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The effects of oestrogens on linear bone growth

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Regulation of linear bone growth in children and adolescents comprises a complex interaction of hormones and growth factors. Growth hormone (GH) is considered to be the key hormone regulator of linear growth in childhood. The pubertal increase in growth velocity associated with GH has traditionally been attributed to testicular androgen secretion in boys, and to oestrogens or adrenal androgen secretion in girls. Research data indicating that oestrogen may be the principal hormone stimulating the pubertal growth spurt in boys as well as girls is reviewed. Such an action is mediated by oestrogen receptors (ER-α and ER-β) in the human growth plate, and polymorphisms in the *ER* gene may influence adult height in healthy subjects. Prepubertal oestradiol concentrations are significantly higher in girls than in boys, explaining sex-related differences in pubertal onset. Men with a disruptive mutation in the *ER* gene (oestrogen resistance) or in the *CYP19* gene (aromatase deficiency) who have no pubertal growth spurt and continue to grow into adulthood due to lack of epiphyseal fusion supports this notion. Furthermore, phenotypic females with complete androgen insensitivity syndrome have a normal female growth spurt despite lack of androgen action. Oestrogens may also influence linear bone growth indirectly via modulation of the GH-insulin-like growth factor-I (IGF-I) axis. Thus, ER blockade diminishes endogenous GH secretion, androgen receptor (AR) blockade increases GH secretion in peripubertal boys, and non-aromatizable androgens [oxandrolone or dihydrotestosterone (DHT)] have no effect on GH secretion. Treatment with aromatase inhibitors reduces circulating IGF-I concentrations in healthy males, and reduces growth in boys with testotoxicosis. Taken together, these findings suggest that oestrogens may, in addition to their direct effects, stimulate GH secretion and thereby increase circulating IGF-I, which in turn may stimulate growth. Thus, oestrogens have important biphasic actions on longitudinal growth in boys as well as in girls. Very low levels of oestrogens may stimulate bone growth without affecting sexual maturation directly at the growth plate as well as through stimulation of the GH-IGF axis, which in turn may stimulate growth. Conversely, higher levels of oestrogens stimulate secondary sexual characteristics and epiphyseal fusion.

Key words: growth factors/hormonal control/insulin-like growth factor/linear bone growth/oestrogen

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Introduction

The importance of oestrogens for female physiology is well established. Furthermore, the importance of oestrogens in male cardiovascular and bone physiology has been recognized recently. Oestrogens appear to be involved in the regulation of linear growth in children and adolescents of both sexes, but the diversity in mechanisms as well as sites of action leave many questions unanswered.

Regulation of linear bone growth in children and adolescents comprises a complex interaction of hormones and growth factors. Traditionally, growth hormone (GH) is considered to be the key hormone regulating linear growth in childhood. The pubertal increase in growth velocity associated with increased GH secretion has traditionally been attributed to testicular androgen secretion in boys, and to oestrogens or adrenal androgen secretion in girls. However, within the past decade, research has indicated that oestrogen may be the principal hormone stimulating the pubertal growth spurt in boys as well as girls.

Historically, oestrogens were supposed to have a growth-repressive role due to closure of epiphyses. In addition to their effects at the growth plate, oestrogens were believed to switch off

GH secretion, and consequently acromegalic patients were treated with oestrogens in the 1940s, with remarkable effects. This led to the treatment of adolescent girls presenting with extreme tall stature with high doses of oestrogen in the 1950s. Treatment with high doses of oestrogens has proven beneficial in terms of final height outcome in girls with constitutional tall stature, although lower doses compared with the initial studies have comparable growth-inhibiting effects, with fewer side effects (Drop *et al*., 1998). In 1965, it was first reported that oestrogen administration at even lower doses was able to increase GH secretion in healthy adults (Frantz and Rabkin, 1965), arguing that previous hypotheses of oestrogens repressing GH secretion was wrong. Since then, the biphasic role of oestrogens on GH secretion has been acknowledged.

In the present review potential mechanisms by which oestrogens may exert their effect on longitudinal growth in children will be discussed.

Longitudinal growth in children

The growth pattern during the prepubertal years is characterized by a relatively constant rate, averaging 5–6 cm per year in both sexes. Prepubertal growth is dependent on normal GH secretion, nutritional and psychosocial factors as well as thyroid hormones. The stable prepubertal growth rate diminishes to a 'preadolescent dip' just before the onset of puberty, whereafter abrupt changes in growth occur. The pubertal onset corresponds to a bone age (biological age) of 11 years in girls, and approximately 13 years in boys. Peak height velocity (PHV) is reached at 12 years of age in girls (mean PHV 9 cm per year), and at 14 years in boys (mean PHV 10.3 cm per year) (Marshall and Tanner, 1969, 1970). The pubertal growth spurt contributes more than 15% to the final height of the individual, and more than doubles the growth rate in prepubertal children when they start sexual maturation. At the same time, epiphyseal fusion is initiated which subsequently terminates linear bone growth. When the epiphyses are closed (fused), growth cannot be further stimulated thereafter. The combination of a longer prepubertal growth period and a higher PHV in males results in their attainment of higher final height compared with females. Development of secondary sexual characteristics is dependent on adrenal androgens (adrenarche) and on gonadal steroids, i.e. testosterone from the testicular Leydig cells in males, and on ovarian oestradiol production in females. The gonadal production of steroids is stimulated by the pulsatile secretion of gonadotrophins from the pituitary. In puberty, increased secretion of LH and FSH, especially at night-time, may be responsible for the very early activation of the gonads. Consequently, increased 24-h gonadotrophin secretion is detectable before physical signs of puberty are apparent (Demir *et al*., 1996; Mitamura *et al*., 1999).

