and diurnal variation. Loss of diurnal variation can be documented by midnight plasma or salivary cortisol determinations (64). Once endogenous glucocorticoid excess is confirmed, the diagnostic evaluation can focus on the specific etiology. In some instances, in the presence of clinical features suggestive of Cushing's syndrome, laboratory studies may need to be repeated to verify endogenous hypercortisolism (65).

Glucocorticoid Resistance

Inherited glucocorticoid resistance is characterized by symptoms due to hyperandrogenism such as PP, hirsutism, oligomenorrhea, and infertility. Hypokalemic hypertension may be noted. Despite elevated cortisol concentrations, symptoms typical of Cushing's syndrome are absent. This autosomal dominant disorder, due to loss of function mutations in the glucocorticoid receptor (*GCCR*) gene, leads to end-organ

insensitivity (66). Homozygosity for a missense mutation in *GCCR* was identified in a girl born with ambiguous genitalia; she also was a heterozygous carrier for a deletion in the *CYP21* gene (67).

The impaired glucocorticoid signal transduction interferes with negative feedback inhibition leading to increased ACTH and cortisol secretion. The net result is a new “set-point” for cortisol concentrations accompanied by excessive adrenal androgen secretion. In some instances, mineralocorticoid secretion is also increased resulting in hypokalemic hypertension. Treatment with dexamethasone is often beneficial to lower adrenal mineralocorticoid and androgen secretion.

RELATIONSHIP OF PP TO PCOS

Some girls with a history of PP manifest persistent hyperandrogenism as gonadarche progresses and develop signs and symptoms consistent with PCOS. PCOS is a familial heterogeneous disorder associated with hyperandrogenism, chronic anovulation, and infertility. Early identification of girls at risk of PCOS is important because women with PCOS have an increased risk of abnormal carbohydrate metabolism and manifest a clustering of cardiovascular risk factors, such as obesity, lipid abnormalities, hypertension, and increased C-reactive protein concentrations (68,69).

Adolescent girls with PCOS have an increased risk of developing impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) (70). Metabolic abnormalities comparable with adult women with PCOS such as insulin resistance, hyperinsulinemia, lower first-phase insulin secretion, LH hypersecretion, dyslipidemia, decreased SHBG concentrations, decreased IGF-1 binding protein-1 concentrations, and increased IGF-1 have been found among adolescent girls with PCOS (71–73). Increased carotid medial thickness, a risk marker for atherosclerosis, was found in 18-year-old to 22-year-old women with PCOS (74). PCOS also adversely affects quality of life for adolescent girls (75). Thus, many of the potential deleterious consequences of PCOS are already apparent in adolescent girls with PCOS.

The relationship between PP and PCOS has been scrutinized among a population of lean Catalunyan girls. At multiple timepoints from presentation with PP to adolescence, these girls are found to have decreased insulin sensitivity, hyperinsulinemia, dyslipidemia, increased central fat distribution, and increased plasminogen activator-inhibitor type 1 (PAI-1) activity (76–78). With increasing gynecologic age, the frequency of anovulation accompanied by secondary amenorrhea increases in this population (79). African-American and Caribbean-Hispanic girls with a history of PP are also reported to have an increased incidence of insulin resistance, hyperinsulinemia, and adrenal hyper-responsiveness (80,81). Post-ACTH stimulation, levels of the $\Delta 5$ steroids, 17-hydroxypregnenolone, and DHEA, may be mildly elevated among girls with a history of PP (82). Comparison of post-menarcheal French girls with a history of PP with healthy control girls showed only increased androstenedione concentrations and decreased SHBG concentrations without apparent differences in insulin resistance (83).

Nevertheless, not all girls with PP develop PCOS. Excessive weight gain during the peripubertal years seems to increase the risk of developing PCOS. But, it is unclear what other factors influence outcome. In the next few sections, items relevant to this association such as ovarian cyst formation, insulin resistance, neuroendocrine changes, genetic factors, and low birth weight will be discussed.

OVARIAN MORPHOLOGY

The description of PCOS by Stein and Leventhal highlighted the association between ovarian cysts and androgen excess (84). Typically, ultrasound examination of a PCOS ovary reveals increased ovarian size, increased ovarian stromal thickness, and accumulation of small follicular cysts—the so-called “string of pearls” pattern. Although ovarian cysts are a common clinical feature of PCOS, their presence is not requisite for diagnosis. Indeed, among healthy women, the prevalence of PCO was reported to be 23% (85). Hence, much discussion has ensued regarding the definition of PCO, their role in the pathophysiology of PCOS, and how they develop.

Confounding the interpretation of ovarian ultrasonography in adolescent girls is the occurrence of multifollicular ovaries (MFO) (86,87). MFO may be normal or slightly increased in size, contain more than six follicles that are 4–10 mm in size, and have normal amounts of stroma. MFO can be seen in adolescent girls, women with hyperprolactinemia, and women recovering from hypothalamic amenorrhea. Distinguishing features between adolescent girls with PCOS and those with MFO are that adolescent girls with PCOS tend to have higher testosterone, LH, and insulin concentrations (88). Hence, women with MFO are generally not hirsute and show normal ovarian morphology when ovulatory cycles occur (89). One feature common to early puberty, hyperprolactinemia, and hypothalamic amenorrhea is a relative gonadotropin deficiency. Thus, GnRH and gonadotropin secretion in these situations may be insufficient leading to impaired follicular maturation and MFO (90).

Ovarian ultrasound is best performed during the first few days of the follicular cycle when the ovary is relatively quiescent. For women with oligo/amenorrhea, the timing may be random. Traditionally abdominal ultrasonography has been utilized. Using a formula to calculate the area of the ovary as a prolate ellipsoid ($0.5233 \times$ maximal longitudinal, anteroposterior, and transverse diameters), a PCO is defined as an ovarian volume $>10 \text{ cm}^3$. Follicle diameter should be estimated and the number of follicles in two planes should be counted. The consensus definition for a PCO includes size ($>10 \text{ cm}^3$) and number of follicles (≥ 12 follicles between 2 and 9 mm in size). Ovaries in women with PCOS tend to have increased stromal hyperechogenicity especially when visualizing by transvaginal ultrasonography. If there is evidence of a dominant follicle (follicle $>10 \text{ mm}$), the scan should be repeated (91,92). Transvaginal ultrasonography provides a more accurate view of the ovaries but may not be practical for young adolescent girls. Newer techniques such as 3D-ultrasound, color Doppler, or magnetic resonance imaging (MRI) may enhance visualization of the ovaries but the need for specialized equipment and the increased cost limit the usefulness of these techniques (93). In an uncontrolled study involving girls with PA, color Doppler ultrasound showed the presence of small ovarian follicles in 10/27 girls (94).

INSULIN RESISTANCE

Insulin resistance/hyperinsulinemia, gauged by decreased insulin-stimulated glucose uptake, increased hepatic glucose production, and increased lipolysis, occurs frequently in women with PCOS. Available data suggest that impaired insulin signal transduction downstream from the insulin receptor plays a role in the insulin resistance of PCOS.

To compensate for the insulin resistance, the pancreatic β -cells secrete more insulin resulting in hyperinsulinemia. Despite the end-organ insulin resistance affecting carbohydrate and fat metabolism, the mitogenic (growth) actions of insulin are generally preserved. Thus, the elevated insulin concentrations act synergistically with LH and/or ACTH, respectively, to provoke excessive ovarian and/or adrenal androgen biosynthesis (95,96). Insulin also inhibits hepatic synthesis of SHBG resulting in increased bioavailable testosterone.

When insulin concentrations are lowered by weight loss or pharmacologic treatment (metformin, troglitazone, D-chiro-inositol, etc.), circulating androgen concentrations decline attesting to the importance of insulin resistance in the pathophysiology of PCOS (97,98). Thus, in PCOS, the steroid-producing tissues, adrenal and ovary, become targets of the metabolic derangements.

Evidence that hyperinsulinemia plays a role in the transition from PP to PCOS comes from recent studies involving the Catalunyan girls. In this population, the use of pharmacologic agents to improve insulin sensitivity has delayed or prevented the onset of hyperinsulinemic hyperandrogenism. Decreased hirsutism accompanied by lower androgen, insulin, total cholesterol, triglyceride, and low-density lipoprotein (LDL) cholesterol concentrations was reported in 10 non-obese Catalunyan girls when treated metformin (99). Among non-obese low birth weight Catalunyan girls with PP, metformin treatment was associated with improvements in SHBG, DHEAS, triglyceride, and adiponectin concentrations and body composition variables; post-pubertal discontinuation of metformin was accompanied by reversal toward basal hormone and adipocytokine values (100).

NEUROENDOCRINE FUNCTION

The onset of puberty is characterized by increased nocturnal LH secretion and accompanied by transient insulin resistance. Pituitary gonadotropin secretion is driven by and reflects GnRH pulse frequency. The normal ovulatory menstrual cycle is characterized by a series of carefully choreographed changes in LH, FSH, estradiol, and progesterone secretion. During the late follicular phase of the normal menstrual cycle, LH pulses occur approximately every hour leading to increased estradiol production, selection of the dominant follicle, the LH surge, and ovulation. Following ovulation, the corpus luteum produces progesterone and estradiol, which slow GnRH pulse frequency to approximately one pulse every 3–4 hours (101).

Women with PCOS demonstrate LH hypersecretion, relative FSH deficiency, and lack of the normal cyclic variation in LH secretion (102–104). This relative FSH deficiency contributes to impaired follicular development, whereas the LH hypersecretion exacerbates ovarian hyperandrogenism generating a vicious cycle characterized by chronic anovulation and hyperandrogenism. Investigations into the mechanism(s) responsible for the initiation of the chronic hyperandrogenic anovulation have failed to identify any primary hypothalamic abnormalities involving neurotransmitters in women with PCOS (105). Although hyperinsulinemia is a common finding among women with PCOS, insulin infusions have not affected LH secretion (106,107). Furthermore, despite improved insulin sensitivity using either metformin or pioglitazone, LH pulse amplitude and pulse frequency were unchanged in women with PCOS

(108,109). Thus, LH hypersecretion does not appear to be a direct consequence of hyperinsulinemia and/or insulin resistance.

Women with PCOS manifest impaired hypothalamic sensitivity to progesterone-mediated LH suppression (110). Restoration of progesterone sensitivity with the use of flutamide to block androgen receptors indicates that hyperandrogenism is responsible for reduced progesterone sensitivity (111). Thus, current evidence suggests that LH hypersecretion is due to impaired sensitivity to progesterone with failure to suppress GnRH pulsatility rather than primary GnRH hypersecretion. Impaired progesterone sensitivity has also been identified in some adolescent girls with hyperandrogenism (112).

GENETICS OF PP AND PCOS

The prevalence of PCOS is higher among family members of women with PCOS than among the general population, 20–40% depending on the study versus 6–8%, respectively (113,114). The inheritance pattern of the phenotype resembles that of an autosomal dominant disorder, but no single PCOS gene has been identified. Similar to other complex genetic disorders, the PCOS phenotype results from a combination of genetic and environmental factors.

In one study in which sisters of women with PCOS were evaluated, a bimodal distribution of the testosterone concentrations was observed (115). Despite poor characterization of the male phenotype, brothers of women with PCOS have higher DHEAS concentrations (116). Ovarian theca cells obtained from women with PCOS show persistently increased androgen secretion, augmented *CYP17* expression, prolonged *CYP17* mRNA stability, and other intrinsic differences in signaling cascades (117). These studies support the hypothesis that there is a major genetic component to the hyperandrogenemia (118).

In addition to high heritability for hyperandrogenemia, genetic factors appear to influence the extent of the insulin resistance, hyperinsulinemia, and β -cell dysfunction. Hyperinsulinemia is common among family members of Australian women with PCOS (119). Mothers, brothers, and sisters of Turkish women with PCOS had greater insulin resistance than population controls (120). Among first-degree relatives of women with PCOS, insulin secretion showed heritability when measured by frequently sampled intravenous glucose tolerance tests (121). First-degree relatives of Catalan girls with PP have an increased prevalence of IGT, T2DM, and gestational diabetes mellitus (GDM) providing additional evidence that genetic factors influence carbohydrate/fat/insulin dynamics in PP and PCOS (122). In a small number of families, the presence of metabolic syndrome in fathers showed a high concordance with the presence of PCO in affected daughters in the absence of maternal PCO supplying more data linking metabolic abnormalities with PCO (123).

With so much evidence that PCOS is familial, numerous linkage analysis and candidate gene association studies have been performed (124). To investigate the genetic basis of PCOS, candidate genes comprising the components of the phenotype such as androgen biosynthesis, androgen metabolism, insulin signal transduction, insulin secretion, and serum inflammatory markers have been scrutinized (125). However, phenotypic and genetic heterogeneity, inconsistent diagnostic criteria, inexact

male phenotype, relative infertility, incomplete penetrance, and epigenetic factors confound the quest for the PCOS genes (126).

Despite these obstacles, some progress has been made to identify relevant genes (127). For example, some children with PP and women with PCOS have ACTH-stimulated 17-OHP and molecular genotype results indicating heterozygosity for *CYP21A2* mutations (128–131). These individuals differ from obligate heterozygote *CYP21A2* mutation carriers ascertained through studies of families with classical CAH where heterozygotic *CYP21* carriers are asymptomatic despite having mildly elevated serum androgen concentrations (132). A common single-nucleotide polymorphism (SNP) in the aromatase (*CYP19*) gene was associated with PP among Catalan girls; this association was replicated in young English women (133). Additional analysis of SNPs in the distal *CYP19* promoter region showed association with testosterone concentrations in these two populations but not with risk of PP among the Catalan girls (134). Mutations in the insulin receptor gene are a rare, but recognized cause of PCOS. Recently, sisters heterozygous for mutations in the insulin receptor gene have been reported. As anticipated, both had hyperandrogenism, hyperinsulinemia, and PCO (135).

Some adolescent girls with PCOS report experiencing PP, whereas other girls present only post-menarche with the clinical features of PCOS. Thus, even among the pediatric population, there may be subgroups of girls with PCOS. These observations trigger speculation that one group of girls with PP maintain the phenotype of excessive adrenal androgen secretion and develop PCOS. There is a second group of girls who have PP without persistent hyperandrogenism and do not manifest the PCOS phenotype. A third group of girls develop features of PCOS post-menarche and have primarily ovarian hyperandrogenism. The differences in the prevalence of *CYP21* heterozygosity among children with PP compared with adult women with PCOS and the observation that increased DHEAS secretion is a stable phenotype in women with PCOS lend credence to this conjecture (136,137). Future studies are needed to investigate the genetic and environmental factors that increase the propensity to develop the PCOS phenotype.

LOW BIRTH WEIGHT

Recent data suggest an association between low birth weight and increased risks of developing PP, PCOS, coronary artery disease, and/or T2DM in adulthood (138–140). These clinical and experimental observations support the concept of the fetal origin of adult disease whereby the intrauterine environment influences postnatal phenotype. This association has been well characterized among Catalan girls in whom low birth weight has been associated with PP, insulin resistance, dyslipidemia, and hyperinsulinemic ovarian hyperandrogenism (141). Among Austrian children with PP, retrospective evaluation showed that 65% were obese, 25% had a history of prematurity, and 35% had been small for gestational age (SGA) (142). However, this hypothesis remains controversial because in another study involving Caucasians of European origin, 130 French women with intrauterine growth retardation and 150 controls, low birth weight was associated with hyperinsulinemia but not hyperandrogenism (143,144). The timing of puberty in boys born SGA appears to be normal (145).

More recent data implicate the transition from low birth weight to greater postnatal catch-up growth with disease risk (146–148). Even in normal children, there appears to be a relationship between birth weight, postnatal growth rate, and the onset of adrenarche in that highest DHEAS concentrations were found among the lowest birth weight children with the greatest rate of postnatal weight gain (149).

In addition to environmental factors, intra-uterine growth retardation, birth weight, and the risk for T2DM may be influenced by genetic factors. This is illustrated by comparison of mothers and infants carrying mutations in the glucokinase (*GCK*) gene, the molecular basis for MODY2, an autosomal dominant monogenic type of diabetes mellitus. When only the mother carries the mutation, the infant is large as would be anticipated for the infant of a diabetic mother. When only the infant carries the mutation, the infant has a lower birth weight, presumably due to lower fetal insulin concentrations, and an increased risk of developing mild diabetes in adulthood (150).

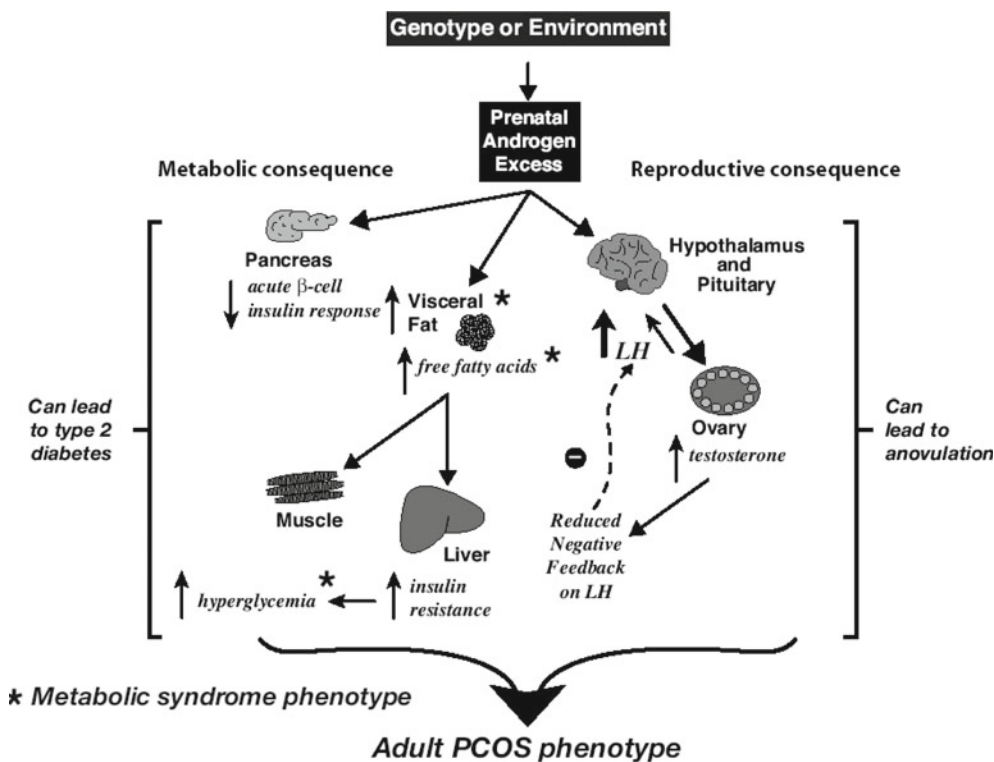


Fig. 1. Summary of the fetal origin hypothesis in which prenatal androgen excess “programs” metabolic and reproductive consequences which lead to the development of polycystic ovary syndrome (PCOS). This hypothesis is based on studies performed in the prenatally androgenized female rhesus monkey, a non-human primate model for PCOS in women. Reproduced with permission (151).

FETAL PROGRAMMING OF OVARIAN DYSFUNCTION

This concept of fetal programming implies that prenatal events trigger permanent changes in organ function or structure (*Fig. 1*). In addition to changes induced by stress, exposure of the female fetus to excessive androgen concentrations may influence hypothalamic, pituitary, and gonadal function. The most obvious example of the consequences of excessive prenatal androgen exposure is the virilized female with ambiguous genitalia due to classical 21-hydroxylase deficiency. In girls with classical forms of CAH, the prenatal androgen exposure may also program the neuro–endocrine axis resulting in LH hypersecretion at puberty (*152*). Another potential consequence of prenatal androgen exposure is gender role in that girls with classical forms of CAH tend to play more with boys’ toys, are more likely to use aggression when provoked, and show less interest in infants than their unaffected sisters (*153–155*). As a group, girls with CAH demonstrate female gender identity and heterosexual orientation. The higher androgen concentrations in pregnant women with PCOS may provide a potential exposure to androgen excess for the female fetus (*156*). This prenatal androgen exposure could contribute to the dominant inheritance pattern described for the PCOS phenotype.

Animal models have been used to investigate the consequences of prenatal androgen exposure to the postnatal ovary. Female rhesus monkeys exhibit hyperandrogenism, anovulation, large MFO, hyperlipidemia, and impaired developmental oocyte competence following prenatal androgen exposure (*157–159*). Curiously, prenatal androgen exposure is also associated with increased ACTH-stimulated DHEA, androstenedione, and corticosterone responses (*160*). Although non-human primates provide the best model for humans, prenatal androgen exposure has been investigated in other species.

Prenatally androgenized mice had elevated LH and testosterone concentrations and demonstrated irregular estrous cycles and flutamide reversible changes in gamma-aminobutyric acid (GABA)-ergic neurons (*161*). Data obtained from prenatally

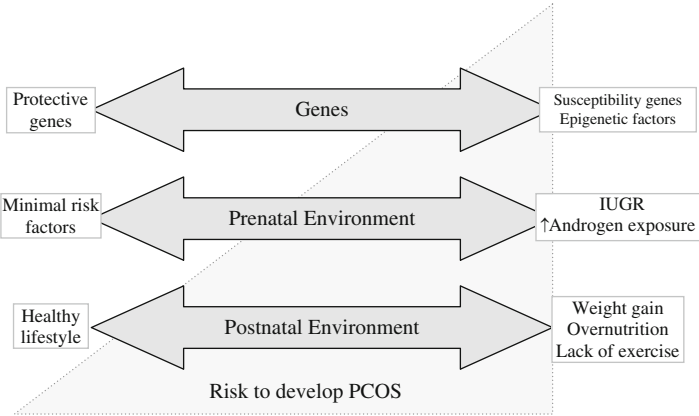


Fig. 2. Factors that potentially influence risk of developing polycystic ovary syndrome (PCOS) in girls with premature pubarche (PP). Genetic susceptibility factors could include genes influencing androgen synthesis, androgen metabolism, androgen action, insulin resistance, insulin secretion, body composition, metabolism, and appetite regulation. The prenatal environment includes intrauterine stress and excessive prenatal androgen exposure. Postnatal environmental factors include food choice, food quantity, weight gain, and activity level.

androgenized rats support the hypothesis that prenatal androgen receptor activation alters the GnRH neurosecretory system by rendering it unable to initiate GnRH surges while accelerating basal GnRH pulse generator activity in adulthood (162). The physiologic basis for this alteration is partly mediated by impaired estrogen-mediated progesterone receptor expression in the hypothalamus (161).

Prenatally androgenized sheep manifest LH hypersecretion, anovulation, and cystic ovarian morphology (163). Prenatally androgenized offspring have reduced birth weight and manifest impaired insulin sensitivity in early postnatal life (164,165).

CONCLUSIONS

PP represents the physical manifestations of the early-onset of pubertal adrenal maturation. For some girls, PP heralds the subsequent development of PCOS and its potential sequelae including increased risks of IGT, T2DM, and the metabolic syndrome. Genetic variants and environmental factors influence the development and progression of PP and PCOS (Fig. 2). Directions for future research efforts include identification of the factors that influence the natural history of PP and development of efficacious therapeutic interventions.

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14

Psychological and Social Problems in Children with Premature Adrenarche and Precocious Puberty

Lorah D. Dorn, PhD

CONTENTS

INTRODUCTION

EMPIRICAL EVIDENCE OF PSYCHOSOCIAL PROBLEMS
IN CHILDREN WITH PA OR PP

HOW CAN WE BETTER UNDERSTAND WHY
PSYCHOSOCIAL PROBLEMS MAY OCCUR IN CHILDREN
WITH PA OR PP?

IMPLICATIONS FOR THE PRACTITIONER REGARDING
PSYCHOSOCIAL ISSUES IN CHILDREN WITH PA OR PP

DIRECTIONS FOR FUTURE RESEARCH

CONCLUSIONS

REFERENCES

Summary

Early puberty, including premature adrenarche (PA) and precocious puberty (PP) can be a challenge for the practitioner as well as the parent and child. This chapter will focus on the Psychosocial problems that may be evident in children with PA or PP. To better Understand these issues, four specific areas will be addressed. First the chapter will focus on the existing empirical evidence of psychosocial problems in children with PA or PP and the limitations of these studies. Second, the chapter will address ways to better understand why certain psychosocial problems may occur in children with early puberty. The theoretical and empirical rationale for such problems will be discussed along with specific actions of hormones that may influence the brain and behavior. Third, implications for the practitioner caring for children with PA or PP will be addressed regarding psychosocial issues. Finally, directions for future research on psychosocial problems in children with PA or PP will be discussed.

Key Words: Premature adrenarche; Precocious puberty; Psychopathology; Hormones; Behavior.

INTRODUCTION

From times as early as Plato and Aristotle to this day, adolescents often have been described in negative terms indicative of moodiness or problematic behaviors.

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The cause of such behaviors was unknown. Before the first hormone, adrenaline, was isolated in 1895, there was awareness that internal secretions influenced body function; however, it was not until 1915 that Walter Cannon showed the connection of the endocrine glands and emotions (1). In 2006, the popular press highlights these hormones as causative factors in adolescent moods and behaviors and often refers to them as “the raging hormones” of adolescence, “testosterone poisoning” in males, and even as moodiness exhibited during certain times of the menstrual cycle, when yet empirically the evidence is not clear.

In the clinical arena, endocrine abnormalities (e.g., hypothyroidism) may be associated with various moods or behaviors reflective of depression and anxiety. Alternatively, psychological disorders may also be associated with various endocrine abnormalities (e.g., thyroid and estrogen). Similarly, in pediatric endocrinology, mood disturbances and negative behaviors have been associated with puberty considered to be off time, such as premature adrenarche (PA) or precocious puberty (PP). It has been hypothesized that such negative behaviors, including aggression, are connected with hormones. As expected, much of this literature has cast a negative shadow on hormones as being implicated in the moodiness and problematic behaviors, particularly of teenagers. The picture is much more complex, however, than simply indicting hormones as the only causal factor in adolescent behavior. It is important to consider true differences in behaviors and mood disturbances between those with early pubertal development and those with on-time development and mechanisms leading to such differences.

This chapter will focus on the psychosocial problems that may be evident in children with PA or PP. To better understand these issues, four specific areas will be addressed. First, the chapter will focus on the existing empirical evidence of psychosocial problems in children with PA or PP and the limitations of these studies. Second, the chapter will address ways to better understand why certain psychosocial problems may occur in children with early puberty. The theoretical and empirical rationale for such problems will be discussed along with specific actions of hormones that may influence the brain and behavior. Third, implications for the practitioner caring for children with PA or PP will be addressed regarding psychosocial issues. Finally, directions for future research on psychosocial problems in children with PA or PP will be discussed.

EMPIRICAL EVIDENCE OF PSYCHOSOCIAL PROBLEMS IN CHILDREN WITH PA OR PP

There is a dearth of literature on the psychosocial aspects of PA or PP, although much of it proves limited in various ways. Of those available papers, many are case studies, whereas others suffer from small sample sizes and/or use of non-standardized instruments. Additionally, many studies prove limited by their sole inclusion of girls. Both PA and PP are more common in girls than in boys, and boys with PP are rarely diagnosed with idiopathic PP, thus distinguishing issues of the disorder between genders. Furthermore, few studies include a control group or follow these patients in a longitudinal fashion. Thus, our information regarding psychosocial aspects of PA or PP is fairly limited. The following section will provide a summary of the empirical evidence of psychosocial problems in children and adolescents with PA or PP.

Children With PA

In a search of Medline and PSYCINFO through 2005, only one study reported on psychosocial issues in children solely with PA. (However, two studies in the section “Children With PP” did include a small number of girls with PA.) The study by Dorn and colleagues (2) was a small pilot study of a PA group of 9 (8 girls and 1 boy) children aged 6–8 and a matched comparison group of 20 (8 girls and 12 boys) on-time prepubertal children. PA children were referred by pediatric endocrinologists, and on-time girls were recruited from the community. On-time girls were matched for age, gender, race, and socioeconomic status (SES) of the PA girls. Girls completed checklists on moods, feelings, and behaviors; had a physical examination for pubertal staging; had their height and weight measured; and had their blood drawn for gonadal steroids, adrenal androgens, and cortisol. Parents reported on their child’s moods and behaviors through interviews (3) and standardized questionnaires (4).

In brief, PA children reported significantly higher scores on the Children’s Depression Inventory (5) than did the on-time children. Scores on the State and Trait Anxiety Inventory (6) were no different between the groups. From parental report using the Diagnostic Interview Schedule for Children,(7) four of nine PA children met criteria for one or more psychiatric diagnoses based on Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised criteria (8). Several of the remaining five children had many symptoms of various psychiatric disorders but missed diagnostic criteria by one or two symptoms. Parents also reported many more behavioral problems in their children with PA compared with those parents whose children were in the on-time group (2). Fig. 1 shows mean group differences on four subscale scores of the Child Behavior Checklist (CBCL), two broadband scores (internalizing and externalizing), and total behavioral problems. In all cases, PA girls had higher scores than on-time girls.

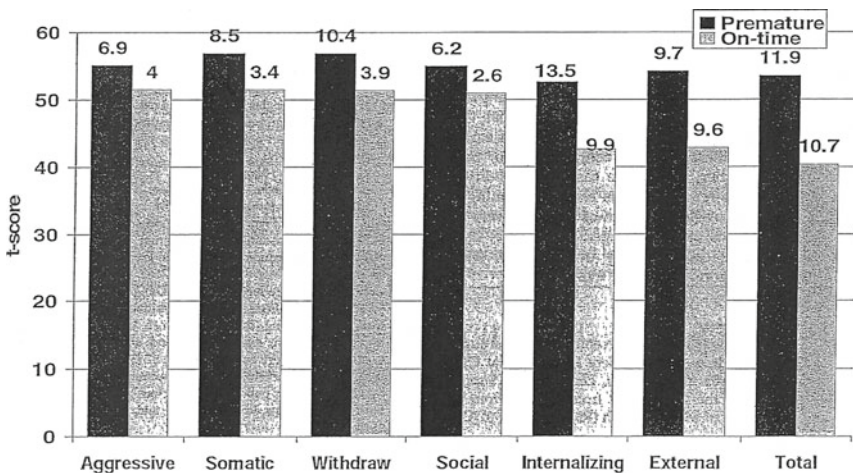


Fig. 1. Group differences in Child Behavior Checklist (CBCL) *T*-scores for girls with premature adrenarche (PA) compared with on-time adrenarche. Standard deviations are reported at the top of each bar. Reprinted with permission (75).

Clearly, not all children had psychopathology, but the anecdotal reports from parents about problems were certainly supported by data. PA girls also had significantly lower IQ scores than on-time girls, but their scores were average and not below normal.

This pilot study had limitations that should be considered (2). First, the sample size was small, and thus one cannot generalize its findings to all PA children. Second, the enrollment criteria specified a diagnosis of PA but did not exclude girls who had since begun breast development. Therefore, the contribution of gonadal axis hormones to behavior could not be eliminated. Third, the PA children were significantly taller and heavier than the on-time children. Having a larger body habitus than one's peer group may influence aggressive behavior. Specifically, when children know they are larger than their peers, they may be aggressive toward others, aware that they are more likely to overpower such peers.

Despite these limitations, the study provided credible evidence to further explore differences in the prevalence of mood and behavioral problems in children with PA compared with those with on-time adrenarche. As this chapter goes to press, a replication of this pilot study was in press (9). This study includes approximately 40 girls, aged 6–8 with PA, who have only adrenal axis activation and no gonadal activation. It also includes an on-time comparison group ($n = 36$) who is matched for body mass index (BMI) in addition to age, race, gender, and SES.

Children With PP

One of the earliest pilot studies examined 16 girls representing premature thelarche ($n = 6$), PA ($n = 4$), or PP ($n = 6$) (10). Clinical interviews and several psychological tests were conducted with both the parent and the girl. Results were expressed as frequencies because the sample size was small. The study reported that either parents were often worried about their daughter acting out sexually or they denied their daughter's sexual development. Girls with PP or premature thelarche presented more embarrassment concerning their development than those with PA, and the PP group presented fewer body image problems compared with the other two groups. Additionally, more aggression toward others was evident in girls with PA compared with the other two groups. One of the conclusions by Solyom and colleagues (10) to explain these findings was that "developmental interferences" may exist for girls with these early pubertal changes based either directly on physical changes or indirectly on parental responses and reactions to this early development.

Ehrhardt and colleagues (11) conducted a follow-up study of 16 girls who began puberty at the age of 2.5–8 years (median 6.5 years) and were diagnosed with idiopathic PP at the age of 7.25 years. Three had been treated with medroxyprogesterone acetate during childhood. At the time of the evaluation, the average age was 17.5 years (± 2.3). A highlight of this study was the inclusion of a comparison group matched for age, sex, race, SES, and menarcheal status. There were no significant ($P < .05$) differences in self-image, self-esteem, or self-concept. Differences in distress symptoms related to the menstrual cycle were noted where PP girls expressed more symptomatology. In parental reports of behavior, there was a trend for PP girls to have more behavioral problems such as conduct and antisocial problems. No group differences in psychiatric diagnoses were noted (11). This study did not report on the psychosocial findings of the girls when they were diagnosed with PA, but rather it conducted the evaluation at an average age of 17.5 years when they were post-pubertal.

In a second report on the same 16 matched pairs, psychosexual development was also evaluated (12,13). All were currently "on time" with their psychosexual milestones,

but PP girls had their first boyfriend and first steady relationship 1 year earlier than did their matched controls. Similarly, sexual experiences were about 1–2 years earlier in the PP girls, and masturbation was about 5.5 years earlier in PP girls as well (12,13).

In a separate study, Galatzer and colleagues (14) explored intellectual functioning in girls with PP. The study included 52 girls with PP (full PP and PA girls), 8 girls with “fast puberty,” and 51 controls with normal development. Significant differences were noted in verbal IQ where those with PP scored higher than the combined group of fast puberty and controls. No differences were noted in performance IQ. This study included a large sample of girls; however, it was difficult to determine exactly how PP was defined (i.e., how it was diagnosed and evaluated) and whether this group was comparable with those in other studies that used idiopathic PP as the definition. Additionally, PA girls were included in the category of PP in this study.

In a cross-sectional study, Sonis and colleagues (15) enrolled 33 girls with PP, aged 6–11 years (mean 8.1 years), and had parents complete the CBCL. Additionally included was a parallel control group on which the CBCL had been normed. Twenty-seven percent of the PP girls had total behavior problem scores in the clinical range (above 98th percentile). The PP girls had higher total behavior problem scores as well as internalizing and externalizing scores compared with the matched standard controls. Similarly, on most of the subscales, the PP girls’ scores were higher. Additionally, the PP girls had lower total social competence scores (15).

In a subsequent study of 77 children, aged 4–11 years (22 boys and 55 girls), the CBCL was also used to examine group differences in behavior (16). This study represented those with idiopathic PP ($n = 35$), those with central nervous system (CNS) lesions ($n = 16$), and those with other forms of PP. No significant differences among the groups were noted in the behavioral ratings.

Jackson and Ott (17) evaluated 22 girls and 6 boys with PP who were 6–14 years of age. Children were diagnosed with PP from 1 to 10 years prior to the psychosocial evaluation. The group of PP children included those with idiopathic PP, CNS lesions, and hormone therapy in infancy. Results were primarily descriptive. Using the Piers-Harris Children’s Self-Concept Scale, there was a great deal of variability across the participants. For example, on the physical appearance cluster, 57% fell within the normal range; on the anxiety-related cluster, more than 25% of the participants scored higher than average; and on the popularity cluster, many had scores that were below normal. A comparison group was not included; therefore, it is difficult to determine how these children may or may not differ from those without PP.

In a more recent study, Xhrouet-Heinrichs and colleagues (18) followed 15 girls with central PP who were treated with triptorelin for 2 years and 5 girls who were not treated. At baseline, girls were at an average age of 8.2 years and ranged between 6.6 and 10.4 years. Analyses did not compare treated versus untreated girls. Semi-structured interviews were completed at 1, 8, 16, and 24 months. Parents reported on their daughter’s behavior using the CBCL at 1 and 24 months, and a self-esteem inventory was administered to the girls at 16 and 24 months. Parents reported that in general girls had exemplary behavior described as model, discrete, and reserved (18). All girls were embarrassed by their pubertal development, with embarrassment subsiding in 6 of 15 following treatment. At baseline, 7 of 20 girls and 8 of 20 girls had high scores on the “withdrawn” and anxiety/depression subscales, respectively, of the CBCL. Six girls had high somatic complaints, and eight parents reported high aggression in their daughters. Changes in these scores (Fig. 2) varied across individuals at the

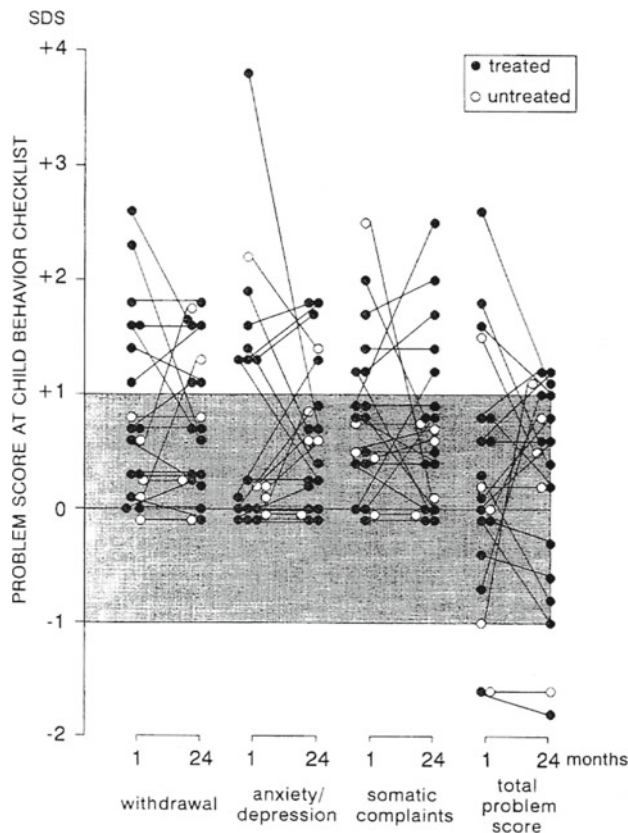


Fig. 2. Changes in problem scores for the Child Behavior Checklist (CBCL) (parent report) on 20 girls with central precocious puberty (PP) at 1 and 24 months. The shaded area represents the normal range. Reprinted with permission (18).

follow-up visits with some scores increasing, some decreasing, and some remaining the same.

