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COMMENTARY

Estrogen: Consequences and Implications of Human Mutations in Synthesis and Action*

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ABSTRACT

Recent developments have advanced our knowledge of the role of estrogen in the male. Studies of the mutations in CYP19, the gene encoding aromatase, in six females and two males and a mutant estrogen receptor α in a man are described. These observations provide illuminating new insights into the critical role of estrogen in the male (as well as female) in the pubertal growth spurt and skeletal maturation, and in the importance of estrogen sufficiency in the accrual and maintenance of bone mass. The weight of evidence supports an effect of androgens on the latter processes, but this effect has not been quantitated.

There is a discordance in the estrogen-deficient male between skeletal growth and skeletal maturation and the accrual of bone mass and density. Estrogen synthesis by the testis is limited before puberty, and estrogen deficiency does not affect the age of pubertal onset. Estrogen deficiency in men leads to hypergonadotropism, macroorchidism, and increased testosterone levels. Estrogen lack has a significant effect on carbohydrate and lipid metabolism, and estrogen resistance was associated with evidence of premature coronary atherosclerosis in a man. These observations have highlighted the role of extraglandular estrogen synthesis and intracrine and paracrine actions.

In the human, in contrast to nonprimate vertebrates, aromatase deficiency and estrogen resistance (α) does not seem to affect gender identity or psychosexual development. The clinical repercussions of mutations in CYP19 on the fetal-placental unit have highlighted the major role of placental aromatase in the protection of the female fetus from androgen excess, thus preventing androgen-induced pseudohermaphrodism and virilization of the mother. These features are compared with the virilization that occurs *in utero* in the female spotted hyena.

The novel features of the aromatase deficiency syndrome in the affected female—in the fetus, during childhood, and at puberty—are discussed, including virilization at puberty and development of polycystic ovaries. The severity of the syndrome correlates with the severity of impairment of aromatase formation in expression systems.

Finally, the structural consequences of missense mutations in CYP19 are described in accordance with a model of the structure of human aromatase. (*J Clin Endocrinol Metab* **84:** 4677–4694, 1999)

LONG HELD concepts of the role and effects of estrogen in the male have been challenged by recent discoveries. Historically, the role of estrogen in the development, growth, and function of the human male has been thought to be relatively unimportant and minor. A remarkable change in perspective has recently emerged. Until 1996, only one estrogen receptor (cloned in the mid-1980s) (1, 2) was thought to mediate the effects of estrogen. Furthermore, even though in the human the enzyme (encoded by the CYP19 gene) P450 aromatase—the critical enzyme responsible for the last and irreversible step in estrogen synthesis from androgens—was recognized to be expressed in multiple tissues in the 1990s (3–5), the significance of estrogen in male physiology had not been appreciated.

New developments have challenged traditional constructs and advanced our understanding of the widespread effects of estrogen in diverse functions in the male, stimulating research ranging from integrative physiology to cell and molecular biology within and outside the reproductive system. Three developments are largely responsible for these advances: 1) description of a man with a null mutation in the ERα that caused estrogen unresponsiveness (6) and of men and girls and women (7–9) with severe estrogen deficiency due to autosomal recessive mutations in the gene encoding aromatase; 2) the concurrent development of mice that lack the estrogen receptor α (αERKO mice) (10–13) or the gene encoding aromatase (ArKO mice) (14); and 3) the discovery of a second widely distributed estrogen receptor, ERβ (15–21), and, most recently, generation of the estrogen receptor β knockout mouse (βERKO) (22) (Fig. 1).

In addition to the relatively slow action of estrogen through classic intracellular steroid receptors (ligand-regulated transcriptional factors that interact with so-called transcriptional coregulatory proteins—coactivators and corepressors to stimulate or inhibit gene expression in target tissues) (23, 24), estrogen also acts through nongenomic processes involving steroid receptors on the cell surface to mediate rapid effects of the hormone on certain cells and tissues (25–29).

Unlike genes for many steroidogenic enzymes, such as CYP17 and CYP21, the large gene (>75 kb) encoding aromatase is expressed in many human tissues (in contrast to the

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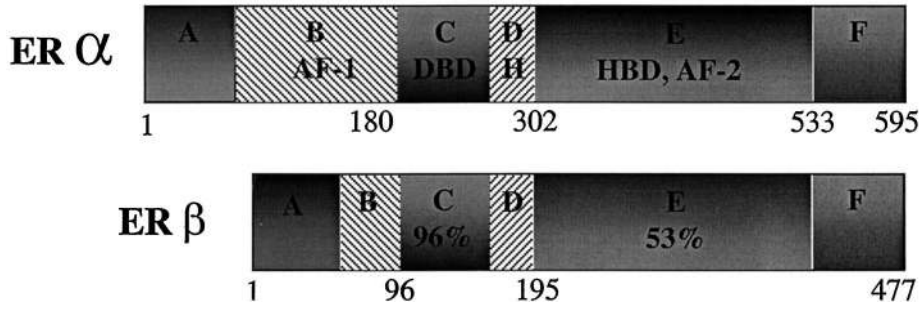

FIG. 1. Human estrogen receptors α and β. The DNA-binding domain (DBD), which contains two zinc fingers, is indicated by C; the hormone-binding domain (HBD) by E which also contains trans-activating function (AF)-2. Domain A/B contains a hormone-independent AF-1, which is distinct in ERα and -β; domain D designates the hinge region (H). The percent homology of estrogen receptor β (which maps to the q25.1 region of chromosome 6) with α (which maps to the long arm of chromosome 14) for the DBD and HBD are indicated. Isoforms have been identified for both receptors; in the 530-amino acid variant of ERβ, which represents the full length amino acid sequence, the A/B domain has an additional 53 amino acids (see text). Each of these receptors has distinct, as well as overlapping, cell and tissue patterns of expression. The estrogen receptors are regulated by phosphorylation of both tyrosine and serine residues, a distinctive property among nuclear receptors. The male with estrogen receptor α resistance had a homozygous cytosine to thymidine transition at codon 157 in exon 2 (AB domain), resulting in a stop codon (Arg157X).

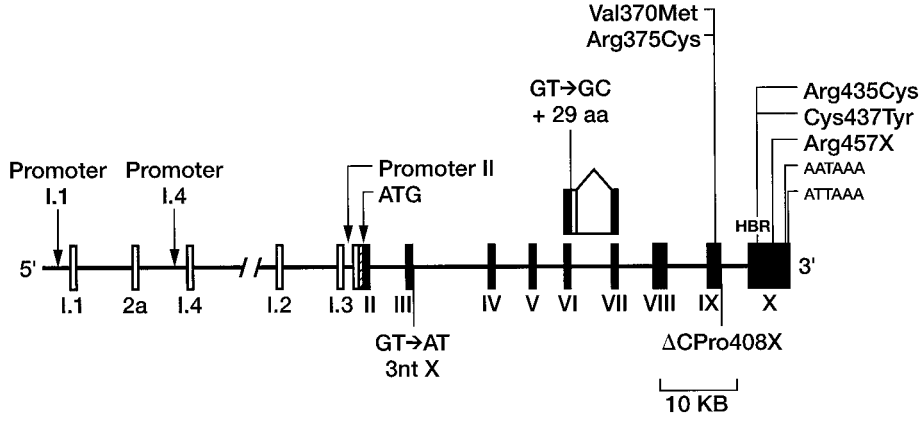

FIG. 2. The structure of the large human CYP19 gene (P450arom) and the mutations that cause aromatase deficiency. The numbered *black boxes* designate translated exons. The septum in the open box in exon II represents the 3′ acceptor splice junction for the untranslated exons. The multiple alternate promoters and the untranslated exons are designated by open boxes. The promoter I.1, which lies more than 40 kb 5′ of the translation start site, is responsible for expression of aromatase in the placenta; promoter I.4 is predominant in adipose tissue and the proximal promoter II is the major promoter in the gonads. The distinct P450 arom promoters provide tissue specific expression by alternate splicing with the common 3′ acceptor splice junction in exon II; irrespective of the choice of the various promoters, only a single aromatase protein is expressed. The known mutations are shown. *X* indicates a nonsense (stop) mutation. Three mutations are in the heme binding region (HBR). GT → GC + 29aa is a thymidine to cytosine transition at the splice junction between exon VI and intron VI, giving rise to a 29 amino acid insert in aromatase. From Grumbach MM, Conte FA. Disorders of sex differentiation. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. Williams textbook of endocrinology, 9th ed. Philadelphia: W.B. Saunders Co. Ltd., 1998; 1303–1425. Modified from Ref. 9.

situation in nonprimate vertebrates). The tissue-specific expression of the enzyme is regulated by means of tissue-specific promoters, but the translated protein is the same in all tissues (see Refs. 3–5) (Fig. 2). These tissues include the placenta and preimplantation blastocyst, the brain, ovary and testis, adipose tissue, fetal liver, muscle, hair follicles, bone, pituitary gland, and the immune system.

Over 25 years ago, Siiteri and MacDonald (30) and Tait and his associates first quantified the extragonadal, extra-adrenal synthesis of estrogen in pregnant and nonpregnant women, in men, and in men and women with a variety of clinical disorders. New developments have highlighted the complexity of extraglandular estrogen synthesis from androgens and androgen precursors (mainly androstenedione, testosterone, and dehydroepiandrosterone and its sulfate) by the aromatase enzyme. These advances focus attention on intracrine mechanisms synthesis (*i.e.* the tissue- and substrate-specific synthesis in peripheral cells from circulating steroid precursors of androgens and estrogens, which act within the specific cell and are not released) and the paracrine and autocrine roles of locally produced estrogen (31–33). A wide variety of cells, for example, contain both aromatase and estrogen receptors (Fig. 3).

In addition, the exceptional tissue distribution and differential actions of ERα and -β receptors and their multiple isoforms (34–36) and their capacity to form heterodimers as well as homodimers (37), combined with the interplay of androgen and estrogen action on target tissues (38–40), provide a new conceptual framework for estrogen action. These advances, coupled with the development of synthetic, tissue-selective nonsteroidal and steroidal estrogen receptor agonists and antagonists–selective estrogen receptor modulators (SERMs)—have important therapeutic applications (24, 41, 42). Table 1 lists the diverse sites of action of estrogen.

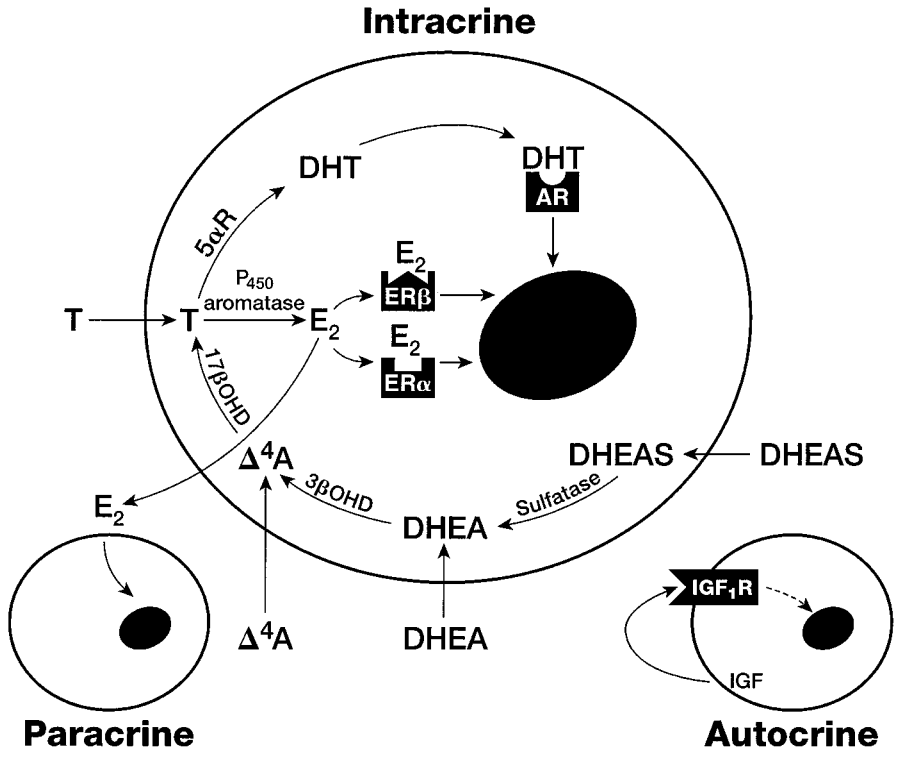

FIG. 3. The complexity of extra-glandular synthesis of estrogen hormones by the conversion of C19 androgens or androgen precursors to aromatic C18 estrogens diagrammatically represented. Intracrine mechanisms (see text) refer, in this instance, to the synthesis in peripheral cells of estradiol from testosterone and other 19-carbon precursors. Testosterone (T) entering the cell from the circulation is converted to dihydrotestosterone (DHT) by 5αR (5α reductase 1 or 2), which acts through binding to the androgen receptor (AR). T is converted to E_2 (17β-estradiol) by CYP19, and the E_2 binds to either the ERα or ERβ forming homo- or heterodimers. Intracellular synthesized T, for example, can arise from Δ^4A (androstenedione) and DHEA or DHEAS. The desulfated DHEA is converted to Δ^4A by 3β-hydroxysteroid dehydrogenase and the Δ^4A is transformed into T by 17β-hydroxysteroid dehydrogenase (*e.g.* Type V isoenzyme), which then can be converted to E_2; or androstenedione can be converted to estrone and then to E_2 by the Type I isoenzyme. Some of the family of 17β-hydroxysteroid dehydrogenase isoenzymes (*e.g.* Type II isoenzyme) can convert E_2 to estrone, providing an additional mechanism of the regulation of estrogen synthesis and metabolism. The E_2 synthesized by intracrine mechanisms can be released to act on a neighboring cell—a paracrine mechanism—through the cell's estrogen receptors; for example, estradiol synthesized by a mesenchymal cell acting on a neighboring epithelial cell. The E_2 also can enter the circulation, an endocrine role. For comparison, an autocrine mechanism is illustrated. IGF-I generated in and released by a peripheral cell can act on the same cell through the cell's surface IGF-I receptors. Recent studies have emphasized the importance of the autocrine/paracrine role of IGF-I in body growth in contrast to the endocrine role of IGF-I synthesized and released into the circulation by the liver, the major contributor to plasma IGF-I. Mice with a selectively and totally deleted hepatic IGF-I gene have greatly reduced circulating IGF-I, but normal postnatal bone and body growth; these observations challenge the widely held somatomedin hypothesis (198, 199). Similarly, even though estrogens produced by extraglandular synthesis are a major source of circulating estrogen in the male and the postmenopausal woman, especially, the intracrine and paracrine role of estrogen in its diverse and specialized functions in specific tissues needs to be considered. Endocrine, paracrine, and intracrine estrogen can also act rapidly on cell surface receptors, a nongenomic action.

