Modulation of growth by aromatase inhibitor treatment in boys:

Efficacy and safety

by

Matti Hero

ACADEMIC DISSERTATION

To be publicly discussed, with the permission of the Medical Faculty of the University of Helsinki,
in lecture hall 3 at Meilahti hospital on 25 May 2007, at 12 noon

HELSINKI 2007
To my family
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1. SUMMARY

Without estrogen action, the fusion of the growth plates is postponed and height growth continues for an exceptionally long time. Aromatase inhibitors, blockers of estrogen biosynthesis, have therefore emerged as a new potential option for the treatment of children with short stature. The aim of the present study was to evaluate the efficacy and safety of an aromatase inhibitor, letrozole, in promoting statural growth in boys with a growth disturbance.

We investigated the efficacy of the aromatase inhibitor letrozole in the treatment of boys with idiopathic short stature (ISS) using a randomised, placebo-controlled, double-blind research setting. A total of 31 boys were initially recruited, of whom 30 completed the two-year treatment. By decreasing estrogen-mediated central negative feedback, letrozole increased gonadotrophin and testosterone secretion in pubertal boys, whereas the pubertal increase in IGF-I was inhibited. Treatment with letrozole effectively delayed bone maturation and increased predicted adult height by 5.9 cm ($P < 0.001$), while placebo had no effect on either parameter. The effect of letrozole treatment on near-final height was studied in another population, in boys with constitutional delay of puberty, who received letrozole ($n = 9$) or placebo ($n = 8$) for one year, in combination with low-dose testosterone for six months during adolescence. The mean near-final height of boys randomised to receive testosterone and letrozole was significantly greater than that of boys who received testosterone and placebo (175.8 vs. 169.1 cm, $P = 0.04$). In addition, at near-final height, the gain in height standard deviation score (SDS) over the pretreatment height SDS was greater in boys treated with testosterone and letrozole than in boys treated with testosterone and placebo (1.4 vs. 0.8 SDS, $P = 0.03$).

As regards safety, treatment effects on bone health, lipid metabolism, insulin sensitivity, and body composition were monitored in boys with ISS. During treatment, no differences in bone mass accrual were evident between the treatment groups, as evaluated by dual-energy x-ray absorptiometry (DEXA) measurements of the lumbar spine and femoral neck. Bone turnover and cortical bone growth, however, were affected by letrozole treatment. As indicated by differences in markers of bone resorption (urine aminoterminal telopeptide of type I collagen, U-INTP) and formation (serum aminoterminal propeptide of type I collagen, S-PINP, and serum alkaline phosphatase, S-ALP), the long-term rate of bone turnover was lower in letrozole-treated boys, despite their more rapid advancement in puberty. Letrozole also appeared to stimulate cortical bone growth in those who progressed in puberty: the metacarpal index (MCI), a measure of cortical bone thickness, increased more in letrozole-treated pubertal boys than in placebo-treated pubertal boys (25% vs. 9%, $P = 0.007$). The change in MCI correlated positively with the mean testosterone-to-estradiol ratio. In post-treatment radiographic evaluation of the spine, a high rate of vertebral deformities - mild anterior wedging and mild compression deformities - were found in both placebo and letrozole groups.

In pubertal boys with ISS, some risk factors for cardiovascular disease were influenced by letrozole treatment. Stimulated testosterone secretion was associated with a decrease in the percentage of fat mass and in HDL-cholesterol, while LDL-cholesterol and
triglycerides remained unchanged. Insulin sensitivity, as evaluated by HOMA-IR, was not significantly affected by the treatment.

In summary, treatment with the aromatase inhibitor letrozole effectively delayed bone maturation and increased predicted adult height in boys with ISS. Long-term follow-up data of boys with constitutional delay of puberty, treated with letrozole for one year during adolescence, suggest that the achieved gain in predicted adult height also results in increased adult height. However, until the safety of the treatment is confirmed, particularly as regards bone architecture, vertebral morphology and fertility, the treatment of short stature with aromatase inhibitors must be considered experimental.
2. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by Roman numerals I-IV:

I  Hero M, Norjavaara E, Dunkel L. Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. J Clin Endocrinol Metab. 2005 90:6396-6402.


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3. ABBREVIATIONS

ACTH  Adrenocorticotropic hormone
ALP  Alkaline phosphatase
ANOVA  Analysis of variance
ArKO  Aromatase knockout
BA  Bone age
BMAD  Bone mineral apparent density
BMC  Bone mineral content
BMD  Bone mineral density
BMI  Body mass index
CAH  Congenital adrenal hyperplasia
CDP  Constitutional delay of puberty
CV  Coefficient of variation
CYP  Cytochrome P450
DEXA  Dual-energy x-ray absorptiometry
ELISA  Enzyme-linked immunosorbent assay
ERαKO  Estrogen receptor α knockout
ERβKO  Estrogen receptor β knockout
FM  Fat mass
FMPP  Familial male-limited precocious puberty
FSH  Follicle-stimulating hormone
G  Genital stage according to Tanner
GH  Growth hormone
GHD  Growth hormone deficiency
GnRH  Gonadotropin-releasing hormone
HDL  High-density lipoprotein
HOMA-IR  Homeostasis model assessment of insulin resistance
IGF-I  Insulin-like growth-factor I
INTP  Aminoterminal telopeptide of type I collagen
ISS  Idiopathic short stature
IVA  Instant vertebral assessment
LDL  Low-density lipoprotein
LH  Luteinizing hormone
MCI  Metacarpal index
P  Pubic hair stage according to Tanner
P  Probability
PINP  Aminoterminal propeptide of type I collagen
pqCT  Peripheral quantitative computed tomography
PAH  Predicted adult height
r  Correlation coefficient
RIA  Radioimmunoassay
SD  Standard deviation
SDS  Standard deviation score
SEM  Standard error of the mean
vBMD  Volumetric bone mineral density
4. INTRODUCTION

Based on findings in men with estrogen deficiency due to disruptive mutations in the aromatase gene (1-4), and in a man with estrogen resistance due to inactive estrogen receptor (5), it has become clear that without estrogen action, growth plates remain open and height growth continues for an exceptionally long time. Consequently, it has become possible to outline and test the hypothesis that treatment with an aromatase inhibitor, a blocker of estrogen biosynthesis, may delay bone maturation, prolong the period of growth, and thereby increase final adult height.