Oestrogens in healthy children

Oestradiol serum concentrations in healthy children

Early oestrogen assays were those that quantitated biological effects of oestrogens, such as vaginal cornification, vaginal metabolic activity, vaginal epithelial thickening or uterine weight. Oestradiol concentrations have been determined by conventional radioimmunoassays in healthy children in numerous studies. The first reports on oestradiol concentrations in serum from prepubertal children by radioimmunoassays were published in the 1970s, with mean concentrations ranging from 22 to 41 pmol/l, and with apparently similar concentrations in prepubertal boys and girls (Jenner *et al*., 1972; Bidlingmaier *et al*., 1973; Angsusingha *et al*., 1974; Baker *et al*., 1976; Ducharme *et al*., 1976). However, as the reported concentrations were very close to the detection limit of the assays, they may represent assay background values, and the results are therefore questionable. Moreover, it appeared that oestradiol was measurable in all prepubertal serum samples, which is likely to be due to an erroneous overestimation of the actual concentrations. Further developments have improved the sensitivities of the commercially available immunoassays, but many problems remain. For example, in a large proportion of samples from prepubertal children the oestradiol concentrations are below the detection limit of current commercially available oestradiol assays. In prepubertal boys aged 6–8 years, 85% had values below the detection limit of the assay (18 pmol/l) (Andersson *et al*., 1997), whereas 60% of prepubertal girls aged 6–8 years had undetectable oestradiol concentrations using this assay (Sehested *et al*., 2000). Especially, in cord blood and infancy such immunoassays may overestimate the concentrations of oestradiol due to cross-reaction with multiple pregnancy-associated steroids. Thus, chromatographic separation of the steroid fractions before the analysis of oestradiol seems mandatory in cord serum, neonates and infants (Figure 1).

It has been suggested that oestrogens exert biological effects at concentrations below the detection limit of conventional immunoassays; this was illustrated by the 'undetectable' oestradiol concentrations determined by a commercially available assay, in girls presenting with thelarche (breast development) (Garibaldi *et al*., 1993). In support of this hypothesis is a recent report (using an ultrasensitive oestrogen assay; see below) detailing increased oestradiol concentrations in girls with premature thelarche compared with unaffected girls (Klein *et al*., 1999). Moreover, the pubertal growth spurt precedes normal breast development in girls (Marshall and Tanner, 1969), which may suggest that increased growth occurs at even lower concentrations of circulating oestrogens compared with development of secondary sexual characteristics. The hypothesis of oestrogens being biologically active at very low concentrations cannot be confirmed by the use of conventional immunoassays, though with the advent of an ultrasensitive oestrogen assay this may now be possible.

Ultrasensitive oestrogen assay

In 1994, Klein and co-workers reported almost 100-fold lower oestradiol concentrations in prepubertal children when determined by an ultrasensitive oestrogen assay compared with previously reported concentrations. These authors reported on an ultrasensitive recombinant cell bioassay for oestrogen using a yeast strain transformed with plasmids containing the human oestrogen receptor (ER) DNA and an oestrogen response element (Klein *et al*., 1994). This assay has a detection limit of 0.07 pmol/l, and is relatively specific for oestradiol. Interestingly, by the use of this assay, significantly lower oestradiol concentrations were reported in prepubertal children (mean concentrations

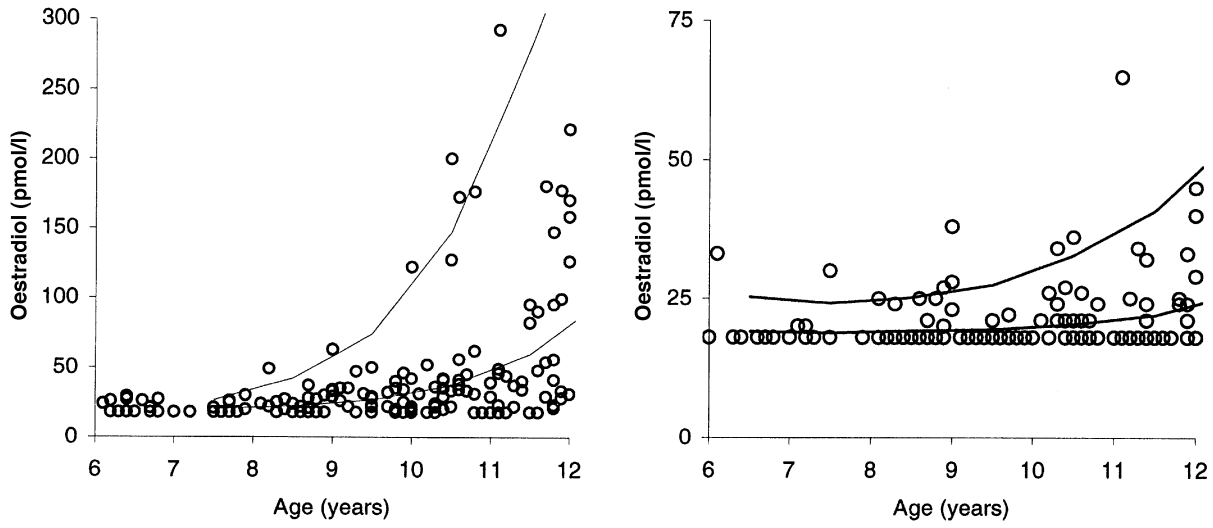

Figure 1. Serum oestradiol (E2) concentrations in healthy girls (left) and boys (right) using a commercially available immunoassay with a detection limit of 18 pmol/l. Lines represent the mean and 95% prediction interval. Note different scales on the y-axes. (Own data, redrawn from Andersson *et al.*, 1997 and Sehested *et al.*, 2000).

of 0.03 pmol/l in prepubertal boys). Moreover, significantly higher oestradiol concentrations were reported in prepubertal girls (mean 1.3 pmol/l) compared with prepubertal boys (approximately 8-fold higher) (Figure 2).