Baumann and colleagues (19) recruited 19 girls who had been treated for PP but were at an average age of 18 years (range 14.3–21.9) at the time of the study. Parental report on the CBCL indicated elevated internalizing and total behavior problem scores compared with norms. Furthermore, there was a positive correlation of age at the onset of PP with both Total Behavior Problem and neuroticism subscales of a personality inventory. From self-report, girls with a history of PP did not report elevated scores on either a personality inventory or a questionnaire that focused on attitudes and awareness of one’s body (19). It is unclear whether the reports of the CBCL requested current behavior of daughters or past behavior during the treatment of PP. Importantly, the age range of the participating girls was outside the range that the parent CBCL was designed for if in fact the parents reported on current behavior.

Several important points should be emphasized regarding the aforementioned studies on PP. First, most of the studies reported were on girls with PP, as idiopathic PP is less common in boys. Studies with boys had smaller sample sizes, were primarily case reports, and included boys with PP due to multiple etiologies rather than idiopathic PP.

Therefore, based on the literature, generalizing behavior in PP boys was much more difficult. Second, it should also be noted from the studies above that PA or PP generally was defined as occurring before the age of 9 in girls and the age of 10 in boys. If one subscribes to more recent literature that states that puberty is now earlier, particularly in girls (20), then many of the studies mentioned on PA or PP were conducted in children who are now seen as developing at a normative age of puberty.

HOW CAN WE BETTER UNDERSTAND WHY PSYCHOSOCIAL PROBLEMS MAY OCCUR IN CHILDREN WITH PA OR PP?

Theoretical Rationale

There are theoretical reasons why psychosocial problems are suspected in children with PA or PP. Off-time puberty has been examined in the psychological literature since the 1930s (21–23). Continuing from that time until now, numerous studies have examined the issue of off-time puberty with respect to psychosocial functioning in adolescents *without* an endocrine disorder. That is, “off time” is defined as relative to peers (e.g., earlier or later than a target group) but not clinically early or late. Much can be learned from these studies through their application in understanding psychosocial functioning in PA and PP children.

From a theoretical perspective, many studies on timing of puberty have utilized specific research frameworks to explain the influence of physical maturation on primarily negative outcomes such as behavioral problems, adjustments, and mood disturbances. First, for example, the *maturational deviance hypothesis* (24–28), also referred to as the *off-time hypothesis*, emphasizes a greater degree of stress and adjustment difficulties experienced when pubertal development is off time (early or late) as compared with on time. This increased difficulty is thought to be a result of fewer available resources, including social support, that are more accessible to children/adolescents who develop in the same fashion as their on-time peer group. A second hypothesis directing research on timing of puberty is the *early maturational or early timing hypothesis* (24–27). In line with this hypothesis, early pubertal development is viewed as a disadvantage, as many of the normal developmental tasks of middle childhood were missed (24). Furthermore, as early maturing adolescents look more physically developed, society may view them as older and, in turn, more developed in the social and cognitive arenas (24). When this mismatch in physical, emotional, and cognitive development occurs, the young adolescent may experience greater social pressures. As a result, the early maturer may engage in more risky behaviors with older peers or may be placed in situations normative for older peers to address but that he/she is too emotionally immature to handle.

The literature is relatively inconsistent regarding gender differences and the effects of pubertal timing on psychosocial and cognitive outcomes. Often this inconsistency is due to the fact that both puberty and pubertal timing are examined using varied methodologies (e.g., self-report, parent report, physical examination, age at menarche, and height velocity), although all methodologies conclude that they reflect pubertal timing. Generally speaking, early pubertal timing in girls and later pubertal timing in boys are seen as more disadvantageous than later timing in girls and early timing in boys. However, there are exceptions to this. It is beyond the scope of this chapter to review all of the timing of puberty literature. For further discussion on

the methodological issue of measuring puberty and for specific studies on timing of puberty, see Dorn and colleagues (29,30). In brief, early pubertal timing in girls generally is viewed as more negative; it is related to more psychopathology (31), more suicide attempts (32), depressed mood, depressive symptoms, or internalizing problems (33–35). Additionally, early maturation in girls has been related to greater dissatisfaction with height and weight, decreased happiness, a more negative body image (24,36–38), more frequent interaction with deviant peers (39), and engagement in more risky behaviors such as drinking, smoking, or earlier sexual experiences (40,41).

For boys, later timing of puberty is often seen as more negative. For example, later maturing boys had lower self-esteem or confidence, less happiness (42,43), lower achievement (44), more psychopathology (31), and more depressed mood (35). There are exceptions, however, and studies can be found reporting no influence of timing of puberty or the opposite influence of puberty than was stated above. For example, participation in deviant or antisocial behavior (37), association with deviant peers (45), and more external hostile feelings (46) were more likely to be reported in early maturing boys, whereas Andersson and Magnusson (47) reported that alcohol use was more common in late and early maturing boys.

From a theoretical perspective, the early timing hypothesis would be particularly relevant to studies with PA and PP as both disorders represent early pubertal development. The earlier empirical findings and theoretical underpinnings can guide future research with PA and PP children. Although there are no external signs of puberty (visible when clothed) when PA initially begins, PA children may be taller or have acne and body odor, indicating that they may be older than their actual chronological age. Parents who have seen their PA children undressed also may begin having higher expectations for their children because they are now more physically mature. In the case of PP, pubertal development is often evident to those interacting with the child because of advanced height and breast development. Thus, interactions and expectations may be different with this early developer than with an on-time developing child. It should be noted that all of the literature on timing of puberty previously mentioned was with young adolescents who were “earlier” or “later” than peers in their timing, not those clinically early or late. One may expect more of a mismatch in the development and behavior of PA or PP children compared with adolescents with PA or PP because the children are even more off time with respect to age-matched peers.

Hormonal Influence on Behavior: Research Frameworks

In PA and PP, one presumes that the early hormonal changes are contributing to behavioral changes that may occur. Over the last 20 years, there have been advances in the understanding of the relationship of hormones and behavior (48,49) (*Fig. 3*). Initially, models of study focused on hormones directly influencing or “causing” behavior (Model a of *Fig. 3*). However, the term causal is relatively strong, particularly because many of the studies were not longitudinal in nature, thus preventing the determination of true causation. Despite this, the literature focused on increases or decreases in hormone concentrations and evidenced changes in the expression of the stated emotion or behavior. For example, in patients with Cushing’s syndrome, higher cortisol was related to more depression. This initial model was also used in the first generation of studies examining hormones and behavior in healthy adolescents (50–56) where specific hormones were related to higher or lower reports and observations of

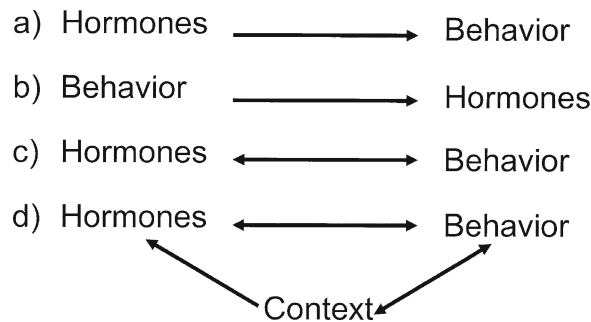


Fig. 3. Advances in the field of hormone behavior research where first hormones were viewed as “causing” behavior changes (Model a), behaviors “causing” hormone changes (Model b), the bidirectional hormone–behavior relationship (Model c), and finally where context is viewed as an important mediator or moderator in the hormone–behavior relationship (Model d).

such things as aggression or depressive symptoms. Although there was a significant relationship between the hormone and the behavior, one hormone usually accounted for less than 5–10% of the variance in the behavior, whereas multiple hormones may have accounted for as much as 50–60% of the variance. Thus, from Model a, explaining the true cause of the behavior remained a mystery.

Studies using Model b of *Fig. 3* focused on a behavior influencing a hormone. For example, testosterone was elevated in men before they competed in a tennis match (57), and when winning a tennis match, testosterone also was increased (58).

Model c of *Fig. 3* increased in complexity and examined the bidirectional hormone–behavior relationship. This model can be illustrated by considering anorexic eating behaviors and how they may change the hormone concentrations reflecting the stress axis. In turn, these higher hormone concentrations may contribute to the altered eating behaviors and to the resultant vicious cycle seen in these girls.

Model d of *Fig. 3* incorporates the notion that context may influence the hormone–behavior relationship. In this case, context may include the environment that the individual is in (e.g., single-parent household and inner-city neighborhood), or it may be more internal and refer to the genetic make-up (e.g., specific genetic markers) of an individual. One excellent example of Model d is the case of psychosocial dwarfism where emotional deprivation may result in delayed puberty and/or short stature (59). Children with psychosocial dwarfism often show signs of depression (60) and decreased growth hormone (GH); however, levels of GH normalize once the child is taken out of the aversive environment (61).

In line with the last model, hormones may also mediate or moderate a behavior. Hypotheses can be tested using mediation or moderation models as described by Baron and Kenney (62) and Holmbeck (63) where hormones or the context may be designated as a mediator or a moderator. It should be noted, however, that even in using the more advanced Model d, hormone–behavior relations can be more complex than simply considering context. Patterns of hormone release may be as important as the concentration itself. For example, change in cortisol to a stress paradigm (cortisol reactivity) or diurnal rhythm of the hormone may be more strongly related to certain behavioral changes than the actual concentration of the hormone in addition to contextual contributions.

Many of the more recent studies with adolescents that examine hormone–behavior relationships now include a contextual variable in the model. Model d also may be useful to studies of PA or PP. Such studies will be more likely to explain the causation of the behavior and factors that may contribute to the expression, or lack thereof, of psychopathology in these children. For example, context in the case of PA may be a genetic variant that is expressed in only some PA children. Thus, in order to exhibit a certain behavior, a certain genetic variant must be present along with other factors or in a specific environment.

Organizational and Activational Effects of Hormones

Hormonal increases evident in PA or PP may be related to behavior dependent on the type of actions of the hormones. Hormones can be considered organizational or activational in nature. Organizational effects are defined as providing in utero programming effects on the brain for a certain physiological or behavioral function that is expressed later in development. Alternatively, activational effects occur postnatally when hormone concentrations change or influence a structure or function of an organ system or a behavior. Hormones that change during puberty or across the menstrual cycle are considered activational.

There is a body of literature that supports organizational effects of hormones that are implicated in later development of adult hypertension and other expressions of the metabolic syndrome. In some children with PA, it is thought that organizational effects of hormones may lead to lower birth weight or small for gestational age, which in turn may present numerous physiologic problems in later childhood or adulthood (64). Seckl provides an excellent review regarding evidence for programming effects of glucocorticoids and their influence on later pathology (65,66). It may be that higher stress (with an increase in glucocorticoids) at a specific time in gestation interrupts or alters the trajectory of development of the brain or hypothalamic–pituitary–adrenal (HPA) axis and consequently predicts pathology in adulthood. Thus, in the case of PA and PP, hormones may be influencing behavior either from an organizational (e.g., glucocorticoids in fetal development) or from an activational (e.g., adrenal androgens at adrenarche) perspective.

Brain Changes During Puberty

Psychosocial and behavioral problems also may occur in vulnerable children with PA or PP because of a mismatch between physical development with emotional and cognitive development throughout the brain's development. To date, the literature does not provide any evidence of how or if the brain is changing in these early (off time) pubertal children. It is still unknown whether changes occurring in the early maturing children are the same as those in their age-matched peers or their pubertal stage-matched peers. One must abstract information from healthy non-endocrine-disordered adolescents that is relevant to changes in the brain during puberty and adolescence. Furthermore, the burgeoning area of research on brain development in adolescence is extensive and a detailed description is beyond the scope of this chapter. Therefore, a brief overview of highlights will be outlined in the next paragraphs of this section. The reader is referred to Dahl and Spear (67), Giedd and colleagues (68), Sisk and Foster (69), and Weinberger and colleagues (70) for a more thorough discussion.

Over the last 10 years or so, dramatic strides have been made in understanding the development of the brain during childhood and adolescence. Animal models have contributed greatly to understanding brain development, as such models offer more opportunity for studying structure and function with more inclusive and invasive methods than those with humans. In children, much of this new information is due to more advanced technology including magnetic resonance imaging (MRI) and functional MRI (fMRI).

Brain development and growth continue beyond infancy and early childhood and into adolescence when very dramatic changes occur. These changes are measurable and occur both in the structural and in the functional processes. One important developmental change that occurs in the brain during adolescence is the concept of “pruning.” Initially, the brain has many more cells and connections than necessary, and a competitive process of elimination of these cells called pruning begins just prior to puberty. Later in adolescence, a second pruning occurs to increase the efficiency of the brain particularly for information processing (70). Myelination also is increased during this time, and there is significant remodeling of synapses.

Changes in the brain occur in many areas. In an excellent longitudinal study, Giedd and colleagues (68) show age-related changes in different parts of the brain from the age of 4 to 22 years.

In *Fig. 4*, peak age of change is denoted by the arrows. For example, note that the frontal gray matter has a later peak in boys (age 12) compared with girls (age 11.5) and that the peak of temporal gray matter is at “greater than 16 years of age,” indicating that different parts of the brain develop at different rates. Additionally, tremendous individual variability is evident in cerebral volume as shown by the top left block in the figure.

Changes in the hippocampus, the amygdala, and the caudate nucleus are seen during adolescence. These areas are important for new memories, learning new routines, and processing emotional information (70). Levels of neurotransmitters also change. One of the last changes in the brain is the maturation of the prefrontal cortex (PFC). This area of the brain is important for higher functions including such aspects as mature decision making, impulse control, social conduct, goal setting, organization, and insight. The interconnectivity among various parts of the brain and the complexity of the brain is increased, which allows for processing more complex information (70). All of these developmental processes of the brain are likely to be influenced by hormones, genes, and/or the environment. The issues that arise in adolescent brain development often concern a mismatch of intellectual capabilities and emotional control/maturity and planful behavior. Additionally, in adolescence, there may be more of a response to stress and less sensitivity to rewards (71). This mismatch and change in sensitivity may lead to poor decision making and engagement in risky behaviors that make adolescents vulnerable to potentially negative outcomes.

To our knowledge, there are no research studies regarding brain changes in children with PA or PP that incorporate the use of the MRI or fMRI. Whether the early organizing or activating effects of glucocorticoids, adrenal androgens, or gonadal steroids change the normative trajectory of brain development remains unknown. The specific role of puberty in brain development is still under exploration, and specifically, the role of early puberty is unknown. The hormones that change in PA and PP may

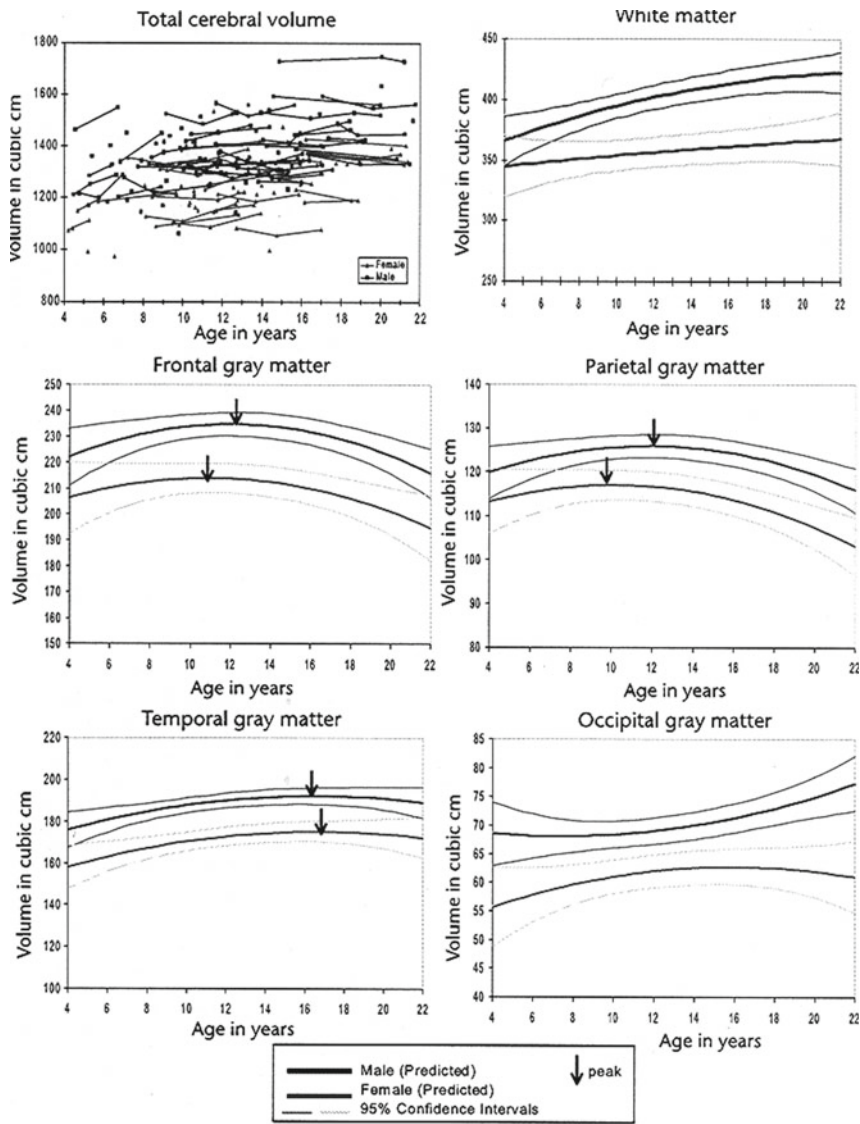


Fig. 4. Predicted size with 95% confidence intervals for cortical gray matter in frontal, parietal, temporal, and occipital lobes for 243 scans from 89 males and 56 females, aged 4–22 years. The arrows indicate peaks of the curves. Reprinted with permission (68).

have activating effects on the brain and in turn influence behavior. The behavioral problems and psychopathology evident in some of these children may be partially explained by the immature brain. However, according to Dahl (72), there are some changes in the brain that occur prior to the endocrine and body changes of puberty, some changes that occur as a consequence of pubertal processes, and some changes that are independent of pubertal processes. Therefore, puberty should not be viewed as entirely responsible for changes in the brain during adolescence or preadolescence.

IMPLICATIONS FOR THE PRACTITIONER REGARDING PSYCHOSOCIAL ISSUES IN CHILDREN WITH PA OR PP

From both theoretical and empirical perspectives, there are reasons to believe that PA or PP may have a psychosocial component. Although there is certainly variability in the frequency of psychopathology in children and adolescents with PA or PP, many would agree that some children may be at risk of psychopathology and thus vigilance is in order. When a primary care provider or an endocrinologist evaluates a child with PA or PP (or a history of such), the following suggestions may be relevant. First, a determination of the child's psychological and social adjustment is necessary. In addition to questioning the parent and child during the examination, there are simple checklists (e.g., CBCL) (4) that could be completed by the parent. These standardized checklists focus on moods, adjustments in the home, relationships with peers, and accomplishments in school. There are also checklists that can be completed by teachers (73) as well as the children themselves (5,6,74). It would also be important to have a comparison of information on how the child was before and after PA or PP was evident. Furthermore, at each follow-up visit, reassessing the psychosocial development of the child would be important. As there are few longitudinal studies that have prospectively followed girls with PA or PP, the long-term ramifications of early development are still unclear.

Second, parents may need information about raising a child with PA or PP from the perspective of pubertal changes, reproductive function, and social changes. Being "off time" from peers can be an adjustment for both parents and children. Parents may not be ready to discuss issues of puberty with their young child, and they may not be knowledgeable about PA or PP and the early maturation that may involve menstruation, sexual maturation, and the like. Parents also need to be aware that some children are at risk of emotional and behavioral problems, or at the least, at risk of adjustment difficulties. Knowing that behavioral problems may be related to internal as well as external changes may be helpful. For example, there is a great deal of variability in the phenotype of PA. Some PA children are much taller, heavier, or more hirsute, whereas other PA children may be more within the normal range of height and weight. Such phenotypic differences may have an impact on behavior because of the responses from peers, teachers, or other adults. Anticipation of what may happen behaviorally and why such behavioral changes occur may be useful for parents.

Third, if a child currently has emotional, behavioral, or achievement problems that interfere with individual development and family functioning, the practitioner may want to consider an appropriate referral to a child psychologist or other specialist for a more complete evaluation. Evaluation and intervention before problems become more serious are more likely to result in positive development later in adolescence and adulthood.

DIRECTIONS FOR FUTURE RESEARCH

Clearly, there is a dearth of confirmatory evidence regarding psychosocial differences between children with PA or PP and those who are developing on time. Based on the limitations of the state of knowledge, the following is suggested for future research.

Inclusion of a Comparison Group

Many studies of these endocrine-disordered groups often did not have a comparison group. Without a comparison group, definitive conclusions cannot be made regarding what normative behavior is for this age group. Comparison group children should be carefully selected. Depending on the hypotheses of the study, children may need to be matched on various factors (e.g., age, race, and BMI). Matching can be difficult and may mean that many additional children must be screened through questionnaire and physical examination in order to determine eligibility. Study costs rise because of the increased number of screens. Also, there have been suggestions to match children based on pubertal stage, but again, the goal of the hypothesis should be addressed prior to making the decision on a criterion for matching. However, when matching on pubertal stage, chronological age would still be a confounding factor.

Multisite Studies

To enroll adequate numbers of children with PA or PP in a research study, it is likely that a multisite study is necessary. Such a study necessitates increased funding that may be more difficult to obtain. Additionally, fewer PA and PP children may be available for research participation, as early puberty is often viewed as normative by parents and many primary care providers. Thus, there may be fewer referrals made to endocrine clinics and to research studies seeking PA or PP participants. However, there is support that referral for further evaluation may be indicated for some of these children with early puberty (2,75,76).

Longitudinal Psychosocial Studies

Prospective, longitudinal studies of children with PA or PP are virtually non-existent. Following these children may yield important changes in psychosocial development as they progress into true puberty and young adulthood. Following children offers an opportunity to identify factors that exacerbate psychosocial problems or factors that may make a child more resilient from psychosocial problems both initially and at follow-up. Clearly, the latter is important, as not all children with PA or PP have psychopathology.

Use of Other Research Methodologies

To examine the psychosocial aspects of PA or PP, it would be ideal to conduct a study simultaneously with either routine clinical care or another study obtaining hormone concentrations. In this case, blood samples could be drawn for a biobehavioral study without an additional venipuncture. In past studies, we have found that some parents and/or children are reluctant to have an additional venipuncture, which results in their elimination from the study.

Newer, less invasive methodologies for examining biological contributions to behavioral changes may also be considered in future studies. Such methodologies, void of a venipuncture, may enhance participant recruitment. For example, there are now many hormones that can be measured successfully in saliva samples (77–80). (See also <http://www.salimetrics.com>.) To our knowledge, a salivary assay sensitive enough to measure prepubertal concentrations of estradiol does not exist, but there is an assay

that has been used for estradiol in both premenarcheal and postmenarcheal females (80). [See Dorn and colleagues (29) for further discussion of the limitation of this assay in prepubertal age boys and girls.] Some hormones can also be measured in urine samples (e.g., cortisol), and urinary gonadotropins have been used in studies of puberty since the 1960s (81,82). Other methodologies for examining biological parameters of development in PA or PP children include MRI and fMRI. These instruments note developmental changes in brain structure and function, all of which may change during puberty.

CONCLUSIONS

Psychosocial problems are evident in some children with PA or PP. Many of the studies regarding psychosocial changes in children with PP or PA are cross-sectional and suffer from many methodological problems. Therefore, the literature is inconclusive regarding the existence of truly psychosocial issues in children and adolescents with PA or PP. Just as in other disorders, be it in an endocrinopathy (e.g., hyperthyroidism) or in a pulmonary disorder (e.g., asthma), signs and symptoms vary among those who are affected. Therefore, one does not expect all PA or PP children to exhibit psychopathology. Based on these observations, further study of psychosocial aspects of development in PA and PP children is necessary. The state of science with respect to both theoretical and empirical findings regarding hormone–behavior relationships further supports continuing research. However, until such studies are completed, there is enough evidence to support primary care providers' role in both vigilant monitoring of the psychosocial development in these children and anticipatory guidance to parents regarding possible psychosocial changes in those with PA or PP.

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V

GONADOTROPIN-DEPENDENT PRECOCIOUS PUBERTY

15

Etiology of Gonadotropin-Dependent Precocious Puberty

*Erik A. Imel, MD and
Kathleen E. Bethin, MD, PhD*

CONTENTS

INTRODUCTION
ETIOLOGY
CONCLUSIONS
REFERENCES

Summary

Precocious puberty is puberty occurring earlier than 2.5 SD below the mean. In boys, this is before age 9. In girls, the age is controversial, but classically, has been considered age 8 for white girls and 7 for African American girls. Precocious Puberty can be centrally or peripherally mediated and is more common in girls than boys. In central precocious puberty (CPP) the concern is to identify whether the etiology is pathological or idiopathic. In girls, the cause of CPP is usually idiopathic but in boys a central lesion is more common. The most commonly identified cause of CPP is the hypothalamic hamartoma. A head MRI should be considered in any child with CPP to rule out occult intracranial lesions.

Key Words: Central precocious puberty; GnRH; Etiology; Gonadotropin –dependent; Idiopathic; Intracranial.

INTRODUCTION

Definition

Normal puberty occurs when gonadotropin-releasing hormone (GnRH) secretion is reactivated. In girls, the first signs of puberty include breast development (thelarche), estrogenization of the vaginal mucosa, uterine maturation, redistribution of fat, growth of the labia minora, growth acceleration, and rarely menarche (1,2). Development of sexual hair (adrenarche) usually occurs around the same time but is the result of androgen production from the adrenal glands. In boys, puberty is manifested by enlargement of the testes to greater than 3 cc in volume or 2.5 cm in length, enlargement

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of the penis, sexual hair development, increase in muscle mass and decrease in body fat (3). In healthy children, the onset of normal puberty may occur as early as 7 years in African-American girls and as late as 14 years in boys.

Precocious puberty is puberty occurring earlier than 2.5 SD below the mean (*Fig. 1*). For girls the lower limit of normal puberty in the United States has been widely debated and is the subject of Part II of this book (4–9). Classically, it had been considered abnormal for Caucasian-American girls to start puberty before the age of 8 (1,10–12). The Lawson Wilkins Pediatric Endocrine Society issued a statement based on the data published by Herman-Giddens et al. that unless there are symptoms suggesting pathology, Caucasian girls who start puberty after the age of 7 and African-American girls who start puberty after the age of 6 should be considered normal (4,7). However, many endocrinologists recommend that Caucasian girls who start puberty before age 8 and African-American girls who start puberty before age 7 be considered precocious (9,13). Boys who begin puberty before age 9 are considered precocious and should be evaluated (10,14).

Incidence

Central precocious puberty (CPP) occurs in about 1/5000 to 1/10,000 children and is 10–23 times more common in girls than in boys (15–17). The most likely etiology of CPP depends on the gender of the child. In girls, the cause is more likely to be idiopathic (70–90%), whereas identifiable lesions of the central nervous system (CNS) account for 60–94% of cases of CPP in boys (15,16,18–21).

Diagnosis

During the evaluation of a child with precocious puberty, it must be determined whether the child has central (activation of the hypothalamic–pituitary–gonadal axis)

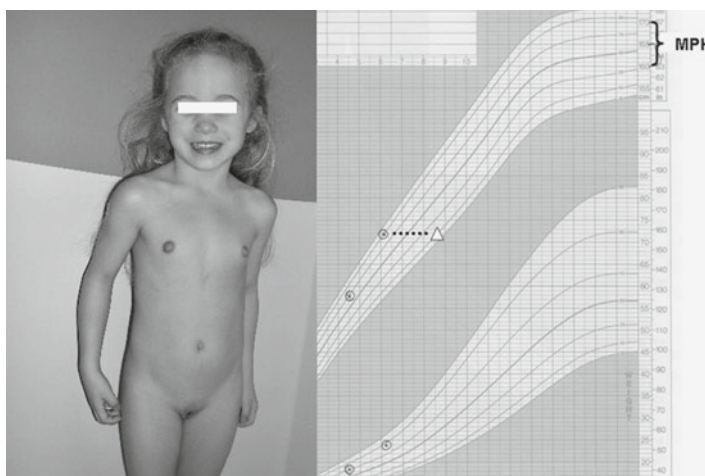


Fig. 1. Photo of 6-year-old female child with idiopathic central precocious puberty. Shown on the right is her growth chart. The dots with circles represent her growth points. The dotted line to the triangle demonstrates her bone age. MPH indicates her midparental height. Picture Courtesy of Dr. Emily Walvoord.

or peripheral (gonadotropin-independent) puberty. In gonadotropin-independent precocious puberty (GIPP), gonadotropins are low while sex steroids are in the pubertal range. However, in CPP, random gonadotropins may still be below the lower limit of detection of standard LH assays. Using ultrasensitive assays, spontaneous levels of luteinizing hormone may help in diagnosing central puberty (22). In this small study, all 39 patients with an ultrasensitive LH (luteinizing hormone) ≥ 0.3 mIU/ml had a stimulated LH ≥ 8 mIU/ml, consistent with central puberty.

Classically, LH, FSH, and estradiol or testosterone are measured after stimulation with GnRH. Patients are considered to have a prepubertal response to GnRH if the peak LH is $< 7 \mu\text{g/l}$ when determined by a third-generation assay (23), < 15 IU/l for girls and < 25 IU/l for boys when measured by radioimmunoassay (RIA) (24), < 5 IU/l when determined by immunochemiluminometric assay (ICMA) (25), or < 6.9 IU/l for girls and < 9.6 IU/l for boys when measured by fluoroimmunoassay (FIA) (26). Since the unavailability of GnRH, stimulation tests with an aqueous GnRH agonist have been used to diagnose CPP (27,28). All of the patients in the Ibanez study with normal puberty or progressive precocious puberty had stimulated LH levels ≥ 8 mIU/ml, measured by immunoradiometric assay (IRMA) (27). However, in the Garibaldi study, a group of patients in early central puberty were identified who had a pubertal, 12–24-h, stimulated estradiol peak ≥ 92 pmol/l but a peak LH response of only 4.8 ± 1 mIU/ml, measured by IRMA (28). Therefore, when doing a GnRH agonist stimulation test, it is important to measure both the stimulated LH and the estradiol or testosterone 12–24 h after stimulation.

In girls, a pelvic ultrasound may be useful in diagnosing CPP. The ovarian volume may be calculated using the ellipse formula. The largest longitudinal diameter (A) is multiplied by the largest anterior–posterior diameter (B), the largest transverse diameter (C), and 0.5233 ($A \times B \times C \times 0.5233$). Prepubertal girls will have an ovarian volume of less than or equal to 1 cm^3 (29,30).

ETIOLOGY

Genetics

The etiology of idiopathic CPP is the subject of many investigations, yet it still remains elusive. In a study of 453 children referred to a center in Israel for precocious puberty, 156 (147 girls and 9 boys) of these children had idiopathic CPP (31). About 27.5% of the children with CPP (42 girls and 1 boy) had a family history consistent with familial precocious puberty and suggestive of autosomal dominant inheritance in the females. Previously, there have been a few reports of familial cases of CPP in girls. The other large study found that a much smaller fraction (5.2%) of cases were familial (32–34). As has been found in the timing of normal puberty, there appears to be a large genetic factor in the etiology of idiopathic CPP (35,36).

Idiopathic Versus Pathological

When CPP is diagnosed, a head MRI is usually done looking for occult intracranial tumors. However, most girls with CPP, and many boys, do not have identifiable intracranial lesions on neuroimaging studies. In a small study in the Netherlands, 30 (29 girls and 1 boy) out of 54 patients being followed for CPP in 1990 with a previously normal head CT agreed to a head MRI (37). In this population of children with

CPP of unknown etiology (i.e., normal head CT), 13% were found to have anomalies on head MRI. Three patients had hamartomas, and one had a low-grade glioma of the optic tract. In a retrospective study of 428 girls in Italy with CPP evaluated between 1988 and 1998, 304 underwent cranial CT scan and/or MRI (19). Neuroimaging abnormalities were present in 18.4%. Grouping the girls with CPP by age, 32.3% under age 4, 18.2% aged 4–6.9, and 16% aged 7.0–7.9 had CNS lesions. Although there was a trend toward an increased chance of finding pathology in the younger girls, this was not statistically significant. In a retrospective single-center study of 67 neurologically normal girls with CPP from the United Kingdom with neuroimaging studies performed between 1990 and 2001, 15% had CNS lesions detectable on MRI (38).

Given that the majority of CPP in girls is idiopathic in nature, investigators have attempted to define a population at higher risk of intracranial lesions to target for imaging studies. In the United Kingdom study, there were no reliable difference between patients with intracranial lesions and those without with respect to age of onset of puberty, stage of puberty at presentation, height or body mass at presentation, bone age advancement, or ultrasound findings (38). In 1997, the LWPES published a statement that white American girls over the age of 7 and African-American girls over the age of 6 who begin puberty without neurological signs, rapid bone age advancement, or rapid progression and with a predicted adult height within their genetic potential need not be considered abnormal (and thus do not need neuroimaging studies). Chalumeau et al. (39,40) evaluated whether applying this criteria to European girls with central puberty would miss any occult intracranial lesions (OICL). They did a retrospective analysis of all girls younger than 8 years of age with CPP seen in a pediatric hospital in Paris between 1982 and 2000 (39). Of the 197 girls who met the inclusion criteria, 11 had a CNS abnormality. Two of the patients with CNS abnormalities (one glioma and one hamartoma) had onset of puberty after age 7, a normal pubertal tempo and normal growth rates between -1.8 and 0.5 SD. In a second retrospective study at seven centers in Europe, all girls younger than 8 years of age with CPP were evaluated (40). Of the 443 girls in the study, 35 (8%) had OICL (hamartoma, $n = 23$; astrocytoma, $n = 7$; arachnoid cyst, $n = 2$; and other, $n = 3$). Nine patients (2%) were found to have incidental findings that the authors felt were not causative (thickening of the pituitary stalk, $n = 3$; empty sella, $n = 2$; pineal cyst, $n = 2$; or pituitary microadenoma, $n = 2$). The authors found that significant predictors of OICL were age of pubertal onset less than 6 years and estradiol concentration greater than the 45th percentile. This combination of age and estradiol concentration provided 100% sensitivity (95% CI, 90–100%) for predicting an OICL in neurologically normal girls (40). However, four of the patients with OICL (11% of the patients with OICL) were 7 years of age or older when puberty began, with only two of the four meeting the criteria for further evaluation based on emotional status and advanced bone age. Therefore, the sensitivity of the LWPES recommendations in this sample population was 94% (95% CI, 81–98%) (40). However, the specificity was low, and the authors were cautious about recommending use of any decision guideline to limit neuroimaging, if MRI is readily available. Although the risk of OICL is low if puberty begins after the age of 6 in girls and the estradiol level is $\leq 45^{\text{th}}$ percentile, a head MRI remains an important part of the evaluation of all children with CPP.

Hypothalamic Hamartoma

The most commonly identified CNS abnormality in CPP is a hypothalamic hamartoma. Although hypothalamic hamartomas are rare, with a prevalence of 1/50,000–1/100,000, they account for 27–56% of identifiable lesions in CPP (16,19,37,41). Hypothalamic hamartomas are congenital malformations consisting of heterotopic masses of normal neurons and glial cells, ectopically located at the base of the third ventricle or attached to the tuber cinereum (42,43). Hypothalamic hamartomas often cause precocious puberty and may be associated with gelastic or other types of seizures. These are nonneoplastic, and as such, they do not tend to grow over time (42). In a review of 277 patients with hypothalamic hamartoma, the number of male patients nearly equaled the number of female patients. Sixty-three percent of the patients had precocious puberty. Sixty-one percent of these patients had presented with seizures and 25% presented with both seizures and precocious puberty. Precocious puberty caused by hypothalamic hamartomas usually occurs before 4 years of age (16,42,44).

Some hypothalamic hamartomas have been shown to contain GnRH-producing neurons (44,45). It has been classically thought that precocious puberty results because these heterotopic GnRH cells are not under the normal inhibitory control of the intrinsic CNS. However, not all hypothalamic hamartomas contain GnRH-positive neurons. In two reported patients with CPP and hypothalamic hamartomas, the hamartomas lacked the expression of GnRH-positive neurons but were found to contain transforming growth factor alpha TGF- α -producing astroglial cells (46,47). In rodents and monkeys, hypothalamic TGF- α expression has been shown to increase during puberty, and implantation of transgenic cells expressing TGF- α into the median eminence induces puberty in juvenile female rats (48). The TGF- α -producing cells induced puberty only when located near GnRH cell bodies or GnRH nerve terminals and not when transplanted in regions of the hypothalamus lacking GnRH neurons. The mechanism by which TGF- α -producing astrocytes induce puberty involves the binding of TGF- α to the TGF- α /epidermal growth factor (EGF)-receptor stimulating a complex signaling cascade inducing cyclooxygenase, which catalyzes the rate-limiting step in prostaglandin synthesis. This results in an increase in PGE₂, which is a potent GnRH secretagogue (49).