Previous studies on the efficacy of aromatase inhibitor treatment in promoting growth have provided mixed results, with some reporting increased predicted adult height (6-8), and some no response (9-11). Many of these studies were uncontrolled, employed old aromatase inhibitors with limited potency, or used adjuvant treatments, thus making it difficult to draw firm conclusions. In a recent randomised, double-blind, placebo-controlled study of boys with constitutional delay of puberty, one year of treatment with the aromatase inhibitor letrozole effectively delayed bone maturation and increased predicted adult height by 5.1 cm (7). Thus far, no data have been reported on the efficacy of aromatase inhibition in increasing final adult height.

Estrogen receptors and the enzyme aromatase are expressed in several tissues and cell types, including osteoblasts and chondrocytes of bone, stromal cells of adipose tissue, Leydig cells and germ cells of the testes, the vasculature smooth muscle, and several areas of the hypothalamus, limbic system, and cerebral cortex of the brain. Therefore, aromatase inhibition may have unwanted effects in several tissues. Previous studies have indeed suggested that, in males, estrogen may be important in the normal accrual of bone mass, the development of bone geometrical properties, lipid metabolism, and insulin sensitivity. However, direct evidence for the significance of estrogen in the regulation of metabolism in different tissues during childhood or adolescence is currently limited to a few studies employing aromatase inhibitors (11,12). These studies have revealed that aromatase inhibitor treatment does not appear to have a significant effect on bone mass accrual, as evaluated by DEXA (11,12), though the treatment may influence lipid metabolism and insulin sensitivity (13).

Idiopathic short stature (ISS) is a diagnostic entity describing children or adolescents with short stature of unknown aetiology. In these individuals, medical treatment options have included growth hormone and the manipulation of puberty with GnRH-agonists. These treatments are costly, however, and have relatively modest efficacy in ISS. Previous studies on the use of aromatase inhibitors in this patient group are not available.

In the present study, the efficacy of a third generation aromatase inhibitor, letrozole, in increasing predicted adult height was studied in boys with ISS, using a randomised, double-blind, placebo-controlled research setting. The impact of letrozole on near-final height, a measure of final adult height, was evaluated in another population, in boys with constitutional delay of puberty, who received letrozole or placebo in combination with low-dose testosterone, for one year during adolescence. As regards
safety, the treatment effects on lipids, insulin sensitivity, bone mineral density (BMD), bone turnover, cortical bone growth, and vertebral morphology were examined in boys with ISS.
5. REVIEW OF THE LITERATURE

5.1 IDIOPATHIC SHORT STATURE

5.1.1 The diagnosis of idiopathic short stature

Historically, several different terms have been used to describe short stature of unknown etiology. In the 1950s, diagnosis of primordial dwarfism referred to short children who were small from birth or early childhood, and who had normal pubertal development (14). In the 1980s and 1990s, after the development of growth hormone (GH) stimulation tests, several new terms that referred to short children with short stature of unknown etiology and normal stimulated GH levels were introduced. These included normal variant short stature, short-normal children, non-GH-deficient short stature, and ISS (14).

Although the definition of ISS has varied in previous clinical studies, currently the following criteria appear generally accepted: Height more than two standard deviations below the mean, absence of (identified) underlying disease, normal birth size for gestational age, normal body proportions, normal nutrition, no psychiatric disorder, and a peak GH response of more than 10 ng/mL in a GH stimulation test (14,15). Thus, ISS is considered a heterogeneous diagnostic entity that permits the inclusion of conditions such as familial short stature and constitutional delay of growth and puberty. Considering the criteria of ISS, its definition may continue to evolve in the future, particularly as new molecular causes of short stature are increasingly identified (16).

5.1.2 Treatment options in idiopathic short stature

Growth hormone

Several studies have established that GH, in the short term, significantly increases growth velocity and height SDS in children with ISS (17), whereas fewer studies have followed the patients until they reached adult height. According to a meta-analysis of controlled and uncontrolled trials published between 1985 and 2000, the average gain in adult height appears to be approximately 4 to 6 cm after a mean duration of 4.7 years of GH (17). Subsequently, a randomised controlled trial (18) and a dose-response study (19) reported significant gains in adult height in GH-treated children with ISS. In the former trial, 68 children with height or predicted adult height at least 2.5 SDS below the mean were initially randomised to receive either GH 0.22 mg/kg per week administered in three weekly injections, or placebo, until they reached near-final height. Adult height measurements were available for 33 patients, who had received treatment for a mean duration of 4.4 years. In GH-treated patients, a 0.5 SDS gain in adult height over that of placebo-treated patients, and a 0.3 SDS gain over the baseline predicted adult height was observed. In the dose-response study (19), the participants were randomised to receive either GH 0.24 mg/kg per week, GH 0.24 mg/kg per week followed by 0.37 mg/kg per week, or GH 0.37 mg/kg per week from the start. Among the 50 patients who completed the follow-up until adult height, a mean height gain of 5.4 cm and 7.2 cm over the baseline...
predicted height was observed in the groups that received GH 0.24 mg/kg per week and GH 0.37 mg/kg per week, respectively, with a mean treatment duration of 6.5 years. The significant difference between outcomes in the low and high dose treatment groups suggested a dose-dependent increase in adult height.

Based on the above-mentioned studies, it has been considered that several years of GH treatment for children with ISS can, on average, produce a 4 to 7 cm increase in adult height (20,21). The safety profile of GH in children with ISS appears similar to that in other pediatric populations, and serious adverse effects appear to be rare (22). Long-term safety data, however, are currently limited. In addition, the use of GH in children with ISS is limited by the need for daily subcutaneous injections, and by the high costs of the treatment. It has been estimated that a one inch gain in adult height in children with ISS treated with GH costs approximately 35 000 US dollars (26 300 euros) (17).