The present discussion focuses on the clinical repercussions of human mutations that lead to estrogen resistance or deficiency in the human and have provided insight into the role of estrogen in the male and the role of the placenta in protecting the female fetus and the mother from virilization. The discovery of a young adult man with a null mutation of ERα clarified the role of estrogen acting through the ERα receptor on bone. The description of two males and six females with estrogen deficiency due to mutations in CYP19 (Fig. 2) has shown the major role of aromatase in the fetal placental unit, protecting the female fetus and mother from androgen excess; the critical role of estrogen in pubertal maturation, in bone accrual and growth, and in skeletal maturation and epiphyseal fusion in both the male as well as the female; and have clarified the action of estrogen in carbohydrate and lipid metabolism. Neither estrogen resistance or deficiency seems to affect gender identity.

Estrogen and Its Effects on Pubertal Growth, Skeletal Maturation, and Bone Accrual

Estrogen has manifold effects on bone including a critical, but incompletely understood, action in the pubertal growth spurt, in skeletal maturation, and in the arrest of linear growth by fusion of the epiphyseal growth plate, in the accrual of peak bone mass, and in its maintenance and repair. These dynamic actions (including interactions with the GH and insulin-like growth factors (IGF) and their binding proteins, other growth and morphogenic factors, thyroid hormone, vitamin D, retinoids, PTH and PTHRP, cytokines, and their receptors among many factors) involve a variety of complex developmental and homeostatic programs (see Ref. 43).

The mechanisms involved in the epiphyseal fusion of long bones–the transformation of the cartilaginous growth plate

into bone–is mediated in large part by the action of estrogen during puberty. This action includes a program of orderly proliferation, maturation, apoptosis of chondrocytes, proteolysis of the cartilage extracellular matrix, and vascular and osteoblast invasion of the growth plate. Among other factors (44), gelatinase B (45) and vascular endothelial growth factor (VEGF) (46) are essential signals in the ossification process. It is not known whether estrogen has an effect on these important factors in osteogenesis. However, in the mammary gland of the baboon, estradiol stimulated VEGF messenger RNA (47) and estradiol increases VEGF messenger RNA and VEGF protein in the human MCF-7 breast carcinoma line (48). Stimulation by estrogen of VEGF in endothelial cells in bone could provide one explanation for its capacity to increase the rate of epiphyseal fusion. Estradiol-17β (but not its inactive isomer) also increases protein kinase C activity in chondrocytes from the rat growth plate, apparently involving a nongenomic action (49).

The constancy of bone mass in maturity is maintained by bone remodeling—the critical balance of bone formation and resorption. Resorption of bone by osteoclasts leads to bone formation by mature osteoblasts, the principal effector cells. Although closely linked, these processes are not necessarily regulated by each other, but can occur independently (50). Estrogen (and androgen) affects bone formation, an anabolic effect, and has a well characterized antiresorptive action that involves its action on local cytokine production (51–53).

In males, constitutional delay in onset of puberty has been advanced as a cause of decreased peak bone mass (54, 55); however, a recent study has disputed this contention (56). On the other hand, sexual infantilism due to hypergonadotropic or hypogonadotropic hypogonadism is associated with decreased peak bone mass. Hypogonadism can also lead to osteoporosis if untreated with estrogen in the female or testosterone in the male; rapid bone loss ensues within 5 yr of castration or the onset of severe hypogonadism. In a long-term study of 72 hypogonadal males, which included serial determinations of volumetric bone mineral density (vBMD) of the lumbar spine, continuous testosterone replacement therapy returned the vBMD into the normal range (57). What mediates this anabolic and antiresorptive effect of testosterone on bone?

Estrogen had been recognized as the major sex steroid in the female, responsible for the pubertal growth spurt, skeletal maturation, and the accrual of bone mass (although some held the view that androgens and androgen precursors secreted by the ovary and adrenal were an important factor in these processes); these effects of estrogen were not thought to be important in the male. At puberty in the female, the increased synthesis and secretion of estrogen by the ovary causes the progressive skeletal maturation that eventually leads to epiphyseal fusion and the termination of linear growth. In the male, on the other hand, received wisdom dictated that testosterone, the corresponding male sex steroid, secreted by the testis was directly responsible for these events during puberty (58) with estrogen having a minor effect, if any.

The reports of a 28-yr-old sexually mature male with tall stature, unfused epiphyses, osteopenia, eunuchoid skeletal proportions, and progressive genu valgum (6) due to an autosomal recessive inherited mutation in the ERα (Table 2) and of two adult males with severe estrogen deficiency due to autosomal recessive transmitted mutations in the gene encoding aromatase (9, 59–61) (Table 2) have changed our thinking. All three men had a normal age of onset of puberty and identical clinical findings despite high or normal testosterone concentrations (Table 3), documenting the critical role in the male of estrogen in skeletal maturation, the accumulation of bone mass, and the pubertal growth spurt. In the two men with aromatase deficiency, but not in the man with estrogen resistance, treatment with estrogen led to rapid skeletal maturation and epiphyseal fusion at the wrist within 6–9 months (59, 60) and a dramatic increase in bone mineralization (61). Fig. 4 shows the growth pattern and bone age and the effect of estrogen treatment in this patient (61). Fig.

TABLE 1. Selected biologic effects of estrogen

Bone
Skeletal maturation
Bone mass accrual
Maintenance of bone mass
Growth: pubertal growth spurt
Gonadotropin regulation
Reproductive system
Cardiovascular system
CHO/lipid metabolism
CNS (and psychosexual development)
Adipose tissue
Skin, hair
Urogenital system
Gastrointestinal tract

TABLE 2. Comparison of estrogen receptor α deficiency (ERKOα) and of aromatase deficiency

28-yr-old male (ERKOα)	24-yr-old male with CYP19 (P450arom)
Height 204 cm, weight 127 kg	Height 204.7 cm (+3.7 SD), weight 135.1 kg
No acromegaloid features	No acromegaloid features
Genu valgum; eunuchoid proportions	Eunuchoid skeletal proportions
Normal age of puberty onset	Normal age of puberty onset
Well masculinized, normal testicular size	Well masculinized; testicular volume 34 ml
Bone age 15 yr	Bone age 14 yr
Severe osteoporosis: increased bone turnover	Severe osteoporosis: increased bone turnover
Psychosexual orientation: heterosexual	Psychosexual orientation: heterosexual
No virilization of mother during pregnancy	Virilization of mother during pregnancy
Insulin resistance; acanthosis	Insulin resistance
No response to high-dose estrogen treatment	Striking response to institution of low dose estrogen therapy
Inheritance: autosomal recessive	Inheritance: autosomal recessive
Mutation: Arg157X (Exon 2) in ERα gene	Mutation: Arg376Cys (Exon IX) in CYP19 gene

TABLE 3. Plasma hormones in a male with aromatase deficiency or αERKO

Plasma	CYP19 deficiency	Estrogen receptor α deficiency	Normal
Testosterone (ng/dL)	2015	445	200–1200
Estrone (pg/mL)	<7	145	30–85
Estradiol (pg/mL)	<7	142	10–50
FSH (mIU/mL)	28	33	5.0–9.9
LH (mIU/mL)	26	37	2.0–9.9

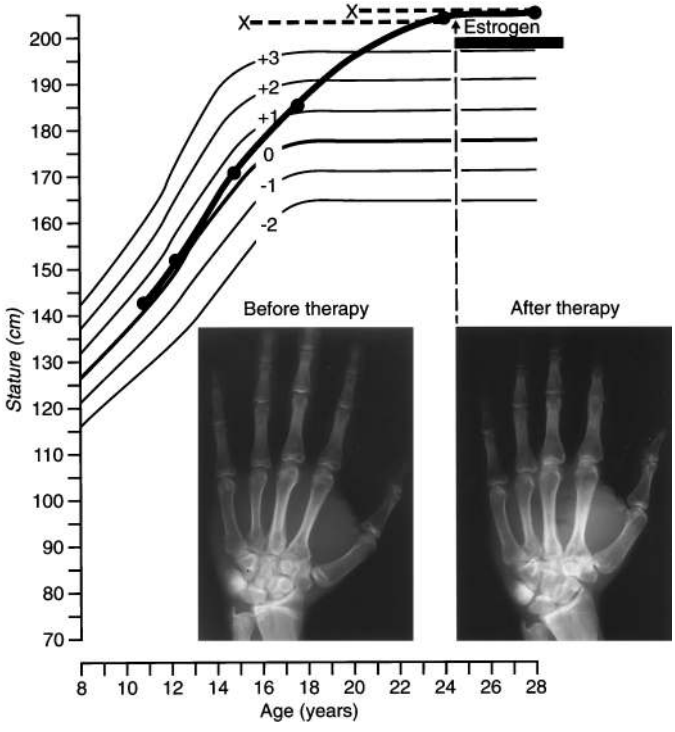

FIG. 4. The linear growth curve (●) and bone age (panels) of the 24-yr-old man with aromatase deficiency whose height was 204.5 cm (+3.7 SD). Note the continued steady growth rate without an apparent pubertal growth spurt, which led to very tall stature and rapid cessation of growth after the institution of estrogen therapy (bar). The X denotes a bone age for the chronological age (*dashed line*). The open epiphyses at the wrist (*left*) closed within 6 months of treatment (*right*). The dark curve is the average normal value for age, and the numerals indicate SD from the mean value (9, 61). The growth curve and, of interest, height and bone age (data not shown), were almost identical in the man with a nonsense mutation in the estrogen receptor α protein (61). From Bilezikian JP, Morishima A, Bell J, Grumbach MM. 1998 Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 339:599–603.

5 presents the effect of estrogen treatment on the accrual of bone mass (61).

These three very remarkable men, together with observations in α and β ERKO and ArKO mice, have led to a revolution in thought about the importance of estrogen in the male, and the recognition of important species differences (see below).

Pubertal growth spurt

Despite normal or increased (the aromatase-deficient men) plasma concentrations of testosterone, none of the men had an apparent pubertal growth spurt (Fig. 4). Whereas the

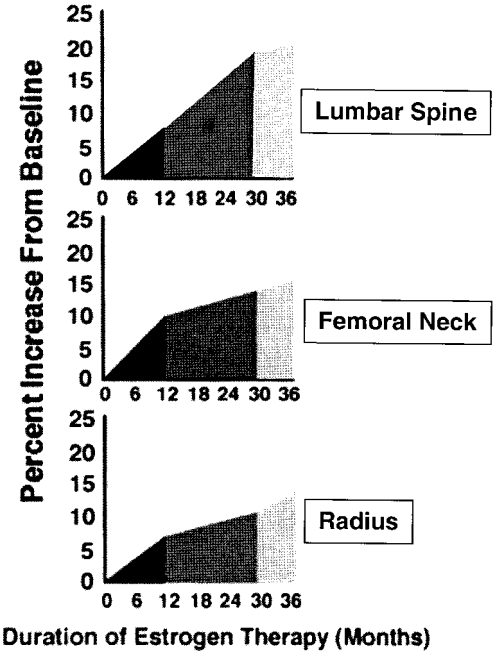

FIG. 5. The dramatic change in bone density during estrogen therapy in the man with CYP19 deficiency. The initial daily dose of 0.3 mg of conjugated estrogens was gradually increased during the first 12 months to 0.75 mg daily, his present dose (see text). On this dosage, the accrual of bone density continued after growth ceased (within 6 months) and increased to within the normal range for adult young men without inducing gynecomastia, impotence, or excessive weight gain. Following baseline studies, bone density was measured at 12, 30, and 36 months (vertical lines) (61). Modified from Bilezikian JP, Morishima A, Bell J, Grumbach MM. 1998 Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med 339:599–603.

evidence provided by examination of their growth curves is not definitive because of gaps in measurement, the data suggest continued steady, slow growth throughout adolescence and the 3rd decade without a "pubertal inflection" or epiphyseal fusion. The delay in skeletal maturation with continued growth leads to eunuchoid skeletal proportions (9). These observations strongly support an essential role for estrogen in the pubertal growth spurt in the male.

Not only does a rise in estradiol concentration correlate with the earlier onset of the pubertal growth spurt in normal girls, but in boys sampled longitudinally before and during puberty, the rise in the estradiol levels as measured by an ultrasensitive bioassay correlated with peak height velocity which occurred, as expected, about 3 yr after pubertal onset (62). Of note, the plasma estradiol concentrations during peak height velocity are similar in boys and girls (about 3–4 pg/mL) and correlate not only with testosterone levels in boys, but with bone age as well.

Additional critical support is provided by the pattern of growth in 46,XY phenotypic females with the complete androgen insensitivity or resistance (CAIS) syndrome. These women have a pubertal growth spurt despite resistance to the action of testosterone owing to a mutation or deletion of the androgen receptor. In the study by Zachmann *et al.* (63), the adult height of CAIS patients was 172.3 ± 4.1 cm SD, 10 cm greater than normal women (162.2 ± 6.0 cm SD), but 2.4

cm less than normal men (174.7 ± 6.7 cm SD). The greater mean height of CAIS than normal women may be related in these 46,XY individuals, in part, to Y-specific growth genes outside of the pseudoautosomal region on the short arm of the Y chromosome. The age of peak height velocity and the pattern of skeletal maturation and epiphyseal fusion were comparable to normal females. Zachmann *et al.* (63) advanced these observations as evidence for the importance of estrogen alone in the female pubertal growth, as opposed to the role of ovarian or adrenal androgens. This study indicated that despite high testosterone concentrations in the presence of physiologically ineffective testosterone action, the pubertal growth in these XY women is attributable to estrogen secreted by the testis and arising from extragonadal conversion of androgens to estrogens (the latter occurs independent of the androgen receptor). Similarly, for example, the autosomal dominant aromatase excess syndrome in prepubertal age boys, in addition to gynecomastia, is associated with an increased rate of growth and skeletal maturation despite a prepubertal concentration of plasma testosterone (64–67). Comparable observations have been reported in boys below age 6 with the Peutz-Jeghers syndrome and estrogen-secreting testicular tumors (68–70).

Other subtle and suggestive, but not definitive, clues to the effect of estrogen on growth and skeletal maturation in the male are listed in Table 4. The low plasma concentrations of estradiol during peak height velocity suggest a potent effect of circulating estradiol on linear growth, but at least two other factors need to be considered in the process: the local aromatization of androgen in bone and the effect with pubertal onset of increased GH secretion and IGF-I generation (reviewed in Refs. 70 and 71). Even though GH secretion increases 2- to 3-fold during puberty, a normal pubertal growth spurt can occur without increasing the dose (per kg) of rhGH therapy in children with severe GH deficiency (58, 72).

In summary, estrogen, not testosterone, has a critical role in the male pubertal growth spurt and in ensuring normal skeletal proportions as exemplified by males with estrogen resistance or deficiency.