These studies indicate that the mechanism for induction of precocious puberty by hypothalamic hamartomas may not only involve direct pulsatile release of GnRH, but also be caused by alterations of the signals controlling hypothalamic GnRH-producing neurons. In addition, it is likely that idiopathic precocious puberty is caused by locally abnormal signaling involving stimulatory or inhibitory pathways without a mass of abnormal cells or abnormally located cells.

Other Intracranial Etiologies

Other intracranial tumors may cause CPP by disrupting the inhibition or by direct stimulation of the GnRH-producing cells. These include astrocytomas, gliomas, ependymomas, craniopharyngiomas, dysgerminomas, neurofibromas, and tuberous sclerosis (50–55). Rarely, a pituitary adenoma may produce pulsatile LH release and precocious puberty (56). Metastatic lesions may cause CPP through similar mechanisms.

Other congenital anomalies including arachnoid cysts, septo-optic dysplasia, suprasellar cysts, and empty sella syndrome have been reported to cause CPP (Table 1) (40,57–59). In addition, tumors, congenital anomalies, inflammatory changes,

Table 1
Causes of gonadotropin-dependent precocious puberty

Idiopathic
Structural
Hypothalamic hamartoma
Tumors
Optic glioma
Astrocytoma
Craniopharyngioma
Dysgerminoma
Ependymoma
Neuroblastoma
Pituitary adenoma
Congenital anomalies
Hypothalamic and pituitary anomalies
Arachnoid cyst
Rathke’s cleft cyst
Hydrocephalus
Inflammatory/scarring
Trauma
Post-surgical
Post-irradiation
Post-chemotherapy
Meningitis, encephalitis
Intracranial abscess
Granulomatous disease
Metabolic
Nonketotic hyperglycinemia
Syndromes
Williams syndrome
Prader–Willi syndrome
Neurofibromatosis, type 1
Pallister–Hall syndrome
Tuberous sclerosis
Klinefelter’s syndrome
Triple X syndrome
Following chronic sex steroid exposure, that is, after treatment for gonadotropin-independent precocious puberty
Migration from underdeveloped to developed countries

or scarring may cause obstructive hydrocephalus, leading to compression of the hypothalamus and precocious puberty (60,61). Other acquired causes of CPP include inflammation, infection (abscess, encephalitis, or meningitis), granulomatous disease (especially tuberculosis), intracranial tumors, chemotherapy, cranial surgery, or cranial irradiation (62). Some cases of CPP, such as those due to obstructive hydrocephalus or intracranial abscess, may be reversible. The exact mechanisms underlying the development of pulsatile GnRH secretion in each of these cases are unclear.

Leptin

Weight outside the normal range also affects puberty. Obesity is associated with earlier normal puberty in girls, and severe weight restriction leads to amenorrhea. Leptin has been postulated to signal the body fat content to the hypothalamic–pituitary–gonadal axis. The leptin knockout mouse (ob/ob) is obese and has hypothalamic hypogonadism, both of which are corrected by leptin replacement, but not by dietary restriction (63–65). In a study of 55 girls and 10 boys with CPP, mean SD score for leptin levels was mildly increased in girls with CPP, but not in boys with CPP (66). This increase could not be explained by increased BMI alone. In another cohort of girls with CPP, there was no significant elevation in leptin SD scores (67). Patients with identifiable neurologic lesions had higher leptin concentrations than those with idiopathic CPP. In a series of patients with CPP treated with GnRH agonists, the girls with CPP had greater leptin levels than the boys with CPP (66). The leptin levels in the girls did not change during the course of treatment; however, the boys with CPP had an increase in leptin levels with GnRH agonist treatment and a decrease in leptin levels after the treatment was discontinued. Thus, although leptin does not appear to cause precocious puberty, it may have a permissive effect allowing the development of puberty at an earlier age.

Adoption from Third-World Countries

An increased incidence of CPP has been reported in young girls following immigration from third-world countries into developed areas when compared with peers in both the country of origin and the country of destination. This may be due to alterations in nutritional status, or possibly due to the presence of environmental endocrine disruptors in their country of origin (36). Rapid catch-up growth after a period of malnutrition has been postulated as a stimulus for precocious puberty in this population (68). It is postulated that estrogenic endocrine disruptor chemicals in the environment stimulate hypothalamic maturation, but also inhibited gonadotropins. On acute withdrawal of these compounds (i.e., by emigration), the hypothalamus begins to stimulate production of gonadotropins and CPP ensues (36). This appears to be a sexually dimorphic phenomenon, as girls are more commonly precocious in this population than boys. A full discussion of endocrine disruptors in puberty is the topic of chapter 20 and is a discussion of precocious puberty in internationally adopted girls is the subject of chapter 21.

Sex Steroid Exposure

Long-term exposure to sex steroids due to GIPP is also a known cause of CPP and is reported in patients with McCune–Albright syndrome, familial male-limited precocious puberty, congenital adrenal hyperplasia, and ovarian or adrenal tumors (69–72). This most commonly occurs following treatment of GIPP and may be the result of priming by the high levels of sex steroids releasing the normal inhibition of GnRH pulses or possibly due to treatment of the primary disorder releasing the negative feedback that the high levels of sex steroids had created. Consequently, treatment of CPP sometimes is necessary following treatment of peripheral forms of precocity.

Syndromes

Several syndromes are noted in association with CPP. Some, such as Pallister–Hall syndrome, neurofibromatosis, and tuberous sclerosis, cause CPP due to characteristic hypothalamic lesions associated with these disorders. In others, the reason for the finding is less clear. Prader–Willi syndrome is usually associated with hypogonadotropic hypogonadism and underdeveloped genitalia (73). However, idiopathic CPP has been reported in a small number of patients (73–77). Likewise, CPP has been reported in two patients with triple X syndrome, which is usually associated with premature ovarian failure (77–79). In a study of 171 female patients with Williams Syndrome the incidence of CPP was 1 in 5 to 1 in 6 (80).

Although Klinefelter’s syndrome is more commonly associated with delayed puberty and hypergonadotropic hypogonadism, an increased incidence of precocious puberty has been described. It has been estimated that precocious puberty occurs in Klinefelter’s syndrome five times more often than in the general population (81). Most cases of precocious puberty in Klinefelter’s syndrome are not centrally mediated but are due to germ cell tumors (81,82). However, CPP has been reported in Klinefelter’s syndrome, either related to a hamartoma or idiopathic in etiology (81,83–85). In the vast experience of one group, CPP occurred in 2/743 males with Klinefelter’s syndrome (83). Some authors have recommended karyotype analysis of all boys with precocious puberty and small testes, especially in the presence of a germ cell tumor (82,85).

Molecular Mechanisms

There are a number of potential mechanisms for CPP. Activating mutations anywhere along the signal transduction pathways of the GnRH neuronal network could lead to premature activation of GnRH release. Any such mechanism would need to be cell specific in order to cause isolated CPP, and possibly sex specific, as CPP is far more common in girls (15). One may also postulate that CPP may be a result of defects in the excitatory and inhibitory neuronal circuits involving the GnRH system. The main stimulatory regulator for GnRH release is glutamate via the N-methyl-D-aspartate (NMDA) receptor (86), while the main inhibitory regulator is the gamma-aminobutyric acid (GABA) receptor (87). These two signals counter-regulate each other, and derangement in these pathways may cause activation of pulsatile GnRH release and precocious puberty. Glycine, along with glutamate, activates NMDA receptors in the brain. High levels of glycine seen in the metabolic disease nonketotic hyperglycinemia may result in NMDA receptor stimulation, resulting in GnRH secretion. Nonketotic hyperglycinemia results from an enzymatic defect in the ability to cleave glycine and results in high CNS levels of glycine, hypotonia, and seizures. In a case report of an infant with nonketotic hyperglycinemia, the infant developed CPP within the first year of life (88). The antiepileptics used to treat this infant were loreclezole, which has stimulatory activity at the GABA receptor, and vigabatrin, an irreversible inhibitor of GABA transaminase. Treatment with these drugs resulted in regression of this child’s puberty. These results led the authors to hypothesize that CNS glycine could potentiate the NMDA-evoked release of GnRH and that the use of GABA agonists inhibited the release of GnRH. Bourguignon et al. (88) tested this hypothesis by incubating rat hypothalamic explants with increasing concentrations of

glycine resulting in a concentration-dependent increase in GnRH pulse frequency. This effect was partially inhibited by glycine antagonists and GABA agonists (88).

Other evidence of a role for GABA in tonically inhibiting prepubertal pulsatile GnRH release comes from studies in monkeys. GABA in the median eminence is higher in prepubertal than pubertal rhesus monkeys. Intraventricular infusion of GABA inhibited GnRH release in pubertal monkeys (87). Intraventricular infusion of a GABA_A receptor antagonist stimulated GnRH release in prepubertal monkeys with a lesser effect in pubertal monkeys. Infusion of a GABA_B receptor antagonist had no effect.

Data from several experiments link TGF- α but not epidermal growth factor (EGF) to the onset of puberty. In female rats, TGF- α , but not EGF, mRNA is detectable in the hypothalamus (49). Median eminence isolated from juvenile 28-day-old female rats incubated with TGF- α or EGF, but not TGF- β 1 or TGF- β 2, caused a concentration-dependent increase in LHRH release. This effect requires the prostaglandin PGE₂ and can be blocked with administration of indomethacin, a nonselective cyclooxygenase inhibitor. Incubation with RG-50864, a selective inhibitor of TGF- α /EGF receptor kinase, inhibits the LHRH release in response to EGF and TGF- α but not to PGE₂. Damage by radiofrequency to the preoptic anterior hypothalamic area of 22-day-old rats results in precocious puberty within 7 days (89). TGF- α mRNA expression (but not EGF) was 3.5-fold increased in the damaged area by 4–6 days after the insult, and reactive astrocytes near the lesions contained TGF- α precursors (89). Infusion of RG-50864 at the lesion site inhibited advancement of puberty after the radiofrequency-induced damage. Hypothalamic injury in 24-day-old rats also triggers a local increase in mRNA for the transcription factor Oct-2, which may up-regulate TGF- α (90). Furthermore, Oct-2 is up-regulated in the preoptic area of the hypothalamus and medial basal hypothalamus of female rats at the time of normal puberty, and infusion into the third ventricle of antisense RNA to Oct-2 significantly delays puberty in rats (90). Many forms of brain injury including trauma, radiation, infection, or inflammation are known to cause CPP. In this setting, activation of local TGF- α from reactive astrocytes is likely to be involved in the pathogenesis of precocious puberty. Activation of TGF- α may be involved in normal puberty as well.

CONCLUSIONS

In summary, CPP may be caused by a variety of insults to the CNS in general and specifically to the hypothalamus. The most commonly identified lesion is the hypothalamic hamartoma, although, in girls with CPP, no specific cause is usually identified. As the most concerning possibility is an intracranial malignancy or other lesion with a mass effect, evaluation should include a detailed neurologic history and physical examination. A head MRI should be strongly considered in all patients with CPP to evaluate for occult intracranial pathology.

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16

Treatment of Gonadotropin-Dependent Precocious Puberty

Zeina M. Nabhan, MD
and Emily C. Walvoord, MD

CONTENTS

INTRODUCTION
DIAGNOSIS OF CPP
INDICATIONS FOR TREATMENT OF CPP
TREATMENT OPTIONS
MONITORING THERAPY
OUTCOMES OF GnRHa TREATMENT
COMBINATION OF GnRHa AND GH
COMBINATION OF GnRHa AND OXANDROLONE
CONCLUSIONS
REFERENCES

Summary

Gonadotropin dependant precocious puberty is a heterogeneous disease with variable clinical presentations ranging from incomplete forms of precocious pubertal development to rapidly progressive forms. Although there is some controversy about the indications for treatment of central precocious puberty, in this chapter we review and clarify the indications as well as summarize the various methods available for monitoring therapy. Gonadotropin releasing hormone agonists (GnRHa) are currently considered the treatment of choice for central precocious puberty (CPP). Long-term outcome studies have shown that GnRHa have completely reversible suppressive effects on the hypothalamic-pituitary-gonadal axis, preserve final adult height, and have no long-term effects on bone mineral density and body composition in children with CPP. Newer therapies for use in CPP such as GnRH antagonists and third generation aromatase inhibitors are currently being studied and may be additional treatment options in the future.

Key Words: Gonadotropin-dependent precocious puberty; GnRH agonists; Oxandrolone; Bone density; Skeletal maturation; Final height; Growth hormone.

INTRODUCTION

Precocious puberty is usually defined as the onset of puberty before the age of 8 years in girls and 9 years in boys (1). Precocious puberty is classified as either central

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(gonadotropin dependent) or peripheral (gonadotropin independent). In this chapter, we review the current indications for the treatment of central precocious puberty (CPP), treatment options, monitoring strategies, and outcomes.

DIAGNOSIS OF CPP

CPP is much more common in girls than in boys (2) with an overall incidence of 1:5000 to 1:10,000 (3). Approximately 20% of boys with CPP have an underlying central nervous system (CNS) lesion (4), whereas less than 5% of girls do (5). In addition to having physical signs of puberty such as rapid growth, breast development in girls, and genital growth and testicular enlargement in boys, patients also need hormonal evidence of CPP prior to the initiation of therapy.

Hormonal verification of CPP involves demonstration of pubertal levels of gonadotropins. The intravenous gonadotropin-releasing hormone (GnRH) stimulation test is considered the gold standard for confirming CPP (6,7). Classic pubertal responses are characterized by a luteinizing hormone (LH) peak above the upper limit of the prepubertal range, with the LH level also being greater than the follicle-stimulating hormone (FSH) level. Using radioimmunoassays (RIAs), a peak LH ≥ 10 IU/l in girls or > 15 IU/l in boys or an LH/FSH ratio higher than 0.66 is diagnostic of CPP (8,9). Using immunofluorometric assays (IFMA), an LH peak > 6 IU/l in girls and > 10 IU/l in boys or an LH/FSH ratio greater than 0.3 is considered diagnostic of CPP (6).

Alternatives to intravenous GnRH stimulation test also exist. A single LH level > 8 IU/l by immunochemiluminometric assay (ICMA) 40 min following 100 mcg of subcutaneous GnRH was found by Eckert et al. (10) to be a valid tool for laboratory confirmation of CPP, whereas Neely et al. (11,12) showed that a random LH ICMA level of > 0.3 IU/l had 100% specificity for diagnosing CPP and correlated very well with a pubertal response to GnRH stimulation testing. Urinary gonadotropins have been studied in the evaluation of CPP; however, further work is needed to determine the feasibility of this test (13).

INDICATIONS FOR TREATMENT OF CPP

Left untreated, patients with progressive CPP will have accelerated growth rates resulting in tall stature during childhood yet decreased adult heights as a consequence of rapid advancement in skeletal age limiting the time for linear growth (14). In addition, children with CPP appear more physically mature than their peers, making interaction with peers and older individuals difficult (14). However, CPP is not a homogeneous disease. On the contrary, it represents a spectrum ranging from normal variants or incomplete forms of precocious pubertal development to slowly progressive or transient forms to rapidly progressive forms (15,16). Therefore, there is no consensus on the indications for the treatment of CPP (17); however, there is consensus that not all patients with CPP need medical therapy to preserve adult height (17–19); thus, the decision to treat should be individualized. *Table 1* provides a summary of the different published guidelines to consider when determining the need to treat CPP.

Behavioral and Psychological Indications

Although there are very few well-designed studies available in the literature, behavioral and psychological problems have been described in children with CPP. Sonis

Table 1
Indications for treatment in children with central precocious puberty (CPP)

Indication for Treatment	Clinical Variable
Precocious puberty/age of onset	Complete and rapidly progressive precocious puberty (17–19,24) Age of onset before 7.5 years (23)
LH response to GnRH stimulation	Pubertal response LH (RIA) \geq 10 IU/l (girls); $>$ 15 IU/l (boys) (6) LH (IFMA) $>$ 6 IU/l (girls) and $>$ 10 IU/l (boys) (8)
Testosterone level (boys)	$>$ 100 ng/dl (24)
Estradiol level (girls)	Not reliable for diagnosing CPP in girls with conventional assays (17,24)
Age at menarche	Before age 7 years (24) Before age 9.5 years (18)
Bone age (BA)	Advanced BA $>$ 2 years over chronological age in patients with pubertal responses to GnRH (19)
Predicted adult height (PAH)	PAH $<$ 152.5 cm (girls) or 155 cm (boys) (17) PAH $<$ 5th percentile (18) PAH falling significantly (18)
Psychosocial factors	Menses in mentally or emotionally immature person (18) Behavioral or emotional disturbance (18)

GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

et al. (20) found that 27% of their patients with precocious puberty appeared to be more depressed, socially withdrawn, aggressive, and moody compared with a control group. However, a follow-up study of girls with idiopathic precocious puberty at 17.5 years of age found no significant lasting psychological effects, except a tendency for excessive psychosomatic complaints (21). More recently, evidence based on scores of internal and total behavior from the Child Behavior Checklist suggests some increased neuroticism, accentuation of physical appearance, and insecurity among girls with CPP (22). Because of individual variation among children in coping skills and readiness for adolescent development, some authors (19,23) do not consider psychological and behavioral problems as potential indicators for the treatment of CPP, whereas others believe that the psychological and behavioral indications for CPP treatment need to be tailored on an individualized basis (14,17,18).

Auxological and Hormonal Indications

With respect to the auxological indications for CPP treatment, there is also no general agreement. Some authors have based their indications on predicted adult height (PAH: $<$ 152.5 cm in girls or 155 cm in boys) (17), age of puberty onset (before 7.5 years) (23), or specific bone age (BA) advancement ($>$ 2 years advanced compared with chronological age) (19), whereas others have used only biochemical criteria such as pubertal gonadotropin levels (LH $>$ 9 IU/l) (19), testosterone levels ($>$ 100 ng/dl)

(24), estradiol levels (17), or if the child's PAH is < 5th percentile (18) or decreasing significantly (17,19).

It is generally agreed that any child with precocious signs of puberty, a significantly advanced BA, a decreased predicted height, and a pubertal response to gonadotropin testing should be treated to suppress pubertal progression and improve adult height (17). However, those children with a borderline evaluation need close follow-up, as treatment is not always necessary. In girls who do not meet classic criteria for treatment, puberty is expected to be slowly progressive or unsustained (25).

TREATMENT OPTIONS

GnRH agonists (GnRHa) are currently considered the treatment of choice for CPP. Drugs such as medroxyprogesterone acetate (MPA) and cyproterone acetate (CPA) are no longer used to treat CPP, as both have been shown to have little or no effect on hormonal suppression (26) and auxological outcome (27) in addition to a relatively high potential for side effects (28).

GnRH Agonists

Continuous stimulation of the pituitary gland by GnRHa causes desensitization and down-regulation of GnRH receptors (29,30). As a result, gonadotropin release is gradually inhibited following an initial stimulatory phase (31). In addition, GnRHa have been shown to alter the transcription of LH α - and β -subunit genes (32). As a result of GnRHa administration, the β -subunit secretion decreases markedly, whereas the α -subunit secretion is stimulated that results in an ineffective LH (32).

Several types of GnRHa are approved for use in children in different countries with variable routes of administration. They are available as daily subcutaneous injections, multiple daily intranasal applications, or monthly intramuscular/subcutaneous depot injections (24). The depot preparations of GnRHa have been found to suppress the pituitary–gonadal axis much better than the daily subcutaneous or nasal preparations (33,34). The most widely used depot preparations are leuprolide acetate (35,36) and triptorelin (37). Both were available as intramuscular injections only and are often very painful for patients. Currently, a less painful subcutaneous route is available. The recommended dosage of leuprolide acetate in the United States is 0.3 mg/kg/month (36). This dosage has been shown to be very efficacious in achieving complete pituitary–gonadal suppression (36).

Other Medication Options

A 3-month depot formulation of leuprolide has been used to treat patients with endometriosis and prostate cancer (38). Few studies are available on the efficacy of this preparation for treating CPP (39,40). In one study of 44 children (40 girls) with CPP, leuprolide 11.25 mg q 3 months was shown to successfully inhibit the pituitary–gonadal axis in 95% of children during a 6-month trial. However, there are currently no studies on the long-term efficacy of this preparation.

Recently, Hirsch et al. (41) demonstrated that subcutaneous implantation of the GnRHa (Histrelin) implant is effective in suppressing clinical and laboratory parameters of puberty for 1 year. This therapy will need to be studied in a larger number of

patients to assess its long-term efficacy, but it is a promising method for the treatment of CPP that decreases the pain and inconvenience of monthly injections.

GnRH receptor antagonists (such as cetrorelix) immediately block the effects of GnRH without causing an initial stimulation of LH secretion (42). Currently, GnRH receptor antagonists are used in assisted reproduction. Aside from one case report (43) and a small study comparing the effects of combination therapy of both GnRH agonist and antagonist on the initial “flare-up (stimulatory) phase” (44), no clinical data exist to show whether GnRH antagonists would also work in CPP. However, studies in the female rat are very promising (45).

Side Effects

In general, side effects from GnRHa therapy in children are considered to be minor. Transient vaginal withdrawal bleeding has been reported to occur in girls following the first injection because of initial stimulation of LH and FSH production with subsequent high levels of sex steroids resulting in the flare-up phase (46,47). Prolonged and recurrent vaginal bleeding has also been reported in one study (48), making one wonder if complete suppression had not been achieved. Other side effects include menopausal symptoms such as headache, hot flushes, and nausea in 2–5% of girls (39,40) and local allergic reactions in 10–13% of patients (49–51). Local reactions often include pain, swelling, erythema, or formation of a sterile abscess at the injection site (49–51). Antibody formation against GnRHa has not been reported even in children with local allergic reactions (52).

MONITORING THERAPY

Therapeutic monitoring can be achieved by following gonadotropin levels either after GnRHa administration or after GnRH stimulation testing or by basal testosterone levels in boys and physical examinations. As some patients require dosage changes following initiation of therapy, it is recommended that patients be evaluated every 3 months during the first treatment year and every 6 months thereafter (6).

Hormonal Monitoring

The effectiveness of GnRHa therapy in CPP depends on suppression of LH secretion. Intravenous GnRH stimulation testing provides the best index of adequate therapy; however, a complete test is not always necessary (6). An abbreviated GnRH test with an LH measurement after only 20–40 min has been reported to be sufficient to indicate adequacy of treatment (36). Lawson et al. (53) demonstrated that 100 mcg of LHRH followed by a single LH measurement 40 min later had 75% sensitivity and 100% specificity compared with the standard intravenous GnRH stimulation test and therefore should be strongly considered as an easier way to monitor GnRHa therapy in children with CPP.

Other tests for evaluating the efficacy of GnRHa therapy have been proposed because of the lack of commercial availability of synthetic GnRH. Salerno et al. (54) found that a single blood sample taken 12 h after the injection of the GnRHa for determination of LH, FSH, and estradiol levels was a simple and inexpensive means for monitoring therapy, although sampling 12 h after the treatment dose is sometimes inconvenient. Recently, Bhatia et al. (55) showed that a single serum LH sample of ≤ 0.83 mIU/ml by

Table 2
Summary of published data on the different tests available for monitoring therapy

<i>Test</i>	<i>Hormone levels consistent with adequate suppression</i>
Intravenous GnRH stimulation test (6)	LH < 4.0 IU/l (RIA); < 1.6 IU/l (IFMA) FSH < 5.0 IU/l (RIA); < 2.0 IU/l (IFMA)
Abbreviated intravenous GnRH stimulation test (36)	20–40 min post GnRH stimulation test
LHRH (100 mcg) (53)	LH (FIA) < 1.75 IU/l FSH (FIA) < 2.5 IU/l 40 min post LHRH stimulation test Single LH (ICMA) < 2.0 IU/l
GnRHa (54)	12 h post GnRHa injection LH (RIA) < 1.6 IU/l FSH (RIA) < 4.0 IU/l Estradiol (RIA) < 73 pmol/l or < 20 pg/ml
GnRHa (55)	30–60 min post GnRHa injection Single LH (ICMA) < 0.83 IU/l
GnRHa (56)	2 h post GnRHa injection Single LH (ICMA) < 6.6 IU/l
Estradiol level (6,57,58)	Not recommended for monitoring therapy with conventional assays
Testosterone level (6)	< 10 ng/ml (< 35 nmol/l)

FIA, fluoroimmunoassay; FSH, follicle-stimulating hormone; ICMA, immunochemiluminometric assay; IFMA, immunofluorometric assay; LH, luteinizing hormone; RIA, radioimmunoassay.

ICMA obtained 30–60 min after depot leuprolide correlated with adequate suppression, whereas others have shown that an LH level by IFMA < 6.6 IU/l 2 h after the depot leuprolide dose indicates appropriate suppression (56). *Table 2* provides a summary of the different tests available to monitor therapy.

Monitoring of girls by following estradiol levels only is inadequate. Intra-individual variations in plasma estradiol are common in girls with CPP. In addition, basal levels tend to overlap between girls with CPP and prepubertal girls; girls with CPP in fact have prepubertal estradiol levels as measured by conventional assays in up to 50% of cases before treatment (6,57,58). In boys, however, a serum testosterone level below 10 ng/ml (< 35 nmol/l) is a clear indication of adequate suppression (6). Urinary gonadotropins levels are still not recommended for use as an alternative to GnRH stimulation testing in children undergoing treatment for CPP because it lacks the sensitivity and the specificity required to evaluate LH suppression (59).

Clinical Monitoring

As a result of LH suppression, deceleration in growth rates, reduction in breast size, ovarian and uterine size in girls, and testicular volume in boys are usually observed. Therefore, careful clinical evaluation is crucial in the follow-up of children

with CPP. Measurement of height, weight, and body proportions and the evaluation of pubertal progression through Tanner staging are recommended every 3 months during the first year of therapy and every 6 months thereafter (6). Growth velocities should be calculated at every visit. Growth rates typically decrease to rates appropriate for skeletal age (14). Deceleration in skeletal maturation, however, is not evident until after 6 months of GnRHa therapy. Skeletal maturity then slows markedly (14) as shown in *Fig. 1*. Ovarian and uterine volumes diminish and return to prepubertal sizes usually within 3–12 months (47).

Timing of Cessation of Therapy

Although the indications for using GnRHa in children with CPP have been widely discussed, the optimal age for completing this therapy has not been widely studied (60). Most authors recommend stopping therapy at a CA of 11 ± 1 years or at a BA between 12 and 12.5 years (60–62). Carel et al. (60) suggested that continuing therapy in girls beyond 11 years of age does not improve final height and could potentially decrease final height by reducing the post-treatment growth spurt. However, Klein et al. reported in a series of 80 girls, of whom 47 were treated with GnRHa until after 11 years of age, a significant improvement in final height with no loss in predicted final height at the end of treatment (63). Thus, the decision to stop therapy must be individualized and should be based on CA, BA, and PAH at the time of discontinuation of GnRHa therapy.

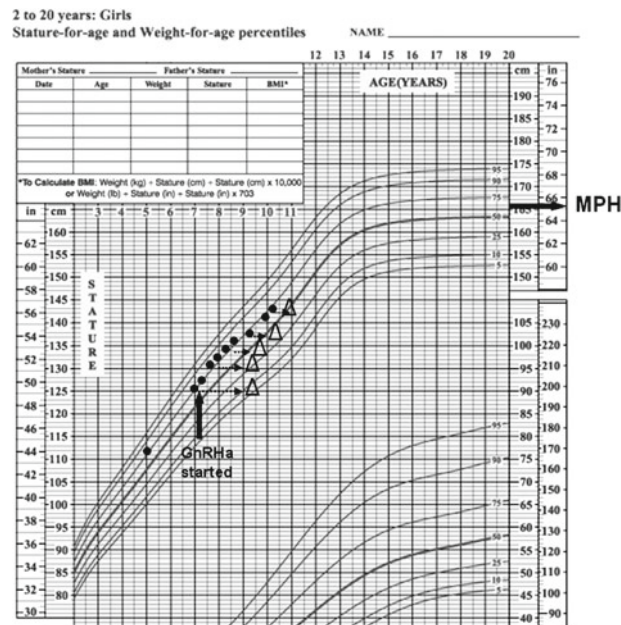


Fig. 1. Growth chart of an Ethiopian girl who presented with idiopathic central precocious puberty at 7 years of age. Note how the advancement in bone age decreased following the initiation of GnRHa therapy. Closed circles represent the height at each visit. Open triangles represent bone age. MPH refers to midparental height.

OUTCOMES OF GnRHa TREATMENT

Pituitary and Gonadal Activity

The suppressive effects of GnRHa therapy on the hypothalamic–pituitary–gonadal axis are completely reversible (64–66). The pulsatile characteristic of gonadotropin secretion resumes, and sex steroid secretion usually reaches pubertal levels within 4 months after discontinuation of the GnRHa treatment (67). Menarche starts within 2–17 months after stopping treatment in the majority of girls (64–66) but has been reported to not occur until as long as 4.5 years (14). Menstrual cycles usually become regular in the first year however; irregular cycles, metrorrhagia, and oligomenorrhea requiring treatment with oral contraceptives have been rarely reported (65).

There is conflicting literature regarding the morphology of the ovaries following the discontinuation of GnRHa therapy. One study by Bridges et al. (68) reported an increased incidence of polycystic ovaries in girls with CPP treated with combined GnRHa and growth hormone (GH). However, polycystic-like ovaries have rarely been reported or not at all in studies with GnRHa alone (65,69,70). The incidence of polycystic ovary syndrome after GnRHa therapy in women treated for CPP has not been evaluated. Although fertility and pregnancy outcomes are not available on a large number of patients after prolonged treatment with GnRHa, several small studies of 15–50 patients have demonstrated that fertility is normal (64,65). Fewer data are available on males, but it is believed that spermatogenesis is unaffected in males treated with GnRHa. Animal studies on non-human primates have shown that spermatogenesis recovers completely after long-term GnRHa treatment (71).

Psychosocial Effects

Few studies are available regarding the long-term emotional and psychosocial effects of precocious puberty. Some studies have shown that precocious development results in behavioral problems such as increased neuroticism, accentuation of physical appearance, and insecurity even years after termination of therapy (22) with some patients requiring psychological support (22,72,73). Other studies, however, have shown no negative effects of early puberty on the quality of life and psychosocial well-being (65). In a study of 50 young women with CPP, all subjects reported good self-esteem and quality of life. None were unemployed or had dropped out of an educational system (65).

Bone Mineral Density

A significant decrease in bone mineral density (BMD) has been reported in women with endometriosis and in men with benign prostatic hyperplasia treated with GnRHa (74,75). In children with CPP, BMD is often increased for CA at the time of diagnosis (76,77). During GnRHa therapy, some studies have shown a decrease in BMD after 6 and 12 months of GnRHa therapy (76,78), whereas others reported no change (79). However, long-term studies following cessation of GnRHa therapy have demonstrated normal BMD and peak bone mass attainment in female patients at final height (65,77, 79–81). In one study (81), calcium supplementation during GnRHa therapy was shown to improve peak bone mass achievement. To date, there is no evidence that long-term GnRHa therapy causes osteoporosis or osteopenia in adults with a history of CPP.

Body Weight, Composition, and Body Proportions

Children with CPP are known to develop obesity at a high rate (65,82–84). The exact reason why so many children with CPP have an increased body mass index (BMI) at initial presentation is unknown. In one study (83), 50% of patients had BMI standard deviation scores (SDSs) above the 85th percentile at the time of diagnosis which remained unchanged during GnRHa treatment. This association of a high BMI thus appears to be unrelated to long-term pituitary gonadal suppression induced by GnRHa administration (65,79,82–84). In fact, the strongest predictor of an elevated BMI SDS after treatment was shown by Palmert et al. (83) to be an elevated BMI SDS before treatment. Arrigo et al. (84) recently demonstrated that GnRHa therapy results in a significant reduction in adiposity in girls with CPP and decreases BMI if treatment is continued for at least 2 years and is accompanied by complete gonadal suppression. Thus, it appears that GnRHa do not cause or aggravate obesity.

In the prepubertal years, the growth of the legs is more rapid than the spine. At puberty, the growth of the spine accelerates, whereas the growth of the legs remains constant without an acceleration phase (85,86). Thus, as a result of early skeletal maturity of long bones, untreated patients with CPP develop characteristic body disproportion with shorter limbs compared with relatively normal trunk length (65,85,86). Heger et al. (65) found that 95% of their patients with CPP achieved normal sitting height/lower leg length ratios following GnRHa treatment, indicating that therapy preserves normal body proportions.

Final Height

To date, a large number of patients with CPP treated with GnRHa have reached adulthood. Although it is well known that adult height (AH) in untreated patients with CPP is 3.5–10 cm shorter than target height (TH) (87,88), there are controversies in the literature regarding the impact of GnRHa on AH.

Multiple factors have been identified as important predictors of greater height gain following GnRHa treatment. A number of groups (63,65,66,89) have reported that a shorter delay in the onset of treatment, longer duration of treatment, and lower BA and CA at the start of treatment are all associated with greater height gains. Arrigo et al. (62), however, showed that BA/CA at the onset of treatment is the only significant factor. Most data indicate that younger girls benefit more from treatment. In a study of 26 girls, Paul et al. (90) demonstrated that girls in whom therapy was started before a CA of 5 years reached a final height of 164.3 ± 7.7 cm, whereas those who were older than 5 years reached a final height of only 157.6 ± 6.6 cm. Similarly, Kletter et al. (91) reported final heights of 160.4 and 157.5 cm for girls younger and older than 6 years at the onset of puberty, respectively. Two studies (92,93) showed no beneficial effects of GnRHa treatment in girls who started puberty between 8 and 10 years of age, whereas Micillo et al. (94) demonstrated improvement in final height in 11 girls treated after the age of 8 years. Given this inconsistency in the literature and the possibility that some of these older girls may not have had truly rapidly progressing precocious puberty, some authors consider it premature to conclude that GnRHa treatment is not beneficial to any girl over 6 years of age (63).

Long-term outcome studies with respect to final height demonstrate that GnRHa therapy preserves AH in children with CPP. However, when final heights are compared with initial height predictions, results are less positive in boys with CPP as compared with girls. This discrepancy has been attributed by some to the height prediction methods used in children (66). In fact, the Bayley–Pinneau height prediction method most widely used in clinical practice (95) has not been validated in patients with CPP and likely tend to overestimate final height.

In summary, more than 75% of patients with CPP will reach their midparental height and more than 90% of treated girls will have a final height > 150 cm with GnRHa treatment (66,96,97). These figures are clearly superior to those of untreated patients (83,87,88). Thus GnRHa therapy is very effective in preserving adult height in children with CPP.

COMBINATION OF GnRHa AND GH

One of the goals of GnRHa therapy is normalization of a child's growth rate while slowing further skeletal maturation in order to allow more time for linear growth. Unfortunately, in some cases, GnRHa treatment results in such significant deceleration of the growth rate that improvements in PAH are not realized despite halting BA advancement.

The pathophysiological cause of the growth impairment seen in some children during GnRHa therapy is not completely understood (98). Studies of changes in the growth axis during treatment have resulted in conflicting data with some investigators finding abnormally low GH and insulin-like growth factor-1 (IGF-1) levels during GnRHa therapy (99,100), whereas others have not detected pathologic effects on the growth axis (101–103). Interestingly, none of the described alterations in GH and IGF-1 levels correlated with changes in growth velocities in individual patients (98,99,104). The only GH-dependent factor that appears to decrease during treatment and parallel the fall in growth velocity is the acid-labile subunit (ALS) of the ternary IGF-1, insulin-like growth factor binding protein-3 (IGFBP-3), ALS complex (105). These inconclusive data have led to speculation that in fact a mechanism separate from the GH–IGF-1 system is responsible for the significant growth deceleration sometimes observed during treatment.

Recently, it was shown in 100 girls with CPP that the BA at the start of GnRHa therapy had a strong inverse correlation with the growth rate seen during treatment. The advanced skeletal maturation was thought to be a surrogate marker of higher levels of estrogen exposure prior to treatment. Other indirect markers of estrogen exposure including duration of puberty and pubertal stage also correlated negatively with growth rate. These investigators hypothesized based on these findings (106), as well as animal data (107), that prior estrogen exposure resulted in accelerated and irreversible growth plate senescence. Senescence of the growth plate refers to the normal programmed decline in growth plate cartilage function with age, leading to slowing of linear growth and eventual epiphyseal fusion. Thus, in those children who are further along in the “senescence progression” because of prolonged estrogen exposure, the growth deceleration is likely a process intrinsic to the growth plate.

Because of this slowing of the growth rate sometimes observed during the treatment of CPP with GnRHa, a number of investigators have sought to determine whether the addition of GH would result in improvements in growth in this subset of patients.

The first two studies were published in 1995 (104,108). Both groups studied girls with idiopathic CPP who had experienced significant slowing of their growth velocity after 1 year of GnRHa treatment. Both found that adding GH for 12 months improved the growth rates of these children. Saggese et al. (104) also showed that mean PAH improved by 4 cm during those 12 months of GH. AH data have only been reported by one group. Pasquino et al. treated 10 girls with CPP who had growth rates below the 25th percentile for age after 1 year of GnRHa therapy with 0.3 mg/kg/week of GH for approximately 3 years. Ten matched girls with the same degree of growth deceleration remained on GnRHa only and served as a control group. The mean AH of the combination group was 7 cm higher than the PAH at the time of addition of GH and 3.5 cm higher than the mean AH of the control group (109). No adverse effects, including excessive BA advancement, were noted from the GH treatment (110). Interestingly, both the GH-treated group and the control group reached adult heights above their calculated genetic THs. Thus, the addition of GH to slowly growing girls on GnRHa may not be necessary to achieve THs in this population, but further controlled trials are needed. However, the combination of GH and GnRHa is likely of substantial benefit in other conditions such as congenital adrenal hyperplasia (111) or in children who develop the paradoxical combination of GH deficiency and precocious puberty following cranial irradiation (112).