Manipulation of puberty with GnRH-agonists

The physiologic acceleration of growth velocity during puberty is associated with enhanced bone maturation, which ultimately results in cessation of growth and epiphyseal fusion. In children with pathologically early puberty growth is prematurely arrested, resulting in adult height shorter than expected. When the onset of puberty is delayed by using gonadotrophin-releasing hormone (GnRH) agonist in these patients, adult height significantly improves (23). Based on these findings, it has been hypothesized that postponing puberty with a GnRH-agonist might increase adult height also in children with ISS. However, GnRH-agonist treatment alone for up to two years does not appear to significantly increase predicted adult height in those with normally timed puberty, since the achieved decrease in the rate of bone maturation is coupled with reduced growth velocity (24,25). The efficacy of longer term GnRH-agonist treatment to improve adult height was recently evaluated in a randomised clinical trial which included both children with ISS and patients with other conditions that affect growth (26). In that study, treatment with a GnRH-agonist for a mean duration of 3.5 years resulted not only in a 4.2 cm increase in adult height, but also in significantly reduced BMD of the lumbar spine, as compared with placebo-treated patients. Taken together, these studies indicate that postponing puberty for several years most probably modestly increases adult height in patients with ISS, but is associated with adverse effects, such as reduced BMD (26), slow growth velocity (24,26), and psychosocial strain caused by different timing of pubertal progression relative to peers (27).

Combination therapy with GH and GnRH-agonist appears to be associated with a somewhat higher growth velocity than GnRH-agonist alone (28,25), and a slower rate of bone maturation than GH alone (26,29), and is thus potentially more effective in promoting growth than each treatment alone. In support of this view, a recent randomised controlled trial of three years of combined treatment found that predicted adult height increased by 10.4 cm in a population of boys with either ISS or intrauterine growth retardation (30). However, uncontrolled studies, including mostly girls, have reported more divergent results (28,31,32). The efficacy of combined GH and GnRH-agonist treatment to increase
adult height is currently unclear. One study of girls with familial short stature found that the increase gained in predicted adult height after 28 months of combined treatment did not translate into greater adult height (33). Another small uncontrolled study of girls with ISS, in turn, found that the gain of 10.5 cm achieved in predicted adult height during 4.6 years of combined treatment preserved almost completely as their adult height was 10 cm greater than the pretreatment predicted adult height (34). In that study, treatment with GH alone resulted in a 6.1 cm height gain over the pretreatment predicted adult height. In conclusion, due to a limited number of controlled clinical studies with long-term follow-up, the superiority of combined treatment to either GH or GnRH-agonist alone has not been confirmed. Only one trial has evaluated the efficacy of combined treatment with GH and a GnRH-agonist to increase adult height in a population including boys with ISS. Three years of combined treatment resulted in approximately 5 cm gain in adult height, as evaluated by the difference in height gain over the pretreatment predicted adult height between the treated and untreated groups (35).

5.2 AROMATASE INHIBITORS IN THE MANAGEMENT OF GROWTH DISORDERS

5.2.1 Expression of P450 aromatase in different tissues
The aromatase enzyme, a member of the cytochrome P450 superfamily, catalyses the aromatisation of C_{19} androgens (androstenedione and testosterone) to C_{18} estrogens (estrone and estradiol). In males, the majority of circulating estradiol is produced by extragonadal tissues, and only approximately 15% is synthesised in the testes (36). Aromatase activity has been detected in various tissues and cell types in males, including osteoblasts and chondrocytes of bone, stromal cells of adipose tissue, Leydig cells and germ cells of the testes, the vasculature smooth muscle, and several areas of the hypothalamus, limbic system, and cerebral cortex of the brain (37-41). Hence, some have postulated that the effects of estrogen produced locally in the tissues in a "paracrine" or "intracrine" manner are more important than those of circulating estrogen in males (42). The regulatory region of the aromatase gene contains several distinct promoters, which are regulated in a tissue- or signaling pathway-specific manner (43). Thus, the expression of aromatase is regulated differentially in different tissues.

5.2.2 Rationale for the use of aromatase inhibitors in the treatment of short stature
After the activation of hypothalamic GnRH secretion at the onset of puberty, the increasing concentrations of sex steroids induce an acceleration in longitudinal growth, the "pubertal growth spurt", and an advancement in bone maturation with subsequent fusion of the epiphyseal growth plates and discontinuation of statural growth. The specific contributions of androgen and estrogen in the regulation of pubertal growth and bone maturation were poorly understood in males until the publication of a case report of a 28-year-old man with an inactivating mutation of the estrogen receptor α (5), and reports of men with estrogen deficiency due to inactivating mutations of the aromatase gene (1, 5)
These men shared a common phenotype characterised by exceptionally tall stature, continuing linear growth, unfused epiphyses, a bone age well below the chronological age, and osteopenia. In addition, although the data are limited, they appeared to have experienced no acceleration in growth during puberty. Estrogen treatment of men with aromatase deficiency induced a rapid closure of their growth plates and discontinued their abnormal growth (2,3). Collectively, these findings indicate that in the absence of estrogen effects, epiphyseal growth plates of the long bones remain open and linear growth continues for an exceptionally long time. In addition, they suggest that the pubertal growth spurt is induced by estrogen in males, whereas linear growth is not influenced by estrogen.

Increased tibial length and growth plate height in peripubertal male mice treated with the aromatase inhibitor letrozole further support a role for estrogen in the regulation of endochondral growth (44). In these mice, letrozole treatment appeared to upregulate the local synthesis of IGF-I in the growth plate and, in keeping with delayed bone age in men with estrogen deficiency, attenuate the final differentiation of the hypertrophic chondrocytes.

5.2.3 Classification of aromatase inhibitors

The first-generation aromatase inhibitor aminogluthethimide became available in the 1970s. However, despite its efficacy, its use was limited by toxicity and a lack of selectivity for the aromatase enzyme (45). Subsequently, second-generation (rogletimide, fadrozole, and formestane) and third-generation (vorozole, anastrozole, letrozole, and exemestane) aromatase inhibitors were introduced. The newer generation aromatase inhibitors are highly selective, and inhibit in vivo aromatisation by approximately 98% (46).

Aromatase inhibitors are also classified as steroidal (formestane, exemestane) and nonsteroidal (aminogluthethimide, rogletimide, fadrozole, vorozole, anastrozole, and letrozole) compounds based on their mechanism of action (46). Steroidal aromatase inhibitors bind irreversibly to the aromatase enzyme, causing permanent aromatase inactivation even after the drug is cleared from the circulation. In contrast, nonsteroidal aromatase inhibitors competitively and reversibly inhibit the conversion of androgens to estrogens.