Bone maturation and the accrual of bone mass during puberty

Aromatase is expressed widely in human and rodent bone, including in osteoblasts (73–79), chondrocytes of articular cartilage and bone adipocytes (73). Both estrogen receptors, α and β, are expressed in human bone; ERα is expressed in osteoblasts (80–82) and in human osteoclasts (52) (although the latter remains controversial; Refs. 82 and 83), and ERβ is expressed in osteoblasts, but less so than ERα (84). ERα is expressed in resting, proliferative, and hypertrophic human growth plate chondrocytes (82); in contrast, ERβ was identified only in hypertrophic epiphyseal chondrocytes (41, 85).

With the onset of puberty there is a rapid increase in bone mass (86–91), which correlates with bone age. The rate of bone mass accrual approaches a peak in girls by 16 yr, about 3 yr after menarche, and in boys by about 17 yr; the rate then decreases, reaching a plateau in the 3rd decade. For example, white girls attained 94% of volumetric bone density by age 17 yr; in contrast, white boys attained only 86% of volumetric bone density by 17 yr of age (90). Bone acquisition during childhood and through puberty is a major determinant of peak bone mass, which is influenced by genetic and a variety of other factors, as well as hormones. In both sexes, this peak occurs after peak height velocity; hence, the attainment of peak bone mass lags behind the increase in linear growth.

The men with estrogen deficiency or estrogen resistance have severe osteopenia as quantified by dual-energy x-ray absorptiometry scans and an increased rate of bone turnover, as evidenced by the markers of osteoblastic and osteoclastic activity (Table 5). Treatment with high doses of estrogen had no effect on growth, the skeleton, or classic estrogen end organs in the man with the null mutation of the estrogen receptor α (6). In contrast, a relatively low dose of conjugated estrogen (0.3 mg/day gradually increased to 0.75 mg/day over 12 months) had a dramatic positive effect on biological markers of bone metabolism, bone mass and maturation, as well as other sites of estrogen action (see below), without evoking gynecomastia (59, 61). Epiphyseal fusion at the wrist was rapid in both men with severe aromatase deficiency (59–61). In the patient described by Morishima *et al.* (9) (who had fused proximal femoral epiphyses but not ossification of the iliac apophyses), epiphyseal fusion occurred by the 6th month of estrogen treatment and, as a consequence, linear growth ceased (Fig. 4), but bone mineral accrual continued (Fig. 5) (61). Within 3 years of initiating estrogen therapy, the markers of bone turnover gradually approached normal values (Table 6). The excretion of urinary calcium fell, and bone mass increased dramatically. Bone mass in the lumbar spine had increased by 20.7%, in the femoral neck by 15.7%, and in the distal radius by 12.9% (61). In this estrogen-treated aromatase-deficient man, all but the radial site was at the mean value for normal young men (Fig. 5).

In the young male adults with aromatase deficiency, estrogen treatment not only prevented increased bone loss but repaired the severe osteoporosis—a striking anabolic effect on bone mineral content and volumetric and areal BMD mediated, at least in part, by stimulation of osteoblastic synthesis of IGF-I and transforming growth factor-β (61, 92). In menopausal women, on the other hand, exogenous estrogen in the traditional dosage range primarily protects against bone loss (61, 93). This difference in estrogen action, an anabolic in contrast to a primarily protective (antiresorptive) effect, in part, may be an age-related (94) or dose-related phenomenon (95). The osteoporotic bone of postmenopausal

TABLE 4. Earlier clinical clues to the effect of estrogen on growth and skeletal maturation in the male

Complete androgen insensitivity (resistance) syndrome is associated with a pubertal growth and epiphyseal fusion
Short-term estradiol administration increases rate of ulnar growth in prepubertal boys
Aromatase-inhibitor decreases rapid growth and skeletal maturation in testotoxicosis, whereas antiandrogen has no effect on skeletal maturation
Aromatase excess syndrome in boys is associated with increased rate of growth and skeletal maturation, elevated plasma estrogen concentrations and prepubertal testosterone values
Estrogen-secreting tumors (adrenal and testicular neoplasms including Peutz-Jeghers syndrome) are associated with rapid growth and skeletal maturation

TABLE 5. Markers of bone homeostasis in men with aromatase deficiency or deficiency in estrogen receptor α

	CYP19 deficiency	Estrogen receptor α deficiency	Normal
Bone formation			
Alkaline phosphatase (U/L)	241	205	35–95
Osteocalcin (ng/mL)	19.8	18.7	3–13
Bone spec Alk phos (ng/mL)	—	34.2	4.3–19.0
Bone resorption			
Pyridinoline (nmol/nmol creat)	101.7	110	20–61
D-Pyridinoline (nmol/nmol creat)	25.3	32	4–19

TABLE 6. Changes in biomarkers of bone metabolism during estrogen therapy in the aromatase-deficient man

	Baseline	18 months	36 months	Normal
Alk Phos	241	264	136	39–117 IU/L
Osteocalcin	19.8	11.6	14.4	3–13 ng/mL
Urine				
Calcium	94	40	61	50–250 mg/g Cr
Pyridinoline	102	76.5	51	20–61 nmol/nmol Cr
Deoxypyridinoline	25.3	16.2	8.9	4–19 nmol/nmol Cr

women apparently has a limited capacity to mount an anabolic response to conventional estrogen replacement (94), unlike that of the young adult.

The two girls with aromatase deficiency (one a sibling of the affected man) we studied during adolescence had a delayed bone age despite increased circulating androgens and virilization at puberty (8, 9). Estrogen therapy induced a pubertal growth spurt and epiphyseal fusion in both girls. A female child with a null mutation in the P450 arom gene (CYP19) had densitometric evidence of osteopenia of the lumber spine at age 3 4/12 yr that improved after 50 days of low-dose estrogen therapy (96).

Thus, in both males and females the lack of estrogen leads to a dissociation between skeletal growth and skeletal maturation and the accrual of bone density and mass.

Prepubertal estrogens may be important in the sex difference in the rate of skeletal maturation. A bone age of 13 yr in boys is equivalent in the standard gender-specific estimates of skeletal age to that of an 11-yr-old girl; the bone maturation of girls in childhood is about 20% more advanced than that of boys. The nature of this difference had not been understood. Whereas RIA were sufficiently sensitive and specific to quantitate the low testosterone levels in prepubertal boys (97, 98), the method of estimating low (pg/mL) amounts of serum estradiol (99), despite many reports to the contrary, was not sufficiently sensitive and specific to detect prepubertal levels in girls, much less in boys even after solvent extraction and thin-layer chromatography (100). Although a prepubertal sex difference in plasma estradiol concentrations was suspected, the hypothesis remained speculative until Klein *et al.* (101) developed an ultrasensitive recombinant cell bioassay for serum estradiol (detection limit 0.02 pg/mL). With this assay, prepubertal girls (age 7.5 $\pm$ 2.1 yr SD) had a mean concentration of serum estradiol of 0.6 $\pm$ 0.6 pg/mL, whereas the level in prepubertal boys was 0.08 $\pm$ 0.2 pg/mL; no correlation with age or body mass index was found. This more than 7-fold difference was proposed to explicate the more advanced skeletal maturation of prepubertal girls compared with boys (101). Moreover, aromatase activity was not detected or present at a low level in the Leydig cells of prepubertal boys (102, 103), nor was aromatase detected in the human fetal testis at 16–18 weeks of gestation (69). Aromatase is not well expressed in the testis until LH rises at the time of male puberty (102–104). Furthermore, the total aromatase activity of adipose tissue and other extraglandular tissues is low in childhood (64, 105). Accordingly, in the male, circulating estrogen and intracrine and paracrine estrogen becomes important in growth and skeletal maturation during puberty, but not at its onset.

These observations indicate that estrogen has an essential role in the pubertal growth spurt, skeletal maturation, and the accrual of a normal peak bone mass in males as well as females and may have an effect on the prepubertal accretion of bone mineral in the female. New therapeutic approaches—the use of nonsteroidal third generation aromatase inhibitors (*e.g.* letrozole, anastrozole) and ERα antagonists, including SERM—to control growth and skeletal maturation in disorders of growth and puberty by suppression of estrogen synthesis or action are suggested by the critical role of estrogen in these processes (Table 7). The use of these pharmacological agents in the male should not delay or affect the appearance of male secondary sex characteristics. Furthermore, they suggest that in the human ERα, but not ERβ, is the principal estrogen receptor that mediates the action of estrogen on bone mass accrual and epiphyseal fusion. Additional support is indicated by the bone findings, still preliminary, in the αERKO (11, 106) and ArKO mouse (Simpson, E.R., personal communication), but there are important species differences. Table 8 summarizes the effect of estrogen in the male and female on pubertal growth and bone.

Androgens, Growth, and Bone Mass Accrual

Androgens can act directly by activating the androgen receptor or indirectly by their conversion by aromatase to estrogen. The observations in the estrogen-deficient and -resistant men have redefined the role of androgens in the skeleton. Despite normal or strikingly elevated testosterone concentration in the men with impaired estrogen synthesis or action, a pubertal growth spurt was not apparent, and a

TABLE 7. Potential use of aromatase inhibitors (or estrogen receptor antagonists) in disorders of growth and sexual maturation to restrain skeletal maturation

Growth disorders or variants of normal growth (to restrain epiphyseal maturation)
Isolated growth hormone deficiency
Genetic short stature/constitutional delay in growth
Sexual precocity
Congenital virilizing adrenal hyperplasia in male and female
To reduce dose of glucocorticoid
To inhibit conversion of 19-carbon steroids to estrogens (or estrogen action)
With/without use of CYP17 inhibitor or anti-androgen
Testotoxicosis
To inhibit conversion of 19-carbon steroids to estrogens (or estrogen action)
McCune-Albright syndrome
To inhibit conversion of 19-carbon steroids to estrogens (or estrogen action)
Gynecomastia
To inhibit estrogen synthesis (or estrogen action)

TABLE 8. The role of estrogen on pubertal growth and bone in boys and girls

Estrogen acting through its α receptor plays a critical role in:
Pubertal growth spurt
Epiphyseal fusion
Accrual and maintenance of bone mineral density
Bone turnover

steady rate of growth that continued into adulthood was associated with lack of epiphyseal fusion, tall stature, eunuchoid skeletal proportions, and marked osteopenia. Rapid fusion of the epiphyses with the initiation of estrogen therapy in the estrogen-deficient men (59–61) was accompanied by the gradual accretion of a normal bone mineral content (61).

It is important to emphasize that establishing a role for estrogen does not exclude a direct effect of androgen on bone in the male (but one less important than traditionally thought). Quite likely, the direct effect of testosterone (not mediated by conversion to estradiol) is small with reference to pubertal growth and epiphyseal fusion. However, the effect of estrogen treatment on bone mass accrual in the aromatase deficient males occurred in the presence of normal or increased testosterone concentrations. A significant body of evidence supports a direct role for androgen on bone mass and in the sexual dimorphic features of the skeleton (107–111). The human androgen receptor is present in a variety of bone cells, including osteoblasts (112), osteoclasts (108, 113), osteocytes (114), and hypertrophic growth plate chondrocytes (114). The androgen receptor is expressed in periosteal bone (114) as well as cancellous bone. That androgens have a significant role in bone mass accrual is supported by evidence reviewed by Hofbauer and Khosla, (111) Orwoll (107), and Kasperk *et al.* (109) in the human and, for example, by Vanderschueren *et al.* (115–119) and Lea and Flanagan (120) in the rat and by Maor *et al.* (121) in the mouse. In many studies in the rodent, aromatizable androgens (*e.g.* testosterone, androstenedione) were used and not dihydrotesterone or synthetic androgens that are not substrates for aromatase, and they confound interpretation of the direct action of androgen on bone (111). However, 5α-dihydrotestosterone and some synthetic androgens can increase the rate of growth, and, synthetic androgens, the rate of skeletal maturation of children, without increasing circulating GH or IGF-I concentrations.

Furthermore, the decline in bone mass in elderly men correlates better with free estradiol concentrations than with free testosterone levels (122, 123). Men have greater cortical bone width and, as would be predicted from their taller size and higher bone mass, testosterone probably stimulates the periosteal growth of cortical bone (124). Of interest, women with ovarian hyperandrogenism have high BMD (125). Moreover, estrogen treatment of male to female transsexuals increases BMD and markers of bone remodeling (126, 127).

As a counterpart to the action of estrogen in the male on pubertal growth and the skeleton, one can turn to the CAIS in the 46,*XY* phenotypic female who is unresponsive to androgens because of a deletion or null mutation in the X-linked gene encoding the androgen receptor. In these women, the testes secrete estradiol (99) and testosterone, and aromatase activity in the testis and in peripheral tissues is intact. As mentioned earlier, these women have a pubertal growth spurt and fuse their epiphyses in the absence of androgen action. The effect of this mutation on bone mass accrual and bone turnover is smaller, but many confounding factors, including castration in childhood or adolescence and the location of the testes, complicate the interpretation of this phenomenon. Estrogen production, at least in some individuals with CAIS, although greater than that in the normal young male adult (99, 128), is less than the estimated mean estrogen production during the normal menstrual cycle. While both volumetric and areal bone density are reduced in CAIS, biochemical markers of bone turnover are normal (129–131). The skeletal mass in these women is similar to that of normal women (130). Nor do these patients seem to have an increased risk of fractures (129). These observations, however, suggest that the production of estrogen by both the testis and peripheral tissues is sufficient to induce a pubertal growth spurt, epiphyseal fusion, and near normal accrual of bone mass for normal women. Parenthetically the androgen-resistant male rat, analogous to the human with partial androgen resistance, has reduced bone remodeling and size, but not decreased bone density (115–117).

In summary, the conventional belief that the human male skeleton accrues greater bone mass than that of the female because of the direct action of testosterone alone seems no longer tenable. Estrogen has an essential role in attaining optimal peak bone mass in the male. Nevertheless, androgen seems to have a direct but unquantitated effect on the skeleton that has not been distinguished unambiguously from the putative skeletal effects of genes on the human Y chromosome, nor from the observation that most of the sex differences and the age-related changes at the end of puberty are attributable to differences in skeletal dimensions than to bone density.

The Testis and Ovary and the Regulation of Gonadotropins

In the man with severe aromatase deficiency whom we studied, circulating levels of testicular androgens and of FSH

TABLE 9. Gonadal hormones, gonadotropins, and testicular size before and after 3 yr of estrogen therapy in the aromatase-deficient male

Serum or plasma	Baseline	After estrogen	Normal range
Estradiol	<7	64	10–50 pg/mL
Estrone	<7	49	10–50 pg/mL
Androstenedione	335	217	30–263 ng/dL
Testosterone	2015	990	200–1200 ng/dL
Dihydrotestosterone	125	79	30–85 ng/dL
LH	28.3	11.3	2.0–9.9 mIU/mL
FSH	28.3	12.7	5.0–9.9 mIU/mL
Testis volume (cc)	34	28	

and LH were elevated (9). In the man reported by Carani *et al.* (60), whose CYP19 mutation was associated with a lesser degree of aromatase deficiency, the serum gonadotropins were slightly increased, but the testosterone level was normal. In the estrogen-resistant man (6), testosterone concentrations were normal, but estradiol and estrone levels were more than twice the upper value for the normal range, and gonadotropin concentrations were increased.