COMBINATION OF GnRHa AND OXANDROLONE

Oxandrolone is a non-aromatizable androgen (113) that has been used to stimulate growth in boys with constitutional delay of growth and puberty (114,115). The exact mechanism of its growth-promoting effects is not completely understood. Some authors believe that oxandrolone acts by activating the somatotroph axis (116,117), whereas others believe that oxandrolone stimulates growth via a GH-independent mechanism by exerting a direct effect on the growth plate (118,119).

Recently, Vottero et al. (120) showed that combination of GnRHa therapy and oxandrolone in girls with idiopathic CPP and severe growth deceleration during GnRHa treatment results in improvement in final AH. In this study, 20 girls with CPP and severe growth retardation on GnRHa therapy were included. Ten girls received combination of GnRHa and oxandrolone (0.06 mg/kg/day), and 10 matched girls with the same degree of growth deceleration remained on GnRHa only and served as a control group. The mean AH of the combination group was 7.8 cm higher than the PAH at the time of addition of oxandrolone and 4.5 cm higher than the mean AH of the control group. No adverse effects were noted from the oxandrolone treatment (120). The results of this study are comparable with those reported by the addition of GH to GnRHa treatment (109,110). Compared with GH, oxandrolone appears equally effective, cheaper, and available as an oral medication that makes it more convenient to patients. However, more studies are needed to establish the efficacy of oxandrolone in a larger number of patients including boys with CPP and growth deceleration on GnRHa.

CONCLUSIONS

GnRHa therapy remains the treatment of choice for children with CPP. Long-term outcome studies have demonstrated that GnRHa therapy is very effective in preserving adult height with the majority of patients reaching a final height near their

individual TH. Studies addressing the long-term effects of GnRHa therapy on BMD, body proportions, BMI, and reproductive function have found no negative effects despite some controversy in the literature. Psychological issues regarding the long-term effects of CPP with or without treatment need to be more thoroughly studied.

Newer therapies for use in CPP are currently being developed. GnRH antagonists are showing promising results in animal models, and their use in the future for the treatment of CPP in order to avoid the initial flare-up phase caused by GnRHa is a definite possibility. Third-generation aromatase inhibitors have been shown to be effective in decreasing skeletal maturation and improving predicted height in adolescent males with advanced BA and limited growth potential (121) and thus may be another treatment option in the future.

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VI

GONADOTROPIN-INDEPENDENT PRECOCIOUS PUBERTY

17

McCune–Albright Syndrome

Pathophysiology, Diagnosis, and Management

Tamara S. Hannon, MD
and Erica A. Eugster, MD

CONTENTS

INTRODUCTION

HISTORY

PATHOPHYSIOLOGY

DIAGNOSIS

TREATMENT OF MAS

CONCLUSIONS

REFERENCES

Summary

McCune-Albright syndrome (MAS) is an extremely heterogenous condition in which a variety of systemic and endocrine abnormalities can occur. It is classically characterized by the triad of peripheral precocious puberty, café au lait skin pigmentation, and polyostotic fibrous dysplasia of bone. It is caused by activating mutations of *GNAS*, the gene encoding the alpha subunit of the stimulatory G-protein ($G_s\alpha$), and result in constitutive, ligand-independent activity in affected cells. The *GNAS* activating mutations lead to a loss of intrinsic guanosine triphosphatase (GTPase) activity of $G_s\alpha$, resulting in an inability to return the cell to its inactive basal state after stimulation by a hormone ligand. Thus, the presence of *GNAS* mutations in endocrine cells is associated with unregulated hormone production and the development of endocrine cells is associated with unregulated hormone production and the development of endocrine hyperfunction. Diagnosis is based on careful physical examination as well as biochemical and radiographic evaluation, while molecular diagnosis is currently best reserved for the research setting. Therapeutic approaches vary widely and must be individualized to target the presiding manifestations of the disorder. Because MAS is both rare and heterogeneous in nature, collaborative and multi-center research efforts are paramount to advance the understanding of the etiology, pathophysiology, diagnosis and treatment of this condition.

Key Words: G-protein-coupled receptor; $G_s\alpha$; *GNAS*; Precocious puberty; Café au lait skin pigmentation; Fibrous dysplasia of bone.

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Fig. 1. Café au lait skin pigmentation noticeable between the buttocks.

INTRODUCTION

Case Vignette

A 2-year-old boy presented to the pediatric endocrinology clinic with failure to thrive. Thyroid function tests performed as part of the medical evaluation revealed a suppressed serum thyrotropin-stimulating hormone level, and the rest of the laboratory evaluation was unremarkable. Subclinical hyperthyroidism persisted without biochemical evidence of Graves' disease. Although he did not present with other manifestations of the disorder, McCune–Albright syndrome (MAS) was included in the differential diagnosis of his hyperthyroidism. A bone scan to screen for fibrous dysplasia of bone was obtained and revealed multiple areas of increased uptake. Subsequently, he was noted to have café au lait skin pigmentation between the buttocks (*Fig. 1*), and a diagnosis of MAS was made.

MAS is classically defined as the clinical triad of precocious puberty (PP), café au lait skin pigmentation, and polyostotic fibrous dysplasia of bone. Scientific advances in the understanding of the molecular basis of the syndrome have provided grounds for clinical insight with regard to the pathophysiology of MAS and the phenotypic heterogeneity among patients. Atypical presentations are increasingly recognized, as was the case in the child described above. As a result, the evaluation and treatment of patients with MAS has evolved and is guided by the knowledge of the specific manifestations of the disease that continue to emerge.

HISTORY

The first published case reports of girls with the constellation of PP, café au lait skin pigmentation, and polyostotic fibrous dysplasia of bone are in the German medical literature from the early 1900s (*1,2*). Drs Donovan James McCune and Fuller Albright first described this clinical presentation as a syndrome at the Society for Pediatric Research Annual Meeting in 1936 (*3*). Here, they reported the case of a 9-year-old girl with a medical history of severe neonatal jaundice, dark brown skin patches, osteitis fibrosa cystica with bony deformities, onset of menarche at the age of 2 years, and hyperthyroidism. Shortly after the original description, case reports of patients with phenotypic evidence of the syndrome were published by McCune (*4,5*) and Albright (*6*), who astutely speculated that the disease was due to a defect in embryological development.

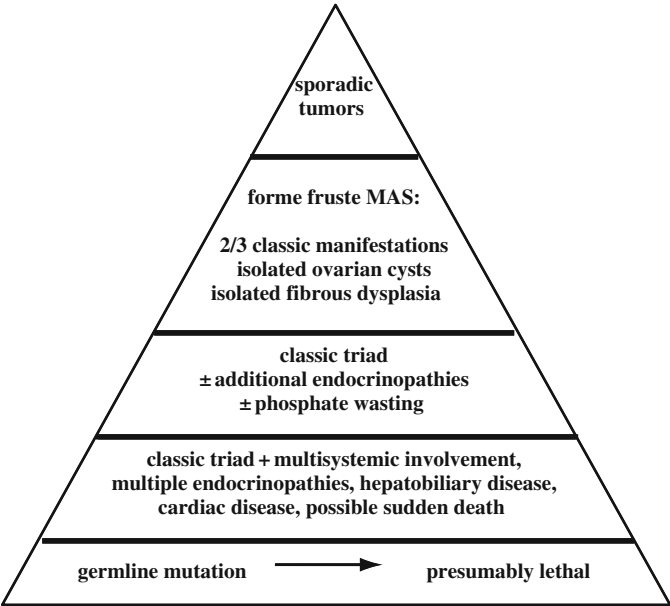


Fig. 2. The clinical spectrum of manifestations of $G_s\alpha$ activating mutations.

It is now understood that the presentation of the underlying defect in MAS ranges from a single manifestation such as isolated ovarian cysts leading to gonadotropin-independent PP (7–9) to widespread multi-system organ failure (10) (Fig. 2). In addition to PP, hyperthyroidism (11,12), growth hormone excess (13,14), hyperprolactinemia (15,16), and hypercortisolism (17–20) can occur. In males, testicular microlithiasis (21) and testicular enlargement due to hyperfunction of Sertoli cells (22) have been described. Non-endocrine manifestations of the syndrome occurring in infancy have included hepatobiliary and cardiac diseases leading to sudden death in the most severe cases (10,23). Most recently, renal phosphate wasting has been added to the spectrum of clinical manifestations of MAS (24–26).

PATHOPHYSIOLOGY

An accumulation of knowledge regarding the intracellular signaling pathways in endocrine tissues led to the discovery that mutations in the heterotrimeric guanine nucleotide-binding protein (G protein) signaling pathway altered hormone production (Fig. 3). In 1991, Weinstein et al. (27) reported their discovery that activating mutations in the gene for the alpha subunit of the stimulatory G protein ($G_s\alpha$) were present in DNA from affected tissues from four patients with MAS. The same group went on to show that the same mutation was present in bone tissue of patients with fibrous dysplasia of bone.

The activating mutations associated with MAS are single amino acid substitutions occurring in exon 8 of gene encoding $G_s\alpha$ (*GNAS*), at the codon for Arg²⁰¹, and result in constitutive, ligand-independent activity in affected cells. The mutations reported most frequently are substitutions of His for Arg²⁰¹ or Cys for Arg²⁰¹ (10,27–29). The replacement of Arg²⁰¹ leads to a loss of the intrinsic guanosine triphosphatase (GTPase)

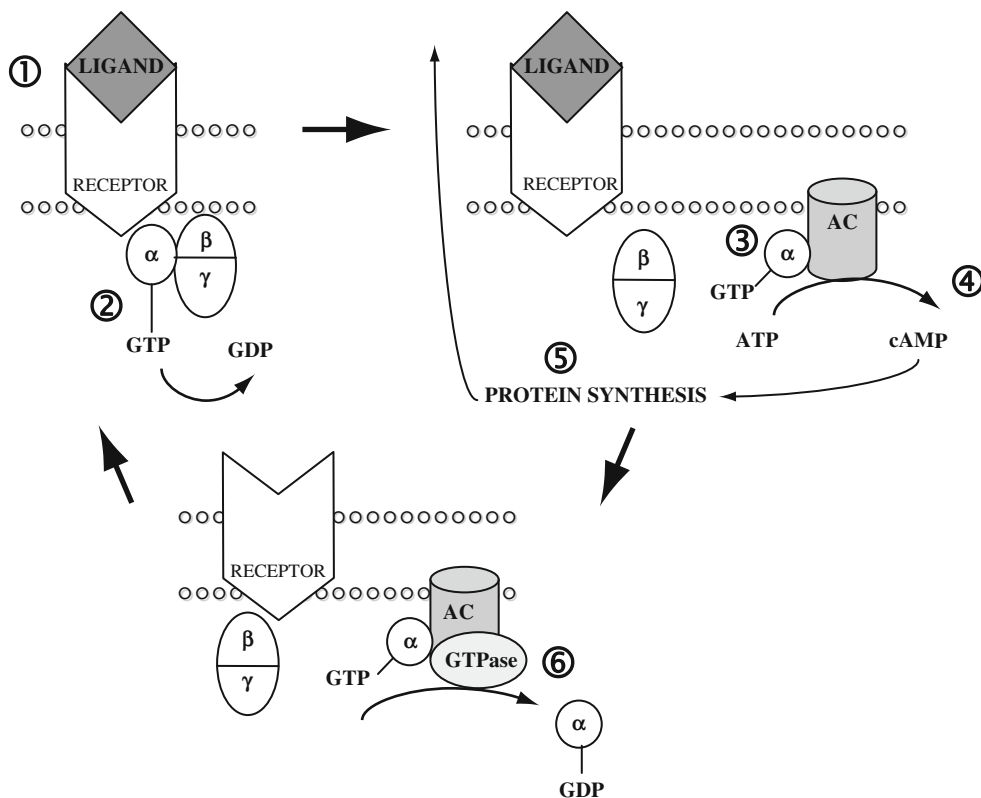


Fig. 3. Basic elements of the G-protein cycle. The ligand–receptor complex activates the G-protein ①, and the α -subunit bound guanosine diphosphate (GDP) is displaced by guanosine triphosphate (GTP) ②. The GTP bound α -subunit activates adenylyl cyclase (AC) ③, resulting in the production of cyclic adenosine monophosphate (cAMP) ④ and the activation of cellular processes leading to increased protein synthesis ⑤. Normally, the intrinsic GTPase in the α -subunit hydrolyzes GTP to GDP ⑥, returning the cell to its inactive basal state. Lack of intrinsic GTPase activity of the α -subunit, as caused by a *GNAS* activating mutation, leads to the production and accumulation of cAMP and unregulated overproduction and secretion of the cellular product.

activity of $G_s\alpha$, resulting in an inability to return the cell to its inactive basal state after stimulation by a hormone ligand. This results in the accumulation of intracellular cyclic adenosine monophosphate (cAMP) associated with unregulated cellular proliferation and overproduction of the cellular product. *GNAS* activating mutations have been found in all affected MAS tissues that have been studied including skin (30), gonad (7,8,22,27), thyroid (27,31), adrenal (27,32,33), pituitary (27,34), liver (23), and bone (35–38).

The deposition of abnormal fibroosseous tissue in fibrous dysplasia is understood to be a consequence of increased rates of cellular proliferation and abnormal differentiation associated with elevated levels of intracellular cAMP in osteoblastic cells containing the MAS *GNAS* activating mutation (29,36,39–41). Serum levels of the phosphaturic factor, fibroblast growth factor-23 (FGF-23), are increased in patients with fibrous dysplasia FD and MAS and correlate with the FD disease burden (24–26,42).

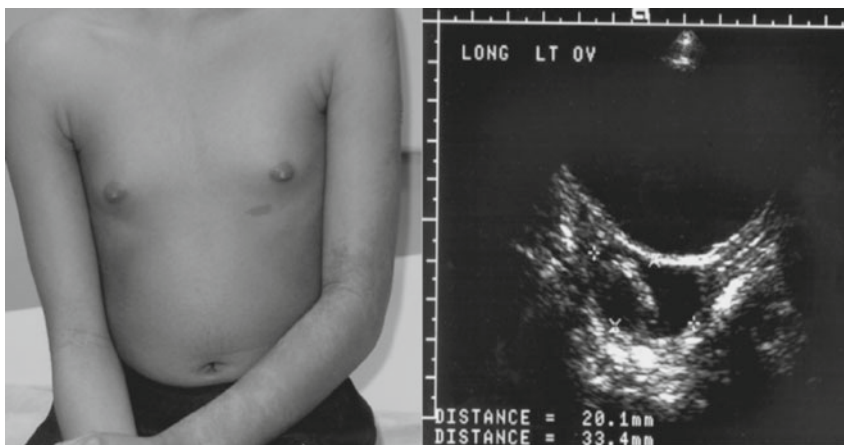


Fig. 4. Café au lait skin pigmentation, premature thelarche, and an ovarian cyst visualized on pelvic ultrasound in a 6 10/12 year-old girl with McCune–Albright syndrome (MAS).

Production of FGF-23 by FD bone lesions contributes to renal phosphate wasting in individuals with MAS, thus increasing the risk of skeletal mineralization defects and bone pathology (25,26).

It is widely believed that the *GNAS* activating mutation causing MAS occurs sporadically within a postzygotic cell line. The timing of the mutational event then determines the extent of the disease such that a mutation occurring earlier in embryogenesis would lead to widespread mosaic distribution, whereas a mutation occurring later would lead to limited tissue involvement. The phenotype is also likely modified by tissue-specific imprinting of the inheritance of *GNAS* (43,44). The observation that the café au lait skin pigmentation found in individuals with MAS respects the midline and often follows the lines of Blaschko (Fig. 4), representing the dorso–ventral outgrowth of two different populations of cells during early embryogenesis, led to the postulate that cells bearing the mutation survive only in the condition of mosaicism (45–47). The classical phenotype of MAS is understood to be caused by the mosaic distribution of the *GNAS* activating mutation in all three germ layers.

Although it is recognized that the true prevalence rate of MAS is unknown, the classic form of the syndrome occurs rarely. There are no known genetic or environmental risk factors associated with the development of the syndrome, and vertical transmission from parent to offspring does not occur. Although rare, sporadic endocrine tumors (31, 34,43,48) and intramuscular myxomas (49) have been associated with *GNAS* activating mutations.

DIAGNOSIS

Precocious Puberty

Peripheral (gonadotropin-independent) PP associated with MAS is most frequently recognized in girls, although the *GNAS* activating mutation in MAS presumably occurs equally in both sexes. PP in girls with MAS typically presents between the ages of 1 and 5 years with acute breast development followed by sudden onset of vaginal

bleeding. This clinical course occurs in response to the development and subsequent resolution of estrogen-producing ovarian cysts (*Fig. 4*). The course of progression of PP is unpredictable and variable, from mild and self-limited to rapidly progressive with recurrent vaginal bleeding, acceleration of linear growth and skeletal maturation, and further development of secondary sexual characteristics (*50,51*). Children with progressive peripheral PP may develop central PP secondary to activation of the hypothalamic–pituitary–gonadal axis because of high circulating levels of sex steroids and skeletal maturation (*51–53*).

Whereas girls with MAS frequently come to attention secondary to PP, boys with MAS typically do not present with PP (*54,55*). Mild testicular abnormalities, including testicular microlithiasis, are likely under-recognized in boys with MAS (*21*). Manifestations of gonadal hyperfunction among boys with MAS vary from mild testicular enlargement due to autonomous function restricted to Sertoli cells (*22*) to the rare occurrence of testicular enlargement and development of secondary sexual characteristics secondary to activation of Leydig cells (*13,56–58*).

LABORATORY EVALUATION

Active peripheral PP is characterized by extreme elevations in sex steroid (estradiol or testosterone) levels and suppressed gonadotropin levels. Pelvic ultrasound, in girls, may reveal ovarian cysts. Testicular ultrasound, in boys, may reveal microlithiasis with or without testicular enlargement.

Fibrous Dysplasia of Bone

Fibrous dysplasia of bone is characterized by the replacement of the normal medullary architecture of bone with abnormal fibroosseous tissue, which radiographically appears cystic (*59*) (*Fig. 5*). The most frequently affected areas are the base of the skull and long bones of the legs, particularly the femur (*60*). The abnormal bone tissue lacks the mechanical properties of normal bone and, depending on the extent of the distribution, can lead to pain, deformity, and pathological fractures (*59,61–64*).

LABORATORY EVALUATION

Bone scan is the most sensitive initial evaluation for determining the presence and extent of fibrous dysplasia (*65–67*). Plain radiographs are indicated to further evaluate fibrous dysplasia lesions of the long bones, which are osteolytic lesions with a “ground glass” appearance (*59*). Bony deformity may also be present and visualized on x-ray. For fibrous dysplasia of the skull, computed tomography (CT) imaging is effective for visualizing and following the progression of these lesions. Lesions in the skull can expand and, in rare cases, impinge on cranial nerves and cause vision or hearing deficits (*68–70*).

Café au Lait Skin Pigmentation

Irregular “coast of Maine” borders that stop abruptly at the midline characterize the café au lait skin pigmentation in MAS. These spots are most commonly found on the forehead/scalp, nuchal area, sacrum, and buttocks (*Fig. 6*). Café au lait skin pigmentation may be identified at birth or may become noticeable as it darkens over time.

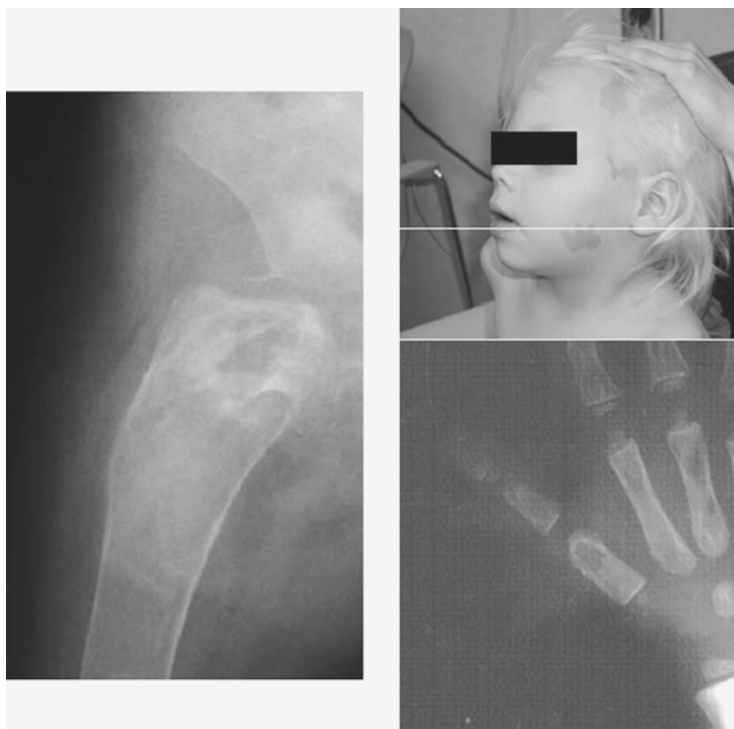


Fig. 5. Radiographs revealing fibrous dysplasia of bone affecting the femur and metacarpals in a boy with McCune–Albright syndrome (MAS) who originally presented with hand pain.

Hyperthyroidism

Elevated serum thyroxine (T4) and/or triiodothyronine (T3) levels with suppressed thyroid-stimulating hormone are indicative of hyperthyroidism caused by the autonomous production of thyroid hormone in MAS. Thyroid function tests should be routinely included in the screening evaluation, as hyperthyroidism occurs commonly in patients with MAS (12).

Atypical MAS

Atypical or “forme frust” MAS refer to cases in which the phenotype includes only a portion of the usual triad. Most commonly, fibrous dysplasia is present with one of the other manifestations (36,71–73). Still, cases of peripheral PP secondary to ovarian cysts, without evidence of fibrous dysplasia of bone, have been confirmed to be associated with the presence of the MAS activating mutation in ovarian tissue/follicular fluid (8).

LABORATORY EVALUATION

Whenever the patient’s clinical phenotype is consistent with the presence of a *GNAS* activating mutation, screening bone scan for fibrous dysplasia is indicated. In turn, screening evaluation for other endocrinopathies associated with MAS [PP, hyperthyroidism, growth hormone excess, hyperprolactinemia, hypercortisolism (in



Fig. 6. Extensive café au lait skin pigmentation in male patient with McCune–Albright syndrome (MAS).

infants), and renal phosphate wasting] is indicated in patients when the diagnosis of fibrous dysplasia of bone is made (74,75).

Molecular Genetics

The diagnosis of MAS has traditionally been made on clinical grounds. However, in cases where the clinical phenotype is not diagnostic, such as isolated peripheral PP and vaginal bleeding suspected to be due to autonomous function of the ovary, there is significant interest in the use of *GNAS* mutation analysis to make a definitive diagnosis. Indeed, cells containing activating *GNAS* mutations have been isolated from ovarian tissue, ovarian cysts, and ovarian cyst fluid in girls with gonadotropin-independent PP (76). The activating *GNAS* mutation has also been detected in peripheral blood leukocytes from patients with a broad phenotypic spectrum of MAS (28,35,36,74,76, 77). Nevertheless, the current ability to detect *GNAS* activating mutations is often positively associated with the prevalence and severity of phenotypic manifestations of MAS. Future improvements in the sensitivity and specificity of mutation analysis from peripheral blood in patients with atypical forms of MAS may improve the diagnostic utility of mutational analysis. Until then, such testing in peripheral blood should

continue to be reserved for research settings. The availability of affected tissue improves detection rates, as would the development of enhanced techniques geared toward illuminating low-level expression of the mutation in genomic DNA.

TREATMENT OF MAS

Therapeutic approaches in children with MAS vary widely and are targeted toward specific manifestations of the disorder. Depending on the individual situation, goals may include amelioration of clinical symptoms, preservation of growth, decreased fracture risk, or improvement in cosmetic appearance. Treatment options for each aspect of MAS are summarized in *Table 1* and are discussed below.

Table 1
Current therapeutic options for the individual disease features of McCune–Albright syndrome (MAS)

Disease feature	Therapeutic options		
	Medical	Surgical	Other
Precocious puberty	Tamoxifen	Ovarian cystectomy	
Hyperthyroidism	Arimidex or letrozole Methimazole	Total thyroidectomy	Radioactive Iodine (RAI) ablation
Growth hormone excess	Propylthiouracil (PTU) Octreotide Long Acting Release (LAR)	Removal of pituitary adenoma	
Cushing’s syndrome	Cabergoline or bromocriptine Metyrapone	Bilateral adrenalectomy	
Hypophosphatemic rickets	Phosphorus and vitamin D supplementation		
Fibrous dysplasia of bone	Intravenous pamidronate	Intramedullary rods Osteotomy and dynamic hip screws	
Café au lait pigmentation	Laser Intense pulsed light		

Endocrine Hyperfunction

PRECOCIOUS PUBERTY

The vast majority of reported cases of PP in the setting of MAS have occurred in girls (76). Therefore, girls have been the focus of most anecdotal reports as well as clinical trials investigating the therapeutic efficacy of many pharmacologic agents. Regardless of the specific strategy, the intent of therapy is to prevent vaginal bleeding and pubertal progression while attenuating the effects of estrogen on growth, skeletal maturation, and final adult height. Relatively non-specific medications used for the treatment of PP in MAS have included medroxyprogesterone (78), cyproterone acetate (79), and ketoconazole (80). However, concerns about efficacy as well as safety have been associated with these drugs. To date, the greatest success in the medical treatment of PP in girls with MAS has been with anti-estrogens in the form of aromatase inhibitors or the selective estrogen receptor modulator (SERM) tamoxifen, both of which are reviewed in the following 2 paragraphs. After primary therapy has been initiated, the addition of a gonadotropin-releasing hormone (GnRH) analog once central puberty has commenced may be beneficial in optimizing outcome (81).

Aromatase Inhibitors. Aromatase inhibitors are non-steroidal compounds that bind reversibly to the p450 portion of the aromatase enzyme, thereby preventing the conversion of androgens to estrogen. First, second, and third generation aromatase inhibitors have been investigated for the treatment of PP in MAS. Although initial reports of the first-generation agent testolactone appeared promising (82), a longer term study involving 12 girls revealed a progressive decline in efficacy over time (51). In boys with MAS and PP, testolactone has also been used in conjunction with an androgen receptor blocker, although only in the setting of isolated case reports (83). The second-generation aromatase inhibitor fadrazole has proven disappointing and has been demonstrated to result in a dose-dependent suppression in adrenal function (84). Although still under investigation, reports of the third-generation drugs anastrozole and letrozole have been decidedly mixed (85). Thus, no single aromatase inhibitor has been identified to date that demonstrates consistent and sustained efficacy for the treatment of PP in girls with MAS.

Tamoxifen. Tamoxifen, a member of the SERM family, has both estrogenic and antiestrogenic effects in various tissues. The first report of the beneficial effect of tamoxifen for the treatment of PP in a girl with MAS appeared in 1999 (52). This was followed by a multi-center 1-year prospective trial of tamoxifen in 25 girls with MAS. Significant reductions in the number of vaginal bleeding episodes, growth velocity, and rates of skeletal maturation were observed (86). However, average uterine volumes were noted to increase during tamoxifen treatment, and the significance of this finding remains unclear. It is also unknown whether efficacy from tamoxifen will be maintained long term in these patients. Therefore, although tamoxifen may now be considered first-line therapy for PP in girls with MAS, further study is needed. No information on the use of other estrogen receptor blockers in MAS is available, although drugs such as the pure anti-estrogen Faslodex theoretically have the potential to be effective for PP in this condition (87).

Surgery. Although ovarian cystectomy in girls with MAS has been performed (88), it is very rarely indicated and is not routinely recommended. Even extremely large ovarian cysts resolve spontaneously (89), although the subsequent decrease in serum estradiol often results in withdrawal of vaginal bleeding. Unfortunately, some girls with MAS undergo surgical removal of an ovary prior to having the correct diagnosis recognized. Oophorectomy in MAS is contraindicated, as the potential for fertility in these patients is normal.

HYPERTHYROIDISM

Therapeutic options for the treatment of hyperthyroidism in patients with MAS are similar to those available for other forms of hyperthyroidism. However, because permanent remission would not be expected, anti-thyroid medications are usually not considered appropriate in this setting (90). Although definitive treatment with radioactive iodine has been used successfully in many patients (12), the recognition of an increased propensity for the development of thyroid cancer in MAS (31) has led to the suggestion that total thyroidectomy be preferentially performed. Regardless of whether treatment with radioactive iodine or thyroidectomy is chosen, lifelong thyroid hormone replacement is necessary following definitive treatment.

GIGANTISM

In patients with MAS and a growth hormone-secreting pituitary adenoma, both medical and surgical approaches have been utilized (91). Although surgery is initially effective, growth hormone excess typically recurs (92). Additional limitations to surgery include frequent involvement of the skull with fibrous dysplasia (93), as well as the fact that many patients have diffuse pituitary hyperplasia rather than a discrete lesion. In these cases, long-acting octreotide has been reasonably effective (83) and is typically combined with bromocriptine or cabergoline to treat the hyperprolactinemia that is nearly always present (14).

CUSHING'S SYNDROME

Cushing's syndrome is a rare but serious form of endocrine hyperfunction in MAS that occurs most commonly during infancy (19). Although this condition may spontaneously resolve, some children have required bilateral adrenalectomy to control their disease. Alternatively, metyrapone has been reported to provide effective therapy in some cases (94).

HYPOPHOSPHATEMIC RICKETS

Hypophosphatemic rickets is a rare complication of MAS, although variable degrees of renal phosphate wasting have been reported in up to 50% of patients with fibrous dysplasia of bone (24). An inappropriately low serum level of 1,25 dihydroxyvitamin D is also observed in patients with MAS and hypophosphatemia (26). As in other forms of hypophosphatemic rickets, primary treatment consists of phosphate and vitamin D supplementation (95).

Fibrous Dysplasia of Bone

Although the extent and rate of progression of MAS-associated bone disease varies considerably, the number of affected bones and the severity of the fibrous dysplasia

tend to worsen over time (54). The decision to treat is based on the presence of bony deformities or symptoms such as fractures or bone pain. Both medical and surgical therapeutic modalities exist. In terms of medical intervention, the advent of bisphosphonates for the treatment of metabolic bone disease in children represents a significant breakthrough. Many small uncontrolled studies have now been conducted using intravenous pamidronate for the treatment of fibrous dysplasia in children with MAS. In the first of these, intravenous pamidronate given to five children every 6 months for 2 years resulted in improvements in mobility and bone pain with a concurrent decrease in markers of bone turnover (96). Similar results were seen in two subsequent trials involving 31 subjects. An additional benefit of pamidronate is that it has been found to decrease FGF-23, the phosphaturic factor implicated in the genesis of renal phosphate wasting in patients with fibrous dysplasia (25). Despite beneficial effects on symptoms and indices of bone metabolism, minimal improvements in bone density and dysplastic lesions have been noted (97,98). Although these initial reports are highly encouraging, questions remain regarding the long-term safety of these agents in pediatric patients. Therefore, it has been recommended that their use be limited to carefully conducted clinical trials with ongoing safety monitoring (99). Surgical approaches such as the placement of intramedullary rods have been used as both a primary treatment and an adjunct to bisphosphonates (100). Alternate techniques including osteotomy and placement of dynamic hip screws have also been shown to be beneficial for the treatment of the Shepard's crook deformity in patients with MAS (101).

Café au Lait Macules

Depending on the distribution and the extent of café au lait skin pigmentation in children with MAS, treatment for cosmetic purposes may be desirable. The only effective therapy is with lasers, intense pulsed light, or a combination of the two (102). As these areas are superficial, two treatments with intense pulsed light alone have been shown to be effective (103). Various lasers have also been used and found to be safe and beneficial for the removal of pigmented lesions in children (104). Interestingly, the presence of a jagged border has been reported to confer a good prognosis for clinical response to the Q-switched laser (105), a characteristic nearly ubiquitous of the "Coast of Maine" café au lait macules in patients with MAS. As these areas tend to become darker and more prominent over time, it would seem reasonable to wait at least until early childhood before embarking on treatment.

CONCLUSIONS

In conclusion, MAS is an extremely heterogeneous condition in which a plethora of systemic and endocrine disorders can be manifest. As we come to understand more about the underlying pathophysiology of MAS, recommendations for the evaluation and clinical care of patients with MAS continue to evolve. There is no unified treatment for MAS that addresses all of the disparate manifestations of the disorder. Whether gene therapy will eventually fulfill its promise of being able to cure genetic conditions remains to be seen. Until that time, each aspect of MAS must be dealt with individually. The rare and heterogeneous nature of MAS makes collaborative and multi-center research efforts paramount. Happily, new areas of investigation and novel therapeutic

approaches continue to be explored, enhancing our knowledge of both the biology and the optimal clinical management of MAS.

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18

Familial Male-Limited Precocious Puberty

Nerissa C. Kreher, MD, MSc

CONTENTS

INTRODUCTION
CLINICAL FINDINGS
LABORATORY FINDINGS
LABORATORY AND RADIOGRAPHIC EVALUATION
ETIOLOGY
ACTIVATING MUTATIONS OF THE LH RECEPTOR
MOLECULAR STUDIES
THERAPY
LONG-TERM COMPLICATIONS AND CONCERNS
CONCLUSIONS
REFERENCES

Summary

Familial male-limited precocious puberty (FMPP), also known as testotoxicosis, is a rare, dominantly inherited disorder that causes gonadotropin-independent sexual precocity in boys. Early signs of puberty, including virilization, growth acceleration, and skeletal advancement, develop in affected boys, usually by 3 years of age. Activating mutations of the human luteinizing hormone (LH) receptor result in increased testosterone production by the Leydig cells despite low LH levels. Diagnosis, molecular analysis, and therapy for FMPP are discussed.

Key Words: Testotoxicosis; LH receptor mutation; Gonadotropin-independent precocious puberty; Biclutamide.

INTRODUCTION

Familial male-limited precocious puberty (FMPP), initially termed “familial testotoxicosis” by Rosenthal et al. (1), is an inherited form of gonadotropin-independent precocious puberty. The underlying pathophysiologic mechanism causing FMPP is an activating mutation of the luteinizing hormone receptor (LHR). These missense mutations are inherited in an autosomal dominant male-limited inheritance pattern (2), in which females carrying the mutation have no recognizable clinical phenotype (3,4).

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Table 1
Clinical Signs of (FMPP)

<i>Acne Oily skin</i>
Facial hair
Deepening of the voice
Axillary hair
Adult body odor
Pubic hair
Scrotal thinning
Penile enlargement
Testicular enlargement (less than expected relative to other signs of pubertal development)
Spontaneous erections
Muscular development
Aggressive sexual behavior
Emotional lability

Although familial forms of this disorder are most prevalent, there are males with LHR mutations who have no family history of the disorder; thus, both sporadic and familial forms of the disorder exist. Several therapeutic strategies have been studied although long-term outcome data remain limited. In this chapter, the clinical features, mutations causing FMPP, treatment modalities, and potential adult complications of the disorder will be discussed.



Fig. 1. Four-year-old male with familial male-limited precocious puberty (FMPP). Note the small testicular size relative to the degree of penile enlargement and pubic hair.

CLINICAL FINDINGS

Clinical features include the expected signs of isosexual pubertal development and usually occur between 1 and 3 years of age (5). Accelerated growth velocity, with growth rates similar to those expected in true puberty, and advanced epiphyseal maturation are noted. Acne, body odor, pubic hair development, and penile enlargement are among the signs that may be noted on physical examination (*Table 1*). A defining feature of the disorder is testicular enlargement that is less than expected for the degree of sexual maturation and skeletal advancement (*Fig. 1*). Unlike other causes of pseudo-puberty such as congenital adrenal hyperplasia (CAH) in which the testicular volume is pre-pubertal, in FMPP the testes enlarge but only into the early pubertal range (4–8 cc) (2,6,7). This degree of testicular enlargement is due to the presence of isolated Leydig cell hyperplasia, which accounts for only 10% of the overall testicular volume (5). There is less increase in Sertoli cell and seminiferous tubule size due to the pre-pubertal levels of follicle-stimulating hormone (FSH) (5). Patients may also exhibit sexual behaviors including masturbation and aggressive sexual interest in females.

LABORATORY FINDINGS

The description of a form of precocious puberty without elevated gonadotropins was described in the 1950's (8–10), but increased investigation of the disorder was accomplished in the 1980s. Several groups described male patients with high testosterone levels in the absence of pulsatile gonadotropin secretion as well as pre-pubertal gonadotropin responses to gonadotropin-releasing hormone (GnRH) agonist (1,2,11–14).

Testosterone levels range from early pubertal to adult levels. Basal gonadotropins are low and may even be suppressed beyond levels expected for pre-pubertal children (15). Overnight monitoring for pulsatile secretion of gonadotropins is not clinically utilized in most contemporary settings but reveals a pre-pubertal pattern indicative of the lack of activation of the hypothalamic–pituitary–gonadal axis (11). Further evidence for a gonadotropin-independent state is the LH response to GnRH-agonist administration. Unlike patients with central precocious puberty (CPP) who have an incremental increase in LH after 100 µg administration of GnRH, patients with FMPP have either no, or only a slight, incremental increase in LH (11).

Histology

Leydig cell proliferation is a key histologic finding in FMPP. Testicular biopsies from patients with FMPP reveal fully differentiated Leydig cells in a distribution characteristic of the normal adult testis (13). Despite low levels of FSH, findings consistent with Sertoli cell maturation are also present. Although the degree of germinal cell maturation is variable, spermatogenesis is also present (13). Premature spermatogenesis in this setting is thought to be secondary to high intra-testicular levels of testosterone (16).