These differences have not previously been detectable using conventional immunoassays, and may well explain the earlier onset of increased growth, earlier pubertal signs and greater rate of skeletal maturation observed in girls compared with boys. Thus, use of ultrasensitive oestrogen assays may be necessary when associations between circulating oestrogens and longitudinal growth is studied.

Pulsatility of oestradiol secretion in children

Another factor which has made evaluation of the correlation between oestrogens and growth difficult, is the pulsatile nature of oestrogen secretion in healthy children. Oestradiol is secreted in a pulsatile manner, and this pulsatility is accentuated with the onset of puberty. Several authors have demonstrated the pulsatile nature of circulating oestradiol concentrations in early-pubertal girls and boys (Goji, 1993; Norjavaara *et al.*, 1996; Albertsson-Wikland *et al.*, 1997). Thus, determination of oestradiol on single blood samples may not be representative of 24-h oestrogen secretion, and thereby not representative of the potential endocrine oestrogen exposure to the growth plate.

Endogenous oestrogen and longitudinal growth in children

Several studies have studied the association between a single circulating oestradiol concentration and growth velocity in healthy children. In a study of healthy girls, oestradiol concentrations were significantly associated with growth velocity (Goji, 1993; Norjavaara *et al.*, 1996). With the use of the ultrasensitive oestrogen assay, the rise in oestradiol concentrations correlated with PHV in a longitudinal study of 23 normally growing boys who progressed through puberty (Klein *et al.*, 1996). The oestrogen concentration correlated positively with growth velocity before the time of PHV, and negatively with growth velocity after PHV. This study is consistent with the hypothesis that oestrogen at low concentrations augments skeletal growth and maturation in boys and girls, and that continued exposure to oestrogen leads to epiphyseal fusion.

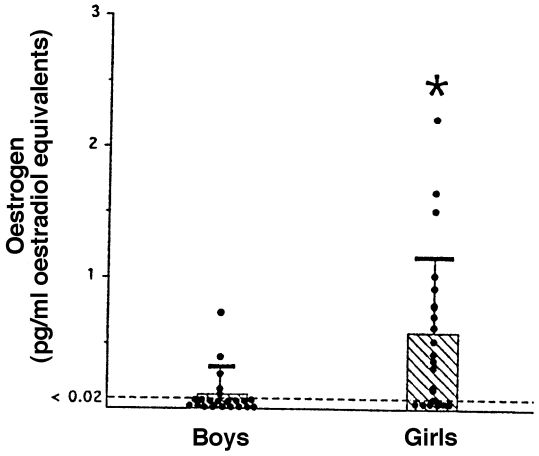

Figure 2. Serum concentrations of oestradiol equivalents in normal prepubertal boys and girls. Error bars represent SD. $*P<0.05$ versus concentrations in boys. (Figure reproduced from Klein *et al.*, 1994, with permission from American Society of Clinical Investigation.)

Thus, many of the problems from previous studies which have evaluated endogenous oestrogens in relation to growth rates may be overcome by the use of an ultrasensitive oestradiol assay and further optimized if the pulsatile nature of oestradiol secretion is also taken into account.

Direct effects of oestrogens on growth

The growth plate consists of chondrocytes which are organized in distinct layers. The chondroblast progenitor cells form the reserve

zone in the epiphysis. According to the dual effector theory (Green *et al.*, 1985), these resting chondrocytes are primed by GH, which prepares them for clonal expansion under the influence of insulin-like growth factor-I (IGF-I) (Isaksson *et al.*, 1982) and form columns in the proliferative zone. The proliferative chondrocytes are subsequently differentiated to hypertrophic chondrocytes, which secrete matrix to be mineralized. As GH and IGF-I receptors are not confined to resting chondrocytes, but are present on resting as well as on proliferative and hypertrophic chondrocytes, it is currently believed that GH and IGF-I exert effects at each stage of chondrocyte differentiation. The chondrocytes in the hypertrophic zone then undergo apoptosis, and thereafter vascularization and invasion of osteoblasts occurs which closes the growth plate (epiphyseal fusion).

Presence of ER in the human growth plate

A prerequisite for a direct oestrogen action at the level of the growth plate is the presence of ER on chondrocytes in the growth plate. Traditionally, oestrogens are thought to act exclusively through intracellular steroid receptors which act to stimulate or inhibit gene expression in target tissues at a relatively slow rate. Two distinct types of ER (ER-α and ER-β) exist, both of which stimulate gene expression by activating protein (AP)-1 sites, though with opposite effects when liganded with oestradiol. Both receptor types have been localized to the human growth plate; ER-α have been localized in all zones of the growth plate, i.e. resting, proliferative and hypertrophic chondrocytes (Kusec *et al.*, 1998), whereas ER-β were expressed in hypertrophic chondrocytes exclusively (Nilsson *et al.*, 1999).

The current understanding of how oestrogens may affect growth is as follows. Oestrogens (at low concentrations) stimulate GH secretion through binding to intracellular ER, and may subsequently stimulate chondrocyte growth in the proliferation zone, but may also potentiate clonal expansion. By contrast, high doses of oestrogens inhibit clonal expansion, and cell proliferation in the hypertrophic zone. Furthermore, high concentrations of oestrogens induce apoptosis of hypertrophic chondrocytes, and stimulate osteoblast invasion in the growth plate. The discrepant localization of the ER subtypes on chondrocytes may suggest separate roles for the ER-α and ER-β in ER-mediated effects on bone growth and maturation, although this is speculative. Furthermore, oestrogens also act through non-genomic pathways that mediate rapid effects of oestrogens in certain tissues (Moss *et al.*, 1997; Revelli *et al.*, 1998). Whether or not the rapid non-genomic actions of oestrogens play a role for longitudinal growth remains to be seen.