In addition, our patient has macroorchidism (he did not consent to provide a semen sample before estrogen treatment). The infertile man reported by Carani *et al.* (60) had small testes and oligospermia with immotile on semen analysis, but he had a brother with a normal CYP19 gene who also was infertile and had azoospermia. The familial occurrence of infertility in this pedigree limits an interpretation of the influence of estrogen deficiency on spermatogenesis. The estrogen-resistant man had normal-sized testes and a normal sperm count with a sperm viability of 18% (normal >50%). However, the male αERKO mouse becomes infertile due, at least in part, to an interruption of fluid resorption by the efferent ductules of the epididymis, which leads to dilatation and disruption of the seminiferous tubules (132), whereas the βERKO male mouse is fertile but less so than the wild type mouse (22). The male ArKO, initially fertile, develops progressive infertility associated with impaired spermatogenesis and a reduction in spermatids (133). In the human male, in contrast to the rodent, the data are insufficient at present to allow an assessment of the action of estrogen on spermatogenesis and fertility.

Treatment with estrogen in the men with aromatase deficiency reduced the elevated gonadotropin values into the normal range; in our patient estrogen treatment decreased the high testosterone and dihydrotestosterone concentrations into the male range and the enlarged testes reduced to normal (9, 61) (Table 9).

These observations have clarified further the role of estrogens on the sex steroid-gonadotropin feedback system in the male (Fig. 6), a site of action for which there was previous evidence (134, 135). Aromatase and ERα predominantly are expressed in the pituitary (136, 137) and the hypothalamus. In the two females with aromatase deficiency the elevated levels of androgens failed to suppress gonadotropins into the normal range after the age of puberty or in infancy and early childhood in the absence of estrogen (8, 9), as was the case after puberty in our male patient. In the male, in the virtual absence of estrogen synthesis, no testosterone, androstenedione or dehydroepiandrosterone (DHEA) and its sulfate

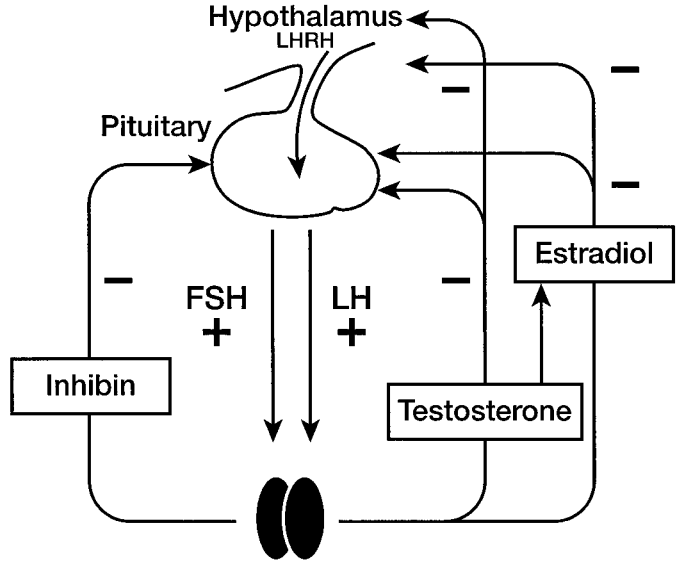

FIG. 6. The male hypothalamic-pituitary gonadotropin-testis axis in the light of observations in aromatase deficiency and defective estrogen receptorα. The observations suggest that estradiol has a significant role in the regulation of FSH and LH. The plasma estradiol arises from the testes and from aromatization of 19-carbon steroids in extra-glandular tissues; in addition, intracrine and autocrine/paracrine roles of locally synthesized estradiol in the pituitary gland and hypothalamus can affect FSH and LH secretion. Accordingly, estradiol, testosterone, and inhibin all play a part in the regulation of gonadotropin secretion in males.

TABLE 10. Glucose and lipid metabolism in men with C_{19} deficiency or estrogen receptor α deficiency

Plasma	P450 Arom	ERKO	Normal
Insulin	52	50	5–25 (uU/mL)
Glucose	70	135	70–105 (mg/dL)
Glycosylated hemoglobin	7.4	9.5	5.1–8.5 (%)
Cholesterol	238	130	<200 (mg/dL)
HDL cholesterol	36	34	36–54 (mg/dL)
LDL cholesterol	139	97	<130 (mg/dL)
Triglycerides	317	97	30–200 (mg/dL)

(DHEAS) were converted to estrogen by the Leydig cells or extragonadal tissues, and the high LH levels stimulated hypersecretion of testosterone by the Leydig cells. We attribute the macro-orchidism in the male patient to the increased level of FSH acting on a functional testes (9, 61); testes volume decreased after estrogen treatment. In two female patients, the chronically increased gonadotropin concentrations led to the formation of multicystic ovaries at puberty that resolved or were prevented from recurring by replacement estrogen and progesterone treatment (8, 9). In the affected female child with a null mutation of the aromatase gene, multicystic ovaries and hypergonadotropism were present by 2–4 yr of age; low-dose estrogen therapy led to normalization of gonadotropins and regression of the ovarian cysts (96).

Carbohydrate and Lipid Metabolism and the Cardiovascular System

The aromatase-deficient man described by Morishima *et al.* (9) had abnormalities of carbohydrate and lipid metabolism

TABLE 11. Metabolic indices before and after estrogen treatment in the aromatase-deficient man

Index[a]	Before	After	Normal
Glucose	70	101	70–105 mg/dL
Insulin	52	15	5–25 uU/mL
Cholesterol	238	234	<200 mg/dL
HDL	36	46	36–54 mg/dL
LDL	139	123	<130 mg/dL
Triglyceride	317	176	30–200 mg/dL

[a] Fasting values.

TABLE 12. Cardiovascular function in the estrogen receptor α-deficient man at age 31 yr

Structural abnormalities of the coronary vasculature were associated with the absence of ER α (in absence of hyperlipidemia but low HDL)
Premature atherosclerotic coronary artery disease as evidenced by coronary calcification by Electron-Beam CT scan ("Ultrafast" CT)
Endothelial dysfunction: absence of flow-mediated vasodilation in brachial artery (endothelium-dependent vasodilation)
Intact rapid vasodilator response (3 min) to estradiol (nongenomic estrogen action)

(61). The insulin resistance and the elevated levels of serum cholesterol, low-density lipoprotein (LDL)-cholesterol triglycerides, and a low concentration of high-density lipoprotein-cholesterol (Table 10) gradually returned to the normal range after the administration of low-dose conjugated estrogen replacement therapy (61) (Table 11). The man reported by Faustini-Fustini *et al.* (92) did not exhibit glucose intolerance but had an abnormal lipid profile (59), which improved as well with estrogen treatment.

The estrogen receptor α-deficient man had axillary acanthosis nigricans, decreased glucose tolerance, insulin insensitivity, and increased glycosylated hemoglobin, which did not respond to high-dose estrogen therapy. This suggests that the insulin resistance was a consequence, at least in part, of the defective estrogen receptor α or was an independent defect. In contrast to the aromatase-deficient men, this man had low serum concentrations of cholesterol, LDL-cholesterol, apolipoprotein (a) and apolipoprotein A1, but normal serum triglycerides (Table 10). This difference in the serum lipid pattern from the pattern described in the estrogen-deficient men may be related, among other factors, to the presence of a functional hepatic estrogen receptor β, which may affect lipid metabolism.

The effect of estrogen on carbohydrate metabolism is unclear; estrogen deficiency is associated with impaired glucose tolerance (138, 139). However, the normal or elevated testosterone concentration acting in the absence of estrogen synthesis or estrogen action by the estrogen receptor α may play a role in this phenomenon.

In summary, men with defective estrogen synthesis or action (ERαR) have a propensity for insulin resistance and dyslipidemia.

Estrogen has a cardioprotective effect (140, 141). This seems to be mediated by two different mechanisms: its effect on serum lipids (142–145) and its direct action on human vascular endothelial and smooth muscle cells and cardiac myocytes and fibroblasts (reviewed in Refs. 146);

TABLE 13. Comparison of clinical features in men with CYP19 deficiency and estrogen receptor α resistance

	Estrogen receptor α resistance	CYP19 deficiency
Stature	Tall	Tall
Eunuchoid proportions	Yes	Yes
Sexual maturation	Normal	Normal
Macroorchidism[a]	No	Yes[b]
Bone age	Severe delay	Severe delay
Bone density	Severe osteoporosis	Severe osteoporosis
Serum FSH/LH	High	High
Plasma testosterone level[a]	Normal	High
Plasma estradiol level[a]	High	Very low
Response to estrogen Rx[a]	None	Positive
Propensity for insulin resistance and abnormal lipid metabolism	Yes	Yes
Serum IGF-I level	Normal	Normal
Psychosexual orientation	Male	Male
Virilization of mother[a]	No	Yes[b]
Inheritance	Autosomal recessive	Autosomal recessive

[a] Distinguishing features.
[b] Depends on the severity of the genetic defect.

TABLE 14. Manifestations of aromatase deficiency in the female

Prenatal
Fetal: masculinization of urogenital sinus external genitalia: androgen-induced female pseudohermaphrodism; low plasma estrogen and very elevated androgen levels
Mother: Virilization, low plasma estrogen and elevated androgen levels
Infancy
Elevated plasma levels; undetectable plasma E_2 (± multicystic ovaries)
Puberty
No female secondary sex characteristics: severe estrogen deficiency
Tall stature
No pubertal growth spurt despite increased serum androgens
Delayed skeletal maturation
Virilization with progressive enlargement of the clitoris
Hypergonadotropic hypogonadism
Increased levels of plasma androgens
Polycystic ovaries
Osteopenia
Female psychosexual orientation

The syndrome is most florid in nonsense mutations of the CYP19 gene.

both estrogen receptors α and β are present in these cardiovascular cells and are widely expressed in the cardiovascular system (147–154). In addition, there are rapid nongenomic actions of estrogen on the vascular system (153, 155, 156) and local estrogen synthesis by aromatase in cardiovascular tissue (157). The man with a null mutation of the estrogen receptor α had an intact rapid (within 5 min) brachial vasodilatory response to sublingual estradiol and an increase in brachial artery flow velocity (a nongenomic action involving calcium-activated potassium channels in vascular smooth muscle cells), but lacked endothelium-dependent vasodilation mediated by endothelial nitric oxide generation (159), a nongenomic effect

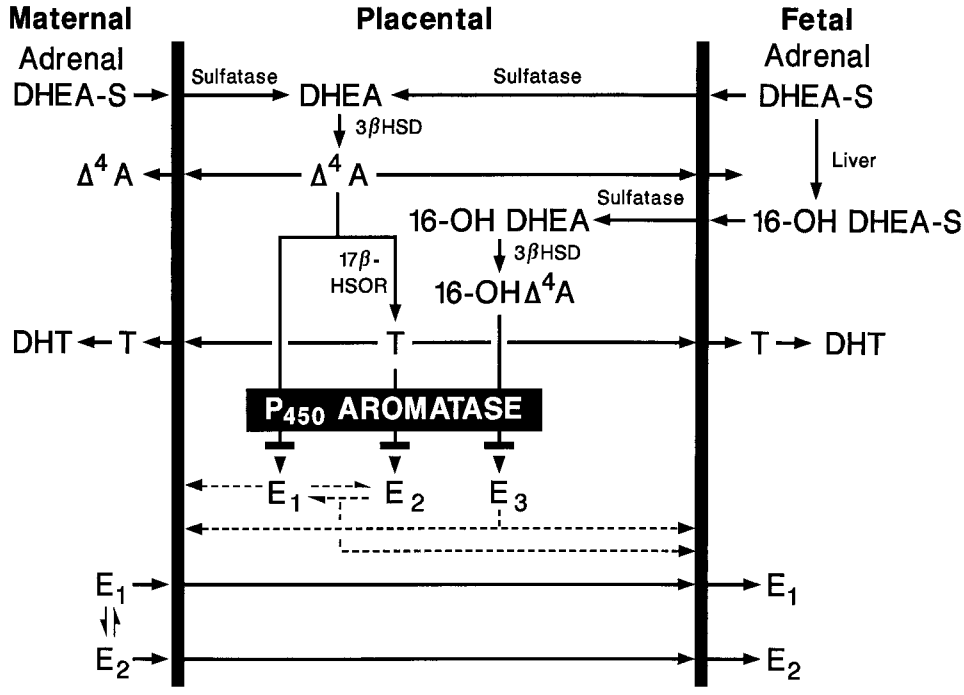

FIG. 7. The placental synthesis of testosterone and 18-carbon steroids from 19-carbon precursors in the CYP19-deficient fetoplacental unit. The placenta lacks the gene encoding CYP17 and hence is unable to convert 21-carbon steroids to 19-carbon steroids. The *black box* indicates the consequences of CYP19 deficiency on the placental synthesis of estrogens; the enzymatic block leads to the overproduction of testosterone and androstenedione. 3β-HSD, 3β-hydroxysteroid dehydrogenase 2; 17-βHSOR, 17β-hydroxysteroid dehydrogenase 3; DHT, dihydrotestosterone; Δ^4A, androstenedione; E_1, estrone; E_2, estradiol; E_3, estrone. (Reproduced with permission from Conte *et al.*, J Clin Endocrinol Metab 1994; 78:1287–1292.)

TABLE 15. Mutations of CYP19: relation between aromatase activity in *in vitro* expression systems and virilization of the mother

	Sex of affected child	Genetic defect	Activity (% of normal)	Mother
Kanazawa, 1992 (182, 183)	F	Splice junction defect (exon VI)	0.3	Virilized
San Francisco, 1993 (7, 8)	F	Arg435Cys	1.1	Not virilized
		Cys437Tyr (exon X)	0	
New York, 1995 (sibs) (9)	F&M	Arg375Cys (exon IX)	0.2	Virilized
Lyon, 1996 (184)	F	Arg457X (exon X)	"0"[a]	Virilized
Bern, 1997 (96)	F	Exon III (splice site)	"0"[a]	Virilized
		Pro408X	"0"[a]	
Modena, 1997 (60)	M	Arg365Gln (exon IX)	0.4	?
Bonn, 1998 (185)	F	Val370Met (exon IX)	ND[b]	Virilized

[a] Presumed lack of activity because of stop codon.
[b] ND, not determined.

on endothelial cells which may be mediated by a cell membrane estrogen receptor encoded by the same transcript as the nuclear receptor (29). By electron-beam computed tomography scan, early coronary artery calcification indicated the presence of coronary atherosclerosis despite a low concentration of serum LDL-cholesterol (159) (Table 12). These observations, although limited to one well-studied patient, are consistent with the importance of estrogen and estrogen receptor α on cardiovascular function and protection from cardiovascular disease.