5.2.4 Pharmacology of the aromatase inhibitor letrozole

Letrozole (Femara®) is a potent and selective nonsteroidal third-generation aromatase inhibitor officially indicated for the treatment of breast cancer in postmenopausal women. It competitively inhibits the conversion androgens to estrogens, specifically the conversion of androstenedione to estrone and testosterone to estradiol. In healthy postmenopausal women, letrozole is rapidly and completely absorbed after oral administration, and the terminal half-life of the compound is approximately 42 hours (47). Letrozole is mainly eliminated via the metabolism by the cytochrome P-450 enzymes CYP3A4 and CYP2A6 into an inactive carbinol metabolite, and excreted via the kidneys (48). Steady state concentrations of letrozole are achieved after two to six weeks of oral daily dosing (49).
suggesting that continuous accumulation of the drug does not occur. No pharmacokinetic studies of letrozole in children have been published.

The efficacy and selectivity of letrozole has been well documented in adult women. According to a study of postmenopausal breast cancer patients, in vivo total body aromatisation is suppressed by more than 99.1% after six weeks of treatment with letrozole (50). Even though letrozole is highly specific, the slight but significant reduction in the ACTH-stimulated mean peak cortisol level after the start of letrozole in postmenopausal breast cancer patients suggests a small effect on adrenal steroidogenesis (51). This is unlikely to have clinical significance.

5.2.5 Previous experience with aromatase inhibitors

Findings in men with estrogen resistance or deficiency suggested that it might be possible to delay bone maturation, prolong the period of growth, and increase adult height in children with short stature or compromised adult height prognosis by inhibiting the peripheral conversion of androgens to estrogens with an aromatase inhibitor (52). To date, several studies using testolactone, fadrozole, anastrozole, or letrozole have reported on the efficacy of aromatase inhibitors in delaying bone maturation and improving predicted adult height in various disorders affecting growth (Table 1). The published data is limited by a shortage of placebo-controlled clinical trials, multiple adjuvant treatments used, and short treatment duration in many of the studies. Overall, these studies suggest that the treatment response may depend on the condition treated, the aromatase inhibitor used, and the treatment duration. In an uncontrolled study of boys with familial male-limited precocious puberty (FMPP), long-term treatment with testolactone, a first-generation aromatase inhibitor, significantly improved predicted adult height (6). In contrast, the first- and second-generation aromatase inhibitors testolactone and fadrozole have not significantly improved predicted adult height in girls with McCune-Albright syndrome (9,10). A two-year treatment with the aromatase inhibitor testolactone combined with the antiandrogen flutamide, in turn, delayed bone maturation in a population of boys and girls with congenital adrenal hyperplasia, but failed to improve their predicted adult height (53).

Two studies employing the more potent and selective third-generation aromatase inhibitor letrozole have reported more beneficial findings as regards predicted adult height. In a double-blind, randomised, and controlled study conducted with 23 boys with constitutional delay of puberty, the boys received low-dose testosterone for 6 months in combination with letrozole, or placebo, for 12 months (7). Letrozole effectively inhibited estrogen biosynthesis, while estrogen levels increased in those who received placebo. Evaluated 18 months after the onset of treatment, bone age advancement was slower in the letrozole-treated boys than in the placebo-treated boys (0.9 vs. 1.7 years, respectively, P < 0.05). Growth velocity was similar in the two groups, except for the first five months during which the placebo-treated boys grew faster. Predicted adult height increased by 5.1 cm in the letrozole-treated boys and remained unchanged in the placebo-treated subjects (P < 0.05). Comparable findings were reported in a retrospective uncontrolled study of letrozole treatment in a population of adolescent males with various growth disorders (8).
Treatment with letrozole for a mean duration of 12 months in this population resulted in a significant deceleration in bone age progression (Δbone age/Δcalendar age 1.5 vs. 0.7 pretreatment and during treatment, respectively, \( P < 0.001 \)), unchanged growth velocity, and a 5.5 cm increase (\( P < 0.001 \)) in predicted adult height.

Only one study has assessed the efficacy of another potent and selective third-generation aromatase inhibitor, anastrozole, in promoting growth \(^{11}\). In that study, a one-year treatment of GH-deficient boys with anastrozole and GH did not decrease bone age progression or improve predicted adult height when compared with GH-treatment alone. However, when the same population was evaluated after two and three years of treatment, predicted adult height had increased by 4.4 and 7.4 cm, respectively, in those who received anastrozole and GH, with no change in those who received GH alone \(^{54}\).

Table 1. Previous studies with aromatase inhibitors in children with disorders affecting growth.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Study design</th>
<th>Compound</th>
<th>Adjuvant treatment</th>
<th>Efficacy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMPP</td>
<td>Uncontrolled clinical trial</td>
<td>Testolactone</td>
<td>Spironolactone, GnRHa</td>
<td>No change in PAH after 2-4.2 years of treatment; 12.9 cm increase in PAH after 6 years of treatment</td>
<td>Laue et al 1989(^{55}), Laue et al 1993(^{56}), Leschek et al 1999(^{57})</td>
</tr>
<tr>
<td></td>
<td>Two case reports</td>
<td>Anastrozole</td>
<td>Bicalutamide</td>
<td>Decrease in BA progression, increase in PAH</td>
<td>Kreher et al 2006(^{57})</td>
</tr>
<tr>
<td>McCune-Albright syndrome</td>
<td>Uncontrolled clinical trial</td>
<td>Testolactone</td>
<td>GnRHa</td>
<td>Decrease in BA progression, no change in PAH</td>
<td>Feuillan et al 1986(^{58}), Feuillan et al 1993(^{59})</td>
</tr>
<tr>
<td></td>
<td>Uncontrolled clinical trial</td>
<td>Fadrozole</td>
<td>GnRHa</td>
<td>No change in BA progression or PAH</td>
<td>Nunez et al 2003(^{60})</td>
</tr>
<tr>
<td>CAH</td>
<td>Randomised clinical trial</td>
<td>Testolactone</td>
<td>Flutamide, hydrocortisone, fludrocortisone, GnRHa</td>
<td>Decrease in BA progression, no change in PAH</td>
<td>Laue et al 1996(^{59}), Merke et al 2000(^{61})</td>
</tr>
<tr>
<td>CDP</td>
<td>Double-blind, placebo-controlled randomised trial</td>
<td>Letrozole</td>
<td>Testosterone</td>
<td>Decrease in BA progression, 5.1 cm increase in PAH</td>
<td>Wickman et al 2001(^{7})</td>
</tr>
<tr>
<td>Mixed population of short adolescent boys</td>
<td>Retrospective uncontrolled study</td>
<td>Letrozole</td>
<td>None</td>
<td>Decrease in BA progression, 5.5 cm increase in PAH</td>
<td>Karmazin et al 2005(^{59})</td>
</tr>
<tr>
<td>GHD</td>
<td>Open label controlled trial</td>
<td>Anastrozole</td>
<td>GH</td>
<td>No change in BA progression or PAH after 1 year; increase in PAH after 3 years of treatment</td>
<td>Mauras et al 2004(^{11}), Mauras et al 2006(^{54})</td>
</tr>
</tbody>
</table>