ER *gene polymorphism and growth*

Several polymorphisms in the *ER* gene have been demonstrated, and are related to osteoporosis, breast cancer susceptibility and hypertension in normal ageing men and in postmenopausal women (Kobayashi *et al.*, 1996; Ongphiphadhanakul *et al.*, 1998; Riggs *et al.*, 1998). Others (Lorentzon *et al.*, 1999) studied *ER* gene polymorphism in 17-year-old boys in relation to their bone mineral density. Interestingly, a significant influence of the *PvuII* genotype on height was demonstrated. These authors found that 20 boys with the *PP* genotype (homozygous) were significantly taller compared with boys with the other allelic variants (Lorentzon *et al.*, 1999). Also *ER*-α gene polymorphisms have been linked to height in adult females (Lehrer *et al.*, 1994), suggesting an impact of *ER* gene polymorphisms on height attainment in otherwise healthy individuals.

Androgen insensitivity and growth

Supporting the importance of oestrogens for the pubertal growth spurt is the fact that phenotypic (46,XY) females with complete androgen insensitivity syndrome (CAIS) have a normal (female) pubertal growth spurt despite a complete lack of androgen action. Their pubertal growth spurt is quantitatively normal, and the age of PHV was comparable with that of normal females (Zachman *et al.*, 1986).

Oestrogen-deficiency syndromes

To date, three men with tall stature due to genetic mutations that lead to lack of oestrogen action have been described, and these cases have dramatically expanded our understanding of the importance of oestrogens in the regulation of linear growth (for review, see Grumbach and Auchus, 1999). One man with a disruptive *ER*-α gene mutation was reported in 1994 (Smith *et al.*, 1994), and one year later a man with similar phenotypic appearance due to a mutation in the aromatase gene (*CYP19*) leading to undetectable oestradiol concentrations was described (Morishima *et al.*, 1995). A similar case of a tall-statured male with oestrogen deficiency due to a aromatase gene mutation was reported later (Carani *et al*., 1997).

ER *gene mutation and growth*

In 1994, a man with complete lack of oestrogen action due to a disruptive mutation in the *ER*-α gene was reported (Smith *et al.*, 1994). This man with oestrogen resistance had normal birth weight, and had experienced a normal prepubertal growth, had a normal onset of secondary sexual characteristics, bilateral axillary acanthosis nigricans, osteopenia and hyperinsulinaemia. He had high oestradiol concentrations, as well as high FSH and LH concentrations (33 IU/l and 37 IU/l respectively). Despite normal masculinization, epiphyseal fusion had not occurred and he continued to grow as an adult. He had no recollection of increased growth at the time of pubertal development, which is also apparent from his growth chart (Figure 3). At 28 years of age the man was 204 cm tall, and presented to an orthopaedic surgeon due to continued growth and progressive genu valgum. Because radiological evaluation demonstrated a bone age of 15 years, he was further evaluated. A homozygous mutation in exon 2 in the *ER*-α gene, with a substitution of thymine with cytosine resulting in a premature stop codon (TGA) instead of an arginine codon (CGA) was demonstrated, leaving him totally oestrogen-resistant (Smith *et al.*, 1994). For the first time it was possible to evaluate the role of testosterone on growth and epiphyseal fusion in the absence of oestrogen action in a male. To date, this is the only report of a patient with a disruptive mutation in the *ER*-gene.

Aromatase gene **(CYP19)** *mutations and growth*

Conversion of C19 steroids to oestrogens is catalysed by the aromatase enzyme (P450arom), which belongs to the cytochrome P450 superfamily. P450arom is encoded by the *CYP19* gene, and localized in a variety of tissues such as the ovary, testis, placenta, testis, fat tissue, in various parts of the brain as well as in bone