Psychosexual Development and the Central Nervous System (CNS)

The role of estrogens on psychosexual development in the human in contrast to other mammals is poorly understood. The men and women with aromatase deficiency owing to a mutation in CYP19 and the man with estrogen resistance due to a homozygous mutation in the estrogen α-receptor had sex appropriate gender identities (6, 8, 9). Taken as a whole, these observations suggest that despite the diffuse distribution of estrogen receptors and the enzyme aromatase in the pre- and postnatal CNS (160–162), and despite well-documented sex differences in human brain functioning, estrogen in the human does not have the critical effect on male gender behavior described in nonprimate mammals (13, 163–169) and supports restraint in extrapolating concepts in this active field from studies in animals to the human (8, 14, 170).

Estrogen receptors and aromatase activity are present in many regions of the CNS and coexist in some neurons (171), and different effects of estrogen on neuronal and glial cell growth and function and cerebral blood flow are mediated by genomic and nongenomic mechanisms (18, 169, 172–177). For example, estrogen-replacement therapy in postmenopausal women may have a putative neuroprotective effect (161, 178) and reduce the risk or delay the onset of Alzheimer's disease (179) (reviewed in Ref. 161). Estrogen decreases the generation of Alzheimer β-amyloid peptides on neuronal cells *in vitro* (180) and may improve short-term memory in postmenopausal women (181).

TABLE 16. The increase in human placental aromatase with gestational age during normal pregnancy

Gestational age (weeks)	Total CYP19 (mg)	Total activity (nmol/min)	Placental wt. (g)
11–18 (4)	1.8 ± 1.2	55 ± 31	63 ± 34
26–34 (4)	15.0 ± 3.4	678 ± 242	383 ± 29
37–39 (5)	29.7 ± 10.5	909 ± 215	624 ± 52

From Kitawaki J *et al.* Endocrinology 130:2751–2757, 1992.

CYP19 concentration increases during pregnancy due to an increase in the amount of CYP19.

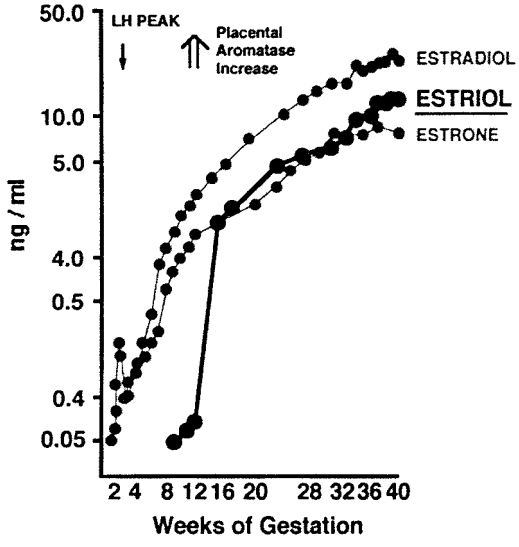

FIG. 8. The pattern of plasma unconjugated estrogens throughout normal gestation. (Note the log scale for plasma concentration; gestational age has been calculated from the last menstrual flow). Estriol is an indirect indicator of the synthesis of DHEA and 16OH DHEA and their sulfates by the fetal adrenal cortex and liver and of the capacity for aromatization of 19-carbon steroids by the placenta. Plasma estriol was first detected at 0.05 ng/mL at 9 weeks gestational age and increased dramatically over the next 7 weeks (shown by ● and thick curve). This is a time in gestation when the fetal adrenal undergoes rapid growth and when placental aromatase activity increases. (Modified from Buster JE. Estrogen metabolism. In: Speroff L, Simpson JL, eds. Reproductive endocrinology, infertility, and genetics, Vol V of Gynecology and obstetrics. Hagerstown, MD: Harper and Row, 1980; 1–11.)

In women, the menopause and adrenopause reduce the availability of 19-carbon steroid substrates for peripheral conversion to estrogen and, accordingly, reduce local estrogen formation and action by paracrine, autocrine, and intracrine mechanisms. In men, 19-carbon precursors of estrogen, mainly of testicular origin (testosterone and androstenedione), gradually decline with age but, nevertheless, are available throughout the aging process for local conversion to estrogen and may provide a mechanism for a persistent neuroprotective effect of estrogen on the CNS. Indeed, this analogy extends to bone; there is a 5-fold greater risk of fracture in the subsequent life of 50-yr-old women as compared with 50-yr-old men.

Table 13 compares and contrasts the salient features of estrogen receptor α-resistance and estrogen deficiency in the male.

TABLE 17. The female spotted hyena (*Crocuta crocuta*)

Exposure to prenatal androgen: genital masculinization, siblicide, female dominance, and aggressiveness
All females are pseudohermaphrodites: females urinate, copulate, give birth through the large penile clitoris and its urogenital canal
Serum androstenedione: females > males
Serum androstenedione and testosterone levels are increased in the pregnant female (ovarian source of androstenedione converted by placenta to testosterone and estrogen)
Placental aromatase activity is 5% of that of human placenta
High level of fetal testosterone may cause formation of cysts in the fetal ovary

TABLE 18. Implications of CYP19 deficiency: the placenta and the fetus

Placenta and fetus: Placental aromatase, by catalyzing the conversion of fetal adrenal androgen to estrogen, protects the female fetus from excess androgen exposure and masculinization of the urogenital sinus and external genitalia and the mother from virilization.
The conceptus can survive in the absence of estrogen synthesis (or estrogen receptor α action) by the implanting blastocyst, fetus, and placenta.
Formation of a female genital tract does not require estrogen
Heterosexual psychosocial development occurs independent of fetal aromatase deficiency (or estrogen receptor α resistance).

Mutations in CYP19 in the Female and Placental Aromatase

A syndrome caused by a variety of autosomal recessive inherited mutations in the CYP19 gene has been described in six females (Table 14) (8, 9, 96, 154, 182, 184, 185). It is characterized by androgen-induced female pseudohermaphrodism and maternal virilization (8, 96, 182), polycystic ovaries, virilization with lack of female secondary sex characteristics at puberty, hypergonadotropism, delayed skeletal maturation, tall stature, and osteopenia (8, 9, 96). The virilization at puberty is associated with greatly augmented LH-stimulated ovarian androgen synthesis (8, 9). Estrogen therapy leads to suppression of virilization, regression of the multicystic ovaries, the development of female secondary sex characteristics, a pubertal growth spurt, epiphyseal fusion, and repair of the osteopenia (Table 9) (8, 9). The clinical features are due to generalized aromatase deficiency.

The lack of placental and fetal hepatic aromatase leads to a failure to convert fetal adrenal androgen precursors to estrogen (Fig. 7) and results in masculinization of the external genitalia in the female fetus and, beginning in the second trimester, virilization of the mother (8, 9, 96, 182), which can be severe. This provides a dramatic illustration of the critical importance of placental and fetal hepatic aromatase in protecting the female fetus and the mother from exposure to large amounts of testosterone synthesized mainly by the placenta (8, 186).

At 36–39 weeks gestation, maternal plasma concentrations of estriol, estrone, and estradiol were strikingly decreased in two mothers with affected fetuses (96, 182). In marked contrast, maternal levels of plasma testosterone, androstenedione, dihydrotestosterone, and DHEAS were increased 4–12 times above the normal mean values (96, 182) [*e.g.* the level

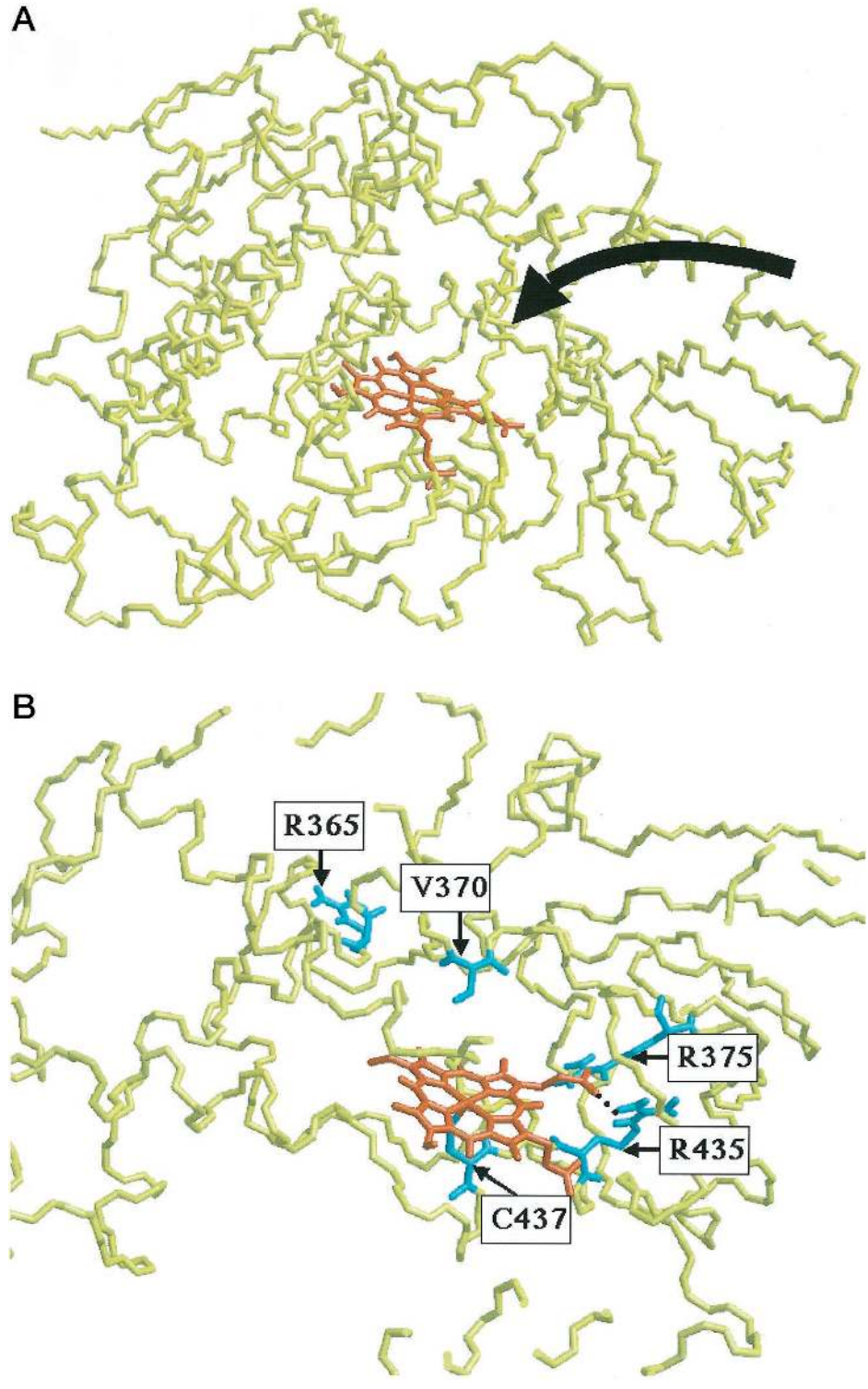

FIG. 9. Structural consequences of human aromatase mutations. A, A tracing of the backbone atoms for a human aromatase model (193). The heme ring is shown in *red*, and the location of bound substrate is indicated by the *black arrow*. B, Amino acid substitution mutations described in patients with aromatase deficiency. This is a coned-down view of the substrate binding site of panel A viewed more from above and to the left of substrate as depicted in panel A. The heme, still shown in *red*, serves to orient the viewer. The majority of the protein is shown in yellow as a tracing of the backbone atoms only, whereas all atoms of the mutated residues arginine 365 (R365), valine 370 (V375), arginine 375 (R375), arginine 435 (R435), and cysteine 437 (C437) are shown in cyan. Note the proximity (*black dots*) between arginine 435 (positive charge) and the carboxyllic acid group of the heme (negative charge).

of plasma-free testosterone was increased 12-fold (96)]. Umbilical cord blood obtained at birth from the two affected female fetuses had similarly elevated testosterone, androstenedione, dihydrotestosterone, and DHEAS levels and decreased estrogen concentrations (96, 182). There is a strong correlation between the aromatase activity of the mutant CYP19 and the masculinization of the female fetus and virilization of the mother (96). As little as 1% of the aromatase activity of wild-type P450arom (in one allele) protected the mother from severe virilization and was associated with less masculinization of the genitalia of the female fetus (Table 15). A pregnant woman with progressive virilization beginning as early as the second trimester and who has high circulating androgens and low plasma and urinary estriol values should be suspected of harboring a fetus (female or male) with a mutation in the CYP19 gene.

These observations provide a dramatic illustration of the critical importance of human placental and fetal hepatic aromatase in protecting the female fetus and the mother from exposure to excessive amounts of testosterone of either fetal or maternal origin (8, 186). The concentration and total amount of placental CYP19 in the syncytiotrophoblast increases during gestation (Table 16). The mean placental level of aromatase is 16-fold greater at 40 weeks gestation than at 10 weeks, and the total aromatase increases 16.5-fold, whereas placental weight increases 9-fold during this interval of gestation (187). In clinical states in which this protective mechanism is overloaded with androgens or androgen precursors either from the fetus or the mother, the female fetus is at risk for androgen-induced female pseudohermaphrodism (see Ref. 186). In women carrying a fetus with congenital virilizing adrenal hyperplasia, for instance, the fetal adrenal enlarges and fetal androgen precursors increase in the first trimester when placental (and quite likely hepatic) aromatase is low (187), as manifested by the relatively low maternal plasma estriol levels at this stage of gestation (Fig. 8), and exceed at these levels of substrate the capacity of the fetoplacental unit for aromatization. The ontogenesis of aromatization during normal gestation is delicately balanced. The mid-first trimester and early second trimester is a critical period for male sex differentiation; at this stage of gestation, the total amount of placental aromatase is relatively low, and, hence, conversion of androgen to estrogen would not compromise the level of fetal plasma testosterone concentrations required for masculinization of the external genitalia. In addition, estrogen synthesis by the fetus is not required for differentiation of the Müllerian ducts or female external genitalia (8).

The female spotted hyena is a female pseudohermaphrodite (in contrast to other members of the hyena family). It urinates, copulates, and gives birth through its large penile clitoris, which encompasses throughout its length a urogenital sinus or canal (188). In this species females usually are dominant, more aggressive, and heavier. One major factor for the masculinized external genitalia and aggressiveness is the relatively high level of secretion of androstenedione from the androgenized ovary, which results in higher circulating androstenedione levels in the adult female than in the male (189, 190). During pregnancy, the spotted hyena placenta converts androstenedione to testosterone and estradiol, especially the former, which is released into the fetal circulation (190). The spotted hyena placenta has a relatively low capacity for aromatization in contrast to the human placenta (191). It would seem that the uniquely elevated ambient androstenedione levels of ovarian origin in the mother present from conception (190, 191) are beyond the capacity of the placenta in this species to aromatize. In aromatase deficiency in the human, the fetal adrenal, not the maternal ovary, is the source of the testosterone precursors. The underlying defect seems to reside in the maternal hyperandrogenism (associated with androgenized ovaries) and the low capacity for placental aromatization of 19-carbon steroids. Still unanswered is a possible contributory role of nonandrogen, nonsex chromosome-mediated genetic mechanisms to the differentiation of the ambiguous female external genitalia (192). Table 17 summarizes some implications of maternal hyperandrogenism fetoplacental aromatase deficiency in the female spotted hyena.