FMPP, familial male-limited precocious puberty; GnRHa, gonadotropin releasing hormone agonist; PAH, predicted adult height; BA, bone age; CAH, congenital adrenal hyperplasia; CDP, constitutional delay of puberty; GHD, growth hormone deficiency; GH, growth hormone.
5.3. SAFETY OF AROMATASE INHIBITION IN MALES

5.3.1 Sex steroids and bone strength

Strength of a bone to resist mechanical loading is largely determined by its size and density (60). In addition, in sites with predominantly trabecular bone, such as the vertebral bodies, the microarchitecture of the trabeculae influences bone strength (60). Thus, large and dense bones, as well as trabecular bone with numerous thick trabeculae with good connectivity, are strong. Since the longitudinal growth of long bones (endochondral ossification), the growth of bones in width (bone modeling), and the maintenance of bone (bone remodeling) are all influenced by sex steroids (61), the modulation of sex hormone production during adolescence may impact bone quantity and quality.

Bone mineralisation

Throughout life, a continuous turnover of calcified material occurs in bones. This bone remodeling results from the coupled action of bone resorbing osteoclasts and bone forming osteoblasts within the basic multicellular units. An imbalance between net bone formation and bone resorption in favour of the latter leads to bone loss and bone fragility. Sex steroids are known to influence bone remodeling in males, although the relative roles of androgens and estrogens are not completely clear (62). Several lines of evidence suggest that androgens directly increase bone mass in men. Hypogonadism in men is associated with high bone turnover and osteopenia, which can be reversed with androgen treatment, although no studies have been performed on nonaromatisable androgens (63). Furthermore, genetic males with androgen insensitivity syndrome due to mutations in the androgen receptor exhibit decreased areal and calculated volumetric BMD, as assessed by DEXA, despite estrogen sufficiency (64,65). In addition, testosterone treatment similarly prevents bone loss induced by orchidectomy in wild type and estrogen receptor α knockout (ERαKO) male mice (66,67). Taken together, these findings suggest that aromatisation is not a necessary requisite for the bone preserving effects of androgen.

Despite the role of testosterone as the dominant circulating sex hormone, estrogen action appears to be important in the accrual or maintenance of bone mass in males, or both. In line with this, observational studies in elderly men have found that serum estrogen levels are associated with DEXA-assessed BMD (68-72) and the rate of bone loss (73). Furthermore, men with congenital estrogen deficiency (1,2,74,75) all had reduced areal (1,2,74,75) or volumetric (4) BMD despite androgen sufficiency, as evaluated by DEXA or peripheral quantitative computed tomography (pqCT), respectively. BMD was unaffected by testosterone treatment in one of the men (74), while estrogen treatment substantially improved DEXA-assessed areal BMD in most cases (1,2,75). Direct volumetric BMD measurements using pqCT before and after estrogen treatment have provided mixed results. In one man with aromatase deficiency, an increase in volumetric BMD was evident (4), whereas in a 17-year-old boy with the same condition, no change occurred (75).
Chemical castration of male-to-female transsexuals and subsequent treatment with high-dose estrogen provides an opposite model for studying the effects of estrogen on BMD. In a retrospective analysis of 40 middle-aged transsexuals, such treatment depleted testosterone production, substantially increased estradiol levels, and significantly increased areal BMD of the lumbar spine and femoral neck (76).

The skeletal findings in aromatase knockout (ArKO) male mice correspond to findings in estrogen-deficient men. As indicated by radiological and direct histomorphometric measurements, male ArKO mice exhibit reduced BMD, particularly at trabecular sites (77). Similar findings were evident when the skeletal effects of the aromatase inhibitor vorozole in growing male rats were evaluated by means of pqCT of the femur (78). In comparison with control mice, vorozole treatment reduced volumetric trabecular BMD, while volumetric cortical BMD was unaffected by the treatment. This impairment of BMD associated with estrogen deficiency appears to be primarily mediated by a lack of ERα activity, since both ERαKO and ArKO male mice show similarly reduced bone mass, while ERβKO mice have no skeletal abnormalities (79). Limited evidence from observational studies support the view that serum estrogen concentration in the lower end of a normal range is associated with decreased volumetric BMD, at least in elderly men (80).

In addition to reduced BMD, the men with estrogen deficiency or resistance had elevated markers of bone turnover (1,2,4,5,74,75), suggesting that estrogen may preserve bone mass by controlling the rate of bone remodeling. In support of this view, a nine-week treatment of elderly eugonadal men with the aromatase inhibitor anastrozole decreased estradiol concentrations by 29% and increased markers of bone resorption and reduced those of bone formation, despite a simultaneous increase in testosterone secretion (81). In another approach, elderly men were rendered temporarily hypogonadal for three weeks with GnRH-agonist treatment, and the sex hormone deficiencies were selectively replaced by using estradiol and testosterone patches in combination with an aromatase inhibitor (82). The study showed that the increases in bone resorption markers induced by hypogonadism were almost completely inhibited by estrogen treatment, but only partially inhibited by selective testosterone replacement. In a more recent study of younger, healthy men, an almost similar pharmacological intervention was employed to induce deficiency of both sex hormones, selective deficiency of estrogen, and sufficiency of both estrogen and testosterone for a 12-week period (83). In that study, androgen sufficiency in the presence of estrogen deficiency partially prevented increases in bone resorption markers after the onset of GnRH-agonist treatment, suggesting a direct anti-resorptive role for androgens as well. In these studies, estrogen alone (82) as well as androgen alone (83) were able to reverse the effect of hypogonadism on markers of bone formation. Thus, in adult men, estrogen may be more important than androgen in controlling bone resorption, while both sex hormones participate in the maintenance of bone formation. In vitro and animal studies suggest that potential mechanisms by which estrogen affects bone resorption include the suppression of osteoclastogenesis (84,85) and the promotion of osteoclast apoptosis (86). Androgens, in turn, have been shown to stimulate the proliferation and differentiation of human osteoblasts (87) and inhibit osteoclast formation (88).
While estrogen deficiency is apparently associated with decreased bone mass in adult men, it is unclear whether estrogen deficiency impairs the achievement of peak bone mass or the maintenance of bone mass during maturity. In most cross-sectional observational studies of healthy males at the age of attainment of peak bone mass, DEXA-assessed areal BMD has not correlated with circulating sex hormone concentrations (88,89), whereas in a recent large study, a positive correlation was found between free testosterone and areal BMD of the spine and femur (90). Instead, when pQCT was employed, volumetric BMD of cortical bone showed a weak positive correlation to free circulating estradiol, while no correlations between free testosterone and volumetric BMD, or between sex hormones and trabecular BMD were found in the same group of boys (90). Thus, within the physiological concentration, limited observational data suggest that estrogen may have a weak positive influence on the gain in cortical BMD in young adult males.