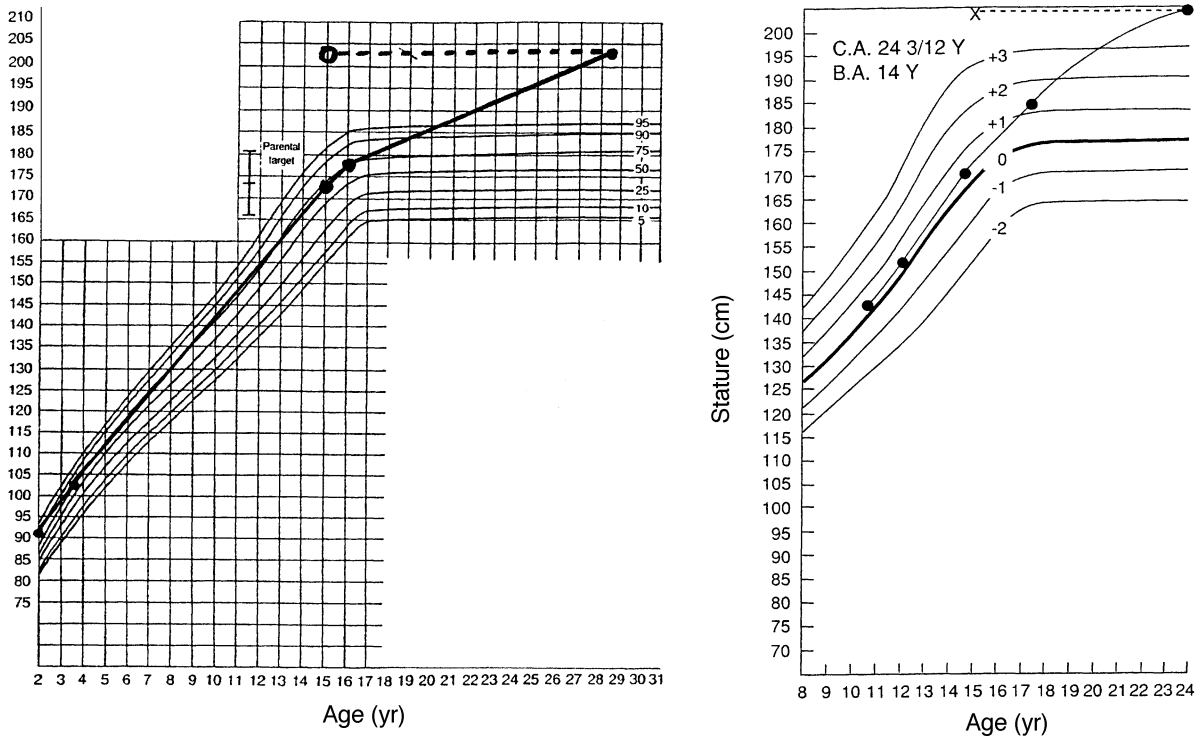

Figure 3. Growth charts from two males with oestrogen deficiency syndromes. Left: a man with a disruptive mutation in the *ER*-α gene. Right: a man with aromatase deficiency due to a mutation in the *CYP19* gene. CA = chronological age; BA = bone age. (The growth charts are adapted from data published in Smith *et al.*, 1994 and Morishima *et al.*, 1995.)

tissue. To date, nine patients with aromatase gene mutations have been described in the literature of which three are males (two adult males and one infant boy). The first patient with aromatase deficiency was described in 1994, a girl who at 14 years of age had high concentrations of androgens and low concentrations of oestrogens. She presented with no breast development, no menarche, abundant pubic and axillary hair, enlarged clitoris and short stature with no pubertal growth spurt and delayed bone age. Following oestrogen supplementation she had a remarkable growth spurt, illustrating the importance of oestrogens for the female growth spurt (Conte *et al.*, 1994).

In 1995, aromatase deficiency in male and female siblings was reported (Morishima *et al.*, 1995); these siblings were from a pedigree indicating a consanguineous marriage between the paternal great great grandfather and his second wife's sister. A point mutation [bp 1123(C→T)] in exon IX of the *CYP19* gene which results in a cysteine instead of arginine in position 375 (R375C) was demonstrated. The clinical features of the affected sister were similar to the girl described by others (Conte *et al.*, 1994). The affected brother was 24 years of age when first examined; he was 204 cm (+3.7 SD), and presented with a bone age of 14 years. His clinical characteristics are shown in Table I. As seen from Figure 3, no pubertal growth spurt was apparent, and he continued to grow in adulthood.

A similar case was reported in 1997 (Carani *et al.*, 1997) in which a patient was first evaluated at the age of 29 years due to infertility (sperm count $<10^6$/ml), and subsequently treated with human chorionic gonadotrophin (HCG) for 4 months, without effect. At the age of 31 years the patient was further evaluated because of continued linear growth, infertility and skeletal pain, especially in the knee (Table I). At the age of 38 years, he was 190 cm (>97th percentile) and his bone age had not changed (Figure 3). He was also found to have a point mutation in the *CYP19* gene [bp 1094 (G→A)]. Both these aromatase-deficient men had undetectable oestradiol concentrations, and relatively high testosterone concentrations (Table I). Administration of oestradiol resulted in fusion of the epiphyses with cessation of linear growth, and the skeletal pain stopped (Carani *et al*, 1997; Bilezkian *et al*, 1998). A recent dose-finding study suggests an appropriate substitution dose of 25 µg/day of transdermal oestradiol to maintain both bone mass and normal a oestradiol concentration in a man with aromatase deficiency (Rochira *et al.*, 2000).

Thus, men with lack of oestrogen action due to either oestrogen resistance or to lack of oestrogen production, has provided valuable insight into the physiological importance of oestrogens in male growth.

Oestrogen-excess syndromes

Precocious puberty

Children with idiopathic precocious puberty (CPP) of central origin are predominantly of the female sex. There is no apparent explanation for this difference according to gender. Children with CPP are characterized by a marked increase in growth velocity, GH secretion and IGF-I concentrations due to elevated oestradiol

Table I. Clinical and biochemical characteristics of three male patients with oestrogen deficiency syndrome due to mutations in the oestrogen receptor (*ER*) gene (oestrogen-resistant) or aromatase (*CYP19*) gene (oestrogen-deficient)

Characteristic	Oestrogen resistance (Smith *et al*., 1994)	Aromatase deficiency #1 (Morishima *et al*., 1995)	Aromatase deficiency #2 (Carani *et al*., 1997)
Mutation	Exon 2 in *ER*-α gene, bp 157 (C→T)	Exon 9 in *CYP19* gene, bp 1123 (C→T)	Exon 9 in *CYP19* gene, bp 1094 (G→A)
Age at examination (years)	28	24 3/12	38
Height (cm)	204 (+3.7 SD)	204 (+3.7 SD)	190 (>97th percentile)
Bone age (years)	15.0	14.0	14.8
Pubertal growth spurt	No	No	No
Testicular volume (ml)	20–25	8	>25
IGF-I conc. (ng/ml)	528	203	332
Testosterone conc. (ng/dl)	445	2015	523
Oestradiol (pg/ml)	119	<7	<10

concentrations (Juul *et al*., 1995). The increased growth often begins 1–2 years before physical signs of puberty. In the untreated state these children end up being very short due to early fusion of the epiphyses (Sigurjonsdottir and Hayles, 1968). However, when treated appropriately with gonadotrophin-releasing hormone (GnRH) agonists by which gonadal suppression is achieved, a significant decline in the accelerated growth is obtained and the rate of bone maturation, GH secretion and IGF-I concentrations are decreased, and as a result final height is markedly improved (Heger *et al.*, 1999). Moreover, a quantitatively normal pubertal growth spurt has been reported in patients with CPP who were also GH-deficient (Attie *et al.*, 1990), indicating a direct role of oestrogens, not mediated by GH, in the pubertal growth spurt.