In the female with CYP19 mutations, the pubertal failure, virilization, multicystic ovaries, and hyperstimulation of the ovaries by the increase of FSH and LH concentrations are the consequence of the inability of the ovary to aromatize androstenedione and testosterone to estrogens (8) (Table 14). The role of estrogen deficiency in folliculogenesis beyond the early antral stage in these patients is uncertain because of simultaneous elevations of plasma gonadotropin and intraovarian androgen levels (9).

The findings in the aromatase-deficient patients and the estrogen-resistant man suggest that estrogen synthesis or action through the estrogen α-receptor in the blastocyst, fetus, and fetal portion of the placenta is not essential for normal embryonic and fetal development (8, 9) (Table 18).

Structural Consequences of CYP19 Mutations

The amino acid substitutions in the CYP19 gene in patients with human aromatase deficiency all lie in regions of the protein critical for enzymatic activity. Fig. 9, A and B, shows the backbone atoms for a model of human aromatase developed by Graham-Lorence *et al.* (193) with the heme shown in *red*. Cysteine 437 donates the axial liganding sulfhydryl to the iron atom in the center of the heme ring; the mutation Cys437Tyr renders the protein unable to incorporate heme and, hence, eliminates all activity. Similarly, arginine 435 provides a positive charge to pair with a negative charge of the heme (*dotted line* in Fig. 9B)—an interaction found in nearly all CYP enzymes, that appears to optimize heme bind-

TABLE 19. Conventional wisdom: prearom deficiency and deficiency of ERα

In the human being
Estrogen synthesis or responsiveness by conceptus is essential for implantation, and survival of the embryo and fetus.
Fetal/placental estrogen is critical in the timing of parturition.
In the human male
Testosterone is the principal sex hormone directly involved in skeletal maturation, accrual of bone mineral, and maintenance of the skeleton (prevention of osteoporosis).
Estrogen is not an important regulator of FSH secretion.
Endogenous estrogen has no role in the regulation of cardiovascular function.
Local conversion of testosterone to estradiol by the fetal CNS has an important effect on psychosexual development.

TABLE 19. New insights: post-arom deficiency and deficiency of ERα

In the human being
~~Estrogen synthesis~~ or responsiveness by conceptus is ~~essential~~ for implantation, ~~and survival of the embryo~~ and fetus.
~~Fetal/placental~~ estrogen is critical in the timing of parturition.
In the human male
~~Testosterone is the principal sex hormone directly involved in skeletal maturation, accrual of bone mineral, and maintenance of the skeleton (prevention of osteoporosis).~~
Estrogen is not an important ~~regulator~~ of FSH secretion.
Endogenous estrogen has ~~no role in the~~ regulation of cardiovascular function.
Local conversion of testosterone to estradiol by the fetal CNS has an important effect on psychosexual development.

ing; the mutant Arg435Cys retains only 1% of wild-type activity. The analogous mutant Arg440His in human CYP17 is also devoid of most enzymatic activity (194).

The mutation Arg365Gln retains less than 1% of enzymatic activity despite the location of this residue on the periphery of the enzyme (Fig. 9B). Arginine 365 is part of a four amino-acid motif Glu-aa2-aa3-Arg (referred to as the ExxR motif) found at the end of an α helix called the "K-helix" in the sequences of all known CYP enzymes. Furthermore, this Glu and Arg form a salt bridge in all reported crystal structures of CYP enzymes, suggesting that this interaction has an essential role in stabilizing the overall protein structure (195, 196). Mutations Val370Met and Arg375Cys, which lie just C-terminal to this ExxR motif, appear to be closer to the active site (Fig. 9B) and may disrupt substrate binding, as well. Mutant Arg375Cys retains $<$ 1% of wild-type P450arom activity, and the activity of mutant Val370Met must be very low since the latter mutation caused severe masculinization of the external genitalia of the affected female fetus and virilization of the mother (185).

Epilogue

Human mutations, through their clinical repercussions, can have a profound effect on our understanding of complex biologic systems. This concept is well illustrated by the rare mutations that impair estrogen synthesis or action. Table 19 lists conventional wisdom before the detection of mutations in human CYP19 and in estrogen receptor α and the challenge to these constructs that have originated from the study of patients with these genetic errors.

We are at an early stage in our understanding of the role of estrogen in the male. The burgeoning and diverse research in this field, with the perspective of provocative recent advances, has the promise of exciting new developments that will contribute to the further clarification of the full spectrum of estrogen-mediated effects in human beings.

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References

1. **Green S, Walter P, Kumar V, et al.** 1986 Human oestrogen receptor cDNA: sequence, expression and homology to v-*erb*-A. Nature. 320:134–139.
2. **Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J.** 1986 Sequence and expression of human estrogen receptor complementary DNA. Science. 231:1150–1154.
3. **Simpson ER, Mahendroo MS, Means GD, et al.** 1994 Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev. 15:342–355.
4. **Simpson ER, Michael MD, Agarwal VR, Hinshelwood MM, Bulun SE, Zhao Y.** 1997 Expression of the CYP19 (aromatase) gene: an unusual case of alternative promoter usage. FASEB J. 11:29–36.
5. **Simpson ER, Zhao Y, Agarwal VR, Michael MD, Bulun SE, et al.** 1997 Aromatase expression in health and disease. Rec Prog Horm Res. 52:185–214.
6. **Smith EP, Boyd J, Graeme RF, et al.** 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med. 331;1056–1060.
7. **Ito Y, Fisher CR, Conte FA, Grumbach MM, Simpson ER.** 1993 Molecular basis of aromatase deficiency in an adult female with sexual infantilism and polycystic ovaries. Proc Natl Acad Sci USA. 90:11673–11677.
8. **Conte FA, Grumbach MM, Ito Y, Fisher CR, Simpson ER.** 1994 A syndrome of female pseudohermaphrodism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). J Clin Endocrinol Metab. 78:1287–1292.
9. **Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K.** 1995 Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab. 80:3689–3698.
10. **Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O.** 1993 Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci USA. 90:11162–11166.
11. **Korach KS, Couse JF, Curtis SW, et al.** 1996 Estrogen receptor gene disruption: molecular characterization and experimental and clinical phenotypes. Rec Prog Horm Res 51;159–186.
12. **Cooke PS, Buchanan DL, Lubahn DB, Cunha GR.** 1998 Mechanism of estrogen action: lessons from the estrogen receptor-α knockout mouse. Biol Reprod. 59:470–475.
13. **Couse JF, Korach KS.** 1999 Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev. 20:358–417.
14. **Fisher CR, Graves KH, Parlow AF, Simpson ER.** 1998 Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the *cyp19* gene. Proc Natl Acad Sci USA. 95:6965–6970.
15. **Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-Å.** 1996 Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA. 93:5925–5930.
16. **Enmark E, Pelto-Huikko M, Grandien K, et al.** 1997 Human estrogen receptor β–gene structure, chromosomal localization and expression pattern. J Clin Endocrinol Metab. 82:4258–4265.
17. **Ogawa S, Inoue S, Watanabe T, et al.** 1998 The complete primary structure of human estrogen receptor β (hER-β) and its heterodimerization with ER-α *in vivo* and *in vitro*. Biochem Biophys Res Commun. 243:122–126.
18. **Kuiper GGJM, Shughrue PJ, Merchenthaler I, Gustafsson J-Å.** 1998 The estrogen receptor β subtype: a novel mediator of estrogen action in neuroendocrine systems. Frontier Neuroendocrinol. 19:253–286.
19. **Mosselman S, Polman J, Dijkema R.** 1996 ERβ: identification and characterization of a novel human estrogen receptor. FEBS Lett. 392:49–53.
20. **Pau CY, Pau K-YF, Spies HG.** 1998 Putative estrogen receptor β and α mRNA expression in male and female rhesus macaques. Mol Cell Endocrinol. 146:59–68.
21. **Brandenberger AW, Tee MK, Lee JY, Chao V, Jaffe RB.** 1997 Tissue distribution of estrogen receptors α (ER-α) and β (ER-β) mRNA in the midgestational human fetus. J Clin Endocrinol Metab. 82:3509–3512.
22. **Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JÅ, Smithies O.** 1998 Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc Natl Acad Sci USA. 95:15677–15682.
23. **Smith CL, Nawaz Z, O'Malley BW.** 1997 Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol. 11:657–666.
24. **Shiau AK, Barstad D, Loria PM, et al.** 1998. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. Cell. 95:927–937.
25. **Ciocca DR, Vargas Roig LM.** 1995 Estrogen receptors in human nontarget tissues: biological and clinical implications. Endocr Rev. 16:35–62.
26. **Ramirez VD, Zheng J.** 1996 Membrane sex-steroid receptors in the brain. Front Neuroendocrinol. 17:402–439.
27. **Moss RL, Gu Q, Wong M.** 1997 Estrogen: nontranscriptional signalling pathway. Rec Prog Horm Res. 52:33–68.
28. **Revelli A, Massobrio M, Tesarik J.** 1998 Nongenomic actions of steroid hormones in reproductive tissues. Endocr Rev. 19:3–17.
29. **Razandi M, Pedram A, Greene GL, Levin ER.** 1999 Cell membrane and nuclear receptors derive from a single transcript: studies of ERα and ERβ expressed in CHO cells. Mol Endocrinol. 13:307–319.
30. **Siiteri PK, MacDonald PC.** 1973 Role of extraglandular estrogen in human endocrinology. In: Greep RO, Astwood EB, eds. Handbook of physiology, Vol II. Female reproductive system, part I. Washington, DC: American Physiological Society; 615–629.
31. **Labrie F, Bélanger A, Simard J, Van L-T, Labrie C.** 1995 DHEA and peripheral androgen and estrogen formation: intracrinology. Ann NY Acad Sci. 774:16–28.
32. **Labrie F, Bélanger A, Cusan L, Candas B.** 1997 Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology. J Clin Endocrinol Metab. 82:2403–2409.
33. **Peltoketo H, Vihko P, Vihko R.** 1999 Regulation of estrogen action: role of 17 beta-hydroxysteroid dehydrogenases. Vitam Horm. 55:353–398.
34. **Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J-Å, Nilsson S.** 1998 Differential response of estrogen receptor α and estrogen receptor β to partial estrogen agonists/antagonists. Mol Pharmacol. 54:105–112.