Differential Diagnosis

The differential diagnosis for FMPP includes all forms of peripheral or gonadotropin-independent precocious puberty that affect males (*Table 2*). CAH is the most common disorder that must be excluded. Other pathology including adrenal, testicular, and

Table 2
Differential diagnosis

Congenital adrenal hyperplasia
Virilizing tumors
Adrenal carcinoma
Adrenal adenoma
Leydig (interstitial) cell tumors
hCG-secreting tumors
Germinomas
Hepatomas
Hepatoblastomas
Teratomas
Choriocarcinomas
McCune–Albright syndrome
Severe hypothyroidism (van Wyk–Grumbach syndrome)
Exogenous androgen exposure

human chorionic gonadotropin (hCG)-secreting tumors must also be considered. Although more common in females, McCune–Albright syndrome remains in the differential diagnosis (17). Exogenous androgen exposure should also be inquired about by the physician. Without confirmation of the diagnosis of FMPP by LHR mutation analysis, the disorder should be considered a diagnosis of exclusion.

LABORATORY AND RADIOGRAPHIC EVALUATION

The initial evaluation of any male patient with clinical evidence of precocious puberty should include testosterone and gonadotropin levels. Measurement of gonadotropins consists of basal ultrasensitive LH-ICMA immunochemiluminometric assay and measurement of LH and FSH after stimulation testing with a GnRH agonist such as leuprolide (18). Advanced skeletal maturation, as determined by a bone age radiograph, suggests radiographic evidence of precocious puberty.

Once the diagnosis of gonadotropin-independent precocious puberty is confirmed, further evaluation to determine the etiology is required. This evaluation should include a 17-hydroxyprogesterone to evaluate for CAH, hCG to rule out tumors such as germinomas, teratomas, hepatomas, hepatoblastomas, and chorioepitheliomas (19), and adrenal androgens, including androstenedione and dehydroepiandrosterone sulfate (DHEA-S), to exclude adrenal tumors. Radiographic evaluation including abdominal and testicular ultrasound and/or computed tomography should also be considered based on laboratory studies. Testicular ultrasound is especially important in boys in whom the diagnosis of male-limited precocious puberty is being considered who lack a family history of the disorder (20). In these cases, excluding Leydig cell adenomas as the etiology of excess androgen secretion is imperative. The finding of fibrous dysplasia of bone on a nuclear bone scintigraphy suggests the diagnosis of McCune–Albright syndrome (21).

ETIOLOGY

Of historical interest, before an activating mutation of the LHR was described as the cause of FMPP, several theories regarding the mechanisms leading to gonadotropin-independent sex steroid secretion were hypothesized. “Testotoxicosis” was initially hypothesized by Rosenthal et al. to be caused by a circulating immunoglobulin with LH-like activity that would act similar to the thyroid-stimulating antibodies found in thyrotoxicosis. However, no such immunoglobulin was demonstrable in their male subjects with gonadotropin-independent precocious puberty (1). In 1991, Manasco et al. (22) presented evidence of a testis-stimulating factor that increased testosterone secretion from the testes was present in the plasma of boys with FMPP; however, this finding was never replicated. Other suggestions for the etiology of FMPP included paracrine-mediated control of gonadal steroid secretion and abnormalities in the local innervation of the gonads (11).

ACTIVATING MUTATIONS OF THE LH RECEPTOR

In 1993, evidence that FMPP was caused by an activating mutation of the LHR was discovered by two groups. Kremer et al. (23) determined that FMPP co-segregated with missense mutations in the sixth transmembrane (TM) domain of the LHR. Further evidence for the relationship was supported by the finding by Shenker et al. of a mutation causing the substitution of glycine for aspartate at position 578 in the sixth TM helix in affected individuals from eight different families. When this mutant LHR was transfected into COS-7 cells, basal cyclic adenosine monophosphate (cAMP) production was elevated despite the absence of ligand (24). Together, these findings suggested that FMPP was caused by a mutation leading to constitutive activation of the LHR.

The LHR is a member of the G-protein-coupled, seven-TM domain receptor family (25). Within this large family of receptors, it forms a subclass of glycoprotein hormone receptors along with the FSH and thyroid-stimulating hormone (TSH) receptors. These receptors have a similar structure including a long N-terminal extracellular domain whose main function is ligand binding, the seven-TM region, and a small intracellular, C-terminal region (26,27). Because all of the receptors in this subclass have a leucine-rich region (LRR), they are also collectively termed the LRR-containing GPCR G protein coupled receptor family (28). The gene for the human LHR is found on chromosome 2p21 (29). Eleven exons encode the LHR, including exons 1–10, which encode the extracellular domain, and exon 11, which encodes the TM and intracellular regions of the receptor (28).

Thus far, 16 activating mutations of the LHR have been described, all of which are found in exon 11 (30,31). However, only 15 of these mutations have been described in patients with familial or sporadic forms of male-limited precocious puberty (Table 3). One mutation (Asp⁵⁷⁸His) has only been described in patients with Leydig cell adenomas (20,32,33). Most of these mutations are found in a “hot-spot” region for amino acid substitutions including areas encoding the third intracellular loop and the sixth TM domain (34). However, other areas of the receptor including the first, second, third, and fifth TM domains also harbor mutations (Fig. 2). Although no activating mutations have been identified in exons 1–10, it is possible they exist; except in rare patients, only exon 11 has been evaluated for mutations in patients with FMPP (3,35).

Table 3
LH Receptor Mutations in FMPP

Nucleotide change	Amino acid substitution	Location	Familial (F) or sporadic (S)	Ligand responsiveness	Reference
T1103C	Leu ³⁶⁸ Pro	TM1	F	Responsive	(31)
C1188T	Ala ³⁷³ Val	TM1	F, S	Responsive	(86)
T1193C	Met ³⁹⁸ Thr	TM2	F, S	Responsive	(36,37,44,52,87,88)
T1370G	Leu ⁴⁵⁷ Arg	TM3	S	Unresponsive	(89)
A1624C	Ile ⁵⁴² Leu	TM5	F, S	Unresponsive	(36,37)
A1691G	Asp ⁵⁶⁴ Gly	IL3	F, S	Responsive	(36,37)
C1703T	Ala ⁵⁶⁸ Val	IL3	F, S	Responsive	(38,89)
G1713A	Met ⁵⁷¹ Ile	TM6	F	Responsive	(23,90)
C1715T	Ala ⁵⁷² Val	TM6	F, S	Responsive	(91)
A1723C	Ile ⁵⁷⁵ Leu	TM6	F, S	Responsive	(35,37)
C1730T	Thr ⁵⁷⁷ Ile	TM6	F	Responsive	(90,92,93)
G1732T	Asp ⁵⁷⁸ Tyr	TM6	S	Responsive	(36,37,42)
A1733G	Asp ⁵⁷⁸ Gly	TM6	F, S	Responsive	(23,24,36,92,93,94)
T1734A	Asp ⁵⁷⁸ Glu	TM6	F	Responsive	(95)
T1741C	Cys ⁵⁸¹ Arg	TM6	F	Unresponsive	(36)

Certain mutations have been described more commonly in various countries or regions, suggesting founder effects for those mutations. In the United States, approximately 90% of all mutations causing FMPP are due to the Asp⁵⁷⁸Gly substitution (36). In contrast, studies completed from European kindreds rarely identified this mutation. This suggests there is a strong founder effect for the Asp⁵⁷⁸Gly mutation in the United States (36). There is also a group of Dutch kindreds in whom the Ile⁵⁴²Leu mutation is common, although this mutation has also been identified in other areas (37). Studies

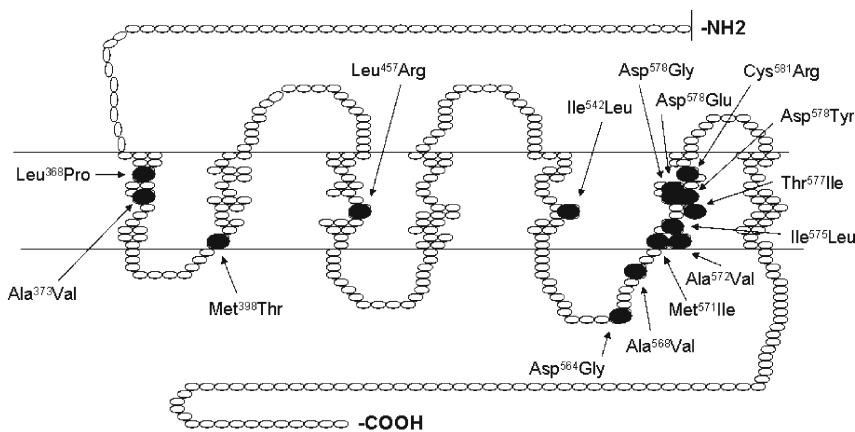


Fig. 2. Diagram of exon 11 of the luteinizing hormone receptor (LHR) demonstrating the positions of the activating mutations that have been described in familial male-limited precocious puberty (FMPP). Adapted with permission (30).

reveal that the most common mutation found in subjects from Brazil is a substitution of valine for alanine at position 568 (38). Thus far, all mutations found in subjects with FMPP have been present in the heterozygous state except for one report of a subject with a homozygous Ala⁵⁶⁸Val mutation. In this case, the homozygous inheritance of the mutation was due to maternal isodisomy, a condition in which both copies of the maternal chromosome are inherited (39).

Genotype–Phenotype Correlation

In general, there is little evidence for a relationship between genotype and severity of phenotype for the mutations causing FMPP (37). However, in vitro studies reveal that the Asp⁵⁷⁸Tyr mutation results in higher basal cAMP production compared with other constitutively activating mutations of the LHR (36,40). Subjects harboring this mutation have an earlier age of onset of the symptoms of testosterone excess in accordance with the in vitro analysis of the mutation (41,42). Three unrelated males have been described with this mutation; all had signs of FMPP by the age of 1 year compared with the usual age of onset, which is between 3 and 5 years of age (36,41–43). Phenotypic heterogeneity has been reported in a family with the Met³⁹⁸Thr mutation and another family with the Ile⁵⁴²Leu mutation. Evans et al. reported three brothers, all of whom had the Met³⁹⁸Thr mutation. Of the three, two were diagnosed with gonadotropin-independent precocious puberty at the ages of 5 and 7 years. However, despite also having an LHR mutation, one brother was 12 years old and had no history of precocious puberty at the time of the report (44). We have identified another family with a history of precocious puberty in males extending through four generations. The index case presented at the age of 3 years with precocious puberty; mutational analysis of exon 11 of the LHR of the subject and his father revealed the Ile⁵⁴²Leu mutation. Despite having the mutation, the father has an interesting history of precocious sexual development that began at the age of 2 years; however, pubertal development was not progressive, and he reached a final height of 73 inches (185.4 cm), well within his predicted genetic height. The paternal uncle of the index case presumably has FMPP, although mutational analysis of the LHR has not been completed. His pubertal development was advanced, and final adult height was compromised compared with his mid-parental height (MPH) and that of his brother's (the proband's father) (45). In the subject with maternal isodisomy identified by Latronico et al. (39), no increase in severity of symptoms or earlier age of onset of symptoms was recognized.

G_sα Mutation

Although mutations that lead to constitutive activation of the LHR are well accepted as the cause of FMPP, a mutation in the G_sα subunit has also been described in a male with gonadotropin-independent precocious puberty with a phenotype comparable with FMPP. Iiri et al. (46,47) described two boys who presented with both testotoxicosis and pseudohypoparathyroidism type 1-a (PHP1-a) who were found to have a substitution of alanine for serine at position 366 (Ala³⁶⁶Ser) in the G_sα subunit. They determined that this is a thermo-labile mutation that is constitutively active at a lower temperature as occurs in the testes, but inactivated at 37°C. The authors demonstrated a three- to four-fold increase in cAMP activity at 33°C compared with 37°C. The mutant G_sα protein

degrades much more rapidly at 37°C than the wild-type protein. This degradation at higher temperature leads to inactivation of G_sα at body temperature, resulting in the concomitant PHP1-a phenotype.

MOLECULAR STUDIES

Pathways Activated by LHR

The effects of ligand binding or constitutive activation of the LHR on Leydig cells are predominantly mediated by signaling through the Gs/adenylyl cyclase/cAMP/PKA pathway (48). However, there is also evidence that the phospholipase C pathway is activated in the presence of high concentrations of LH or hCG; it has been proposed that the activation of the phospholipase C pathway may only play a role in females during the pre-ovulatory surge or during pregnancy (49). Ascoli et al. (28) also suggested there may be a role of phospholipase C signaling during pregnancy when high levels of maternal hCG are present.

Because the cAMP-signaling pathway is the predominantly activated pathway during LHR activation, functional studies evaluating the various activating mutations of the LHR have focused on measuring the increase in cAMP in the basal state as well as during addition of the LHR ligand, hCG. The activating mutations causing FMPP consistently lead to increased basal cAMP production; however, the response to ligand (hCG) is variable, with some mutations leading to an increase in cAMP production over basal production and others having no further increase above the basal cAMP increase. There is discrepancy in the literature regarding activation of the phospholipase C/inositol triphosphate (PLC/IP3) pathway. The sensitivity of the method utilized to measure IP3 activity compared with that used to measure cAMP may contribute to the discrepant results (28). The Asp⁵⁷⁸His mutation that is present in patients with Leydig cell adenomas, but not identified in patients with FMPP, exhibits increased cAMP and IP3 signaling, whereas the Asp⁵⁷⁸Tyr mutation present in Leydig cell hyperplasia leads to increased cAMP production but only very little IP3 production (20). The combination of increased IP3 signaling and cAMP activity with the Asp⁵⁷⁸His mutation has been suggested as a contributing factor for the development of Leydig cell adenomas as opposed to FMPP (20).

Structural Changes Leading to Constitutive Activation

The structural mechanisms that lead to constitutive activation of the LHR involve interactions between the TM3 and TM6, TM3 and TM7, and TM6 and TM7 regions. These interactions play an obligatory role in stabilization of the receptor in an inactive state. The majority of amino acid residues susceptible to activating mutations of the LHR are either adjacent to, or in, the interfaces between these TM regions (28). In the human LHR, the amino acid residue at position 464 is involved in the formation of a salt bridge that has been shown to stabilize the receptor in an inactive state. Amino acid substitutions that weaken the salt bridge interaction with this Arg⁴⁶⁴ residue lead to constitutive activation (50). Zhang et al. (51) revealed that not only is constitutive activity of the LHR due to release of intramolecular interactions, it can also be attributed to the formation of links between TM domains. In reciprocal mutation studies, they demonstrated that constitutive activity only occurs in the presence of certain amino

acid residues. These residues lead to strong structural links between TM domains, suggesting that constitutive activation can occur because of new structural interactions and not solely due to release of intramolecular interactions.

Certainly large strides have been made in understanding the molecular mechanisms underlying the clinical features of FMPP; however, much is left to be deciphered regarding the structural interactions that are disrupted by the activating mutations leading to the disorder. Advances in bio-informatics and computer modeling will undoubtedly continue to advance our knowledge in this area.

THERAPY

Treatment of FMPP is important to prevent premature epiphyseal fusion, which ultimately will result in severe short stature. Untreated, the adult height reported in males with FMPP is 158.9 ± 8.5 cm (52).

GnRH Analogs

The underlying pathophysiology of the disorder suggests that GnRH analog therapy would not be effective; this has been proven true in separate studies utilizing two different GnRH analogs. Testosterone production continued unsuppressed after prolonged treatment with LRF-A (D-Trp⁶-Pro⁹-NEt-LRF) (1,11) and buserelin (53). GnRH analog therapy has been utilized as an adjunctive therapy when CPP develops in these patients Spironolactone and Testolactone.

Medroxyprogesterone Acetate

Medroxyprogesterone Acetate (MPA) is a progestin compound, known to inhibit gonadal steroidogenesis through the inhibition of pituitary gonadotropin secretion. There is also in vitro and in vivo evidence that MPA may directly inhibit testicular steroidogenesis (54–56). Limited reports of the use of MPA in the treatment of FMPP are available. Rosenthal et al. (56) described two boys with FMPP who were treated with MPA. One subject was treated for 5 years, and the other was still receiving MPA at the time of the report. In these two subjects, testosterone levels fell below 100 ng/dl, decreasing approximately 60% compared with their pre-treatment values. Growth velocity also decreased in both patients during treatment. No information regarding improvement in predicted height or final adult height is provided, and in one subject, the bone age at a chronologic age of 10 years was 17 years.

Cyproterone Acetate

The anti-androgen, cyproterone acetate, has been reported to have a suppressive effect on testosterone levels and growth velocity in one patient with FMPP who was treated for a period of 4 years (57). Prior to treatment, the patient's testosterone level was approximately 275 ng/dl, whereas on cyproterone acetate at doses ranging from 50 to 250 mg/day, the testosterone level decreased to < 100 ng/dl. In this report, the authors state that the bone age continued to advance during treatment; however, no specific information regarding skeletal maturation or predicted adult height (PAH) is provided (57).

Based on the limited data available and lack of long-term follow-up, it is difficult to make conclusive statements regarding the efficacy of either MPA or cyproterone

acetate in patients with FMPP. These treatments are mainly of historical interest, as better therapeutic choices are currently available.

Ketoconazole

Ketoconazole is an imidazole derivative, mainly utilized as an anti-fungal drug. Because the drug is a P450 cytochrome inhibitor, it also inhibits gonadal and adrenal androgen biosynthesis, predominantly through the inhibition of C17-20 lyase activity (58,59). Ketoconazole also inhibits other steps in adrenal and gonadal steroidogenesis including cholesterol side chain cleavage, 17-, 11-, and 18-hydroxylase activity (7,41). As ketoconazole inhibits testosterone production, efficacy can be evaluated based on decreasing testosterone levels. In fact, in patients with FMPP treated with ketoconazole, testosterone levels fall rapidly, usually within the first 24 hours (60). Multiple reports of the successful use of ketoconazole in FMPP are available, although results are variable depending on the age of therapy initiation as well as the degree of skeletal maturation at the time therapy was begun (53,60). Holland et al. initially reported three boys, ages 3.3–3.9 years, treated with ketoconazole for 9–12 months. During that brief period of treatment, none of the subjects developed the “escape phenomenon,” in which gonadotropin levels rise and the patients develop “secondary” CPP that has been described in some FMPP patients. In a subsequent report by the same investigators, three older subjects (5.0–7.4 years old) with more advanced skeletal maturation (mean bone age 13.2 years) were treated with ketoconazole. After a very short period of treatment (1–3 months), all three subjects had an elevation in testosterone, LH, and FSH and required the addition of a central GnRH agonist (buserelin) to control the clinical signs and symptoms of androgen excess. This “escape phenomenon” has been attributed to the more advanced age and bone age at the time of treatment initiation (60). It has been proposed that skeletal age may be involved in the onset of central puberty and maturation of the hypothalamic–pituitary–gonadal axis, thus explaining the onset of central puberty in the subjects in this report (60).

Final adult height data are available from five patients treated with ketoconazole for a period of 5–10 years (61). Median adult height (173 cm) obtained was similar to median target height (175 cm) and was significantly improved compared with median pre-treatment PAH (165 cm). Individual improvements in patients’ final adult heights compared with pre-treatment PAHs ranged from 5 to 13 cm. As expected, testosterone levels were significantly decreased during ketoconazole treatment. None of the patients exhibited signs of the “escape phenomenon” described by Holland et al.; thus, treatment with long-acting GnRH agonist was not utilized.

Concerns regarding ketoconazole therapy include liver toxicity and adrenal insufficiency secondary to the inhibition of adrenal steroidogenesis. The literature available does not support cause for significant concern regarding adrenal insufficiency. In one series, diurnal cortisol levels remained normal while peak cortisol response to ACTH stimulation testing was decreased but still within normal range (60). No specific recommendations regarding evaluation or treatment of adrenal insufficiency in patients on ketoconazole exist. In their report, Soriano-Guillen et al. had one patient with an elevated ACTH level and normal morning cortisol level that they opted to give stress dose hydrocortisone treatment during times of illness (61). If ketoconazole is chosen as a treatment option for FMPP, the health care provider should be cognizant of

the potential for adrenal insufficiency and instruct the patients and parents of such a possibility as well.

The more concerning side effect associated with ketoconazole therapy is the potential for liver insufficiency and even acute liver failure that can occur at any time during treatment. Severe hepatotoxic reactions occur in approximately 0.1–1% of subjects treated with ketoconazole (62). Other side effects include gastrointestinal symptoms and skin rash (63). Although ketoconazole has reportedly been well tolerated in patients with FMPP (53,60,61), acute liver failure in a boy with FMPP treated with high doses of ketoconazole has been reported (41). As compared with the usual adult dose of 400–800 mg per day, this patient was receiving 1200 mg per day for 1 year when he presented with pneumonitis and renal and liver failure. At the time of presentation, his alanine aminotransferase (ALT) was 924 U/l and the aspartate aminotransferase was 1028 U/l. Full recovery occurred after discontinuation of ketoconazole, and re-initiation of ketoconazole at a maximum dose of 600 mg per day resulted in no further pulmonary or kidney abnormalities and his liver enzymes remained lower than two times the normal limit (41). Based on this case, caution should be taken in treating FMPP patients with ketoconazole, and if such a decision is made, utilizing high doses (more than 600–800 mg per day) should be avoided. Some authors suggest that ketoconazole be discontinued if serum ALT levels increase above three times the normal limit (64,65).

Spironolactone and Testolactone

The combination of spironolactone, an anti-androgen, and testolactone, a first generation aromatase inhibitor, is the most extensively studied therapy in patients with FMPP. Initially, the drugs in this combination were evaluated individually without substantial success, and significant gynecomastia was noted as a side effect of spironolactone alone (66). Combination therapy is necessary because spironolactone does not block androgen production; instead, it is an anti-androgen due to antagonism at the level of the androgen receptor. Because androgens remain present for aromatization to estrogens, and estrogens can result in epiphyseal maturation (67,68) and gynecomastia (69), an aromatase inhibitor is necessary to inhibit estrogen synthesis. As described in the largest series of patients treated, a long-acting GnRH agonist is added when patients develop CPP (70).

In a 6-year follow-up, Leschek et al. described a decrease in growth velocity and skeletal maturation that resulted in an increase in PAH as compared with pre-treatment PAH (70). After just 1 year of treatment, growth velocity SDS standard deviation score decreased from 6.9 cm year \pm 1.0 cm year to 1.1 cm year \pm 0.4 cm year and remained in the normal range for age throughout the entire treatment period. Secondary to a decrease in the rate of skeletal maturation, PAH increased throughout the successive years of treatment. Following 6 years of treatment, the PAH increased from 160.7 \pm 4.6 cm to 173.6 \pm 3.2 cm (Fig. 3). Although no comparison with MPH is provided in this series, the average PAH achieved with this therapy is approximately the 30th percentile for an adult male. MPH predictions are often difficult to utilize in patients with FMPP if the mutation was inherited from the father. The true genetic height potential may be underestimated depending on whether or not the father was treated and if so, the mode of therapy. During this 6-year treatment period, all of the subjects developed CPP and were also treated with deslorelin. On average, deslorelin was started 2.6 \pm 1.3 years

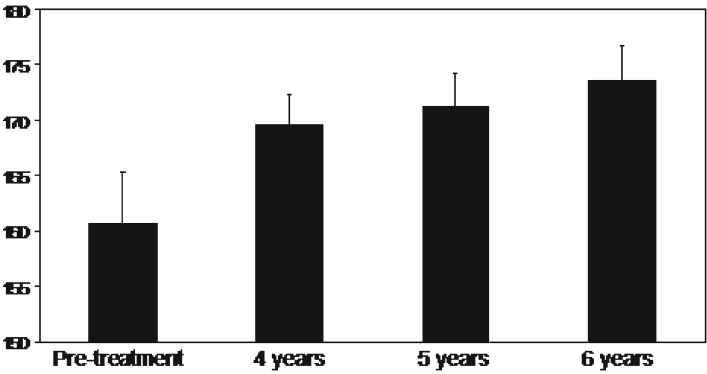


Fig. 3. Increase in predicted adult height during years 4–6 of spironolactone and testolactone therapy. Adapted with permission (70).

after the start of spironolactone and testolactone, and by the fifth year of the study, all of the patients were being treated with deslorelin. Final adult height data have been reported in 13 subjects treated with this combination (71). Final height results are similar to the predicted height results reported after 6 years of treatment. Compared with pre-treatment PAH (164.5 ± 10.9 cm), the final adult height was significantly improved (173.8 ± 8.4 cm) (71).

Spironolactone and testolactone are generally well tolerated. Testolactone is associated with gastrointestinal side effects (72), which were occasionally reported by the subjects in this series; however, therapy was not discontinued, nor was dosage adjustment required (5). To avoid electrolyte abnormalities, specifically hyponatremia, it is recommended that the spironolactone be withheld during acute gastrointestinal illnesses (5). The major drawback of this therapy is the frequency of dosing required. Spironolactone is given twice daily and testolactone must be given either three or four times per day. Adherence to such a dosing schedule may affect compliance and ultimate clinical outcome.

Newer Generation Androgen Receptor Antagonists and Aromatase Inhibitors

Since the combination of spironolactone and testolactone was first utilized in FMPP, improvements in androgen receptor blockers and aromatase inhibitors have been achieved. Overall efficacy, side effect profiles, and pharmacokinetics that allow for once daily dosing represent significant advances in both classes of pharmacotherapies. The most recently developed class of androgen receptor blockers currently available includes nilutamide, flutamide, and bicalutamide. All of these drugs exhibit potent androgen receptor blockade and in general have an excellent safety profile. Liver function abnormalities have been reported and usually occur within the first 6 months of therapy; thus the recommendation for frequent surveillance during this time period has been made. Post-marketing surveillance demonstrated an association of this class of drugs with interstitial pneumonitis. This condition has been reported most frequently with nilutamide (73).

The third generation aromatase inhibitors, letrozole and anastrozole, represent a significant advance in aromatase inhibition. As compared with the first generation aromatase inhibitors, the third generation inhibitors, anastrozole and letrozole, inhibit whole body aromatization by greater than 96% (74). This class of medications also has few adverse effects, and published experience in children allows for evaluation of its efficacy and safety in that population as well (75–78).

Experience with these agents in FMPP is limited. However, treatment with the combination of bicalutamide and anastrozole has been reported in two patients with FMPP (79,80). Both patients have had an impressive decrease in growth velocity and skeletal maturation, the latter resulting in an increase in PAH. Signs of androgen excess, including amount of pubic hair, acne, and sexual behaviors, also markedly improved. The first patient, treated for 44 months, had a decrease in growth velocity from 9.1 cm/year (+3.3 SD) to 4.3 cm/year (–1.1 SD). The rate of skeletal maturation, calculated as the change in bone age divided by the change in chronologic age ($\Delta\text{BA}/\Delta\text{CA}$), decreased from 3.4 to 0.77, preserving his PAH (182.1 cm) within the range of his MPH (179 cm). The second patient received treatment for 22 months with similarly impressive results. His growth velocity decreased from 13.4 cm/year (+6.0 SD) to 7.0 cm/year (+1.3 SD) and skeletal maturation ($\Delta\text{BA}/\Delta\text{CA}$) decreased from 4.7 to 0.3. His PAH (180.8 cm) is within the MPH range (179 cm). Depot-leuprolide therapy was added in the second patient after 1 year of bicalutamide and anastrozole therapy because of an increase in testicular volume, testosterone levels, and leuprolide stimulated LH levels. Both patients have tolerated the therapy without adverse side effects. Hepatic transaminases have remained normal in both patients.

Undoubtedly, more potent therapies that inhibit sex steroid production or action have the potential to improve the treatment of FMPP. However, because of the rarity of the disorder and the long-term follow-up required to assess efficacy, results will require years of clinical evaluation. Effects of these therapies on adult outcomes such as bone mineral density, lipid profiles, and fertility must also be considered.

LONG-TERM COMPLICATIONS AND CONCERNS

Fertility

No large-scale studies to investigate potential complications in adult males with FMPP have been conducted. Several early studies suggested that some adult men with FMPP may be at increased risk of developing infertility in adulthood. However, fertility is obviously achieved in a large number of males with FMPP; otherwise, the mutation would not be propagated through father-to-son transmission. In the cases of infertility in males with FMPP that have been reported, the adult males are fully virilized but have small testicular volume and oligospermia. FSH levels were consistently elevated in these patients with infertility (2,6,81). No obvious relationship to the type of therapy received can be made due to the small number of patients reported. Whether the high levels of testosterone or the degree of FSH suppression during childhood play a role in germ cell damage is unknown.

Tumors

Both somatic and germline activating mutations of the LHR have been described in testicular tumors (3,20,82,83). Therefore, patients with FMPP are theoretically at risk of

the development of testicular tumors. Despite this concern, only two confirmed reports of testicular tumors in patients with FMPP exist. A testicular seminoma occurred in a 35-year-old man with a history of FMPP (83). He had a germline mutation resulting in the substitution of glycine for aspartic acid at position 578, a commonly found mutation in patients with FMPP. Leschek et al. (82) reported a patient with FMPP who developed nodular Leydig cell hyperplasia at the age of 10 years. This patient had a germline substitution of glycine for aspartic acid at position 564. In patients with germline LHR mutations, a causative relationship for the development of tumors cannot be proven; however, the possibility that high levels of sex steroids during childhood played a role certainly exists (3,84). Certainly, the awareness of the possibility for testicular tumors in patients with FMPP is important. Patients should be counseled about this theoretical risk and taught testicular self-examination during adolescence and adulthood.

Somatic mutations of the LHR have also been described in patients with Leydig cell adenomas (20,32,85). The mutation described in those patients was a substitution of histidine for aspartic acid at position 578. This mutation has thus far not been described in patients with FMPP.

CONCLUSIONS

FMPP is a rare form of gonadotropin-independent precocious puberty that leads to phenotypic findings exclusively in males. The disorder is caused by constitutively activating mutations of the LHR and is inherited in an autosomal dominant manner. Therapy is aimed at decreasing signs of virilization and preserving height potential by delaying premature epiphyseal maturation. Adult height outcomes obtained after treatment with the most common therapies have only recently been published. Long-term complications of the disorder, as well as of therapies utilized during childhood, in adult men with FMPP remain an area requiring further investigation.

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19

Gonadal and Adrenal Tumors

Inessa M. Gelfand, MD
and Nadine G. Haddad, MD

CONTENTS

GONADAL TUMORS

ADRENAL TUMORS

REFERENCES

Summary

Ovarian and adrenal tumors are rare causes of precocious puberty. The pubertal progression is typically more rapid than is observed in central precocious puberty. Juvenile granulosa cell tumors are the most common ovarian tumors associated with premature sexual development, whereas Leydig cell tumors are the most common hormone-producing tumors of the testis. A variety of other sex cord tumors and less commonly germ cell tumors can be associated with premature pubertal development. These tumors can secrete androgens, estrogens, and a multitude of other hormones. Adrenocortical tumors (ACTs) are characteristically functional in children. Virilizing signs are the most common feature, although a mixed hormonal syndrome is often present. The association of ACT with several genetic syndromes has increased our understanding of the molecular mechanism of these tumors. Genetic abnormalities of chromosomes 11 and 17 are the most common finding. Complete surgical resection is potentially curative. Advances in therapy are still needed to improve outcome.

Key Words: Granulosa cell tumor; Leydig cell tumor; Sertoli cell tumor; Gonadoblastoma; Adrenal rests; Adrenocortical tumor; *p53*; IGF-2; Mitotane.

GONADAL TUMORS

Hormone-secreting ovarian and testicular tumors cause signs of precocious sexual development through the direct effects of estrogens and androgens on target tissue (1). During normal embryogenesis, ovarian granulosa and testicular Sertoli cells are derived from embryonic sex cords, whereas ovarian theca and testicular Leydig cells arise from stromal cells. Sex cord–stromal cell tumors are the most common hormone-secreting tumors of ovaries and testes and may contain a variety of cell type combinations (2). Precocious puberty caused by gonadal neoplasms is generally characterized by a more rapid progression than is observed in idiopathic central precocious puberty (3). The premature sexual development can be isosexual or heterosexual; the latter is characterized by signs of feminization in boys and virilization in girls. Central (or true,

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gonadotropin dependent) precocious puberty may develop in patients with histories of peripheral (or gonadotropin independent) precocity (4,5).

Ovarian Causes of Precocious Puberty

OVARIAN CYSTS

In the fetal and neonatal periods, ovarian cysts are caused by maternal and fetal gonadotropin stimulation in utero and therefore are physiologic. Simple cysts less than 2 cm in diameter are generally considered physiologic. Small, nonfunctioning cysts are observed in about 2–5% of asymptomatic girls between the ages of 2 and 8 years (6). The majority of ovarian cysts are nonfunctioning (7); however, up to 1.5% can be associated with precocious puberty (6). Periodic vaginal bleeding has been described in girls with recurrent benign ovarian cysts and is the result of estrogen withdrawal upon involution of the cyst (8).

Common clinical manifestations of ovarian cysts include abdominal pain, premature breast development, accelerated growth, advanced bone age, and menstrual irregularities. An elevated serum estradiol in the face of suppressed gonadotropins is the typical laboratory finding in symptomatic prepubertal girls with ovarian cysts (9). Spontaneous regression of the ovarian cyst with resolution of signs of estrogenization is observed in most patients, and surgical resection is rarely required. The diagnosis of McCune–Albright syndrome (MAS) should be entertained in all girls with recurrent ovarian cysts.

OVARIAN TUMORS

Ovarian tumors are divided into three major categories: common epithelial tumors, sex cord–stromal tumors, and germ cell tumors. Of these, only sex cord–stromal tumors and germ cell tumors can cause precocious puberty. Tumors that do not clearly fit into these categories are classified separately (*Table 1*). Abdominal pain, distension, and a palpable mass are the most common presenting signs and symptoms of ovarian neoplasms. When associated with torsion, the pain can be acute in onset. Other symptoms include nausea, vomiting, fever, vaginal bleeding, and sexual precocity (10–12). Only tumors with endocrine function will be discussed in the following sections.

Sex Cord–Stromal Cell Tumors. Sex cord–stromal cell tumors are commonly associated with endocrine manifestations, both estrogen and androgen related. The diagnosis is confirmed by specific immunohistochemical findings characterized by negative epithelial membrane antigen, and positive inhibin and calretinin (13). These tumors are typically unilateral, and limited surgery is usually curative.

Granulosa–Stromal Cell Tumors: Juvenile granulosa cell tumors and granulosa cell tumors—The granulosa cell tumor (GCT) was first described by von Kahliden in 1895. Juvenile GCT (JGCT) is more common in the pediatric population and is a distinct entity from adult GCT with different pathological and clinical features (14). Up to 10% occur in prepubertal girls and the incidence significantly increases after the age of 4 years. Approximately 10% of JGCTs are bilateral (15). Sexual precocity is seen in up to 70% of JGCTs and adult GCTs (16). Clinical findings include premature

Table 1
Classification of ovarian tumors

Common epithelial tumors
Serous tumors
Mucinous tumors
Endometrioid tumors
Clear cell tumors
Brenner tumors
Mixed epithelial tumors
Undifferentiated carcinoma
Unclassified epithelial tumors
Sex cord–stromal tumors
Granulosa–stromal cell tumors
Sertoli–Leydig cell tumors
Mixed form/unclassified
Germ cell tumors
Dysgerminoma
Endodermal sinus tumor
Embryonal carcinoma
Polyembryoma
Choriocarcinoma
Teratoma
Mixed form
Lipid cell tumors
Gonadoblastoma

Adapted with permission (52).

breast development, vaginal discharge and bleeding, pubic and axillary hair growth, accelerated linear growth, and advanced bone age (17). Uterine bleeding is a later finding, and it is usually irregular. Both adult GCT and JGCT can be associated with significant androgen production (18). Clinical manifestations of hyperandrogenism include clitoromegaly, hirsutism, deepening of the voice, male escutcheon, temporal hair recession, acne, and amenorrhea or irregular menses (19).

The androgenic effects of GCT and JGCT are a source of much controversy. Clinical features of androgen excess have been observed in patients with normal serum androgen levels. It has been postulated that high estrogen levels could stimulate androgen-responsive end organs (20). Alternatively, measurable progesterone and testosterone levels were seen in tumor extracts (21), and increased testosterone levels were detected in ovarian veins (22), suggesting that hypersecretion of androgens may be responsible for the development of signs of virilization (19). Secretion of androstenedione, inhibin, insulin-like growth factor (IGF)-1, and mullerian inhibitory substance has also been described (23,24).

Unilateral salpingo-oophorectomy is considered curative, and careful follow-up is recommended. Postoperatively, patients commonly develop uterine bleeding due to estrogen withdrawal. Regression of secondary sexual characteristics is seen in younger children, and they later have onset of menarche at appropriate age (15). Androgenic

manifestations of JGCT also regress after curative surgery, although some features, such as deepening of the voice and hirsutism, may persist (19). Although activating mutations of the Gs- α and follicle-stimulating hormone (FSH) receptors were not found to be a major cause of GCT (25), the expression of G-protein-coupled receptor kinases (regulators of signal transduction) was altered in GCT indicating a possible involvement in the pathogenesis of these tumors (26).

Granulosa–theca cell tumors: Granulosa–theca cell tumors are sex cord–stromal tumors that consist of more than 25% of granulosa and theca cells (27). Common presenting features include premature sexual development, menstrual abnormalities, and abdominal mass (27). Signs of virilization, accelerated growth, and advanced bone age are frequently seen (28–31). Biochemical evaluation reveals elevated serum levels of estrogen, progesterone, testosterone, and α -fetoprotein (AFP) (28,29,31).

Theca cell tumors: Theca cell tumors are sex cord–stromal tumors with less than 25% granulosa cells (27). Therefore, GCTs, theca cell tumors, and granulosa–theca cell tumors probably represent a distribution along a spectrum. Theca cell tumors cause precocious puberty and menstrual abnormalities related to estrogen production that is seen in over half of these tumors (27,32).

Sertoli–Leydig Cell Tumors: Sertoli–Leydig cell tumors (formerly known as androblastoma or arrhenoblastoma) account for less than 0.5% of ovarian tumors (33). Most common presenting features are abdominal pain, distension, and a palpable abdominal mass (34). Signs of androgen excess are present in 35–50% of patients (34,35). Infrequently, these tumors cause isosexual precocity in girls (36,37).