Interventional studies with aromatase inhibitors in pubertal males have not confirmed the crucial role of estrogen in bone homeostasis suggested by adult and animal studies. When calcium kinetics were analysed in late pubertal and young adult males before and after a short-term treatment with the aromatase inhibitor anastrozole, no evident changes in bone calcium deposition or resorption occurred (91). More recently, the effects of a one-year treatment with anastrozole in combination with growth hormone were studied in a group of GH-deficient adolescent boys (11). In comparison with GH alone, no differences in DEXA-assessed BMD of the whole body or lumbar spine, or in serum markers of bone formation were reported, despite significant reductions in serum estradiol levels in those receiving anastrozole. Furthermore, when boys with delayed puberty were treated with low-dose testosterone for 6 months, in combination with the aromatase inhibitor letrozole or placebo for 12 months, no significant differences were noted between the treatment groups in BMD of the lumbar spine or femoral neck, as assessed by DEXA (12). However, an increase in calculated volumetric BMD of the lumbar spine was observed in boys treated with testosterone plus placebo 12 months after the beginning of the treatment, whereas in boys receiving testosterone plus letrozole, volumetric BMD increased only 6 months after the cessation of treatments.

In summary, published data indicate that the lack of estrogen effects mediated through the ERα is associated with impaired accrual or maintenance of bone mass in males. The bone-preserving effects of estrogen appear to relate to its anti-resorptive influence on bone. The limited data available, however, suggest that estrogen suppression, at least when accompanied by stimulated testosterone secretion, has no major harmful influence on BMD in adolescent boys. Also androgens appear to directly stimulate bone mineralisation. Previous studies in humans have specifically studied neither BMD in different compartments of bone nor bone geometrical properties during modulation of the sex hormone environment.

Bone geometric properties

Bone structural properties, such as bone size, cortical bone thickness, and the quality of the trabecular network influence bone strength independently of BMD (60,92,93). A well
characterised sex difference in bone geometry in favour of men develops during puberty, when the thickness of cortical bone increases through stimulated periosteal apposition in males and stimulated endocortical apposition in females (94). As a result, the long bones of adult men are wider than those of adult women, with slightly thicker cortices placed further away from the neutral axis of the bone (94). Based on animal studies, and on the difference in exposure to sex hormones between men and women, high exposure to androgen may stimulate periosteal bone formation, whereas high exposure to estrogen may inhibit periosteal bone formation and stimulate endocortical growth (95). In keeping with this, in healthy adolescent males, free estradiol was found to be an independent negative predictor and free testosterone a positive predictor of cortical bone size, as evaluated by pqCT in a large cross-sectional study (90). However, no prospective reports on the effects of sex hormone treatment, or sex hormone modulative treatment, on bone geometry in adolescent males are currently available. In addition, recent findings in a 17-year-old aromatase deficient boy with androgen sufficiency suggest a more complicated regulation of bone expansion by sex steroids. In this boy, estrogen treatment increased bone cross-sectional area and cortical thickness, but not volumetric BMD, as evaluated by pqCT (75). This suggests that, in males during adolescence, a threshold-level of estrogen is needed for androgen-induced bone expansion to occur. Limited evidence from an animal study suggests that the anabolic effect of estrogen on bone may be mediated partly by the IGF-I receptor (96). Sex steroids influence bone mass and architecture at least partly by modulating the osteogenic response of bone to mechanical loading (97,98). Strain imposed by mechanical loading on bone stimulates the proliferation of osteoblast-like cells (99), and increases bone mass and cortical thickness (100). As suggested by findings in mice lacking functional ERα, this anabolic response of bone to strain may require a certain level of ERα activity in bone tissue (99). Androgens, in turn, may increase the strain imposed on bone by increasing muscle mass, and may thereby indirectly stimulate bone mass accumulation and bone expansion.

Little is known about the specific effects of testosterone or estradiol on trabecular bone structure in males. According to a community-based cross-sectional study of adult men, no correlation between trabecular parameters and circulating sex steroid levels were found in younger males, whereas in older males, circulating bioavailable testosterone correlated positively with trabecular thickness, and bioavailable estradiol correlated with both trabecular thickness and trabecular number (80). Thus, both sex steroids may participate in the maintenance of trabecular bone microstructure, at least in aging males.

5.3.2 Sex steroids and lipid metabolism
Adult men in general have lower concentrations of HDL-cholesterol and higher concentrations of total cholesterol and LDL-cholesterol than do age-matched women. This relatively proatherogenic lipid profile in men potentially explains their increased risk of cardiovascular disease in comparison to premenopausal women. The gender difference in
lipid profile develops during puberty, and has been attributed to differences in sex hormone concentrations (101).

Lipids and male puberty

Observational studies have demonstrated that, in boys during puberty, the level of HDL-cholesterol decreases whereas those of triglycerides and, during late puberty, LDL-cholesterol slightly increase (102-106). The level of HDL-cholesterol decreases in an inverse relationship with serum testosterone (106-108) and follows both spontaneous progression of puberty and induction of puberty by exogenous testosterone treatment (109). The close inverse relationship between serum testosterone and HDL-cholesterol level during exogenous testosterone administration, and the dose-dependent effect of testosterone on HDL-cholesterol suggest a causal relationship (109). A potential mechanism by which increasing androgen concentrations decrease HDL-cholesterol is increased catabolism of HDL-cholesterol through stimulation of the hepatic lipase (110).