35. **Brzozowski AM, Pike ACW, Dauter Z, et al.** 1997 Molecular basis of agonism and antagonism in the oestrogen receptor. Nature. 389:753–758.
36. **Sharpe RM.** 1998 The roles of oestrogen in the male. Trends Endocrinol Metab. 9:371–377.
37. **Cowley SM, Hoare S, Mosselman S, Parker M.** 1997 Estrogen receptors α and β form heterodimers on DNA. J Biol Chem. 272:19858–19862.
38. **Weatherman RV, Fletterick RJ, Scanlan TS.** 1999 Nuclear-receptor ligands and ligand-binding domains. Ann Rev Biochem. 68:559–581.
39. **Katzenellenbogen JA, O'Malley BW, Katzenellenbogen BS.** 1996 Tripartite steroid hormone receptor pharmacology: interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. Mol Endocrinol. 10:119–131.
40. **Yeh S, Miyamato H, Shima H, Chang C.** 1998 From estrogen to androgen receptor: a new pathway for sex hormones in prostate. Proc Natl Acad Sci USA. 95:5527–5532.
41. **Nilsson S, Kuiper G, Gustafsson J-A.** 1998 ERβ a novel estrogen receptor offers the potential for new drug development. Trends Endocrinol Metab. 9:387–395.
42. **Paige LA, Christensen DJ, Grøn H, et al.** 1999 Estrogen receptor (ER) modulators each induce distinct conformational changes in ER α and ER β. Proc Natl Acad Sci USA. 96:3999–4004.
43. **Stevens DA, Williams GR.** 1999 Hormone regulation of chondrocyte differentiation and endochondral bone formation. Mol Cell Endocrinol. 151:195–204.
44. **Baron J, Klein KO, Yanovski JA, et al.** 1994 Induction of growth plate cartilage ossification by basic fibroblast growth factor. Endocrinology. 135: 2790–2793.
45. **Vu TH, Shipley JM, Bergers G, et al.** 1998 MMP-9/Gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell. 93:411–422.
46. **Gerber H-P, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N.** 1999 VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med. 5:623–638.
47. **Nakamura J, Lu Q, Aberdeen G, Albrecht E, Brodie A.** 1999 The effect of estrogen on aromatase and vascular endothelial growth factor messenger ribonucleic acid in the normal nonhuman primate mammary gland. J Clin Endocrinol Metab. 84:1432–1437.
48. **Ruohola JK, Valve EM, Karkkainen MJ, Joukov V, Alitalo K, Harkonen PL.** 1999 Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. Mol Cell Endocrinol. 149:29–40.
49. **Sylvia VL, Hughes T, Dean DD, Boyan BD, Schwartz Z.** 1998 17β-Estradiol regulation of protein kinase C activity in chondrocytes is sex-dependent and involves nongenomic mechanisms. J Cell Physiol. 176:435–444.
50. **Corral DA, Amling M, Priemel M, et al.** 1998 Dissociation between bone resorption and bone formation in osteopenic transgenic mice. Proc Natl Acad Sci USA. 95:13835–13840.
51. **Turner RT, Riggs BL, Spelsberg TC.** 1994 Skeletal effects of estrogen. Endocr Rev. 15:275–300.
52. **Sunyer T, Lewis J, Collin-Osdoby P, Osdoby P.** 1999 Estrogen's bone-protective effects may involve differential IL-1 receptor regulation in human osteoclast-like cells. J Clin Invest. 103:1409–1418.
53. **Manolagas SC, Jilka RL.** 1995 Bone marrow, cytokines, and bone remodeling: emerging insights into the pathophysiology of osteoporosis. N Engl J Med. 332:305–311.
54. **Finkelstein JS, Klibanski A, Neer RM.** 1996 A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty. J Clin Endocrinol Metab. 81:1152–1155. (See also Finkelstein JS et al. 1999 J Clin Endocrinol Metab. 84:3400–3401.)
55. **Moreira-Andrés MN, Cañizo FJ, de la Cruz FJ, Gómez-de la Cámara A, Hawkins FG.** 1998 Bone mineral status in prepubertal children with constitutional delay of growth and puberty. Eur J Endocrinol. 139:271–275.
56. **Bertelloni S, Baroncelli GI, Ferdeghini M, Perri G, Saggese G.** 1998 Normal volumetric bone mineral density and bone turnover in young men with histories of constitutional delay of puberty. J Clin Endocrinol Metab. 83: 4280–4283. (See also Bertelloni S *et al.*, J Clin Endocrinol Metab 1999; 84:3403–3404.).
57. **Behre HM, Kliesch S, Leifke E, Link TM, Nieschlag E.** 1997 Long-term effect of testosterone therapy on bone mineral density in hypogonadal men. J Clin Endocrinol Metab. 82:2386–2390.
58. **Attie KM, Ramirez NR, Conte FA, Kaplan SL, Grumbach MM.** 1990 The pubertal growth spurt in eight patients with true precocious puberty and growth hormone deficiency: evidence for a direct role of sex steroids. J Clin Endocrinol Metab. 71:975–983.
59. **Morishima A, Grumbach MM, Bilezikian JP.** 1997 Estrogen markedly increases bone mass in an estrogen deficient young man with aromatase deficiency (abstract). J Bone Miner Res. 12:S126.
60. **Carani C, Qin K, Simoni M, et al.** 1997 Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med. 337:91–95.
61. **Bilezikian JP, Morishima A, Bell J, Grumbach MM.** 1998 Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. N Engl J Med. 339:599–603.
62. **Klein KO, Martha PM Jr, Blizzard RM, Herbst T, Rogol AD.** 1996 A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. II. Estrogen levels as determined by an ultrasensitive bioassay. J Clin Endocrinol Metab. 81:3203–3207.
63. **Zachmann M, Prader A, Sobel EH, et al.** 1986 Pubertal growth in patients with androgen insensitivity: indirect evidence for the importance of estrogen in pubertal growth of girls. J Pediatr. 108:694–697.
64. **Hemsell DL, Edman CD, Marks JF, Siiteri PK, MacDonald PC.** 1977 Massive extraglandular aromatization of plasma androstenedione resulting in feminization of a prepubertal boy. J Clin Invest. 60:455–464.
65. **Bulun SE, Noble LS, Takayama K, et al.** 1997 Endocrine disorders associated with inappropriately high aromatase expression. J Steroid Biochem Mol Biol. 61:133–139.
66. **Stratakis CA, Vottero A, Brodie A, et al.** 1998 The aromatase excess syndrome is associated with feminization of both sexes and autosomal dominant transmission of aberrant P450 aromatase gene transcription. J Clin Endocrinol Metab. 83:1348–1357.
67. **Shozu M, Takayama K, Bulun SE.** 1998 Mutation in 5′-flanking region of CYP19 gene causes excessive peripheral aromatase expression in boy with gynecomastia (Abstract OR50–2). Proc 80th Annual Meeting of The Endocrine Society, New Orleans, LA.
68. **Coen P, Kulin H, Ballantine T, et al.** 1991 An aromatase-producing sex-cord tumor resulting in prepubertal gynecomastia. N Engl J Med. 324:317–322.
69. **Bulun SE, Rosenthal IM, Brodie AM, et al.** 1993 Use of tissue-specific promoters in the regulation of aromatase cytochrome P450 gene expression in human testicular and ovarian sex cord tumors, as well as in normal fetal and adult gonads. J Clin Endocrinol Metab. 77:1616–1621.
70. **Grumbach MM, Styne DM.** 1998 Puberty: ontogeny, neuroendocrinology, physiology, and disorders. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR, eds. Williams textbook of endocrinology, 9th ed. Philadelphia: W.B. Saunders; 1509–1625.
71. **Clark PA, Rogol AD.** 1996 Growth hormones and sex steroid interactions at puberty. Endocrinol Metab Clin North Am. 25:665–681.
72. **Grumbach MM, Bin-Abbas BS, Kaplan SL.** 1998 The growth hormone cascade: progress and long-term results of growth hormone treatment in growth hormone deficiency. Horm Res. 49(Suppl 2):41–57.
73. **Sasano H, Uzuki M, Sawai T, et al.** 1997 Aromatase in human bone tissue. J Bone Miner Res. 12:1416–1423.
74. **Schweikert HU, Wolf L, Romalo G.** 1995 Oestrogen formation from androstenedione in human bone. Clin Endocrinol. 43:37–42.
75. **Saito H, Yanaihara T.** 1998 Steroid formation in osteoblast-like cells. J Int Med Res. 26:1–12.
76. **Bruch HR, Wolf L, Budde R, Romalo G, Schweikert HU.** 1992 Androstenedione metabolism in cultured human osteoblast-like cells. J Clin Endocrinol Metab. 75:101–105.
77. **Jakob F, Siggelow H, Homann D, Köhrle J, Adamski J, Schütze N.** 1997 Local estradiol metabolism in osteoblast- and osteoclast-like cells. J Steroid Biochem Mol Biol. 61:167–174.
78. **Nawata H, Tanaka S, Tanaka S, et al.** 1995 Aromatase in bone cell: association with osteoporosis in postmenopausal women. J Steroid Biochem Mol Biol. 53:165–174.
79. **Shimodaira K, Fujikawa H, Okura F, Shimizu Y, Saito H, Yanaihara T.** 1996 Osteoblast cells (MG-63 and HOS) have aromatase and 5 alpha-reductase activities. Biochem Mol Biol Int. 39:109–116.
80. **Eriksen EF, Colvard DS, Berg NJ, et al.** 1988 Evidence of estrogen receptor in non-human osteoblast-like cells. Science. 241:84–86.
81. **Komm BS, Terpening CM, Benz DJ, Graeme KA, O'Malley BW, Haussler MR.** 1988 Estrogen binding receptor mRNA and biological response in osteoblast-like osteosarcoma cells. Science. 241:81–84.
82. **Kusec V, Virdi AS, Prince R, Triffitt JT.** 1998 Localization of estrogen receptor-α in human and rabbit skeletal tissues. J Clin Endocrinol Metab. 83:2421–2428.
83. **Collier FM, Huang WH, Holloway WR, et al.** 1998 Osteoclasts from human giant cell tumors of bone lack estrogen receptors. Endocrinology. 139:1258–1267.
84. **Arts J, Kuiper GGJM, Janssen JMMF, et al.** 1997 Differential expression of estrogen receptors α and β mRNA during differentiation of human osteoblast SV-HFO cells. Endocrinology. 138:5067–5070.
85. **Nilsson LO, Boman A, Savëndahl L, et al.** 1999 Demonstration of estrogen receptor-β immunoreactivity in human growth plate cartilage. J Clin Endocrinol Metab. 84:370–373.
86. **Bonjour JP.** 1996 Bone mineral acquisition in adolescence. In: Marcus R, Feldman D, Kelsey J, eds. Osteoporosis. San Diego: Academic Press; 465–476.
87. **Fan YC, Bachrach LK.** 1997 Bone mineral acquisition during adolescence. Curr Opin Endocrinol Diabetes. 4:85–90.
88. **Bachrach LK, Hastie T, Wang M-C, Narasimhan B, Marcus R.** 1999 Bone mineral acquisition in healthy Asian, Hispanic, Black, and Caucasian youth: a longitudinal study. J Clin Endocrinol Metab. In press.
89. **Theintz G, Buchs B, Rizzoli R, et al.** 1992 Longitudinal monitoring of bone mass accumulation in healthy adolescents: evidence for a marked reduction after 16 years of age at the levels of lumbar spine and femoral neck in female subjects. J Clin Endocrinol Metab. 75:1060–1065.

90. **Magarey AM, Boulton TJC, Chatterton BE, Schultz C, Nordin BEC, Cockington RA.** 1999 Bone growth from 11 to 17 years: relationship to growth, gender and changes with pubertal status including timing of menarche. Acta Paediatr. 88:139–146.
91. **Lu PW, Cowell CT, Lloyd-Jones SA, Briody JN, Howman-Giles R.** 1996 Volumetric bone mineral density in normal subjects, aged 5–27 years. J Clin Endocrinol Metab. 81:1586–1590.
92. **Faustini-Fustini M, Rochira V, Carani C.** 1999 Oestrogen deficiency in men: where are we today? Eur J Endocrinol. 140:111–129.
93. **Riggs BL, Khosla S, Melton LJ.** 1998 A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J Bone Miner Res. 13:763–773.
94. **Patel S, Pazianas M, Tobias J, Chambers TJ, Fox S, Chow J.** 1999 Early effects of hormone replacement therapy on bone. Bone. 24:245–248.
95. **Tobias JH, Compston JE.** 1999 Does estrogen stimulate osteoblast function in postmenopausal women? Bone. 24:121–124.
96. **Mullis PE, Yoshimura N, Kuhlmann B, Lippuner K, Jaeger P, Harada H.** 1997 Aromatase deficiency in a female who is compound heterozygote for two new point mutations in the P450arom gene: impact of estrogens on hypergonadotropic hypogonadism, multicystic ovaries, and bone densitometry in childhood. J Clin Endocrinol Metab. 82:1739–1745.
97. **August GP, Tkachuk M, Grumbach MM.** 1969 Plasma testosterone-binding affinity and testosterone in umbilical cord plasma, late pregnancy, prepubertal children and adults. J Clin Endocrinol Metab. 29:891–899.
98. **August GP, Grumbach MM, Kaplan SL.** 1972 Hormonal changes in puberty. III. Correlation of plasma testosterone, LH, FSH, testicular size, and bone age with male pubertal development. J Clin Endocrinol Metab. 34:319–326.
99. **Kelch RP, Jenner MR, Weinstein R, Kaplan SL, Grumbach MM.** 1972 Estradiol and testosterone secretion by human, simian, and canine testes, in males with hypogonadism and in male pseudohermaphrodites with the feminizing testes syndrome. J Clin Invest. 51:824–830.
100. **Jenner MR, Kelch RP, Kaplan SL, Grumbach MM.** 1972 Hormonal changes in puberty. IV. Plasma estradiol, LH, and FSH in prepubertal children, pubertal females, and in precocious puberty, premature thelarche, hypogonadism, and in a child with a feminizing ovarian tumor. J Clin Endocrinol Metab. 34:521–530.
101. **Klein KO, Baron J, Colli MJ, McDonnell DP, Cutler GB Jr.** 1994 Estrogen levels in childhood determined by an ultrasensitive recombinant cell bioassay. J Clin Invest. 94:2475–2480.
102. **Brodie A, Inkster S.** 1993 Aromatase in the human testis. J Steroid Biochem Mol Biol. 44:549–555.
103. **Inkster S, Yue W, Brodie A.** 1995 Human testicular aromatase: immunocytochemical and biochemical studies. J Clin Endocrinol Metab. 80:1941–1947.
104. **Saraco N, Berensztein EB, Rivarola MA, Belgorosky A.** Lack of expression of the aromatase (CYP19) gene in human prepubertal testis: transient stimulation of estradiol secretion *in vitro* at the onset of steroidogenesis (Abstract P2–72). Proc 80th Annual Meeting of The Endocrine Society, New Orleans, LA.
105. **Siiteri PK.** 1978 Endogenous estrogen production in the young. Pediatrics. 62:1134–1137.
106. **Kimbro KS, Taki M, Beamer WG, Korach KS.** Ovariectomized female estrogen receptor-α knockout and wild type mice respond differently to estrogen and dihydrotestosterone treatment (Abstract OR07–4). Proc 80th Annual Meeting of The Endocrine Society, New Orleans, LA.
107. **Orwoll ES.** 1996 Androgens as anabolic agents for bone. Trends Endocrinol Metab. 7:77–84. (See also Wiren K et al. 1999 Endocrinology. 140:3114–3124.)
108. **Bellido T, Jilka RL, Boyce BF, et al.** 1995 Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. J Clin Invest. 95:2886–2895.
109. **Kasperk C, Helmboldt A, Boercsoek I, et al.** 1997 Skeletal site-dependent expression of the androgen receptor in human osteoblastic cell populations. Calcif Tissue Int. 61:464–473.
110. **Vanderschueren D.** 1996 Androgens and their role in skeletal homeostasis. Horm Res. 46:95–98.
111. **Hofbauer LC, Khosla S.** 1999 Androgen effects on bone metabolism: recent progress and controversies. Eur J Endocrinol. 140:271–286.
112. **Colvard DS, Eriksen EF, Keeting PE, et al.** 1989 Identification of androgen receptors in normal human osteoblast-like cells. Proc Natl Acad Sci USA. 86:854–857.
113. **Mizuno Y, Hosoi T, Inoue S, et al.** 1994 Immunocytochemical identification of androgen receptor in mouse osteoclast-like multinucleated cells. Calcif Tissue Int. 54:325–326.
114. **Abu EO, Horner A, Kusec V, Trifitt JT, Compston JE.** 1997 The localization of androgen receptors in human bone. J Clin Endocrinol Metab. 82:3493–3497.
115. **Vanderschueren D, Van Herck E, Suiker AM, et al.** 1993 Bone and mineral metabolism in the androgen-resistant (testicular feminized) male rat. J Bone Miner Res. 8:801–809.
116. **Vanderschueren D, Jan I, Van Herck E, Moermans K, Verhaeghe J, Bouillon R.** 1994 Time-related increase of biochemical markers of bone turnover in androgen-deficient male rats. Bone Miner. 26:123–131.
117. **Vanderschueren D, Van Herck E, Geusens P, et al.** 1994 Androgen resistance and deficiency have different effects on the growing skeleton of the rat. Calcif Tissue Int. 55:198–203.
118. **Vanderschueren D, Van Herck E, Nijs J, Ederveen AGH, De Coster R, Bouillon R.** 1997 Aromatase inhibition impairs skeletal modeling and decreases bone mineral density in growing male rats. Endocrinology. 138:2301–2307.
119. **Vanderschueren D, Boonen S, Bouillon R.** 1998 Action of androgens *vs.* estrogens in male skeletal homeostasis. Bone. 23:391–394.
120. **Lea CK, Flanagan AM.** 1999 Ovarian androgens reduce bone loss, to a minor but significant degree, in rats treated with the anti-oestrogen ICI 182, 780. J Endocrinol. 160:111–117.
121. **Maor G, Segev Y, Phillip M.** 1999 Testosterone stimulates insulin-like growth factor-I and insulin-like growth factor-I-receptor gene expression in the mandibular condyle—a model of endochondral ossification. Endocrinology. 140:1901–1910.
122. **Slemenda CW, Longcope C, Zhou L, Hui SL, Peacock M, Johnston CC.** 1997 Sex steroids and bone mass in older men. Positive associations with serum estrogens and negative associations with androgens. J Clin Invest. 100:1755–1759.
123. **Khosla S, Melton LJ III, Atkinson EJ, Klee GG, O'Fallon WM, Riggs BL.** 1998 Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab. 83:2266–2274.
124. **Seeman E.** 1997 From density to structure: growing up and growing old on the surfaces of bone. J Bone Miner Res. 12:509–521.
125. **Simberg N, Tiitinen A, Silfvast A, Viinikka L, Ylikorkala O.** 1996 High bone density in hyperandrogenic women: effect of gonadotropin-releasing hormone agonist alone or in conjunction with estrogen-progestin replacement. J Clin Endocrinol Metab. 81:646–651.
126. **Van Kesteren P, Lips P, Gooren LJG, Aescheman H, Megans J.** 1998 Long term follow-up of bone mineral density and bone metabolism in transsexuals treated with cross-sex hormones. Clin Endocrinol. 48:347–354.
127. **Reutrakul S, Ongphiphadhanakul B, Piaseu N, et al.** 1998 The effects of oestrogen exposure on bone mass in male to female transsexuals. Clin Endocrinol. 49:811–814.
128. **MacDonald PC, Madden JD, Brenner PF, et al.** 1979 Origin of estrogen in normal men and in women with testicular feminization. J Clin Endocrinol Metab. 49:905–916.
129. **Bertelloni S, Baroncelli GI, Federico G, et al.** 1998 Altered bone mineral density in patients with complete androgen insensitivity syndrome. Horm Res. 50:309–314:1998.
130. **Munoz-Torres M, Jodar E, Quesada M, Escobar-Jimenez F.** 1995 Bone mass in androgen-insensitivity syndrome: response to hormonal replacement therapy. Calcif Tissue Int. 57:94–96.
131. **Mizunuma H, Soda M, Okano H, et al.** 1998 Changes in bone mineral density after orchidectomy and hormone replacement therapy in individuals with androgen insensitivity syndrome. Hum Reprod. 13:2816–2818.
132. **Hess RA, Bunick D, Lee KH, et al.** 1997 A role for oestrogens in the male reproductive system. Nature. 390:509–512.
133. **Robertson KM, O'Donnell L, Jones MEE, et al.** 1999 Impairment of spermatogenesis in mice lacking a functional aromatase (*cyp 19*) gene. Proc Natl Acad Sci USA. 96:7986–7991.
134. **Finkelstein JS, O'Dea LS, Whitcomb RW, Crowley WF.** 1991 Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and GnRH deficient men. J Clin Endocrinol Metab. 74:621–628.
135. **Bagatell CJ, Dahl KD, Bremner WJ.** 1994 The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. J Androl. 15:15–21.
136. **Scully KM, Gleiberman AS, Lindzey J, Lubahn DB, Korach KS, Rosenfeld MG.** 1997 Role of estrogen receptor-α in the pituitary gland. Mol Endocrinol. 11:674–681.
137. **Shughrue PJ, Lane MV, Scrimo PJ, Merchenthaler I.** 1998 Comparative distribution of estrogen receptor-α (ER-α) and β (ER-β) mRNA in the rat pituitary, gonad, and reproductive tract. Steroids. 63:498–504.
138. **Godsland IF, Gangar KF, Walton C, et al.** 1993 Insulin resistance, secretion and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. Metabolism. 42:846–853.
139. **Sharp SC, Diamond MP.** 1995 Sex steroids and diabetes. Diabetes Rev. 1:318–342.
140. **Barrett-Connor E.** 1997 Sex differences in coronary heart disease: why are women so superior? The 1995 Ancel Keys Lecture. Circulation. 95:252–264.
141. **Collins P.** 1996 Vascular aspects of oestrogen. Maturitas. 23:217–226.
142. **The Writing Group for the PEPI Trial.** 1995 Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women. JAMA. 273:199–208.
143. **Bagatell CJ, Knopp RH, Rivier JE, Bremner WJ.** 1994 Physiological levels of estradiol stimulate plasma high density lipoprotein$_2$ cholesterol levels in normal men. J Clin Endocrinol Metab. 78:855–861.
144. **Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM.** 1991 Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med. 325:1196–1204.