Oestrogen suppression by aromatase inhibitors

Familial gonadotrophin-independent male precocious puberty (testotoxicosis) is a condition of a constitutively activating mutation in the LH receptor; this leads to continuous activation of the LH receptor, resulting in high testosterone concentrations despite low gonadotrophin concentrations. These boys experience increased growth in addition to their sexual precocity. Both blockade with an anti-androgen (spironolactone) and inhibition of aromatase activity with testolactone alone were unsatisfactory in reverting the skeletal growth to the prepubertal rate in these patients, while a combination of these two drugs was effective in restoring the growth rate (Laue *et al.*, 1989), suggesting that aromatization of the increased Leydig cell production of testosterone is important for the increased growth rate in these boys. On the other hand, it showed that the effect of testosterone on growth may not be purely oestrogenic.

Oestrogen suppression in adult males with a new potent aromatase inhibitor (Arimidex; Anastrazole, AstraZeneca, Wilmington, DE, USA) resulted in a significant reduction in circulating concentrations of IGF-I without any effect on GH secretion (Mauras *et al.*, 2000). Future studies should evaluate if the clinical use of aromatase inhibitors is valuable when trying to increase the height potential of short-statured boys by delaying epiphyseal fusion.

Aromatase excess

More than 20 years ago, the extragonadal, extra-adrenal production of oestrogen was quantified in men and women (Siiteri and MacDonald, 1973), opening the possibility of locally produced oestrogen having local actions. Aromatases are present in numerous tissues, including human bone tissue (Sasano *et al.*, 1997), and therefore local production of oestradiol from aromatized androgens within the growth plate could have important local auto- or paracrine actions on longitudinal growth.

Boys with aromatase excess syndrome, in which inappropriately high peripheral aromatase expression (and thereby increased conversion of testosterone to oestradiol) leads to feminization (gynaecomastia) are also characterized by increased longitudinal growth as well as bone maturation. In these boys, elevated circulating concentrations of oestradiol and oestrogen precursors were reported (Hemsell *et al.*, 1977; Bulun *et al.*, 1997; Sher *et al.*, 1998; Stratakis *et al.*, 1998). Boys with idiopathic gynaecomastia are generally of tall stature, and characterized by relative obesity (Sher *et al.*, 1998). As adipose tissue and muscle are important sites for extraglandular aromatase activity, it is possible that these individuals have a higher than average conversion of androgens to oestrogens, responsible for their gynaecomastia and increased linear growth. Similarly, increased growth and gynaecomastia was reported in three boys with Peutz–Jeghers syndrome due to increased aromatase expression in oestrogen-secreting testicular tumours (Coen *et al.*, 1991; Bulun *et al.*, 1993).

Indirect effects of oestrogens on growth

Oestrogens have indirect actions on growth via the entire GH-IGF axis.

Endogenous sex steroids and GH secretion

ER-α and ER-β are present in the pituitary gland, as well as in the hypothalamic preoptic area. In the pituitary gland, the ER-α predominates, whereas ER-β predominates in the preoptic area (Shughrue *et al.*, 1997).

Prepubertal girls have higher mean 24-h GH concentrations or total GH secretory rate compared with prepubertal boys in most (Costin *et al.*, 1989; Merimee *et al.*, 1991; Veldhuis *et al.*, 2000), but not all (Albertsson-Wikland *et al.*, 1994) studies, which may suggest that very low concentrations of oestrogens in prepubertal girls could stimulate GH secretion. In puberty, the pulsatile GH secretion increases 2- to 3-fold, with girls increasing 1–2 years before boys (Rose *et al.*, 1991; Albertsson-Wikland *et al.*, 1994),

which is likely to be due to stimulation of the hypothalamopituitary axis by sex steroids. In young healthy menstruating females, mean GH concentrations were higher compared with young healthy males (Ho *et al.*, 1987). Similar findings were reported in another study in which the higher mean GH concentrations in females were found to be largely due to an amplitude-specific divergence in the pulsatile mode of GH secretion (van den Berg *et al.*, 1997). Furthermore, the influence of oestradiol on integrated GH secretion is evidenced by the finding of higher GH concentrations in the late follicular phase of the menstrual cycle, when oestradiol concentrations are high compared with the early follicular and luteal phases (Faria *et al.*, 1992). In pre- and postmenopausal women, oral oestrogen administration stimulates GH secretion (Dawson-Hughes *et al.*, 1986; Weissberger *et al.*, 1991; Bellantoni *et al.*, 1996; Friend *et al.*, 1996). Not only does oestradiol affect GH secretion, but the metabolic clearance of GH also seems to be dependent on oestradiol concentrations (the half-life is positively associated with oestradiol) in men (Holl *et al.*, 1993).