Sertoli–Leydig cell tumors secrete inhibin, AFP, testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP), estrogen, and progesterone (34,35,38–40). Conservative surgical management with unilateral salpingo-oophorectomy is preferred in the majority of patients (41).

Sertoli Cell Tumors: Sertoli cell tumors of the ovary sometimes have been included under the category of Sertoli–Leydig cell tumors. However, unlike the latter, these tumors lack the Leydig cell component and the immature neoplastic stroma. Their origin is unknown, and it is speculated that they arise from ovarian cells that retained the potential to differentiate toward Sertoli cells (42). Clinical features include isosexual precocity, signs of virilization, and menstrual abnormalities (42). The majority of patients present with a palpable abdominal mass. The tumors are usually unilateral and confined to the ovary (43), although extra-ovarian spread at presentation has been described (44).

Sertoli cell tumors secrete estrogen, progesterone, testosterone, inhibin, calretinin, vimentin, and keratin, all of which can be used as tumor cell markers (43,44). The prognosis of patients with disease confined to the ovaries is generally good.

Sex Cord Tumors with Annular Tubules: Sex cord tumors with annular tubules are associated with Peutz–Jeghers syndrome in about one-third of cases (45). Their origin (Sertoli versus granulosa cell) is uncertain; however microscopic appearance strongly suggests Sertoli cell nature, with a testicular direction of differentiation (46). The tumor can be associated with precocious puberty, advanced bone age, menstrual irregularities, and false-positive pregnancy test (47).

When associated with the Peutz–Jeghers syndrome, these tumors tend to be multifocal, bilateral, calcified, and clinically benign, whereas isolated sex cord tumors with annular tubules are unilateral and large with a malignant potential (45). Elevated serum levels of estradiol, sex hormone-binding globulin (SHBG), prolactin, and progesterone are frequently observed (48,49).

Germ Cell Tumors. Germ cell tumors are the most common ovarian tumors in children, but they are rare causes of precocious puberty (2). Historically, germ cell tumors have been associated with poor survival. However, with the recent introduction of combination chemotherapy regimens, the prognosis of the affected patients has markedly improved (50,51).

Dysgerminoma: Dysgerminomas are the most common ovarian germ cell tumors (52). They resemble primordial germ cells in their morphological and histological characteristics and are analogous to testicular seminomas (14). Ten percent of dysgerminomas occur in children under the age of 10 years (53). The contralateral ovary is involved in 10–15% of cases (54). Pure dysgerminomas are not hormonally active. However, tumors containing teratomatous components may cause signs of virilization and precocious puberty (53). Elevated levels of human chorionic gonadotropin (hCG), placental alkaline phosphatase, and lactic dehydrogenase (LDH) can be detected (55,56).

Teratoma: Teratomas are germ cell tumors containing components from all three embryonic layers. Teratomas are categorized as mature or immature, cystic or solid. Mature cystic teratomas, or dermoid cysts, are the most common ovarian neoplasm in children (57), and they are benign. Immature solid teratomas have a high probability of malignancy (58). Teratomas containing trophoblastic or chorionepithelial components can secrete estrogen and hCG and cause sexual precocity (59,60).

Embryonal carcinoma: Embryonal carcinoma of the ovary is a rare malignancy that resembles the embryonal carcinoma of adult testes. It is associated with hCG and AFP secretion and positive urine and serum pregnancy tests (61). Presenting features include precocious puberty, menstrual abnormalities, and hirsutism (62).

Steroid Cell Tumors. Steroid cell tumors are composed of cells that resemble lutein cells, Leydig cells, and adrenal cortical cells. They may be characterized as lipid-free or lipid-rich. Tumors containing predominantly lipid-rich cells have been called lipoid or lipid cell tumors, adrenal rest cell tumors, or adrenal-like tumors (52). The steroid cell tumors can be hormone secreting and infrequently cause precocious puberty, virilization, and cushingoid features (63,64). These tumors can secrete a variety of hormones including progesterone, testosterone, 17-OHP, androstenedione, estradiol, and dehydroepiandrosterone sulfate (DHEAS) (65). The tumor cells have been shown to express adrenocorticotrophic hormone (ACTH) receptors, as well as P450c11 β and P450c21 markers, supporting the adrenal origin of these ovarian tumors (65).

Gonadoblastoma. Gonadoblastoma is a rare tumor that occurs almost exclusively in dysgenetic gonads containing Y chromosomal material, such as 45 XO/46 XY Turner's syndrome and 46 XY gonadal dysgenesis (Swyer syndrome) (66–68), although rare cases of gonadoblastoma-like tumors developing in normal 46 XX females have been

described (69,70). A susceptibility region has been mapped to the pericentromeric region on the short arm of the Y chromosome and has been named gonadoblastoma gene on the Y chromosome (*GBY*) (71). Five functional genes are present in this region. One, testis-specific protein Y-encoded (*TSPY*) gene, is expressed in gonadoblastoma, suggesting possible involvement in its oncogenesis (72). Histologically, gonadoblastomas consist of nests of dysplastic germ cells mixed with sex cord derivatives resembling immature Sertoli or granulosa cells (73) (*Fig. 1*). Although gonadoblastomas are not metastatic, the germ cell component of these tumors may give rise to malignant germinomas, such as seminomas. Clinical presentation includes precocious puberty and signs of virilization in phenotypic female patients (74). Elevated gonadotropin levels are characteristic of the primary condition of gonadal dysgenesis (75). However, these levels may be not elevated in patients with gonadal dysgenesis and peripheral precocious puberty caused by this tumor.

Testicular Tumors

Testicular tumors cause isosexual precocious puberty through the direct action of testosterone secreted by the tumor or indirectly through the stimulation of testosterone production by Leydig cells. Sex cord–stromal tumors secrete androgens and directly stimulate the end organ tissues, whereas germ cell tumors secrete hCG (1,76), which stimulates Leydig cell androgen secretion by cross-reacting at the level of the LH receptor (77). Because germ cell tumors of the testis are extremely rare in children, only sex cord–stromal tumors will be described in this chapter.

SEX CORD–STROMAL CELL TUMORS

Sex cord–stromal tumors account for up to 40% of testicular neoplasms in children. Inhibin A is secreted by the majority of these tumors and serves as a reliable tumor marker in differentiating them from germ cell tumors (78).

Leydig Cell Tumors. Leydig cell tumors (also known as interstitial cell tumors) are rare testicular malignancies; however, they are the most common hormone-producing

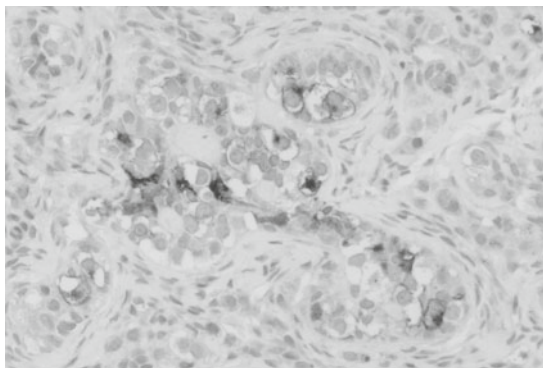


Fig. 1. Multiple foci of gonadoblastomas composed of placental alkaline phosphatase (PLAP)-positive dysplastic germ cells admixed with smaller supporting sex cord cells in an ovarian-like stroma (PLAP immunohistochemistry, 40 \times).

tumors of the testes (79). Up to 18% of cases are reported in children under the age of 10 years, and they are almost always unilateral. Prepubertal boys typically present with isosexual precocity, manifested by pubic and axillary hair growth, acne, penile enlargement, deepening of the voice, accelerated growth, and advanced bone age (79,80). Occasionally, gynecomastia may be present and results from estrogen production by the tumor and/or increased aromatization of testosterone (81). Physical examination usually reveals asymmetric testis, although this may not always be evident due to some contralateral testicular enlargement (82) or a small tumor size (83). Testicular ultrasound is an excellent screening tool and should be used in all patients with evidence of gonadotropin-independent precocious puberty and negative family history of familial male-limited precocious puberty (FMPP). Recently, a somatic activating mutation of the LH receptor has been described in Leydig tumor cells. This mutation was not detected in peripheral lymphocytes (83,84). Differentiation from FMPP may prove to be a challenge. However, patients with the latter condition commonly present at a younger age, typically before the age of 4 years (85).

Elevated serum levels of testosterone and estradiol (86) as well as high urinary 17-ketosteroids and 17-hydroxycorticoids (79) are characteristic of Leydig cell tumors. Leydig cell tumors are typically benign in children (87), and orchiectomy is considered curative (88). Overall, a benign behavior is associated with a younger age at presentation and positive hormonal activity of the tumor (89).

Sertoli Cell Tumors. Pure Sertoli cell tumors of the testes are extremely rare. A distinct type, the large cell calcifying Sertoli cell tumor, was described in a predominantly pediatric population, and it is rarely associated with precocious puberty (90,91). Gynecomastia is a common presenting feature (92) that can be observed in up to 30% of cases (1). It has been attributed to increased testicular aromatase activity. Interestingly, Sertoli cell tumors are bilateral in about 40% of cases, and generally they are benign (93).

These tumors are associated with increased secretion of testosterone, estradiol, androstenedione, and SHBG (92,94). Sertoli cell tumors have been associated with pituitary adenomas and bilateral adrenal hyperplasia (90). Sertoli cell tumors are also seen in patients with Peutz–Jeghers syndrome (95) and Carney complex (96).

ADRENAL REST TUMORS

Adrenal rest tumors are tumors of aberrant adrenal tissue that occur in the clinical scenario of high levels of ACTH, such as in primary adrenal insufficiency (97). Adrenal rest tumors are prevalent in patients with congenital adrenal hyperplasia due to 21- β -hydroxylase and 11- β -hydroxylase deficiency (98,99). Clinical presentation includes testicular enlargement with palpable masses and precocious puberty due to suboptimal Congenital adrenal hyperplasia control (100–102). Adrenal rest tumors originate in the hilar region of the testicle and extend peripherally. They contain large cells that resemble Leydig cells. Sonographically, these tumors appear as multiple round hypoechoic nodules that are frequently bilateral (103). Adrenal rest tumors usually regress with adequate glucocorticoid therapy (104). However, they can also be present in patients with appropriate Congenital adrenal hyperplasia control, indicating the need for frequent careful examination of the testis in patients with CAH (98). Intratesticular expansion of adrenal rest nodules destroys the testicular parenchyma resulting in low

testosterone levels and infertility (98,105). Infertility can be reversed with appropriate glucocorticoid suppressive therapy (106). Intensification of the glucocorticoid therapy is the preferred treatment modality in patients with adrenal rest tumors, whereas testis-sparing surgical procedures are reserved for steroid unresponsive cases (107). Serial sonograms are useful to document tumor regression.

ADRENAL TUMORS

Epidemiology

Adrenocortical tumors (ACTs) are an uncommon cause of peripheral precocious puberty. They are rare in children and account for 0.2% of all childhood malignancies, with a peak incidence between ages 0 and 4 years (108). Mild female predominance has been reported (109,110). For unclear reasons, southern Brazil has one of the highest incidences of childhood ACT, occurring 10–15 times more frequently than worldwide estimates. This unique epidemiologic finding provided grounds for research to unravel the genetic mechanism of childhood ACT.

Genetics

ACTs and adrenal nodules occur in several genetic syndromes, including the Li–Fraumeni syndrome, congenital hemihypertrophy, Beckwith–Wiedemann syndrome (BWS), Carney complex, multiple endocrine neoplasia type-1 (MEN1), and congenital adrenal hyperplasia (111). Studying such patients helped increase our understanding of the molecular pathogenesis of the more common sporadic ACTs.

A genetic link was first suggested by Miller, who observed the familial occurrence of ACTs (112). Later, Li and Fraumeni noted a high frequency of ACTs in children from families with a wide variety of cancers inherited in an autosomal dominant pattern (113,114). Germline mutations of the *p53* gene located on chromosome 17p were later identified in patients with this syndrome (115), as well as in children with sporadic ACTs (116).

p53 is a tumor suppressor gene that leads to cell cycle arrest and apoptosis in response to genotoxic stress. It is the most commonly mutated gene in human cancers (117). In a study of 55 patients with ACTs, Latronico et al. identified a germline mutation of the *p53* gene (Arg337His) in 78% of children with both malignant and benign sporadic tumors (118). This mutation was also identified in asymptomatic older first-degree relatives, indicating that this specific inherited mutation may have a low penetrance for predisposition to the development of ACT. A high incidence of loss of heterozygosity was also observed in these tumors resulting from somatic loss of the second allele, by either deletion or a second mutational hit (118).

ACT can occur in association with BWS, a rare syndrome characterized by macrosomia, visceromegaly, macroglossia, abdominal wall defects, and increased risk of abdominal tumors, with Wilms' tumors and adrenal carcinoma being most common (119). Genetic abnormalities of the 11p15 locus, which includes the insulin-like growth factor 2 (*IGF-2*) and *p57/KIP2* genes, have been described in this syndrome (120). These genes are imprinted, whereby only one parental allele is expressed and the other is silenced. Children with BWS often have uniparental isodisomy of chromosome 11, leading to overexpression of *IGF-2*. Overexpression of *IGF-2* is observed in several

sporadic malignancies, including Wilms' tumor, hepatoblastoma, and ACTs (111,121). Structural abnormalities of the 11p15 locus leading to overexpression of *IGF-2* and inactivation of the *p57KIP2* gene, a tumor suppressor gene, have been observed in children and adults with sporadic ACTs (109,122,123). Both events favor cell proliferation in malignant ACT and suggest that *IGF-2* is a tumor progression factor rather than an initiating factor (111).

Congenital hemihypertrophy is a rare condition characterized by asymmetric growth of the cranium, trunk, and limbs with or without visceral involvement (124). Similar to children with BWS, children with hemihypertrophy are at risk of developing abdominal tumors including Wilms' tumors, hepatoblastoma, and ACTs, suggesting that this condition may represent one end along the spectrum of BWS (124,125).

MEN1 is a rare familial cancer syndrome. Adrenal nodules and tumors are common in this condition (126). The gene responsible for MEN1 is *menin*, a tumor suppressor gene located at 11q13. Although loss of heterozygosity of 11q13 occurred frequently in sporadic ACTs, this finding was typically not associated with a MEN1 mutation, suggesting that this gene does not play an important role in the pathogenesis of sporadic ACTs and that a different tumor suppressor gene on this chromosome may be involved (127,128).

Carney complex is a rare hereditary syndrome characterized by primary pigmented adrenocortical disease, spotty skin pigmentation, atrial and peripheral myxoma, and other endocrine tumors (129). Inactivating mutations of *PRKARIA*, a tumor suppressor gene encoding protein kinase A regulatory subunit 1 α , was found in a subset of patients with Carney complex (130). Recently, somatic mutations of *PRKARIA* were identified in a small percentage of patients with sporadic hormone-secreting ACTs (131).

ACTs are described rarely in patients with congenital adrenal hyperplasia, an inherited condition caused most commonly by a deficiency of the steroidogenic enzyme 21-hydroxylase. However, mutations in the 21-hydroxylase gene were detected in a small subset of patients with sporadic ACTs, suggesting that this specific genetic defect is a rare cause of ACTs (128,132). Similarly, nodular adrenocortical disease was described in MAS, a sporadic genetic condition characterized by pigmented café-au-lait macules, fibrous dysplasia of bones, and endocrine hyperfunctioning, including precocious puberty, Growth hormone-secreting pituitary adenomas, thyroid nodules and thyrotoxicosis, and Cushing syndrome caused by adrenocortical nodules. MAS results from a somatic mutation of *GNAS1*, the gene encoding for the Gs- α stimulatory subunit of the G-protein involved in intracellular signaling of several hormones. However, mutations of the G-protein genes Gs- α and the cyclic adenosine monophosphate (cAMP) inhibitory Gi2- α were not found to be a common cause of ACT (133,134).

Comparative genomic hybridization studies in sporadic ACTs revealed complex findings, indicating that more than one mechanism of tumorigenesis exist. For instance, chromosomal instability involving chromosomes 2, 9, and 11 was found in a significant percentage of patients with ACT and loss of chromosome 17 (carrying the *p53* gene) suggesting a complex genetic interaction (135). Furthermore, a consistent increase in the DNA copy number of chromosomal region 9q34 has been frequently described in childhood ACT (136,137). Recently, amplification of the steroidogenic factor 1 (*SF-1*) gene located in this chromosomal region was detected in the majority of children with

ACT and consistent gain of 9q (138), indicating a novel mechanism of oncogenesis and adding to the complexity of the etiopathogenesis of ACT.

Clinical Manifestations

The majority of childhood ACTs occur in the first 4 years of life and onset in the neonatal period has been reported (109,110). In contrast to adults, the majority of ACTs in children are functional (hormone-producing) (109,110,139). ACTs may autonomously secrete an excess of one or more of the three major corticosteroids: glucocorticoids, mineralocorticoids, and sex steroids. A mixed hormonal syndrome is often present. Signs of virilization are the most common clinical manifestation, resulting from overproduction of adrenal androgens including DHEA, its sulfate (DHEAS), and androstenedione, which may be converted to testosterone. Features of androgen excess include pubic and axillary hair growth, facial acne, clitoromegaly, adult body odor, muscle hypertrophy, penile enlargement, and growth acceleration (*Fig. 2*).

Valuable clinical information is available through the International Pediatric ACT Registry. The registry has compiled data on 254 children worldwide (110). In this study, signs of virilization were present in 84% of children, either alone or in association with signs of glucocorticoid excess. Overproduction of glucocorticoids alone was present in only 5.5% of patients (110). Classic features of cortisol excess include obesity, linear growth delay, moon facies, plethora, muscle wasting, generalized hirsutism, striae, hypertension, and glucose intolerance. Primary hyperaldosteronism and pure feminization are rare (110,139). Hypertension is present in a significant percentage of children and is most commonly related to glucocorticoid excess and/or hyperaldosteronism (110). These findings are consistent with those from other studies (109,110,139).

Most patients have localized or regional disease at the time of diagnosis, with metastatic disease present in less than 5% of cases (110). The liver and lungs are the most common sites of metastasis.



Fig. 2. Pubic hair growth and clitoromegaly in a child with an adrenocortical tumor.

Laboratory Evaluation

Because ACTs can secrete a variety of adrenal hormones, these hormone levels can be used as tumor markers. Initial laboratory testing should establish the endocrine profile of the tumor and rule out other causes of hyperandrogenism such as congenital adrenal hyperplasia.

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are the major sex steroids produced by the adrenal gland, whereas testosterone is secreted in minimal amounts. Under pathological conditions, such as in ACTs, significant amounts of testosterone and its precursors are produced. Therefore, initial screening tests should include measurement of plasma testosterone and its precursors androstenedione, DHEA, and DHEAS. Urinary 17-ketosteroids, a nonspecific measure of androgenic metabolites, are also elevated (139). Hypercortisolism can be detected with measurement of 24-hour urine excretion of free cortisol, which will be greater than 80 μg per m^2 in the majority of children with Cushing syndrome (140). Concurrent measurement of ACTH concentration shows a level consistent with ACTH-independent hypercortisolism. A midnight salivary cortisol is a simple and accurate way to screen for hypercortisolism. A level greater than 7.5 nmol/l is indicative of Cushing syndrome (141).

Imaging studies are important for initial diagnosis, disease staging, and surgical planning. Ultrasound is an ideal modality for screening the adrenal gland but cannot reliably identify small lesions as accurately as computerized tomography (CT) (*Fig. 3*). CT and magnetic resonance imaging (MRI) can comparably detect lesions greater than 1 cm, whereas MRI may have the advantage of assessing vascular invasion and thrombosis (142). Positron emission tomography (PET) with FDG (2-[fluorine-18]-fluoro-2-deoxy-D-glucose) was studied in adults with ACT and was helpful to differentiate malignant from benign tumors. Therefore, it may have the advantage of detecting distant metastasis that is not readily detected by CT or MRI (143).

Histopathology

Pediatric ACTs consist of two main histopathological subtypes: adenoma and carcinoma. Carcinomas are more common in children. The differentiation of benign from malignant ACTs solely based on histological findings is difficult. Several

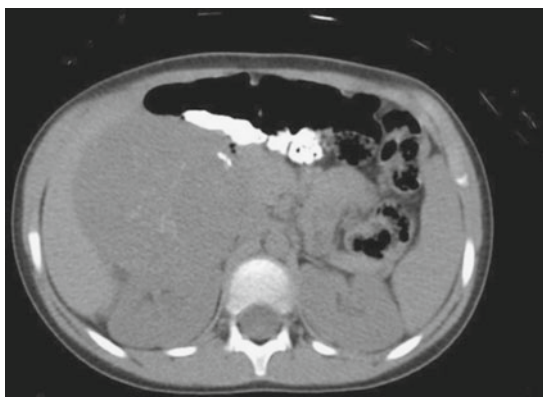


Fig. 3. Computerized tomography appearance of a large adrenocortical tumor.

macroscopic and microscopic criteria are used to define the malignancy of ACTs. Macroscopically, adenomas tend to be well demarcated and relatively small, whereas carcinomas tend to be large with marked lobulation and necrosis rendering them very friable and at risk of rupture during surgery. Microscopically, architectural disarray, increased mitotic index, marked cellular pleomorphism, nuclear atypia, and venous and capsular invasion predict malignancy (139,144).

Treatment

Complete surgical resection is the cornerstone of successful treatment and is potentially curative. Outcome is stage dependent. Patients with a small and completely resected tumor without evidence of metastasis have an excellent prognosis, with a 5-year event-free survival rate of greater than 90% (110), whereas patients with unresectable disease have a dismal outcome (Fig. 4). Age, tumor size, and resectability are the most important prognostic indicators (110,145).

The role of chemotherapy has not been systematically studied in childhood ACTs. Mitotane (1,1-dichloro-(2-*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane; *o*, *p*'-DDD), an insecticide derivative, causes adrenocortical necrosis and is the most commonly used agent in the treatment of ACT. It has been frequently used as an adjuvant therapy to treat residual and metastatic disease in conjunction with surgery and other chemotherapeutic agents. In the pediatric population, approximately a third of the patients respond to mitotane (146,147), but long-term survival has been reported in few children treated with high doses of mitotane (148). Higher serum levels of mitotane may induce more rapid remission. However, this is often curtailed by the development of severe side effects, including most commonly gastrointestinal and neurologic toxicities (149). Side effects appear to be dose related and include nausea, vomiting, anorexia, diarrhea, somnolence, mental confusion, ataxia, blurred vision, developmental delay, and renal and hepatic dysfunction (108,113,148). Monitoring of mitotane serum levels has been recommended to avoid toxicity (150). Because of its adrenolytic effect, all patients treated with mitotane should be assumed to have adrenal insufficiency and should receive adequate glucocorticoid and mineralocorticoid replacement therapies. Mitotane has been shown to induce the hepatic p450 enzyme system and increase

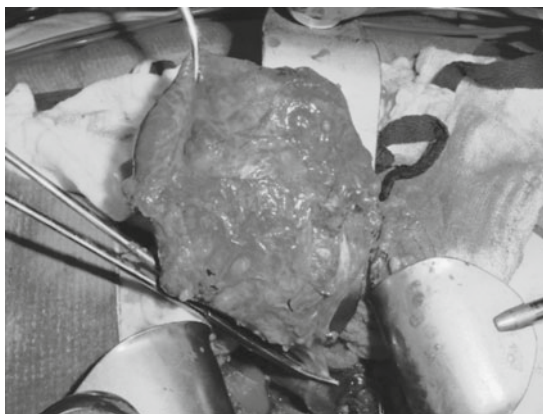


Fig. 4. Intraoperative appearance of a large and friable adrenocortical tumor.

clearance of glucocorticoids. Therefore, high-dose steroid replacement is needed in children treated with mitotane (151).

A variety of chemotherapeutic agents have been used, most commonly cisplatin, etoposide, doxorubicin, cyclophosphamide, and 5-fluorouracil. The response to chemotherapy is poor overall, with few patients experiencing complete remission (152,153), although recent reports from the Pediatric ACT Registry indicate that a subset of children with ACT are sensitive to chemotherapy (110). Chemotherapeutic agents are often used in combination with mitotane, partly due to its ability to reverse multidrug resistance (154). ACT is generally resistant to radiation therapy.

In conclusion, childhood ACTs are typically functional, with signs of virilization being the most common presenting features. These tumors tend to occur at a young age. Because of these findings, it has been suggested that ACTs may arise in utero from the fetal zone of the adrenal cortex, which is oriented toward androgen production (110).

New genetic discoveries have increased our understanding of the pathogenesis of these tumors. *p53* is the most commonly mutated gene in ACT, but other genes are also involved. Genetic testing and counseling should be offered to families of young children with ACT, which may be the first manifestation of Li–Fraumeni syndrome in a family. Complete surgical resection remains the most effective therapy of ACT. Advances are still needed in designing more effective medical therapies to improve outcome.

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VII

ENVIRONMENTAL EFFECTS ON PUBERTY

20

The Role of Endocrine Disruptors in Pubertal Development

Todd D. Nebesio, MD
and Ora H. Pescovitz, MD

CONTENTS

SECULAR TRENDS AND THE ONSET OF PUBERTY
ENDOCRINE DISRUPTORS AND INSIGHT FROM ANIMAL
EXPERIENCE
GENITAL AND REPRODUCTIVE HEALTH CONCERNS
ASSOCIATED WITH ENDOCRINE DISRUPTORS
ALTERATION IN THE SEX RATIO
ENDOCRINE DISRUPTORS AND THE ONSET OF EARLY
PUBERTAL DEVELOPMENT
PHYTOESTROGENS
NATURAL ESTROGENS
PESTICIDES
INDUSTRIAL CHEMICALS AND PHTHALATES
CONCLUSIONS
REFERENCES

Summary

Recent studies have suggested that the age of pubertal onset in children is occurring earlier than previously reported. The precise trigger for the onset of puberty is not known, but it is believed to be a complex interaction between genetics, hormones, and environmental influences. Endocrine disruptors are natural or synthetic environmental chemicals that may result in adverse health by disturbing normal endocrine function through agonistic or antagonistic actions. Endocrine disruptors may result in an altered hormonal status through interference with cell signaling pathways or altering the action of hormone receptors as coactivators or corepressors (1). Several agents have been classified as endocrine disruptors, including phytoestrogens, topical and natural estrogens, pesticides, industrial chemicals, and phthalates. The potential role of endocrine disruptors in reproductive development has been a controversial topic since the early 1990s (2). Although there is a paucity of definitive data to support a role for endocrine disruptors in disturbing the onset and timing of human puberty, several environmental factors may putatively play a role. In this chapter, we discuss the compounds that have been implicated in altering the age of normal puberty, the role of these potential endocrine disruptors in pubertal development, and the implications in genital and reproductive function.

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SECULAR TRENDS AND THE ONSET OF PUBERTY

In the landmark papers by Marshall and Tanner, the normal age for onset of puberty in Britain was defined as 8.5–13 years in girls (3) and 9.5–13.5 years in boys (4). Similar data were later obtained from children in the United States, which indicate that normal girls began puberty between the ages of 8.0 and 14.9 years and boys between the ages of 9.7 and 14.1 years (5). However, in a more recent study in 1997 involving the Pediatric Research in Office Settings (PROS) network, investigators noted that breast and pubic hair development in White and African-American girls is occurring approximately 1 and 2 years earlier, respectively, than previously reported (6). As a result of this report, pediatricians and endocrinologists have increasingly debated the normal age of onset and timing of puberty in girls and boys and have attempted to identify factors that may have contributed to this apparent secular trend.

Following the PROS report, the Lawson Wilkins Pediatric Endocrine Society re-evaluated its recommendations and age limits in defining girls with precocious puberty, suggesting that diagnostic evaluation of girls with early breast and/or pubic hair development need not be performed in most cases for White girls older than 7 years of age or for African-American girls older than 6 years of age (7). However, this statement has not been universally endorsed, as physicians have questioned the validity of the PROS report because of concerns of study design flaws, including its nonrandom sample population, the involvement of multiple observers, and pubertal staging by visual inspection rather than palpation, which may have led to an overestimation of breast development (8). In addition, pubertal girls in the PROS study had an increased body mass index compared with prepubertal girls, which may have led to further overestimation of breast development by classifying adipose tissue as true breast development (9).

In a similar study in 2001 evaluating boys in the United States, investigators noted that the median age of onset of genital and pubic hair growth was lesser than in previous studies (10). When compared with the original study by Marshall and Tanner (4), White boys in the United States had an earlier mean age of onset of genital development by 1.5 years. When the mean age of genital and pubic hair growth was evaluated by ethnicity, African-American boys exhibited earlier maturation than White and Mexican-American boys (10). Despite reports that puberty may be occurring at an earlier age in both girls and boys, a study of Greek children in the 20th century suggested that the secular trend toward earlier pubertal maturation affects girls more than boys (11).

Despite the above reports that puberty is beginning at an earlier age, the tempo or rate of pubertal progression in children has not increased (12). Studies have shown that the average age of menarche gradually decreased by approximately 2 months per decade over the past century and a half, and it has leveled off and remained stable at 12.8 years over the last half of the twentieth century (6,13). More recent studies have indicated that the average age of menarche in the United States has declined to 12.34 years (14). However, when the age at menarche was examined within each race or ethnicity, the declines in age at menarche were considerably smaller. Therefore, some have questioned whether real differences exist regarding earlier pubertal maturation

and age at menarche or whether such data merely reflect differences in collection or analytic techniques (15). In the past, the secular trends toward earlier menarche have been attributed to environmental influences such as socioeconomic status and nutrition (16). However, recent attempts to determine whether the secular trends toward an earlier onset of puberty are related to improved health, nutrition, and socioeconomic status have not been verified (17).

A number of genes have been found to regulate the development and function of the hypothalamic–pituitary–gonadal axis (18) (see Chapter 3). However, with the current evidence that the age of onset of puberty may be decreasing without a clear etiology, physicians and researchers have raised concern that environmental endocrine disruptors could play an important role in the timing and disturbance of normal puberty (19).

ENDOCRINE DISRUPTORS AND INSIGHT FROM ANIMAL EXPERIENCE

Endocrine disruptors can be classified according to their mechanism of action or by their functional role (*Table 1*), and new chemicals and compounds are frequently being discovered. The hormonal activities of certain endocrine disruptors appear to occur through a functional, rather than a structural, mechanism (1). There is also evidence that some endocrine disruptors interfere directly with the hypothalamic–pituitary–gonadal axis, specifically on neurons that produce gonadotropin-releasing hormone (GnRH) (20). Endocrine disruptors are naturally produced in some plants and by humans in the form of industrial chemicals, pesticides, herbicides, fungicides, and certain pharmaceuticals (21). Because endocrine disruptors can accumulate in the environment, the effects may be subtle or even delayed in onset, and theoretically, effects may not be manifest in the exposed individual but rather become apparent in later generations (22). In theory, humans may be exposed to endocrine disruptors daily through the water supply, air, food sources, or various exposures in the work place and home environment. Additionally, endocrine-disrupting chemicals have been shown to transfer to fetuses through the placenta or to infants through breast milk (23–25), as well as potentially altering the normal function of the placenta (26).

Information about endocrine disruptors and the effects on developmental and reproductive abnormalities has been gained through observations in various animal species. In an early report from the 1940s, an outbreak of infertility in Australian sheep was attributed to ingestion of large amounts of subterranean clover (27). This epidemic, known as “clover disease,” was attributed to the estrogenic effects of phytoestrogens in the clover (28). Other animal reports include studies of the bald eagle and various industrial chemicals and pesticides (29–31). Exposure to high doses of dichlorodiphenyltrichloroethane (DDT) and other chemicals resulted in estrogen disruption with development of thin-shelled, nonviable eggs and subsequent reproductive dysfunction in the bald eagle. Exposures to other industrial chemicals and pesticides are well known to cause toxicity and reproductive abnormalities in other wild birds (32).

A more recent experience involves male alligators in Lake Apopka, Florida. In the 1970s, the Tower Chemical Company produced large amounts of pesticides, including DDT and dicofol, which spilled into and contaminated Lake Apopka. Researchers

Table 1
Examples of chemicals classified by their endocrine-disrupting function

Estrogenic
Dichlorodiphenyltrichloroethane (DDT)
Phytoestrogens (at high concentrations)
Polychlorinated biphenyls (PCBs)
Bisphenol A
Anti-estrogenic
Phytoestrogens (at low concentrations)
Androgenic
Testosterone
Trembolone acetate
Anti-androgenic
Phthalates
Dichlorodiphenyldichloroethylene (DDE)
Vinclozolin

found that young male alligators developed significantly smaller phallus size, decreased serum testosterone levels, and abnormal gonadal morphology, in association with elevated levels of DDT metabolites (33). It is presumed that these alligators acquired impaired endocrine and reproductive function because of *in utero* exposure to the endocrine-disrupting effects of DDT and its metabolites. Similar observations have been documented in other animal populations in south Florida, including a group of endangered panthers (34).

As there is evidence that environmental endocrine disruptors play a role in abnormal pubertal and reproductive development in animals, physicians and scientists have focused on potential chemicals that may play a similar role in humans.

GENITAL AND REPRODUCTIVE HEALTH CONCERNS ASSOCIATED WITH ENDOCRINE DISRUPTORS

Since the 1980's there have been many reports documenting the increased prevalence of genital and reproductive problems in males throughout the world. Studies from Europe have noted an increased incidence of hypospadias (35–38), and most recently, in the Netherlands, a 4-fold higher-than-expected hypospadias rate was documented (39). In a study from North America, doubling of the hypospadias rate was noted in all regions of the United States in the 1970s and 1980s. Furthermore, the number of severe cases increased while the ratio of mild to severe cases decreased (40). Researchers have hypothesized that exposure to some type of endocrine disruptor with estrogenic or anti-androgenic qualities may be the reason for this apparent rise in hypospadias (41).

Another reproductive health concern that appears to have increased since the 1950's in males is the incidence of cryptorchidism (42,43). However, this increase may be exaggerated because the definition of cryptorchidism varies between studies, and the inclusion of boys with retractile testes may account for at least some of the apparent increased incidence (44). As it is known that some pesticides have estrogenic properties (45), epidemiological studies in Denmark have evaluated farmers and gardeners, who

are exposed through their work to various pesticides. A significantly increased risk of cryptorchidism, but not hypospadias, in the sons of female gardeners was noted. The risks were not increased in sons of men working in the same environments (46). Although not conclusive, this report suggests an association between prenatal and *in utero* exposure to estrogenic compounds and the development of cryptorchidism. An additional study from Spain reported that cryptorchidism and orchidopexy rates were higher in districts of intensive farming and pesticide use (47).

Infertility in men, due to a decline in sperm motility and concentration, has been noted over the latter part of the twentieth century throughout the world as well (48–50). Close inspection of these data reveals that these findings are likely not due to artifact of bias, confounding factors, or statistical analysis (51). In adult male rat studies, exposure to estrogenic chemicals during pregnancy resulted in reduced mean testicular size and reduced daily sperm production (52). A study in adult men showed increased levels of endocrine disruptors, specifically polychlorinated biphenyls (PCBs) and *p, p'*-dichlorodiphenyldichloroethylene (*p, p'*-DDE), in men with abnormal sperm motility, concentration, and morphology (53). In an additional report of men from Missouri, decreased semen quality was associated with elevated pesticide metabolite levels, specifically herbicides and insecticides, in urine samples when compared with control subjects (54).

The incidence of testicular cancer, both teratomas and seminomas, in adolescent and adult men has also increased throughout the world (55). Some studies have reported ethnic differences in the development of testicular cancer, with White men having significantly higher incidence rates than African-American and Hispanic men (56). Researchers have proposed that testicular cancer is initiated during the fetal period through exposures to endocrine disruptors (57). In a study of Swedish men and their mothers, concentrations of organochlorines were higher in mothers of patients with testicular cancer (58). Although such findings are intriguing and suggestive, a true causal effect has not yet been verified.

In an attempt to collectively explain with a single hypothesis the reported increased incidence of hypospadias, cryptorchidism, infertility, and testicular cancer, the term “testicular dysgenesis syndrome” has often been used (57,59). According to this hypothesis, *in utero* exposure to some environmental factors results in disturbed Sertoli cell function and decreased Leydig cell function. As a result of abnormal Sertoli cell function, impaired germ cell differentiation occurs, which leads to reduced semen quality and eventual testicular cancer. As a result of impaired Leydig cell function, androgen insufficiency occurs, which results in hypospadias, cryptorchidism, and decreased spermatogenesis (57). Various reports have suggested a common etiology for the testicular dysgenesis syndrome, supported by the findings of abnormal semen characteristics in men who later developed testicular cancer (60) and the association between cryptorchidism and testicular cancer development later in life (61).

Further support for the testicular dysgenesis syndrome has been generated in studies in which pregnant rats were transiently exposed to endocrine-disrupting chemicals during the time of gonadal sex determination. In rats exposed to vinclozolin, which is an anti-androgenic compound, a greater than 2-fold increase in spermatogenic cell apoptosis was seen, sperm count was decreased by 20%, and sperm motility was 25–35% lower in male offspring. The frequency of the phenotype in the male rat was

greater than 90% in all subsequent generations. This study is the first to demonstrate that endocrine disruptors have the ability to reprogram the germ line by an epigenetic alteration of DNA methylation patterns, rather than a true genetic mutation, and thus promote disease in subsequent generations (62). Although the doses used in the above experiment were much higher than the typical exposure levels in humans, this study has been used to explain why male genital and reproductive problems have been increasing throughout the world. However, these results need to be replicated and confirmed by other investigators (63).