145. **Walsh BW, Spiegelman D, Morrissey M, Sacks FM.** 1999 Relationship between serum estradiol levels and the increases in high-density lipoprotein levels in postmenopausal women treated with oral estradiol. J Clin Endocrinol Metab. 84:985–989.
146. **Mendelsohn ME, Karas RH.** 1999 The protective effects of estrogen on the cardiovascular system. N Engl J Med. 340:1801–1811.
147. **Iafrati MD, Karas RH, Aronovitz M, et al.** 1997 Estrogen inhibits the vascular injury response in estrogen receptor α-deficient mice. Nature Med. 3:545–548.
148. **Foegh ML, Ramwell PW.** 1998 Cardiovascular effects of estrogen: implications of the discovery of the estrogen receptor subtype β. Curr Opin Nephrol Hypertens. 7:83–89.
149. **Karas RH, Patterson BL, Mendelsohn ME.** 1994 Human vascular smooth muscle cells contain functional estrogen receptor. Circulation. 89:1943–1950.
150. **Lindner V, Kim SK, Karas RH, Kuiper G, Gustafsson J-Å, Mendelsohn ME.** 1998 Increased expression of estrogen receptor β mRNA in male blood vessels after vascular injury. Circ Res. 83:224–229.
151. **Kim-Schulze S, McGowan KA, Hubchak SC, et al.** 1996 Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells. Circulation. 94:1402–1407.
152. **Venkov CD, Rankin AB, Vaughan DE.** 1996 Identification of authentic estrogen receptor in cultured endothelial cells: a potential mechanism for steroid hormone regulation of endothelial function. Circulation. 94:727–733.
153. **Goetz RM, Thatte HS, Prabhakar P, Cho MR, Michel T, Golan DE.** 1999 Estradiol induces the calcium-dependent translocation of endothelial nitric oxide synthase. Proc Natl Acad Sci USA. 96:2788–2793.
154. **Grohé C, Kahlert S, Löbbert K, et al.** 1997 Cardiac myocytes and fibroblasts contain functional estrogen receptors. FEBS Lett. 416:107–112.
155. **Farhat MY, Lavigne MC, Ramwell PW.** 1996 The vascular protective effects of estrogen. FASEB J. 10:615–624.
156. **White MM, Zamudio S, Stevens T, et al.** 1995 Estrogen, progesterone and vascular reactivity: potential cellular mechanisms. Endocr Rev. 16:739–751.
157. **Grohé C, Kahlert S, Löbbert K, Vetter H.** 1998 Expression of oestrogen receptor α and β in rat heart: role of local oestrogen synthesis. J Endocrinol 156:R1–R7.
158. **Sudhir K, Chou TM, Messina LM, et al.** 1997 Endothelial dysfunction in a man with disruptive mutation in oestrogen-receptor gene. Lancet. 349:1146–1147.
159. **Sudhir K, Chou TM, Chatterjee K, et al.** 1997 Premature coronary artery disease associated with a disruptive mutation in the estrogen receptor gene in a man. Circulation. 96:3774–3777.
160. **Sasano H, Takashashi K, Satoh F, Nagura H, Harada N.** 1998 Aromatase in the human central nervous system. Clin Endocrinol. 48:325–329.
161. **McEwen BS.** 1999 The molecular and neuroanatomical basis for estrogen effects in the central nervous system. J Clin Endocrinol Metab. 84:1790–1797.
162. **Stoffel-Wagner B, Watzka M, Steckelbroeck S, et al.** 1998 Expression of CYP19 (aromatase) mRNA in the human temporal lobe. Biochem Biophys Res Commun. 244:768–771.
163. **Meisel RL, Sachs BD.** 1994 The physiology of male sexual behavior. In: Knobil E, Neill JD, eds. The physiology of reproduction, 2nd ed, Vol. 2. New York: Raven Press. pp. 3–106.
164. **Hutchison JB, Wozniak A, Beyer C, Karolczak M, Hutchison RE.** 1999 Steroid metabolising enzymes in the determination of brain gender. J Steroid Biochem Mol Biol. 69:85–96.
165. **Beyer C.** 1999 Estrogen and the developing mammalian brain. Anat Embryol 199:379–390.
166. **Pilgrim C, Hutchison JB.** 1994 Developmental regulation of sex differences in the brain: can the role of gonadal steroids be defined? Neuroscience. 60:843–855.
167. **Wersinger SR, Sannen K, Villalba C, Lubahn DB, Rissman EF, De Vries GJ.** 1997 Masculine sexual behavior is disrupted in male and female mice lacking a functional estrogen receptor α gene. Horm Behav. 32:176–183.
168. **Ogawa S, Lubahn DB, Korach KS, Pfaff DW.** 1997 Behavioral effects of estrogen receptor gene disruption in male mice. Proc Natl Acad Sci USA. 94:1476–1481.
169. **Balthazart J, Ball GF.** 1998 New insights into the regulation and function of brain estrogen synthase (aromatase). Trends Neurosci. 21:243–249.
170. **Resko JA, Roselli CE.** 1997 Prenatal hormones organize sex differences of the neuroendocrine reproductive system: observations on guinea pigs and nonhuman primates. Cell Mol Neurobiol. 17:627–648.
171. **Naftolin F, Horvath TL, Jakab RL, Leranth C, Harada N, Balthazart J.** 1996 Aromatase immunoreactivity in axon terminals of the vertebrate brain. An immunocytochemical study on quail, rat, monkey, and human tissues. Neuroendocrinology. 63:149–155.
172. **Lephart ED.** 1996 A review of brain aromatase cytochrome P450. Brain Res Reviews. 22:1–26.
173. **Lephart ED.** 1997 Molecular aspects of brain aromatase cytochrome P450. J Steroid Biochem Mol Biol. 61:375–380.
174. **Shughrue PJ, Lane MV, Scrimo PJ, Merchenthaler I.** 1997 The comparative distribution of estrogen receptor-α and β mRNA in the rat central nervous system. J Comp Neurol. 388:507–525.
175. **Shughrue PJ, Lubahn DB, Negro-Vilar A, Korach KS, Merchenthaler I.** 1997 Responses in the brain of estrogen receptor α-disrupted mice. Proc Natl Acad Sci USA. 94:11008–11012.
176. **Shughrue P, Scrimo P, Lane M, Askew R, Merchenthaler I.** 1997 The distribution of estrogen receptor-β mRNA in forebrain regions of the estrogen receptor-α knockout mouse. Endocrinology. 138:5649–5652.
177. **Watson CS, Gametchu B.** 1999 Membrane-initiated steroid actions and the proteins which mediate them. Proc Soc Exp Biol Med. 220:9–19.
178. **McEwen BS, Alves SE.** 1999 Estrogen actions in the central nervous system. Endocr Rev. 20:279–307.
179. **Tang M, Jacobs D, Stern Y, et al.** 1996 Effect of oestrogen during menopause on risk and age of onset of Alzheimer's disease. Lancet. 348:429–432.
180. **Xu H, Gouras GK, Greenfield JP, et al.** 1998 Estrogen reduces neuronal generation of Alzheimer β-amyloid peptides. Nat Med. 4:447–451.
181. **Shaywitz SE, Shaywitz BA, Pugh KR, et al.** 1999 Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. JAMA. 281:1197–1202.
182. **Shozu M, Akasofu K, Harada T, Kubota Y.** 1991 A new cause of female pseudohermaphroditism: placental aromatase deficiency. J Clin Endocrinol Metab. 72:560–566.
183. **Harada N, Ogawa H, Shozu M, Yamada K.** 1992 Genetic studies to characterize the origin of the mutation in placental aromatase deficiency. Am J Hum Genet. 51:666–672.
184. **Portrat-Doyen, Forest MG, Nicolino M, Morel Y, Chatelain PC.** 1996 Female pseudohermaphroditism (FPH) resulting from aromatase (P450 arom) deficiency associated with a novel mutation (R457) in the CYP19 gene. Horm Res. 46(Suppl):14.
185. **Ludwig M, Beck A, Wickert L, et al.** 1998 Female pseudohermaphrodism associated with a novel homozygous G-to-A (V370-to-M) substitution in the P-450 aromatase gene. J Pediatr Endocrinol Metab. 11:657–664.
186. **Lo JC, Schwitzgebel VM, Tyrrell JB, et al.** 1999 Normal female infants born of mothers with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab. 84:930–936.
187. **Kitawaki J, Inoue S, Tamura T, et al.** 1992 Increasing aromatase cytochrome P-450 level in human placenta during pregnancy: studied by immunohistochemistry and enzyme-linked immunosorbent assay. Endocrinology. 130: 2751–2757.
188. **Frank LG.** 1996 Female masculinization in the spotted hyena: physiology, evolution and behavioral ecology. In: Gittleman J, ed. Carnivore behavior, ecology and evolution, Vol II. Cornell University Press; 78–131.
189. **Glickman SE, Frank LG, Pavgi S, Licht P.** 1992 Hormonal correlates of masculinization' in the female spotted hyaena (*Crocuta crocuta*). I. Infancy through sexual maturity. J Reprod Fertil. 95:451–462.
190. **Licht P, Hayes T, Tsai P, et al.** 1998 Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). 1. Urogenital morphology and placental androgen production during fetal life. J Reprod Fertil. 113:105–116.
191. **Yalcinkaya TM, Siiteri PK, Vigne JL, Licht P, Pavgi S, Glickman SE.** 1993. A mechanism for virilization of female spotted hyenas *in utero*. Science. 260:1929–1931.
192. **Drea CM, Weldele M, Forger NG, et al.** 1998 Androgens and masculinization of genitalia in the spotted hyaena (*Crocuta crocuta*). Effects of prenatal antiandrogens. J Reprod Fertil. 113:117–127.
193. **Graham-Lorence S, Amarneh B, White RE, Peterson JA, Simpson ER.** 1995 A three-dimensional model of aromatase cytochrome P450. Protein Sci. 4:1065–1080.
194. **Fardella CE, Hum DW, Homoki J, Miller WL.** 1994 Point mutation Arg440 to His in cytochrome P450c17 causes severe 17α-hydroxylase deficiency. J Clin Endocrinol Metab. 79:160–164.
195. **Hasemann CA, Kurumbail RG, Boddupalli SS, Peterson JA, Deisenhofer J.** 1995 Structure and function of cytochromes P450:a comparative analysis of three crystal structures. Structure. 2:41–62.
196. **Graham-Lorence SE, Peterson JA.** 1996 Structural alignments of P450s and extrapolations to the unknown. Methods Enzymol. 272:315–326.
197. **Grumbach MM, Conte FA.** 1998 Disorders of sex differentiation. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR (eds). Williams Textbook of Endocrinology, 9th ed. Philadelphia: W.B. Saunders, pp. 1303–1425.
198. **Sjögren K, Liu J-L, Blad K, et al.** 1999 Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci USA. 96:7088–7092.
199. **Yakar S, Liu J-L, Stannard B, et al.** 1999 Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci USA. 96:7324–7329.
200. **Buster JE.** 1980 Estrogen metabolism. In: Speroff L, Simpson JL, eds. Reproductive endocrinology, infertility, and genetics, Vol V of Gynecology and obstetrics. Hagerstown, MD: Harper and Row; 1–11.