Significant correlations between oestradiol, but not testosterone, and integrated GH secretion have been reported in adult males (Ho *et al.*, 1987). In peripubertal males, testosterone stimulated the pulsatile release of GH, but oxandrolone (a synthetic derivative of testosterone which does not aromatize to oestradiol) did not affect integrated GH secretion (Link *et al.*, 1986). This may be due to the dose used, or alternatively to the fact that aromatization of androgens is important for the stimulatory effect on pituitary GH release. Similarly, testosterone, but not dihydrotestosterone (DHT), stimulated the pituitary GH secretion and disorderliness of GH release (ApEn) in boys with constitutionally delayed puberty, suggesting the necessity for aromatization of androgen to modify pituitary GH output (Veldhuis *et al.*, 1997). Interestingly, therapy with testosterone as well as with DHT for 2.5 months in boys with constitutional delay in growth and adolescence, stimulated height velocity into the peak pubertal range, without an increase in GH secretion or IGF-I (Keenan *et al.*, 1993). This suggests that GH may not be the primary regulator of the increased growth in puberty.

Effect of sex steroid receptor blockade on GH secretion

Three days of treatment with flutamide (Eulexin; Schering Corp., Kenilworth, NJ, USA), a potent non-steroidal competitive inhibitor of binding of androgen to its receptor, enhanced 24-h mean serum GH concentration and enhanced GH production, without alteration in metabolic clearance of GH, in six late pubertal males. Flutamide had no effect on IGF-I concentrations, however (Metzger and Kerrigan, 1993). The stimulatory effect of flutamide on GH secretion may occur as a result of increased stimulation of ER-mediated pathways during AR blockade. Alternatively, androgens may exert a tonic inhibition of GH secretion which can be abolished by AR blockade.

Conversely, ER blockade with tamoxifen (Nolvadex; ICI Pharma, Wilmington, DE, USA) significantly diminished the endogenous GH secretion in 10 late pubertal males after 4 days of treatment (Metzger and Kerrigan, 1994) in normal adult males, as well as in testosterone-treated hypogonadal males (Weissberger and Ho, 1993). This supports the importance of oestrogens for the stimulation of endogenous pituitary GH secretion in males. Tamoxifen significantly decreased IGF-I concentrations in 68 healthy middle-aged women participating in a primary breast cancer prevention trial (Decensi *et al.*, 1999), as well as in pubertal boys (Metzger and Kerrigan, 1994) and adult men (Weissberger and Ho, 1993). Thus, ER blockade down-regulates the entire GH–IGF axis.

Effects of oestrogen administration on IGF-I concentrations

In general, it is believed that IGF-I serum concentrations are stimulated by sex steroids due to an increased GH secretion. However, direct effects of sex steroids (oestrogens in particular) on the hepatic (as well as locally produced) IGF-I secretion, which is not mediated by GH, are probably also important. The effect of oestrogen administration to healthy adult women on IGF-I is dependent on route. Several studies have demonstrated decreasing serum IGF-I concentrations after administration of oral oestrogens (despite increased integrated GH secretion), but unchanged or even increasing serum IGF-I concentrations after transdermal or intranasal administration of oestrogens (Dall'Aglio *et al.*, 1994; Bellantoni *et al.*, 1996; Raudaskoski *et al.*, 1998; Garnero *et al.*, 1999; Vestergaard *et al.*, 1999). Moreover, IGF-I generation following a single dose of GH was significantly lower in elderly females who were receiving oral oestrogen therapy compared with untreated females (Lieberman *et al.*, 1994).

As oral and dermal administration of oestrogens have similar efficacy in preventing postmenopausal bone loss, the differences cannot be attributed to different 'oestrogenicity' of the differently administered oestrogen compounds, but rather to hepatic first-pass effects of orally administered oestrogens on hepatic IGF-I production. In hypopituitary GH-deficient women, oral administration of oestrogens resulted in a significant decrease in IGF-I, insulin-like growth factor binding protein (IGFBP)-3 and acid-labile subunit (ALS), suggesting direct hepatic effects of oestrogens on IGF-I generation (Cook *et al.*, 1999; Kam *et al.*, 2000; Span *et al.*, 2000). Furthermore, a switch from oral to transdermal oestradiol therapy increased serum IGF-I concentrations in GH-substituted GH-deficient women (Cook *et al.*, 1999; Janssen *et al.*, 2000).

In summary, puberty is characterized by dynamic interactions between the GH–IGF axis and gonadal steroids. Although these hormones exert independent action on skeletal growth and development of secondary sexual characteristics, their interaction is crucial for normal growth and development. The role of androgens versus oestrogens on the pubertal stimulation of GH secretion has been studied intensively, and it is now believed that the facilitatory role of ER-mediated processes on pituitary GH secretion is of major importance. It is believed that oestrogens modulate the basal, entropic and rhythmic modalities of GH secretion, which in turn affects IGF-I secretion. In addition, direct actions on hepatic IGF-I production have been shown. Whether or not these changes in the GH-IGF axis are of importance for the pubertal growth spurt remains controversial.