Less is known about the regulation of serum triglyceride, LDL-cholesterol, and lipoprotein (a) concentrations during puberty. While the level of serum testosterone has not correlated with serum triglycerides or LDL-cholesterol in most studies, some have found a favourable negative correlation of serum estradiol to triglycerides and LDL-cholesterol, suggesting a role for estrogen (106,111). However, concomitant to advancing pubertal maturation, a relative increase in lean body mass and a decrease in percentage body fat occurs in adolescent males (112). This change in body composition probably reflects the increasing influence of androgens (113) and appears to contribute to the regulation of lipid metabolism during male puberty: BMI and central obesity are positively associated with LDL-cholesterol and triglycerides, and negatively with HDL-cholesterol in adolescent boys (106,111,114). In accordance with this concept, weight reduction has been shown to improve lipid profiles in obese children (115). The impact of puberty on lipoprotein (a) concentration is poorly characterised in males. According to a cross-sectional study, males have slightly lower concentrations of lipoprotein (a) than do females during adolescence (116). Levels of lipoprotein (a), however, vary tremendously among individuals, and more depend on genetic factors, particularly on apoliprotein (a) size, than on hormonal factors (117).

The role of sex steroids in the regulation of lipid metabolism appears to change after the completion of puberty, since findings in observational studies of healthy adult males suggest a more favourable effect of testosterone on the lipid profile. In contrast to adolescent boys, high endogenous testosterone concentrations in adult males are associated with high HDL-cholesterol, low LDL-cholesterol, and low triglyceride concentrations, whereas no consistent associations have been found between circulating estradiol and lipoproteins (118-123).

By design, observational studies are not ideal for studying the relative contributions of androgens and estrogens to the regulation of lipid metabolism, since the circulating levels of testosterone and estradiol are closely correlated in males during puberty (111). Moreover, circulating levels of sex hormones may inaccurately reflect the
hormonal status at the tissue level due to local aromatisation of androgenic precursors \(^{(42)}\).

**Models of sex steroid deficiency**

Findings in men with congenital estrogen deficiency due to defective aromatase enzyme \(^{(1\text{-}4,74)}\), or with estrogen resistance caused by defective ER\(\alpha\) \(^{(5)}\), have provided new insight into sex steroid regulation of lipid metabolism in males. These men shared a common phenotype of truncal obesity, and most of them had impaired lipid profiles characterised by low HDL-cholesterol, elevated LDL-cholesterol, and elevated triglyceride levels \(^{(2\text{-}4,74)}\). The causal relationship between estrogen deficiency and impaired lipid metabolism is supported by the improvement of lipid abnormalities in most men with aromatase deficiency after the beginning of estrogen treatment \(^{(2\text{-}4,74)}\). However, an elevated testosterone or testosterone-to-estradiol ratio may at least partially explain impairments in the lipid profile in these men. Accordingly, improvements in the lipid profile after the beginning of estrogen treatment may have been induced by reduced testosterone secretion or normalisation of the testosterone-to-estradiol ratio \(^{(3,4)}\). The proatherogenic influence of congenital estrogen deficiency and estrogen resistance was recently confirmed by evidence of early atherosclerosis in two affected men \(^{(74,124)}\).

The phenotype of ArKO male mice with estrogen deficiency due to disrupted aromatase gene is in many respects similar to that of aromatase-deficient men: They exhibit elevated gonadotrophin and testosterone levels, loss of bone mass, and they accumulate excessive intra-abdominal adipose tissue gradually during sexual maturation \(^{(125)}\). In addition, ArKO male mice develop hepatic steatosis which, along with obesity, can be reversed by estrogen treatment \(^{(126)}\). The lipid profile of ArKO male mice, however, differs from that of aromatase-deficient men, since these mice exhibit elevated concentrations of HDL-cholesterol \(^{(127)}\). Despite the differences in lipids, findings in LDL-receptor-deficient male mice treated with an aromatase inhibitor \(^{(40)}\) and the limited evidence available from human males with congenital estrogen deficiency \(^{(74,124)}\) suggest that lack of estrogen effects may predispose males to early atherosclerosis. In male mice, this protective effect of physiological estrogen may be attributable to direct effects in the vascular wall rather than to effects on lipid metabolism \(^{(40)}\).

**Modulation of sex steroid levels and lipid metabolism**

In adult and aging males, hypogonadism is associated with adverse lipid profile, including low HDL-cholesterol, high LDL-cholesterol, and high triglyceride levels \(^{(128)}\). Correction of the hypogonadal state with transdermal or intramuscular aromatisable androgen either has no effect on the lipid profile, or may result in a modest decrease in both HDL-cholesterol and LDL-cholesterol, when standard doses are used \(^{(129)}\). Conversely, administration of supraphysiological doses of aromatisable exogenous androgen to adult men induces a dose-dependent decrease in HDL-cholesterol, leaving LDL-cholesterol and triglycerides unaffected \(^{(130)}\). As suggested by a study using an aromatase inhibitor to prevent the corresponding increase in estradiol levels, this HDL-reducing influence of high androgen concentrations is mediated by direct androgen effects \(^{(131)}\). In line with this,
(peroral) treatment of adult men with high doses of nonaromatisable androgen results in a substantial reduction in the HDL-cholesterol level, particularly in the HDL2-cholesterol subclass, and an increase in LDL-cholesterol \(^{(132,133)}\). However, a certain physiological level of estradiol may also be required for the maintenance of normal HDL-cholesterol level in males \(^{(134)}\). Thus, androgens decrease and estrogens may increase the level of HDL-cholesterol in males. Sex hormones probably regulate HDL-cholesterol by influencing its catabolism, since androgens stimulate hepatic lipase and estrogens inhibit the activity of this enzyme \(^{(110,131,135)}\).