Because there are reports that endocrine disruptors can freely cross the placenta in humans (24,64), there is concern that prenatal exposures to such chemicals in male offspring may result in reproductive and genital problems. A classic, historic example of this type of exposure is the experience with diethylstilbestrol (DES), a synthetic estrogen previously used in pregnant women to prevent miscarriages until it was banned in 1971. Clear cell adenocarcinoma of the vagina in young women whose mothers used DES during their pregnancy has been well documented (65). Less well-known, male offspring of women treated with DES were also reported to have abnormalities including an increased incidence of testicular cancer as well as cryptorchidism (66). However, the association between DES exposure and testicular cancer remains controversial, and recent reports have not supported a greatly increased risk among male offspring of women treated with DES during pregnancy (67).

Phthalates are chemicals used in the manufacturing of many consumer products including solvents, lubricants, and plastics. The ability of phthalates to suppress androgen synthesis during development has implicated phthalates as potentially playing a critical role in the testicular dysgenesis syndrome (68). This concern has been raised from studies that have evaluated male offspring of mothers exposed to supposedly safe environmental levels of phthalates. Using the anogenital distance (AGD), a measure often used to evaluate fetal androgen exposure (69), a positive correlation was found between phthalate metabolites in mothers' prenatal urine and a shorter anogenital index (AGI or AGD divided by weight) in their sons (70). This is based on the sexual dimorphism of AGD that exists and is characterized by newborn males having a 2-fold greater AGD (mean of 22 mm) than newborn females (mean of 11 mm) (71). In a recent study of 85 mother-son pairs, boys with the greatest phthalate exposure had, on average, an 18.3% (range of 10–32%) shorter-than-expected AGI (70). In addition, boys with a shorter-than-expected AGI had an increased likelihood of testicular maldescent, small and indistinct scrotum, and smaller penis size. Although such findings may seem only minor, the prolonged and ubiquitous exposure of certain endocrine disruptors in the environment, and the potential to harm future generations, has provided further support for the proposed testicular dysgenesis syndrome hypothesis.

ALTERATION IN THE SEX RATIO

The sex or gender ratio, which is the proportion of male to female births, varies by country throughout the world. The sex ratio at birth ranges from 1040 male births for every 1000 female births in Belgium to 1092 male births for every 1000 female births in Singapore. In the United States, the sex ratio has varied between 1046 and 1059 for from 1940 to 2002 (72). Although trends in the sex ratio have been fairly constant

over the last part of the twentieth century, there have been incidents where endocrine disruptors have been implicated in altering this ratio.

The most infamous event of an industrial chemical exposure implicated in altering the sex ratio involved 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, also known as TCCD or dioxin, after an explosion at a herbicide manufacturing plant in Seveso, Italy. Researchers showed that exposure of men, but not women, to TCCD was associated with a lowered male-to-female sex ratio. In addition, a trend of increasing serum TCCD concentrations in fathers was associated with a decrease in the proportion of male births. In those men with the highest TCCD serum concentrations, the sex ratio was 0.383, that is, 620 male births for every 1000 female births (73). These results suggest that males may be selectively sensitive to dioxin toxicity before and during puberty, whereas women may be insensitive to any such disrupting effect. Further support of a limited influence of dioxin exposure in females comes from a study of women who were exposed to high levels of TCCD in Seveso and were investigated 20 years later. Individual serum TCCD measurements from the time of the exposure were not significantly correlated to age at menarche (74).

Chemicals similar to dioxin, such as PCBs and polychlorinated dibenzofurans (PCDFs), were studied in Taiwan after rice cooking oil contaminated with these compounds was ingested. Despite high exposure to these endocrine-disrupting chemicals, the sex ratio was not altered in Taiwan (75). Evidence for endocrine disruption and alteration of the sex ratio has been observed in other species, including fish that were exposed to industrial chemicals and pesticides (76,77). Although such reports are of concern, it is difficult to ascertain the significance of these results because similar observations have not been universally replicated.

ENDOCRINE DISRUPTORS AND THE ONSET OF EARLY PUBERTAL DEVELOPMENT

We have discussed the possibility that various endocrine disruptors may affect male genital and reproductive function, as well as potentially altering the male-to-female sex ratio. Few studies have been performed to examine directly whether endocrine disruptors can either advance or delay the onset of puberty. In one report, investigators studied the effects of PCBs on sexual maturation in adolescents in a Belgian industrial setting (78,79). In boys, the probability of slowed genital development and pubic hair growth was greater in those with higher serum concentrations of marker PCBs. Furthermore, testicular volume was smaller in boys from the polluted areas compared with those from the control area. In girls, the probability of slowed breast development was correlated with elevated serum concentrations of dioxin-like compounds. Additional studies of men exposed to PCBs and their heat-degradation product, PCDFs, after ingesting contaminated rice oil in Taiwan have revealed that boys have reduced penile length, and men have abnormalities of sperm morphology and motility (80).

With the concern that the age of onset of puberty may be occurring earlier than previously reported, many studies have suggested that endocrine disruptors might be factors in this process. Several epidemics of premature sexual development have previously been described. Although the etiologies were never definitively proven, endocrine disruptors were implicated (81) (*Table 2*).

Table 2
Examples of endocrine disruptors and events associated with precocious sexual development

<i>Endocrine disruptor</i>	<i>Events associated with precocious sexual development</i>
Phytoestrogens	Puerto Rico (88,90,92)
Topical preparations	Estrogen topical creams (95–99) Hair-care products in African Americans (101–103) Androgen topical creams/sprays (104)
Contaminated food/meats	Puerto Rico (88–92) School in Milan, Italy (106–108) Immigrant children in Belgium (110)
Pesticides	Fishing community near Lake Michigan (111) Jerusalem, Israel (112)
Industrial chemicals	Michigan food chain contamination (113)
Phthalates	Puerto Rico (117)

PHYTOESTROGENS

Phytoestrogens, which have a chemical structure similar to that of estradiol, are plant compounds with varying biologic potency of both estrogenic and anti-estrogenic activities (28). Phytoestrogens are classified as isoflavones, coumestans, or lignans. Some phytoestrogens have biphasic effects and exhibit anti-estrogenic activity, or aromatase inhibition, at low concentrations and estrogenic activity at higher concentrations (82).

Physicians and parents have been concerned about phytoestrogen exposure for many years because phytoestrogens are known to cross the placenta (64), and substantial quantities have been identified in the amniotic fluid during the second trimester (83). There has been concern that phytoestrogen exposure in infants fed soy-based formula, soymilk, or cereals may alter endocrine and pubertal development. In 1998, it was reported that the use of soy protein-based infant formulas had nearly doubled and achieved 25% of the infant formula market in the United States (84). In a recent review of isoflavones in soy infant formula, it was noted that an exclusively soy-fed infant likely has the highest exposure to estrogen activity when comparing all non-pharmacologic sources of exogenous compounds in the general population (85). However, despite this increased exposure, no definitive proof exists that phytoestrogen ingestion through soy-based infant formulas results in endocrine disruption.

A retrospective study compared adult men and women who were fed soy-based formula as infants to a similar, control population fed cows' milk-based formula (86). In the majority of outcomes examining overall health and reproduction, the two groups did not differ. There was no difference in age at menarche, breast development, or male pubertal maturation. One statistically significant difference was that women who had been fed soy formula as infants reported slightly longer duration of menstrual bleeding and greater discomfort with menstruation. In other studies, some researchers have suggested that nuts and seeds, which contain phytoestrogens, may play a role in the regulation of puberty and menstruation (87).

One of the most well-known clusters of premature sexual development was initially reported in Puerto Rico in the late 1970s. The first report noted 272 children, including 121 cases of premature thelarche, 30 cases of premature adrenarche, and 121 cases of precocious puberty, in boys and girls over a 10-year period (88). Food contamination or another environmental chemical disruptor was strongly suspected. Another report from the same year noted an increased incidence of premature thelarche and ovarian cysts in young girls in Puerto Rico (89).

Subsequent reports on the epidemic in Puerto Rico have been published with suggestions that environmental endocrine disruptors, including phytoestrogen exposures, may have played an important role. Significant quantities of estrogens were detected in the food supply, including poultry and meats (90). Theories were proposed for possible sources of exposure, including ingestion of estrogen-containing plants (i.e., phytoestrogens) by animals or contact with waste contamination from pharmaceutical manufacturers. Further studies supported these ideas after discovering significant levels of estrogens contained in chicken, pork, and beef samples (91). After withdrawing suspected contaminated foods from the local diet, ovarian cysts disappeared or decreased significantly, and involution of breast tissue occurred by 6 months in 58% of the children studied. Additional research has also shown positive statistical associations between development of premature thelarche before the age of 2 years and consumption of various meat products, as well as soy-based formulas containing phytoestrogens, in Puerto Rican girls (92).

NATURAL ESTROGENS

Estrogens may be encountered through topical preparations or contamination of various foods, as noted in the examples in the previous section. In addition, children may be exposed to considerable amounts of estrogens through cows' milk, which is typically obtained from heifers in the latter half of pregnancy when estrogen levels are markedly elevated (93). Historical reports describe infants and children who have been in contact with estrogen-containing or estrogen-like-containing topical preparations, such as cosmetics, shampoos, or hair conditioners, and who later were noted to have early pubertal development (94).

In 1969, a 3-year-old girl with premature breast enlargement, sparse suprapubic hair, and vaginal bleeding was admitted to a hospital in Hartford, Connecticut, for the presumed diagnosis of a functional ovarian tumor. Despite denying any exposure to exogenous estrogens, it was eventually discovered that the child was repeatedly pacified by being allowed to play with a discarded jar of her grandmother's facial "hormone" cream, which contained 10,000 units of estrogen per ounce. Once this exposure was eliminated, secondary sexual characteristics regressed (95). Other reports in the literature have noted precocious sexual development in female infants and children exposed to topical estrogen compounds (96,97). Another 4-year-old girl with breast and pubic hair development had an exploratory laparotomy because of concern of a possible solid tumor of the left ovary. However, at surgery, only normal, pubertal ovarian tissue was present. Further questioning revealed that the child had presumably, although it was never definitively proven, been exposed to her mother's stilbestrol (98). These cases emphasize the importance of careful questioning of family members for

potential exposures, which may ultimately prevent unnecessary medical and surgical procedures.

There are also reports of indirect exposure to maternal estrogen creams in young boys that resulted in prepubertal gynecomastia (99). The United States Food and Drug Administration does not closely regulate estrogen-containing cosmetics, and many of these products may contain enough estrogen or estrogen-like compounds to cause precocious puberty in children, gynecomastia in boys or men, and postmenopausal bleeding in women, although the amounts of estrogen are small (100).

Hair-care products may also contain estrogen or placental extract that can result in early sexual development in children. In a report of 102 consecutive referrals for the evaluation of sexual precocity, eight African-American children were identified who were using hormone-containing hair-care products (101). A similar report identified four African-American girls with premature breast and/or pubic hair, which occurred between 2 and 24 months after starting to use placenta-containing or estrogen-containing hair-care products (102). With the concern of the secular trend of early puberty, specifically in African-American children, it has been postulated that estrogen-containing hair-care products may be contributing to the earlier onset of pubertal maturation in this select population (103).

Parents are often unaware of the dangers of such household products, and many do not realize that actual hormones or hormone-like compounds are found in these products. Besides the above reports of passive transfer of estrogens, topical androgen use in parents has also been noted to result in virilization of both boys and girls (104). Five children were described as exhibiting pubic hair development, clitoral enlargement, growth acceleration, and/or skeletal advancement after exposure to compounded testosterone creams, gels, or androgen sprays (Androsol). None of the adults were aware that the use of topical androgens could be associated with the risk of passive transfer of hormones to other family members. Although the majority of adults who were using topical androgens were male, it is important to note that one adult was a mother, who was using topical testosterone cream for female testosterone deficiency (104). This case again highlights the importance of questioning all family members about potential exogenous exposures in the home when investigating children with early sexual development.

As noted in the reports of premature sexual development in Puerto Rico, food contamination through animal husbandry with the use of various chemicals, including estrogenic compounds, has also been implicated in cases of premature sexual development (88–92). Researchers have raised concern about potential adverse effects on human health from the consumption of meat from estrogen-treated animals (105). Other reports from around the world have also noted premature sexual development in children suspected of ingesting meat products contaminated with estrogens. In November 1977, at a small private school in Milan, Italy, an outbreak of breast enlargement in boys and girls was first noted (106). Breast enlargement occurred in 51% of boys between 3 and 10 years of age and in 51% of girls between 3 and 7 years. Only 3–8% of age-matched and sex-matched children at control schools, who were between 3 and 10 years of age, had breast enlargement (107). In children at the affected school, gynecomastia and thelarche were no greater than Tanner stage II and resolved after 8 months. No breast enlargement occurred in any siblings who did

not attend the affected school. Sources of potential estrogen exposure were investigated, including the water supply, cafeteria cooking oils, and art supplies, but none were found. However, researchers suspected an uncontrolled supply of poultry or veal, resulting in a transient exposure to an endocrine disruptor, may have been responsible. Follow-up studies have been done on this population 20 years later to determine late effects from this earlier event (108). Although detailed data are not clearly described, puberty was noted to begin earlier in exposed females, and completion of puberty occurred sooner in exposed males. Furthermore, exposed males were noted to have reduced testicular volume and decreased fertility. Additional studies have suggested that breast enlargement is a common entity in early childhood in Milan, where gynecomastia was noted in 21% of boys less than 2 years of age and premature thelarche was evident in 37% of girls less than 2 years (109).

PESTICIDES

In addition to the reports of abnormal reproductive behavior and development associated with pesticide exposure, a few reports have associated pesticides with alterations in the timing of normal puberty. One study from Belgium investigated precocious puberty in children and the possible relationship to pesticides. Foreign children, or those who immigrated to Belgium from a developed country, were found to have an 80-fold higher prevalence of precocious puberty as compared with native Belgian children. Furthermore, levels of a DDT metabolite were detectable in 81% of foreign children with precocious puberty as compared with being measurable in only 13% of native Belgian children with precocious puberty. The investigators suggested an association between a transient exposure to an endocrine disrupter, such as DDT, and precocious puberty (110). Although these results are intriguing, there was no control population of immigrated children with normal pubertal development. Therefore, further studies are indicated in order to validate these findings and to exclude potential genetic differences.

An additional study investigated fishermen and their spouses living near Lake Michigan. Researchers found that the higher level of DDE, a metabolite of DDT, exposure in utero was associated with a younger age at menarche. An increase in the *in utero* DDE exposure of 15 µg/l reduced the age at menarche by 1 year. No association was found with maternal PCB exposure, a common industrial chemical, and age at menarche (111).

In a retrospective epidemiological study, records of girls presenting with physical signs of precocious sexual development, including premature thelarche, premature pubarche, and central precocious puberty, in Jerusalem over a 10-year period were reviewed (112). A significant increase in the incidence of premature thelarche and central precocious puberty was noted in the spring months of April through June. Despite an extensive search for possible etiologies for the increase in pubertal development during the spring season, a specific cause was not found. However, the authors suggested that some environmental source, such as a seasonal pesticide, might be involved.

INDUSTRIAL CHEMICALS AND PHTHALATES

Many industrial chemicals have been implicated to account for the apparent decline in male reproductive health over the part of the twentieth century (44). In addition, some industrial chemicals have also been implicated in the apparent earlier age of onset of puberty. For example, polybrominated biphenyls (PBBs) have been reported to be associated with an accelerated onset of puberty. In 1973, an accidental contamination of the Michigan food chain with PBBs occurred after a flame retardant, FireMaster, was mistakenly added to livestock feed. More than 4000 individuals were exposed to PBBs after eating animal and dairy products that had been contaminated with PBBs. Researchers have examined the female offspring of mothers who were registered as being contaminated with PBBs (113). Breastfed girls exposed to high levels of PBBs *in utero* had an earlier age of menarche (mean age 11.6 years) than did either breastfed girls exposed to moderate or low levels of PBBs *in utero* (mean age 12.2 and 12.6 years, respectively) or girls who were not breastfed (mean age 12.7 years). Other findings included earlier pubic hair development in breastfed girls, but no association was found with breast development. The findings from this study are particularly confusing because it is difficult to explain the dissociation between secondary sexual development, menarche, and chemical exposures.

Phthalates, which are used to create flexibility in polyvinyl chloride products, are widely distributed environmental contaminants that are found in some medical devices, clothing, cosmetics, and children's toys. Phthalates accumulate in the environment through manufacturing and are able to leach out of plastic products when in contact with lipophilic substances (114). Safety data and risk to humans are difficult to interpret because most of the information gathered on phthalate exposure has been collected from animal models. Phthalate esters have been shown to have weak estrogenic activity *in vitro* (115,116), but there have been no definitive *in vivo* studies confirming these results.

In the case of early pubertal changes in girls from Puerto Rico, a possible link to phthalate exposure has recently been described. In a study of 41 serum samples from young girls from Puerto Rico with premature thelarche, phthalate esters were detected at significant concentration levels in 68% of serum samples. As a comparison, 35 control samples were analyzed and only one showed significant levels of phthalate esters (117). However, the authors noted that phthalate exposure could not be the single cause of premature sexual development in Puerto Rico. Some have questioned the validity of these results because phthalates are rapidly metabolized and excreted, and the high phthalate levels reported were likely due to contamination of the collected serum samples from the use of flexible vinyl in laboratory equipment and tubing (118). Other research has shown that high levels of some phthalate esters may have anti-androgenic effects (119), which theoretically could cause an imbalance between androgens and estrogens resulting in premature thelarche. It is suggested that the cause of premature thelarche in Puerto Rico is multifactorial with other environmental disruptors, as discussed earlier, all playing a possible role in the early pubertal development reported in Puerto Rico.

CONCLUSIONS

There continues to be a concern that pubertal development is occurring at an earlier age in both boys and girls throughout the world. Environmental effects from endocrine-disrupting chemicals, including phytoestrogens, topical and natural estrogens, pesticides, industrial chemicals, and phthalates, have been proposed to play an important part in the secular trend of early pubertal development. In addition, many of these chemicals have also been implicated in the reports of decreasing male reproductive health, including hypospadias, cryptorchidism, infertility, and testicular cancer. However, most of the research thus far is speculative with many questions remaining unanswered. Further studies investigating the effects of endocrine disruptors on the timing and development of normal puberty in humans are needed before a true cause-and-effect relationship can be declared.

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21

Precocious Puberty in Internationally Adopted Girls

*Grete Teilmann, MD,
Anne-Simone Parent, MD,
Niels E. Skakkebak, MD, PhD
and Jean-Pierre Bourguignon, MD, PhD*

CONTENTS

INTRODUCTION
PRECOCIOUS AND EARLY PUBERTY OF CENTRAL/PERIPHERAL ORIGIN
PUBERTAL DEVELOPMENT IN ADOPTEES
PUBERTAL DEVELOPMENT IN NON-ADOPTED MIGRATING CHILDREN
GEOGRAPHICAL DIFFERENCES AND SECULAR CHANGES IN THE TIMING OF PUBERTY
GENETIC FACTORS
AGE AT ADOPTION
EARLY GROWTH DEPRIVATION AND CATCH-UP GROWTH
NUTRITIONAL STATUS
INTRAUTERINE CONDITIONS
ENVIRONMENTAL CHEMICAL EXPOSURES
PSYCHOLOGICAL ASPECTS
FINAL HEIGHT
HORMONAL TREATMENT OF ADOPTED CHILDREN WITH PRECOCIOUS OR EARLY PUBERTY
FUTURE CLINICAL AND RESEARCH PERSPECTIVES
REFERENCES

Summary

In internationally adopted girls a high frequency of precocious puberty has been reported in series of patients, and a low age at menarche has been reported from retrospective cohort studies. Data estimating the risk of precocious pubertal development suggests a highly increased risk in internationally adopted

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girls, possibly related to country of origin and age at adoption. Genetic factors, intrauterine growth retardation, nutritional status prior to adoption, pre- and post adoption growth patterns as well as environmental exposures and psychological stress has been hypothesized to trigger early puberty. However, the mechanism behind precocious and early puberty in adopted girls is still unknown, and central as well as peripherally mediated puberty has been suggested. In daily clinical practice, the challenge is therefore to establish guidelines that ensure appropriate recognition of those adopted children who need medical examinations for precocious puberty and to identify the subgroup of patients who will benefit from treatment with GnRH analogs. Prospective, longitudinal studies on growth and development in internationally and domestically adopted children are needed to disentangle these questions and to understand the pathophysiology.

Key Words: Precocious puberty; Internationally adopted children; Catch-up growth; Nutrition; Growth; Environmental exposures.

INTRODUCTION

Thousands of children are adopted every year, mainly from underdeveloped countries to western countries. The proportion of internationally adopted children in the general childhood population is now as high as 1–1.5% in some European countries (1) (Danish National Board of Adoption, 2005, personal communication). The health and development of adoptees is naturally becoming an increasingly important issue (2–4). Although the majority of adopted children are healthy and thrive well, some specific health problems have been recognized. These include acute and chronic infections, untreated medical diseases, growth retardation, developmental delays, and behavioral deviations (2,5). In childhood and early adolescence, an increased frequency of early sexual maturation has been observed. Sexual precocity in adopted girls was first reported in 1991 (6) and has claimed attention from adoptive parents and pediatricians because of concerns about psychosocial maladjustment, increased risk-taking behavior, and low final height (FH) following early pubertal development. In addition, the phenomenon is an opportunity to expand our knowledge of genetic and environmental factors that trigger puberty. In this chapter, we aim to review the current knowledge about pubertal development in adopted children in a broad perspective and to address present research needs and areas of uncertainty.

PRECOCIOUS AND EARLY PUBERTY OF CENTRAL/PERIPHERAL ORIGIN

In this context, only isosexual precocity will be discussed: namely, early feminization in girls and virilization in boys. Isosexual precocity may be either central [i.e., resulting from the activity of the hypothalamic–pituitary–gonadal (HPG) cascade] or peripheral (i.e., resulting from gonadotropin-independent secretion of sex steroids peripherally or exogenous substances) (Fig. 1).

Traditionally, the term *precocious puberty* refers to the onset of puberty before 8 years of age in girls and 9 years in boys. The term *early puberty* describes the onset of puberty in girls between 8 and 9 years of age and in boys between 9 and 10 years of age. This term was introduced to stress the need of medical awareness on pubertal development in adopted children and others with a borderline-normal pubertal maturation (7). Studies on the epidemiology of precocious puberty are sparse. In Belgium, it was estimated that children with precocious puberty represented 0.01%

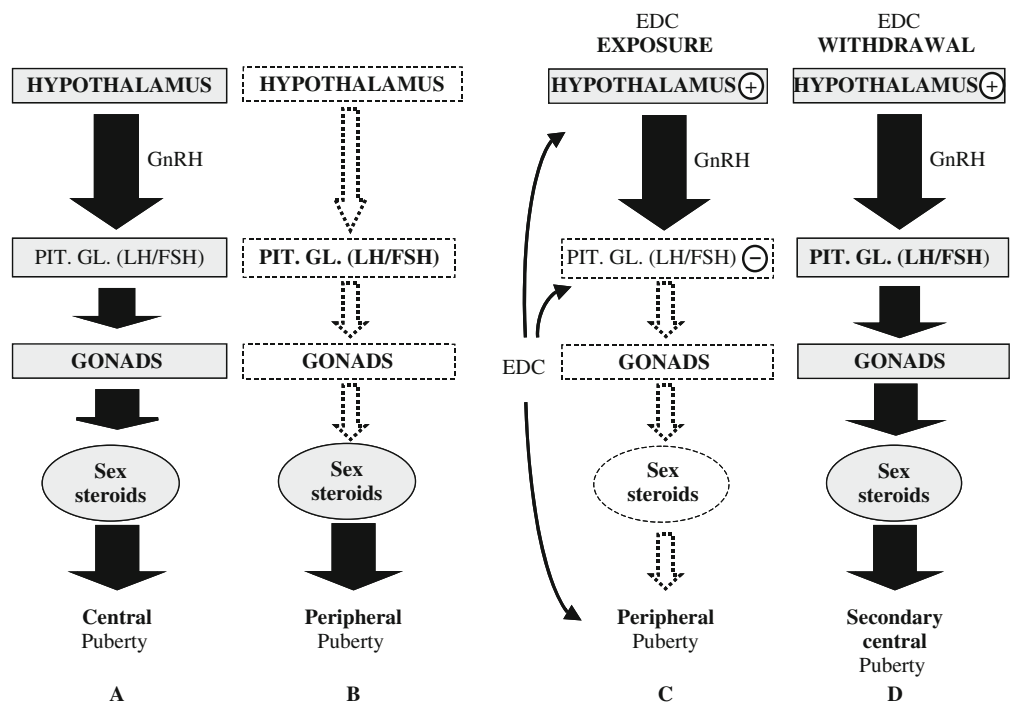


Fig. 1. Schematic presentation of central and peripheral mechanisms to sexual maturation (A and B), and putative effects of exposure to endocrine-disrupting compounds (EDCs) and subsequent withdrawal from these substances (C and D).

of the cumulated children population in a 9-year period (8). A nationwide study from Denmark showed an overall prevalence of precocious pubertal development in 20–23/10,000 girls, whereas the prevalence was much lower among boys (<5/10,000 boys) (9).

The etiology of central precocious puberty (CPP) is commonly idiopathic, although it may be caused by congenital malformations or tumors in the central nervous system. Alternatively, CPP can occur secondary to peripheral hormone producing tumors or to steroid hypersecretion resulting from defects in the biosynthesis. More rarely, mutations in reproductive hormones or their receptors are causal of peripheral precocity (10,11). The etiology behind premature sexual maturation in adopted children is yet to be understood, but it is likely to involve a central component that could be initially triggered by a peripheral component. An indirect argument for the central component comes from the gender distribution with the female predominance as for other forms of CPP.

PUBERTAL DEVELOPMENT IN ADOPTEES

A number of studies have been focusing on sexual development among children of foreign origin adopted to western countries (6,8,12–14). To our knowledge, no studies have yet focused on pubertal development in domestic adoptees (8). In the following, we therefore refer only to internationally adopted children, unless otherwise

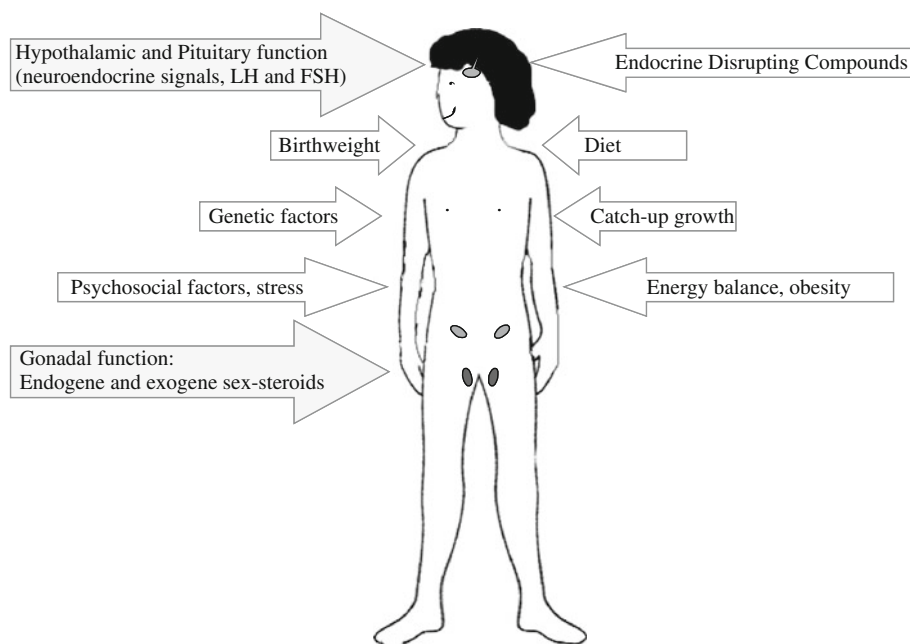


Fig. 2. An overview of proposed hypothesis for early sexual development in internationally adopted children.

stated. In cohort studies, the age at menarche has been studied retrospectively through questionnaire studies, showing that on average menarche occurs considerably earlier among adopted girls than would be expected in their country of origin as well as in the foster country (6,13,15). Among 107 Indian girls adopted to Sweden, the mean age at menarche was 11.6 years, which was below the reference values of 12.8 years in well-off Indian girls living in India and 13 years in Swedish-born girls (6). A larger study including 446 adopted girls originating from Colombia, India, Indonesia, and South Korea found a low mean age at menarche (12.0 years) with significant differences in age at menarche depending on the country of origin. The age at menarche varied from 11.2 years (95% CI 10.9–11.5) to 12.4 years (12.2–12.6) in girls born in India and South Korea, respectively (15). In a survey of children adopted from Eastern Europe to the United States, the parent-reported mean ages at the onset of breast development and menarche were 8.8 (± 2.5) years and 10.5 (± 2.6) years, respectively. The most recent corresponding American reference data are 9.96 years for mean age at breast development and 12.88 years for menarche (16), indicating that puberty started slightly earlier, progressed more rapidly, and was completed at an earlier age in the group of Eastern European adoptees compared with non-adopted White American girls (13,17).

Over-representation of adopted children has been reported in series of patients suffering from precocious and early puberty (8,12,18). The vast majority of adopted children included in these studies have been girls, and to date, very little is known about pubertal development in adopted boys. Epidemiological risk estimates have been calculated in Belgium and Denmark. Based on Belgian registries, the incidence of precocious puberty was calculated to be 0.8% in adopted children in contrast to 0.01% in native Belgian children, suggesting an 80-fold increased risk of precocious

pubertal development in adopted children, independent of the ethnic background of the adopted child (8). However, this risk estimate was based on an adoption registry database that was considered to be incomplete because of foreign adoptions through organizations not accounted by the state authorities (19). In a nationwide registry-based study from Denmark, including 655 unselected cases of precocious puberty, it was found that the relative risk of developing precocious puberty was highly increased in internationally adopted children compared with non-adopted Danish-born children. The overall incidence rate ratio (IRR) was 10.6 (95% CI 8–14.2), and the risk was high in children adopted from South America [IRR 20.5 (12.8–32.8)], India [IRR 14.9 (8.4–26.5)], Asian countries [IRR 18 (9.3–34.7)], and from “other countries”, including Eastern Europe and Africa [IRR 17.1 (8.1–36.1)], whereas the risk was not significantly increased in the large group of children adopted from South Korea [0.6 (95% CI 0.1–4.1)]. On average, adoption after 2 years of age increased the risk of developing precocious puberty 7.6 times, ranging from 3 times (India) to 10 times (other Asian countries) (20,21).

PUBERTAL DEVELOPMENT IN NON-ADOPTED MIGRATING CHILDREN

A new perspective was opened when it was suggested that non-adopted children, who immigrate to western countries together with their original families, also might have an increased risk of precocious sexual development (8). However, no increased risk of precocious puberty was found in children who immigrated to Denmark with their families, in contrast to what was found in internationally adopted children in Denmark (20). This could be explained by the fact that immigrants originated from other regions of the world than adoptees and had a lower risk of precocious puberty involving genetic or environmental factors. Possibly, children who immigrate with their family will maintain their original dietary habits after migration and thereby do not experience a marked shift in nutritional context although they become exposed to nutrients raised in different conditions. Under-reporting of cases of precocious puberty among immigrants due to different use of health services, language barriers, and others might also occur as opposed to possible over-reporting among adopted children who are taken care of by scrutinizing parents.

There are few studies on pubertal development in immigrants and their descendants in the literature, but historical data showed that Japanese girls born and reared in California, United States, experienced menarche more than 1.5 years earlier than Japanese girls living in Japan (22). In a more recent study among Indo-Pakistan girls, living in London, the mean age at menarche was 13.06 years, which was similar to values reported for urban and well-off populations in India (23). In a population of Turkish girls living in Germany (two-thirds immigrants and one-third descendants of immigrants), the age at menarche was close to that of Turkish girls living in Turkey (12.9 vs. 12.8 years) but lower than in native German girls (13.3 years) (24). The latter two studies support that the age at menarche is not markedly accelerated in immigrants or descendants of immigrants and that genetic rather than environmental factors determine the timing of puberty at the population level. However, no equivalent populations have been studied at the same time in the countries of origin, which make direct comparisons of studies uncertain.

GEOGRAPHICAL DIFFERENCES AND SECULAR CHANGES IN THE TIMING OF PUBERTY

The appearance of breast tissue (transition from Tanner stage 1 to Tanner stage 2 breasts) defines the onset of puberty in girls. However, the age at menarche is often used as a marker for age at pubertal maturation, because it is a well-defined milestone in female reproductive life. The significance of geographic and socio-economic factors on sexual maturation has been acknowledged since Eveleth and Tanner (25) demonstrated worldwide variations in the timing of puberty. Comparisons between such studies should be interpreted with care, as the demographics of the study population, ongoing secular trends, as well as the practical and statistical methods may greatly influence the result (26). Recent data from around the world show that in well-off conditions the average age at menarche varies between 12.0 and 13.5 years, whereas the menarcheal age in underprivileged settings varies from 12.9 to 16.1 years (19). Variations are still prominent when comparing populations living in the same country under different living conditions (19). These findings emphasize that extrinsic factors such as nutrition, energy expenditure, and general living conditions have a major impact on puberty timing.

In Western Europe, the age at menarche has fallen approximately from 17 to 13 years between 1850 and 1950 presumably because of improved living standards (27). There seems to have been a halt in this secular trend in some European countries, reflected by unchanged ages at the onset of breast development and menarche after the 1960s (27). In contrast to this, female sexual maturation in American girls is beginning earlier now than in the 1950s: 10% of White and 38% of Black girls do now have breast development at the age of 8 years (16). This has raised concern about the etiology behind accelerated sexual maturation (16,28–30) and the deleterious effects of such a trend (31). It is, however, still a matter of debate whether American data are sufficient to establish if the age at menarche has decreased as well and whether the timing of pubertal development in boys has changed recently (26). In many developing countries, a secular shift toward earlier age at menarche has been reported (32–36), and it must be taken into consideration that ongoing trends in the foster country may affect the timing of puberty, even after adoption.

GENETIC FACTORS

Genetic factors are strongly involved in determining the age at onset of puberty. Mother/daughter and twin correlation studies indicate that 70–80% of the variance in pubertal timing can be explained by genetic factors (19,37). Population studies show that the age at puberty varies among ethnic groups, suggesting a genetic control of puberty as well. The variance in pubertal timing is likely to be a complex polygenic trait (37), and the genes involved in this process still remain to be discovered. They might play a role at different levels of the neuronal and astroglial networks involved in the central activation of puberty or at the level of the upstream transcriptional factors regulating those networks. The genetic control of the timing of puberty could, at least partially, explain racial differences in age at onset of puberty and thus in children from different ethnic backgrounds. One could also hypothesize that some genetic polymorphisms could explain differences such as sensitivity to some exogenous factors and thus the difference in the age at onset of puberty between populations.

AGE AT ADOPTION

It is noteworthy that birth date is often not accurate in foreign adopted children. Assessment of bone maturation at arrival does not solve this question because under/malnutrition, frequent infections and chronic diseases may account for some delay in bone age.

In their retrospective study of age at menarche in 107 girls adopted from India, Proos and colleagues (6) reported that girls who were adopted at a later age tended to be younger when menarche occurred. Later multiple regression analysis revealed that height at arrival was better correlated with age at menarche than age at arrival (38). This puts emphasis on the effect of earlier life conditions before migration that can be reflected by height at arrival. In Denmark, a registry-based study showed that the risk of developing precocious puberty was increased 3–10 times in children who were adopted after 2 years of age, compared with children adopted earlier in life (20). In a study reporting on 19 adopted girls with early puberty, no associations between age at adoption and onset of puberty were found (12). The correlation with age at adoption could involve biasing genetic factors, if children who are adopted late are those genetically more prone to develop precocious puberty. It seems more likely that environmental factors, which are associated with the duration of the pre-migration period, play a key role although they could have stimulatory or inhibitory effects or both coexisting.

Age at adoption is a parameter of equivoqual significance. For instance, if stimulatory factors are interacting in the country of origin, a longer period before migration would be expectedly associated with more rapid development of puberty after migration. If inhibitory factors are interacting in the country of origin, earlier migration would be associated with greater advancement in the age at onset of puberty. In addition, data should be corrected by the age-related increased likelihood to start puberty.

EARLY GROWTH DEPRIVATION AND CATCH-UP GROWTH

A proportion of foreign adopted children have suffered from multifactorial deprivation and thus usually catch-up in different functions after migration. Some of these functions are exquisitely complex; others are much more simple such as growth. Growth is often retarded at arrival to the foster country (12,39–41), indicating the presence of growth limiting factors or the lack of growth stimulating factors prior to adoption. Such factors involve undernutrition and malnutrition, as well as acute and chronic infections. In addition, the effect of poor stimulation and other psychosocial issues may be considered, as seen in the syndrome of psychosocial short stature. In psychosocial short stature, removal from a stressful environment is commonly followed by a period of catch-up growth, but in a study of non-adopted children with a history of psychosocial short stature, mean FH was lower than mid-parental target height, suggesting long-term effects of early psychosocial deprivation on statural growth (42,43). The effect of institutionalization on growth has been studied in Romanian orphanages, where growth stunting was correlated with serum cortisol and length of stay in the institution, suggesting that the hypothalamic–pituitary–adrenal axis may be a link between environmental stress and physical and developmental responses (44,45). Growth retardation has been correlated with the age at adoption, pointing out the time-dependent cumulative influence that environmental factors may exert on the growing

child (39,41). During the first years after arrival to the foster country, catch-up growth is commonly observed, with significant increases in height and weight *z*-scores (14,38).

Severe malnutrition in childhood delays the timing of puberty at least in girls (46–48), and chronic malnutrition throughout childhood retards pubertal development (49), providing observational support that children compensate for incomplete growth attained earlier in life due to a maturational delay of 1.5–2 years allowing prolonged growth. Little is known about the effect of recovering from malnutrition in the prepubertal period on the timing of puberty.

Catch-up growth, as seen in the majority of adoptees, might trigger endocrinological responses to unphysiological levels, for example, increased levels of insulin-like growth factor 1 (IGF-I) and/or leptin, which in turn could stimulate the hypothalamus and/or the gonads to produce sex hormones (50).