Effects of oestrogen therapy on growth

Turner syndrome girls

Several drugs have been used to improve linear growth in Turner syndrome such as GH, anabolic steroids (oxandrolone; BTG Pharmaceutical, Iselin, NJ, USA) and oestrogens, as well as

various combination therapies. Initial studies of oestrogen therapy in Turner syndrome girls indicated a biphasic dose–response relationship between oestrogen and short-term ulnar growth (Ross *et al.*, 1983). Ulnar growth, as determined by a device consisting of a fixing apparatus which immobilizes the forearm in a reproducible manner, was determined 4 weeks after beginning the oestrogen treatment. The authors showed that maximal growth was stimulated at a dose of 100 ng/kg per day ethinyl oestradiol (EE) (equivalent to 4 µg EE/day in a 40 kg girl), which is well below the doses conventionally used to induce puberty. Higher doses resulted in no stimulation of ulnar growth compared with baseline. Interestingly, the stimulatory effect of the low-dose EE occurred without any significant sexual maturation or circulating IGF-I concentrations in these girls (Ross *et al.*, 1983). Using a similar low-dose EE approach in a subsequent 6-month trial of 16 Turner syndrome girls resulted in sustained significant increases in growth rate, with the majority of the girls experiencing no breast development (Ross *et al.*, 1986). In line with this finding, others (Rosenfield *et al.*, 1998) demonstrated that low-dose depot oestradiol administration to nine patients with Turner syndrome (aged 12–15 years) resulted in an increase of 5.8 cm in final height, as predicted by the Bailey-Pinneau method (Bailey and Pinneau, 1962). In this study, oestrogen was administered as intramuscular depot oestradiol, using a starting dose of 0.2 mg/month, this being increased at 6-month intervals to 0.4, 0.6 and 0.8 mg/month (Rosenfield *et al.*, 1998). However, others found that bone age advancement during 18 months of oestrogen therapy precluded any effect on final height using equally low-dose EE regimens (2–4 µg/day) compared with the initial short-term studies (Martinez *et al.*, 1987). Furthermore, addition of extremely low doses of oral EE (0.25–1.0 µg/day) to daily subcutaneous GH administration had no beneficial effect on growth velocity in 40 girls with Turner syndrome (Vanderschueren-Lodeweyckx *et al.*, 1990). Thus, controversy exists on whether or not it is possible to promote growth at an earlier age without affecting sexual maturation using a proper timing as well as low-dose oestrogen therapy in Turner syndrome girls. However, the important issue is the potential effect on final height. A recent study clearly demonstrated beneficial effects on final height in Turner syndrome girls treated with GH, when the introduction of oestrogen supplementation was postponed to 15 years of age. On the other hand, late pubertal maturation may affect the psychosocial development as well as bone mineral density of the patient, which is why earlier introduction of GH is recommended, combined with low-dose oestrogen that will bring the patient into the normal range for pubertal age (Chernausek *et al.*, 2000).

Healthy boys

In early pubertal boys constant intravenous infusions of EE were given (4, 20 or 90 µg/day) for 4 days in a cross-over study, and ulnar growth was registered over the following 3-week periods (Caruso-Nicoletti *et al.*, 1985). It was demonstrated that the low-dose EE (4 µg/day) significantly increased ulnar growth compared with the baseline situation, whereas the two higher doses did not significantly alter short-term growth rate (Figure 4).

By contrast, circulating IGF-I concentrations were not changed during the low-dose period, whereas significant elevations were reported only during intermediate or high doses of EE which failed to induce significant changes in growth rate. This speaks in favour of direct oestrogen action at the level of the growth plate (independent of circulating IGF-I), but it cannot be excluded that locally produced IGF-I may contribute to the effect of oestradiol on growth.

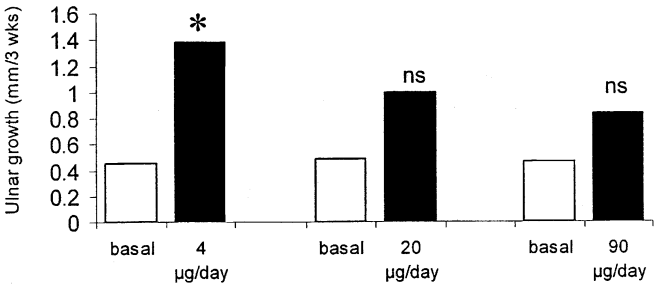

Figure 4. The effects of 4-day ethinyl oestradiol intravenous infusions at different doses on the 3-week ulnar growth in prepubertal or early pubertal boys. Results represent mean values. ns = not significant; * = significant increase, $P < 0.05$. (Figure redrawn from Caruso-Nicoletti *et al.*, 1985.)

Conclusions

In summary, data exist to support a hypothesis of direct, as well as indirect, effects of oestrogens on longitudinal bone growth:

1. Oestrogen receptors are present in the human growth plate, and polymorphism in the *ER* gene influences adult height in healthy subjects.
2. Prepubertal oestradiol concentrations are significantly higher in girls compared with boys when determined by an ultrasensitive assay, explaining differences in pubertal onset between boys and girls.
3. Men with a disruptive mutation in the *ER* gene (oestrogen resistance) or in the *CYP19* gene (aromatase deficiency) have no pubertal growth spurt, and continue to grow into adulthood due to lack of epiphyseal fusion.
4. Phenotypic females with complete androgen insensitivity syndrome have a normal female growth spurt, despite lack of androgen action.
5. ER blockade diminishes endogenous GH secretion, and AR blockade increases GH secretion in peripubertal boys, and non-aromatizable androgens (oxandrolone or DHT) have no effect on GH secretion.
6. Treatment with aromatase inhibitors reduces circulating IGF-I concentrations in healthy males, and reduces growth in boys with testotoxicosis.
7. Low-dose EE treatment (4 µg/day as constant intravenous infusion) results in elevated ulnar growth in Turner syndrome girls, as well as in healthy peripubertal boys; in contrast, high doses inhibit growth, supporting the biphasic dose-response relation between pubertal growth and oestrogens.

Thus, oestrogens are important for normal pubertal growth in boys as well as in girls. Very low concentrations of oestrogens may also stimulate bone growth without affecting sexual maturation directly at the growth plate, through stimulation of the GH–IGF axis, whereas higher concentrations of oestrogens stimulate secondary sexual characteristics and epiphyseal fusion.

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