NUTRITIONAL STATUS

The causal–consequential relation between obesity and the timing of puberty is a two-way complex issue. It has been intensely debated after the publication of data from the United States, showing a secular decline in age at breast development (16,28) together with a trend toward an increased incidence of childhood obesity (51). Although it is well established that girls who have started puberty have higher body weights than age-matched prepubertal mates, the causal relation between prepubertal body composition and the timing of puberty has been subject to limited research. Higher prepubertal body mass index (BMI) has been associated with earlier age at peak height velocity in boys (52) and girls (53) and lower age at menarche in girls (54). In contrast, no association with childhood BMI and age at menarche was found in a large follow-up study (55).

The role of leptin, a hormonal signal secreted by adipose tissue, is still controversial. Leptin signals nutritional status to the hypothalamus to regulate appetite and energy balance. Whether leptin acts as a trigger or permissive factor in pubertal development remains to be clarified, but human studies have provided some evidence that leptin is regulated by GnRH (56). Moreover, Bouret et al. (57) have recently shown that leptin plays a neurotrophic role during the development of the hypothalamus. To date, we do not know whether the accumulation of adipose tissue during critical periods in childhood influences the risk of precocious puberty in adopted children, but such a relation can be hypothesized.

INTRAUTERINE CONDITIONS

Associations between low birth weight and early pubertal development indicate that prenatal conditions may have long-term effects on different functions including puberty and reproduction (58–62), maybe as an adaptive response to intrauterine conditions (63). Although such associations have not yet been studied in adopted children, it can be speculated that many adopted children have been exposed to less favorable intrauterine conditions, as low birthweights are frequently reported (64). The issue of intrauterine weight gain is obviously linked with maternal nutritional conditions, as well as other possible etiological factors such as smoking, preeclampsia, and prenatal health care. Prenatal exposure to polychlorinated biphenyls (PCBs) and lead has indicated an

association with reduced birth weight in some studies (65–68), but a recent large-scale prospective study did not confirm this, as no association between birth weight and maternal serum levels of PCB during pregnancy was found (69). Exploring the field of long-term consequences of intrauterine growth retardation seems to be of major importance, and one of the difficulties is the lack of information about prenatal and perinatal conditions in adopted children.

ENVIRONMENTAL CHEMICAL EXPOSURES

There is increasing consciousness of the possible impact of endocrine disruptors on human health and pubertal development. New understanding of the activity and sensitivity of the HPG axis during infancy and in the prepubertal years has attracted special concern directed against fetal and childhood exposures, as children are uniquely vulnerable individuals (70,71). Focus has especially been on substances with direct estrogen-like action, but a number of these compounds have subsequently been shown to possess anti-androgenic as well as anti-estrogenic properties, emphasizing the complex mechanisms of action on the endocrine system. An example of this are the phthalates to which humans are ubiquitously exposed through a wide range of industrial products including cosmetics, clothing, toys, and paint. Phthalate metabolites have been detected in amniotic fluid, breast milk, serum, seminal fluid, saliva, and urine, and elevated urinary levels of some phthalate metabolites in children have indicated that the average exposure might be higher for children than for adults (72,73). Experimental exposure of rats to phthalates has shown dose-dependent adverse effects on reproductive health in male offspring, whereas female offspring are predominantly unaffected (74). In line with these animal studies are two recent human studies indicating that testicular development might be susceptible to perinatal phthalate exposure mediated by an anti-virilizing effect (75,76), but to date, there are no well-designed studies assessing the possible effect on pubertal timing. Associations between the timing of human puberty and exposure to a wide range of chemical factors such as pesticides [mainly dichlorodiphenyldichloroethylene (DDE), a metabolite of DDT], dioxin-like compounds, endosulfan, lead, polybrominated biphenyls (PBBs), and PCBs have been studied (77,78). Different effects on growth and puberty timing have been found in boys and girls (30,79), and some studies have suggested an acceleration of female puberty with increasing levels of exposure to PBBs and DDE (78,80), whereas others have found indications of delayed puberty in girls exposed to lead and PCBs (79,81,82). One study has addressed this issue in migrating children: In 26 patients of foreign origin with precocious puberty (15 adopted and 11 non-adopted), the mean serum concentration of the *p*, *p'*-DDE, a long-lasting DDT metabolite, was 10 times higher than the detection limit, whereas the levels were below this limit in 13 of 15 Belgian native patients. It was proposed that exposure to estrogenic substances early in life would promote hypothalamic maturation while it exerts an inhibitory effect at the pituitary level. Subsequent migration would interrupt this exposure and could result in a withdrawal of the negative feedback of the endocrine-disrupting compounds at the pituitary level and/or an accelerated hypothalamic maturation (8). This pathophysiological mechanism is analogous to the situation in congenital adrenal hyperplasia, where precocious puberty occurs after treatment with hydrocortisone is started and the exposure to steroids is interrupted (*Fig. 1*). Peripheral puberty becomes

central secondarily. DDT is only one among the compounds possibly involved in the pathophysiological process. It could be studied due to the presence of the persisting derivative *p, p'*-DDE while this does not exclude the effects of other compounds. A stimulatory effect at the hypothalamic level is suggested by experimental studies in rodents. Estradiol increases the frequency of pulsatile GnRH secretion from hypothalamic explants of 15-day-old female rats. Moreover, when estradiol is administered in vivo between day 5 and day 10, pulsatile GnRH secretion is accelerated, which is typical of the process of hypothalamic maturation, and precocious puberty is observed with early vaginal opening and first estrus (83,84).

Taken together, our present knowledge about the possible effects on pubertal timing after prenatal and postnatal exposures to endocrine disruptors is still sparse and to some extent obscure. However, existing studies do suggest that even low doses of exogenous hormones can exert significant effects on the HPG axis (85), and we cannot exclude that changed patterns of environmental exposures or increased susceptibility to exogenous hormones affect the pubertal maturation after adoption.

PSYCHOLOGICAL ASPECTS

Precocious and early-onset puberty are frequently associated with psychological and behavioral problems in childhood as well as later in life (16,31,86–89), and it may aggravate the feeling of being different from peers and enhance negative emotional reactions (90). However, positive association between early puberty and cognitive abilities has been found (89), and no consistent emotional or behavioral abnormalities were found in two psychological studies of adopted children treated for early puberty (91,92). No untreated adoptees with early sexual development were evaluated as controls in these studies. Nevertheless, adopted children are subject to multiple stressful changes of their lives, and precocious puberty may represent an additional risk for psychological vulnerability. At the time of adoption, psychomotor development might be delayed, and behavioral and mental health concerns, including attachment difficulties, frequently affect adopted children and their families (5,93). Increased risk of severe mental health problems and social maladjustment during adolescence and young adulthood was found in adoptees in a large-scale epidemiological study from Sweden (1), and this undesirable tendency was confirmed in a recent meta-analysis on behavior problems and mental health referrals of adoptees, including 25,281 cases and 80,260 controls. More total problem behavior and more frequent referral to mental health services were found in international adoptees than in non-adopted children (5).

Psychological stress might also affect the timing of puberty. Belsky and colleagues (94) have proposed that girls reared in a stressful environment will develop in a manner that accelerates pubertal maturation. In studies designed to test the predictive value of Belsky's theory, it was concluded that positive familial relationships were associated with later pubertal timing, but it could not be ruled out that this was the result of a genetic transmission (95,96). A recent American study supported the hypothesis that certain stressful psychosocial factors in infancy and childhood may lead to earlier pubertal maturation and showed associations between the absence of the father and the age at menarche (97). Although the understanding of the underlying mechanisms is still very weak, it could be proposed that adopted children carry a burden of stressful

traumas, making them susceptible to precocious pubertal maturation. In support to this theory is the observation that age at adoption affects the risk of developing precocious puberty, as early adopted children are expected to have experienced fewer stressful events compared with children adopted at older ages. In addition, the finding that Korean children seem to have a low risk of precious puberty is interesting, as the Korean children are adopted at young ages (in Denmark 99% arrive before 1 year of age) and often are placed in foster care prior to adoption (Danish National Board of Adoption, 2005, personal communication). Studies on behavior have indicated that Korean children fare better than adoptees from other countries, maybe due to an effective adoptive arrangement (4).

FINAL HEIGHT

Final height is lower than would be predicted from parental height in patients with precocious puberty, and in historical series of untreated patients, a mean FH around 152 cm has been reported (98,99). Adopted girls living in the Netherlands reached a mean FH of 156 cm, and in adopted Indian girls living in Sweden, the mean FH was 154 cm. These studies showed that the age at menarche corresponded best to that of the most privileged girls in the country of origin, whereas FH resembled that of the least privileged part of the population (15,100). Although many adoptees catch-up to age norms and thus have promising predictions on FH, precocious or early onset of puberty is associated with low FH in many cases (14,38). In a sample of 34 adopted children, in whom growth could be followed throughout childhood and adolescence, Oostdijk (14) and colleagues demonstrated that height loss occurred in the period between the start of puberty and the attainment of FH. Because puberty is early instead of being very precocious in many adopted children, the consequence on short adult height is still uncertain, and longitudinal studies of growth and pubertal development are needed to determine the associations between the onset of puberty and FH.

HORMONAL TREATMENT OF ADOPTED CHILDREN WITH PRECOCIOUS OR EARLY PUBERTY

Medical arrest of puberty and postponement of further development with the aim of increasing adult height by the use of gonadotropin-releasing hormone agonists (GnRHa) has been reported in series of adopted girls with early or precocious puberty. In further attempt to increase FH in short adopted children with early puberty, the effect of adding growth hormone (GH) to GnRHa treatment has been studied in two randomized trials (101,102). The results are equivocal and do not provide strong arguments for further treatment attempts, as only slightly higher FH was found in the Swedish trial including 24 adopted girls treated with a combination of GnRHa and GH compared with 22 adopted girls treated with GnRHa alone (FH 158.9 compared with 155.8 cm) (101). In a similar Dutch study including a total of 26 adopted girls with early puberty, there was no difference in FH (FH 155.0 cm in both groups) (102). Reliable anthropometric data are important clinical tools to evaluate indications and possible outcomes of GnRHa and GH treatment, and careful registration of all available growth data from birth through childhood is highly recommended to health caretakers and adoptive parents. The other issue is the use of GnRHa alone in foreign adopted patients with precocious

puberty. The vast majority enters puberty relatively close to physiological age limits, and treatment in such conditions has been shown not to result in significant increase in FH. Thus, with our present knowledge, the most important indication for GnRH α treatment seems to be the psychosocial aspects, which should be carefully evaluated.

FUTURE CLINICAL AND RESEARCH PERSPECTIVES

At present, we do not know the relative contribution of the different mechanisms that trigger premature onset of puberty in adopted children, and we are unable to prevent it. In daily clinical practice, the challenge is therefore to establish guidelines that ensure appropriate recognition of those adopted children who need medical examinations for precocious puberty and to identify the subgroup of patients who will benefit from treatment with GnRH analogs. Growth curves, obtained through regular measurements of height and weight throughout childhood, are essential to evaluate prepubertal growth and to discover signs of early growth acceleration. Adoptive parents and adoption agencies should be informed about the importance of getting hold of all available pre-adoption anthropometric data.

Both genetic and environmental factors can account for early sexual maturation in adopted girls, although there is little doubt that environment in a broad sense plays a role. However, whether it is caused by the process of adoption or changes in lifestyle is not known. Prospective, longitudinal studies on growth and development in internationally and domestic adopted children, including data on exposure through diets and endocrine disrupting chemicals, will help us disentangle these questions and understand the physiopathology.

Early or precocious puberty is only one among many problems encountered by internationally adopted children. Integrating pediatric endocrinology in this broad context can potentially support adopted children and their families to manage some of these problems better.

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INDEX

- abnormal serum phosphorylation, 261
- abscess, 181
- ACE inhibitors, 202
- adenohypophysis, 8
- adenosine triphosphate (ADP), 8
- adiposity, 228
- adrenal androgen production
 - regulators of, 224–225
 - basic fibroblast growth factor (bFGF), 224
 - IGF-I, 224
 - IGF-II, 224
 - Interleukin-6 (IL-6), 225
 - pituitary hormone ACTH, 224
 - transforming growth factor β 1 (TGF- β 1), 225
- adrenal cortex
 - steroidogenic pathways in, 222
- adrenal medulla and adrenal cortex, interaction
 - between, 225–227
- adrenal steroidogenesis, 268
 - adrenal steroid biosynthetic pathways
 - in zona reticularis, 257
- adrenarche, 143, 217–235, *see also* normal
 - adrenarche; premature adrenarche
 - in individuals born SGA, 256–262
 - Insulin, 258
 - IGF-I, 258
- adrenocortical tumors, 200–201
- adrenocorticotrophic hormone (ACTH), 218, 232–233, 268, 272
 - in adrenal androgen production, 224
 - in adrenal steroid biosynthetic pathways in zona reticularis, 257
 - in adult adrenal gland growth, 220
- adrenomedullary chromaffin cells, 225
- African-American girls genetic control of pubertal onset in, 84
- alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA), 6
- amenorrhea, 59
- γ -Aminobutyric acid (GABA), 42, 91
 - in CNS, as a major inhibitory neurotransmitter, 42, 64
 - GnRH neurons, in the transsynaptic and glial control of, 7, 20
- Amiodarone, 202
- Amphetamines, 202
- Anabolic steroids, 202
- androgen and estrogen physiology in males, 195–196
 - Leydig cells, 195
 - luteinizing hormone (LH), 195
 - testosterone, 195
- Angelman syndrome, 19
- animal models
 - in pubertal timing studies, 90–94
 - chromosome substitution strains (CSSs), 91–92
 - congenic strains, 93–94
 - mouse models, advantages of, 91
- Anosmin-1, 52
- anteroventral periventricular nucleus (AVPV)
 - region, 5–7, 14, 54, 65
- antiestrogens, 189–210
- apes (Simiidae), 36
- appropriate for gestational age (AGA), 251–253, 258
- arachnoid cyst, 64
- arginine vasopressin (AVP), 17–18
- aromatase excess syndrome, 201
- aromatizable androgens, 202
- Atrazine, 19
- Avon longitudinal study of parents and children (ALSPAC), 259
- axo-dendritic synapses, 36
- basal metabolic rate (BMR), 61
- basic fibroblast growth factor (bFGF), 7–9, 12, 224
 - (move to S) small for gestational age (SGA), pubertal consequences, 247–266
 - adrenarche in, 256–262, *see also under* adrenarche
 - and gonadal function, 260–262
 - metabolic and endocrine consequences, 249
 - puberty in individuals born SGA, 250–256
 - animal studies, 256
 - bone age evaluation in children, 253–254

- pubertal tempo and characteristics, 252–254
- timing of, 250–252
- benign ____ (what tissue/organ?) masses, 180
- benign premature thelarche (BPT), 174–177
 - etiology of, 175
 - hypotheses, 175
- Berardinelli–Seip syndrome, 61
- 3-beta-hydroxysteroid dehydrogenase (3 β -HSD)
 - expression, 218–223, 271–272
- bisphenol, 19
- Black girls, pubertal data for, 121
- BMI (body mass index), onset of puberty and, 137–150
 - body fat and, 144–146
 - in boys, 143–144
 - leptin and insulin resistance role in, 146–147
 - obesity in US children, 138
 - epidemiological evidence, 138–142
 - as a risk factor for early adrenarche, 143
 - studies from other countries
 - linking earlier puberty and increased BMI, 142–143
- body mass index, *see under* BMI
- Bogalusa Heart Studies (1973–1994), 111, 153
- breast development
 - early breast development, 174–177
 - benign premature thelarche, 174–177
 - neonatal gynecomastia, 174
 - growth hormone/insulin-like growth factor axis, 173
 - increased breast tissue, nonhormonal causes of, 180–181, *see also under*
- nonhormonal causes
 - physiology of, 170–173
 - hormonal control, 170–172
 - normal morphogenesis, 170
 - and pubic hair development, 117
 - stages of, 172
 - birth, 172
 - embryogenesis, 172
 - pregnancy, 172
 - puberty, 172
- Britain, pubertal studies in, 105–133
- Bronchogenic carcinomas, 201
- Buserelin, 61
- candidate genes for early puberty, resequencing
 - of, 86–87
- canalization, 4
- candidate genes, 87
- cannabis, 202
- catecholamines, 226
- catechol-O-methyltransferase (COMT), 88
- Centers for Disease Control and Prevention (CDC), 152
- central nervous system (CNS) control of
 - gonadotropin secretion, 57
- cholesterol side-chain cleavage enzyme (CYP11A), 219, 221
- chromosome substitution strains (CSSs), 91–93
 - and QTL identification, survey of, 92–93
- Clomiphene citrate, 207
- CNS inhibitory mechanism, intrinsic, 62–63
 - components of, 63–66
- complex traits, 86
 - genes underlying, 86
 - association studies, 86
 - genomewide linkage analyses, 86
 - resequencing of candidate genes, 86
- congenic strains, 93–94
- congenital adrenal hyperplasia (CAH), 203, 267–284
 - enzymes and genes in, 269
 - 3 β -HSD deficiency, 272
 - 11 β -hydroxylase deficiency, 276–279
 - 17-hydroxylase/17,20-lyase deficiency, 273–275
 - 21-hydroxylase deficiency, 275–276
 - 3 β -hydroxysteroid/ Δ 4,5-isomerase deficiency, 271–273
 - lipoid adrenal hyperplasia, 268–271
 - lipoid CAH, 270
- control of puberty
 - in humans, 51–81, *see also under* humans
 - in non-human primates, 35–50, *see also under* non-human primates
 - in rodents, females, 3–33
 - endocrine disruptors, 18–19
 - female sexual development in rodents, 4
 - GnRH secretion, central control of, 6–10, *see also under* gonadotropin-releasing hormone
 - neuroendocrine changes, 10–12, *see also separate entry*
 - neuronal–glial control, potential defects in, 19–21, *see also separate entry*
 - onset of puberty, 12–18, *see also separate entry*
 - pheromones and rodent puberty, 18
 - postnatal development, phases, 4, *see also separate entry*
 - prepubertal changes in gonadotropin secretion, 4–5: FSH levels and, 4
 - steroid feedback mechanisms, 5–6, *see also separate entry*
- cortical–chromaffin crosstalk, 226
- corticotrophin-releasing factor (CRF), 52

- corticotropin-releasing receptor-1 (CRHR-1)-null mice, 227
- cyclic adenosine monophosphate (cAMP), 9
- cyclic gonadotropin secretion, 56
- cyclic nucleotide-gated cation channels, 53
- cyclic secretion of gonadotropins, 56
- cytochrome P450c17 α (CYP17), 89
- Cytosol receptor assays, 180
- Danazol, 207
- decapeptide, 37
- dehydroepiandrosterone (DHEA), 177, 217–233, 271
- dehydroepiandrosterone sulfate (DHEAS), 217–233, 256–259
 - and chronological age (CA), 229
 - and serum insulin-like growth factor I (IGF-I), 230
- delayed puberty
 - defects causing, 21
- dentition, 130
- deoxycorticosterone (DOC), 268–269, 275–279
- dichlorodiphenyltrichloroethane (DDT), 19
- dihydrotestosterone (DHT), 196, 207
- dizygotic twins (DZ), 85
- dopamine- β -hydroxylase, 226
- ‘doughnut’ sign, 205
- dosage-sensitive sex-reversal adrenal hypoplasia congenital (DSS-AHC), 87
- ductal ectasia, 181
- earlier puberty
 - evidence supporting, 153–154
 - in 21st century, 105–136, *see also under* BMI; United States
 - age and onset of puberty, 108
 - cross-sectional and longitudinal designs, 107
 - data on sexual maturity stages, 106–107
 - decreasing secular trend in, 131
 - menarche trend in, 109
 - possible causes, theories and speculations, 107
 - pubertal milestones, historical data on, 109–110
 - secular changes, effects on, 110–129
 - supporting evidence for, 129–130; dentition, 130; environmental factors role in, 130; growth studies, 129–130; obesity epidemic, 130
 - Tanner stage data, 108
 - validity of the indicators, 107–108
 - socioeconomic differences in, 157–158
 - topics that confound claims of, 154–163
 - racially diverse historical comparison data, lack of, 154–157, *see also separate entry*
 - Tanner staging, drawbacks, 154
- ectopic GnRH pulse generator, 64
- end-feet, 9
- endocrine disruptors, 18–19
- environmental factors
 - in earlier puberty, 130
- epidermal growth factor (EGF), 8, 53, 173
- erythroblastosis oncogene B (ERBB), 173
- estradiol feedback
 - negative, 5
 - positive, 5–6
- estrogens, 170–173, 195, 202–203
 - on hypothalamus, in GnRH release, 11
- Ethionamide, 202
- exaggerated thelarche, 177
- exogenous estrogens, exposure to, 178–180
 - cosmetics, 179
 - Isoflavones, 179–180
 - medications, 178–179
 - Puerto Rican experience, 180
 - medications, 202–203
- fat
 - body fat and onset of puberty, 144–146
- Fels Institute subjects, 108, 110–111
- fetal programming, 249
- fetal salvage hypothesis, 254
- fibroadenomas, 180
- fibroblast growth factor receptor 1 (FGFR1), 52, 66, 87
- fibrous phase, of gynecomastia, 194
- Finasteride, 202
- florid phase, of gynecomastia, 194
- flutamide, 202
- follicle-stimulating hormone (FSH), 195–196
 - and gonadotropins secretory patterns, 56–58
 - gynecomastia evaluation, 206
 - in prepubertal changes, 4–5
- Functional ovarian hyperandrogenism, 261
- genetic factors, identifying approaches, 86–90
 - animal models, 90–94, *see also separate entry*
 - association studies, 86, 88–90
 - genomewide linkage analyses, 86
 - linkage analysis, 88
 - resequencing of candidate genes, 86–87
 - of pubertal timing variation, 83–95
- genistein, 19, 179
- genome-wide linkage analysis, 87
- gestational period of rodents, 4

- Ghrelin, 61
- girls' studies, 117–123
- breast and pubic hair development, 117
 - menarche, 117, *see also separate entry*
- glial control, of GnRH, 8–10
- glial erbB receptors, physiological role of, 9
- glial output, change in, 15
- glial–neuronal communication, defects in, 21
- glutamate, 6, 39
- glutamic acid decarboxylase (GAD), 64
- glutamatergic control, 12–13
- gonadal function SGA and, 260–262
- gonadarche, 36
- gonadotropin secretion
- pattern of, 56–58
 - cyclic, 56
 - tonic, 56
 - prepubertal changes in, 4–5
- gonadotropin-dependent precocious puberty (GDPP), 169, 176–178, 181–183
- gonadotropin-dependent processes, 178
- central precocious puberty, 178
- gonadotropin-independent precocious puberty, 177–178
- exaggerated thelarche, 177
 - McCune–Albright syndrome (MAS), 177–178
 - ovarian cysts and tumors, 178
- gonadotropin-releasing hormone (GnRH), 4
- first preovulatory surge of, 11
 - glial cells regulating, 8
 - GnRH green fluorescent protein model, 53
 - neuronal network
 - defects within, 19–20
 - transcriptional control of, 17
 - GnRH neurosecretory neurons, autorhythmicity in, 53
 - glycine/NMDA-mediated stimulation of, 20
 - hypothalamic release of, pubertal changes in, 41
 - inhibitory transsynaptic control of, 8
 - ovary-independent activation of, 10
 - pulsatile GnRH release
 - hypothalamic neural network responsible for generating, 36–37
 - ontogeny of, 37
 - regulatory pathways controlling, 20
 - release
 - aberrant migration of, 52
 - developmental pattern, 43
 - hypothalamic neural network for, 36–37
 - restraint of, neurobiology underlying, 39–43
 - secretion, central control of, 6–10
 - glial control, 8–10
 - transsynaptic and glial control, genes in, 7
 - transsynaptic control, 6–8, 20–21
 - secretion pattern, 56–58
 - transcriptional control of, 17
 - defects in, 20–21
- growth hormone–insulin-like growth factor I (GH–IGF-I) system, 230
- gynecomastia
- in adolescent males, 190–191
 - androgen and estrogen physiology in males, 195–196
 - androgen-to-estrogen ratios in, 197
 - definition, 189
 - developmental anatomy and pathology, 193–194
 - drugs associated with, 202
 - endocrine changes during, 196–198
 - epidemiology, 191–193
 - estrogen exposure, 203
 - algorithm for, 206
 - imaging studies, 205–206
 - laboratory evaluation, 205
 - physical examination, 204–205
 - florid phase of, 193
 - in prepubertal population, 192
 - phases of, 193–194
 - fibrous, 194
 - florid, 194
 - intermediate, 194
 - sex steroids, production and interconversion of, 196
- Clomiphene citrate, 207
- Danazol, 207
- surgical treatment, 208
- Tamoxifen, 207
- Haloperidol, 202
- Heroin, 202
- High Performance liquid chromatography (HPLC), 179
- Hispanic Health and Nutrition Examination Survey (HHANES), 112, 114–117, 129, 152–153, 156, 160–163
- human chorionic gonadotropin (hCG), 200–201, 205, 271
- humans, puberty control in, 51–81
- fetal and neonatal growth affecting, 61
 - GnRH neurons migration, 52–53
 - intrinsic CNS inhibitory mechanism, 62–63
 - KiSS-1, role of, 53
 - negative feedback mechanism, 62–63
 - neural control, 58
 - pituitary and gonadal sensitivity to tropic stimuli, 66–67

- positive feedback mechanism, maturation of, 67–68
- sleep-associated LH release and onset of puberty, 66
- timing and onset of puberty, 58–62
- 11 β -hydroxylase deficiency, 276–279
- 17 α -hydroxylase/17, 20-lyase deficiency, 273–275, 279–281
- 21-hydroxylase deficiency, 275–276, 279–281
- 17-hydroxyprogesterone (17-OHP), 271
- 3 β -HSD deficiency, 223, 271–273
- hyperglycemia, nonketonic, 20–21
- hyperinsulinemia, 232
- hyperprolactinemia, 174, 203
- hyperthyroidism, 203
- hypogonadism, 199–200
 - primary hypogonadism, 199–200
 - secondary hypogonadism, 200
- hypogonadotropic hypogonadism (HH), 84
 - sexual infantilism due to, 60–61
- hypothalamic astrocytes, 10
- hypothalamic neural network, 36–37
- hypothalamic pituitary axis (HPA), 255, 259
- hypothalamic–pituitary–gonadal (HPG) axis, 84, 89, 91, 151, 250, 254–262
 - activation of, 158–159
- idiopathic hypogonadotropic hypogonadism (IHH), 54
- IGF system
 - insulin sensitivity, 228–232
- immune system and adrenal androgen production, 227–228
- infantile period, 4
 - infantile–juvenile transition, 5
- insulin sensitivity, 229
- insulin-like growth factor-1 (IGF-1), 8, 44 in puberty control, 9
- Interleukin-6 (IL-6), 225
- intermediate phase, of gynecomastia, 194
- Intrauterine growth retardation (IUGR), 248
- Isoflavones, 179–180
- Isoniazide, 202
- juvenile period, 4, 37–40
 - estrogenic–androgenic control during, 5
 - GnRH secretion during, 6–10, 41, 44, 55
 - hypothalamic–pituitary axis during, 38
 - hypothyroid state during, 43
 - infantile–juvenile transition, 5
 - juvenile pause, 91
 - in the monkey, 37
 - Oct-2 mRNA levels during, 16
 - plasma FSH during, 5
 - prepubertal brake on pulsatile GnRH release during, 37–39
- KAL1 mutations, 58
- Kallmann syndrome, 200
- Ketoconazole, 202
- KISS-1 gene, 6, 53–55, 59
 - proteolytic cleavage, 6
 - role of, 53–54
- kisspeptin, 6–7, 39, 54
 - kisspeptin/GPR54 system, 11, 14
 - kisspeptin/metastin immunoreactivity, 7
- Klinefelter syndrome, 199
- lean body mass (LBM), 61
- Lee Study, 111, 117
- Leptin, 59–60
 - in BMI and the timing of puberty relationship, 146–147
- Leydig cells, 57, 67, 195
- linkage analysis, 88, 89
- lipoid adrenal hyperplasia, 268–271
- luteinizing hormone (LH), 195
 - in agonadal juvenile male monkeys, 40
 - in female sexual development in rodents, 4
 - at the onset of puberty, 10–12
 - in positive feedback mechanism, 67–68
 - sleep-associated LH release, 66
- luteinizing hormone-releasing hormone (LHRH), 52
- magnetic resonance imaging (MRI), 58
- major histocompatibility complex (MHC), 227
- malignant tumors, 180
- Marshall and Tanner's 1970 study, 108, 110
- McCune–Albright syndrome (MAS), 177–178
- medial preoptic area (MPOA), 55
- menarche, 117
 - earlier menarche, 156
 - population-based studies on, 117–122
 - pubertal data for white and black girls simplified, age of onset, 121
 - on specific populations, 122–123
 - trend in earlier puberty, 109
- Mendelian traits, 86
- 'the metabolic syndrome', 247
- Metastin, 6–7, 39
- Methadone, 202
- Methotrexate, 202
- methoxychlor, 19
- Methyldopa, 202

- Metronidazole, 202
- Mexican-American girls, genetic control of
 pubertal onset in, 84
- mini-puberty of infancy, 195
- monozygotic twins (MZ), 85
- mouse models
 in pubertal timing studies, 91
- National Health and Nutrition Examination Survey (NHANES)
 NHANES I, 114, 153
 NHANES II, 114, 138, 153
 NHANES III, 84, 107–108, 114–115, 127, 129, 137–150
- National Health Examination Survey (NHES), 105, 112, 114–116, 121–132, 141, 152–163
 NHES I, II, 114, 137–150
- neonatal gynecomastia, 174, 199
- neonatal period, 4
- neural cell adhesion molecules (NCAMs), 52
- neural control of puberty, factors affecting, 58
- neuregulins (NRGs), 9
- neuroendocrine changes at the onset of puberty, 10–12
 ovary-dependent activation of GnRH, 10–12
 ovary-independent activation of GnRH and gonadotropin secretion, 10
- neuronal–glial control, defects in, 19–21
 GnRH neuronal network, defects within, 19–20
- neuropeptide Y (NPY), 41, 53
- nitric oxide (NO), 53
- N-methyl-d-aspartate (NMDA), 6
- non-Hispanic White, genetic control of pubertal onset in, 84
- non-human primates
 control of puberty in, 35–50, *see also under* control of puberty
 hypothalamic neural network in, 36–37
- norepinephrine (NE), 17, 226
- normal adrenarche, 219–233
 adrenal androgen production, 224–225, *see also separate entry*
 adrenal medulla and adrenal cortex, interaction between, 225–227
 human adrenal gland, development and zonation, 219–221
 IGF system and insulin sensitivity, 228–232
 immune system and adrenal androgen production, 227–228
- nutrition, puberty timing and, 59
 onset of puberty and, 138, *see also under* BMI
 puberty timing and, 59
- Oct-2 gene, 15–16
- Omeprazole, 202
- onset in females, 155
- onset of puberty
 and BMI, 137–150, *see also under* BMI
 humans, timing and, 58–62
 mechanism, 12–18
 excitatory transsynaptic output, increase in, 12–14: glutamatergic control, 12–13; kisspeptin-dependent control, 14
 glial output, change in, 15
 inhibitory transsynaptic output, decrease in, 14
 metabolic signals and, 18
 neuron-to-neuron and neuronal–glial communication, integration of, 15–16
 puberty clock, 17–18
 sleep-associated LH release and, 66
 opioid peptides, 8, 42
 orchidectomized pattern, 38
 ovariectomized pattern, 38
 ovarian cysts and tumors, 178
 ovary-independent activation of GnRH and gonadotropin secretion, 10
- ovulation, 4
- oxytocin, 172
- P450 oxidoreductase (POR), 222, 257
 P450c17, 222
 P450c21, 223
- paraventricular nucleus (PVN), 55
- Pediatric Research in Office Settings (PROS)
 study, 107–113, 116–119, 121, 123, 129, 132, 137, 139–145, 153, 155
- Pelvic ultrasonography, 183
- peripubertal period, 4
- Phenothiazines, 202
- phenylethanolamine N-methyltransferase (PNMT), 226
- Phenytoin, 202
- pheromones and rodent puberty, 18
- phthalates, 19
- physical pubertal changes
 age of, 155–157
 early physical pubertal changes, 159
 and onset of puberty, 158
- pituitary and gonadal sensitivity to tropic stimuli, 66–67
- plasma LH levels, 4–5
- polycystic ovarian syndrome (PCOS), 217, 219, 232–233, 247, 260–262
- obesity
 epidemic, 130

- polymerase chain reaction (PCR), 55
- polypeptide YY (PYY), 61
- polysialic acid neural cell adhesion molecule (PSA-NCAM), 42–43
- positive feedback mechanism, maturation of, 67–68
- postnatal development, phases, 4
 - infantile period, 4
 - juvenile period, 4, *see also separate entry*
 - neonatal period, 4
 - peripubertal period, 4
- Prader–Willi syndrome (PWS), 21
- precocious puberty, 19–21, *see also under*
 - neuronal–glial control
 - defects causing, 20–21
- preoptic area (POA) tissues, 17
- premature adrenarche, 217–235
 - association with the risk of chronic disease as adult, 232–233
 - definition, clinical and laboratory characteristics, 231
 - differential diagnosis of, 231–232
- premature thelarche (PT), 169–188
 - algorithm for the work-up of, 181
 - breast development, physiology of, 170–173, *see also under* breast development
 - diagnostic approach, 181–183
 - exogenous estrogens, exposure to, 178–180, *see also separate entry*
 - gonadotropin-dependent processes, 178
- preoptic area (POA), 17
- prepubertal changes in gonadotropin secretion, 4–5
- prepubertal children, 62
- primary breast carcinomas, 180
- progesterone, 170–173
 - progesterone receptor (PGR), 88
- prohormone convertase 1 (PC1), 87
- Prolactin, 203
- protein kinase A (PRKARIA), 180
- pubarche, 219
- Puerto Rican experience, 180
- pulsatile GnRH release,
 - on–off–on pattern of, 43–44
 - ontogeny of, 37
 - prepubertal brake on, 37–39
- push–pull perfusion experiments, 8
- quantitative trait loci (QTLs), 91
- racially diverse historical comparison data, lack of, 154–157
 - physical pubertal changes, age of, 155–157
 - menarche, earlier, 156
 - onset in boys, 157
 - onset in females, 155
- refeeding mechanism, 177
- Reserpine, 202
- retroperitoneal tumors, 201
- Rhabdomyosarcoma, 180
- rhesus macaque (*Macaca mulatta*), 36
- rostral (lamina terminalis), 36
- rostral preoptic area (RPOA), 55
- septo-optic dysplasia, 63–64
- Sertoli cells, 58
- sex hormone-binding globulin (SHBG) gene, 89, 197–198, 203, 260
- sex steroids
 - production and interconversion of, 196
- sexual infantilism, 60
- single-nucleotide polymorphisms (SNPs), 87
- sleep-associated LH release and onset of puberty, 66
- small for gestational age (SGA), 247, *see also under* being born small for gestational age
- socioeconomic status (SES), 151–183
 - in assessing the girls' studies, 117–123, 155
 - in study groups from the United States, 157–158
- Spironolactone, 202
- steroid feedback mechanisms,
 - development, 5–6
 - estradiol negative feedback, 5
 - estradiol positive feedback, 5–6
- steroidogenic acute regulatory protein (StAR), 257
- sterol regulatory element binding factor 2 (SREBF2), 88
- suprachiasmatic nucleus (SCN), 17–18
- Tamoxifen, 207
- Tanner staging
 - data, 108–110
 - drawbacks, 154
 - of the secular trend and ages of menarche, 1840–1970, 109
 - Tanner genital stage 2, (G2), 151–183
- Tanocytes line, 9
- testosterone, 195
- tetrahydro-11-deoxycorticosterone (TH-DOC), 279
- tetrahydro-11-deoxycortisol (THS), 279
- thelarche, 169–188, *see also* premature thelarche
 - definition, 169
 - as first sign of normal puberty, 178
- thyrotropin-releasing factor (TRF), 52
- tonic gonadotropin secretion, 56
- transforming growth factor α (TGF α), 42
- transforming growth factor β 1 (TGF- β 1), 225

- transmission disequilibrium test (TDT), 89
 transsynaptic control, of GnRH, 6–8
 defects in, 20–21
 transsynaptic output
 excitatory, 12–14
 inhibitory, 14
 TTF-1, homeobox gene, 16
 tumors, 200–201
 adrenocortical tumors, 200–201
 hormone-producing tumors, 201
 testicular tumors, 200
 tumors mimicking gynecomastia, 201
 Turner's syndrome, 177
 twin studies with respect to age of menarche, 85
 tyrosine hydroxylase, 226
- UBE3A gene, 19
 United States (US) puberty studies, *see also under*
 earlier puberty
 assessing boys' studies, 123–129, *see also*
 under boys' studies
 assessing the girls' studies, 117–123, *see also*
 under Girls' Studies
 cross-sectional studies, comments on, 114–116
- Bogalusa Heart Study, 116
 government surveys, 114–115
 PROS Study, 116
 in the earlier 21st century, 105–136
 with examiner assessments since the 1940s,
 111–112
 from the 1950s, 110–129
- vaginal mucosa estrogenization, 174
 vaginal opening, in rats, 4
 vasoactive intestinal polypeptide (VIP), 17–18
 vesicular GABA transporter (VGAT), 7, 65
 vesicular glutamate transporter 2 (VGLUT2), 65
 Vinca alkaloids, 202
- White boys, pubertal data for, 124–129
 White girls, pubertal data for, 121
- zona reticularis (ZR), 217–234
 adrenal steroid biosynthetic pathways in, 257
 putative regulation for, 220
 in steroidogenic pathways in the adrenal
 cortex, 222