Only a few studies have addressed the influence of aromatase inhibitor treatment on lipid metabolism in adolescent boys. In a recent study of boys with constitutional delay of puberty, treatment with low-dose testosterone in combination with letrozole reduced HDL-cholesterol, whereas treatment with testosterone and placebo had no effect on HDL-cholesterol, LDL-cholesterol, or triglycerides \(^{(13)}\). The concentrations of testosterone rose to clearly supraphysiological levels in letrozole-treated boys, and an inverse relationship between the treatment-induced changes in testosterone and HDL-cholesterol was observed \(^{(13)}\). Of note, despite high concentrations of testosterone and low concentrations of estradiol in letrozole-treated boys, LDL-cholesterol and triglycerides remained at the pretreatment level, arguing against significant sex hormone-related regulation of these lipids in males during adolescence. Studies with another third-generation aromatase inhibitor, anastrozole, have reported more favourable effects on lipid profiles in adolescent males. The treatment of healthy \(^{(91)}\) or GH-deficient \(^{(11)}\) adolescent boys with anastrozole does not appear to significantly influence HDL-cholesterol, LDL-cholesterol, or triglyceride levels.

The sex hormonal regulation of lipoprotein (a) differs from that of other lipoproteins, since both testosterone and estrogen appear to decrease lipoprotein (a) concentrations \(^{(136)}\). The oral administration of a nonaromatisable androgen, stanozolol, to postmenopausal women with osteoporosis \(^{(137)}\) as well as the parenteral treatment of healthy adult men with an aromatisable androgen \(^{(138)}\) reduced lipoprotein (a) concentrations. Conversely, orchidectomy increased lipoprotein (a) levels by approximately 20% in men with prostatic cancer \(^{(138)}\). A previous study with an aromatase inhibitor supports a dominating role for a direct androgen effect in the sex hormonal regulation of lipoprotein (a) in males; in normal men, administration of testosterone alone resulted in a mean reduction of 37% in lipoprotein (a), while a combination of testosterone and the aromatase inhibitor testolactone reduced the level of lipoprotein (a) by 28% \(^{(139)}\). While the lowering effect of oral estrogen on lipoprotein (a) is well established in postmenopausal women \(^{(140,141)}\), data on the effects of estrogen treatment in males are limited and diverging. Administration of estrogen orally and intramuscularly reduced concentrations of lipoprotein (a) considerably in men with prostatic carcinoma during a six-month follow-up \(^{(142)}\), while another study found that parenteral treatment with estrogen induced no changes in lipoprotein (a) levels in a corresponding group of patients \(^{(138)}\). Currently, no data from interventional studies are available on the effects of androgens or estrogens on lipoprotein (a) in males during puberty.
5.3.3. Sex steroids and insulin sensitivity

Insulin resistance of puberty

Puberty is associated with a state of physiological insulin resistance, which appears to be slightly more pronounced in females than in males (143). Following an otherwise similar pattern in boys and girls, insulin resistance of puberty begins in the early stages of puberty and resolves by the completion of pubertal development (143,144). Insulin resistance of puberty is restricted to peripheral glucose metabolism with relative sparing of amino acid metabolism, and may thus serve to facilitate protein anabolism during this period of rapid growth (145). Although sex hormone concentrations increase simultaneously with the developing insulin resistance, a direct causal relationship between the two appears unlikely; sex hormone concentrations remain elevated while the insulin resistance resolves after puberty, and concentrations of estradiol or testosterone have not been shown to associate with markers of insulin resistance in most studies (144,146). Limited evidence from interventional studies further argues against the significant direct regulatory role of androgens or estrogens, at least in males. In adolescent boys with delayed puberty, neither treatment with low-dose testosterone (147) nor with nonaromatisable dihydrotestosterone (148) influenced insulin sensitivity, as analysed by the hyperinsulinemic, euglycemic clamp procedure. However, when biosynthesis of estrogen was inhibited with an aromatase inhibitor during testosterone treatment in a respective group of adolescent boys, a reduction in fasting insulin levels occurred despite substantial increases in testosterone concentrations (13). Interestingly, in that study, the treatment-induced change in fasting insulin correlated positively with the change in IGF-I, while no correlations were observed between insulin and testosterone, or between insulin and estradiol. Together these data suggest that in males during puberty, androgens do not directly contribute to the regulation of insulin sensitivity while estrogens may play a role through stimulation of the GH-IGF-I axis (149).

A substantial body of evidence suggests an important role for the GH-IGF-I system in the development of insulin resistance during puberty. The anti-insulin action of GH is well documented (150), while IGF-I, the levels of which are primarily regulated by GH (151), has insulin-like properties (152). In correspondence with changes in insulin sensitivity, the levels of GH and IGF-I increase during puberty and decrease at the end of puberty in both sexes (153,154). In particular, a close association between the rise and fall in IGF-I level and the rise and fall in insulin resistance, as analysed by euglycemic hyperinsulinemic clamp studies, exists during puberty in boys and girls (154). Several cross-sectional studies have found that measures of insulin sensitivity correlate negatively with GH secretory status during puberty in males (155-158).

A profound change in body composition occurs in males during puberty: lean body mass rapidly increases and the amount of fat mass in relation to total body mass decreases (112). This presumably has a beneficial impact on insulin sensitivity, since measures of obesity such as BMI (143,158), total fat mass as estimated by underwater weighing (146), and particularly the amount of visceral and intramyocellular fat (159,160) are negatively associated with insulin sensitivity in males during puberty. Therefore, the
change in body composition hardly explains the developing insulin resistance in males. In line with this, a cross-sectional study showed a similar negative relationship between BMI and insulin sensitivity in boys during prepuberty and at each stage of puberty (143). Furthermore, the only available longitudinal follow-up study with a limited number of male patients reported that the fall in insulin sensitivity during pubertal maturation was not associated with changes in total body or visceral fat, as assessed by DEXA (144).

Adiponectin is an adipocytokine exclusively produced by the adipose tissue and possesses antidiabetic properties (161). Its concentrations are low in obese and high in lean adolescents (162), and the levels increase after weight reduction (163), indicating that the degree of obesity, particularly in the visceral compartment (160), is closely linked to adiponectin secretion. Low levels of adiponectin are associated with both peripheral and hepatic insulin resistance in adolescents, even after controlling for obesity (162), which supports a protective role for adiponectin as regards insulin sensitivity. During puberty, the levels of adiponectin decrease in an inverse relationship with serum testosterone in males while the levels of females remain unchanged, resulting in a gender difference in adiponectin concentrations by completion of puberty (164). Indeed, as indicated by a cross-sectional study of 200 non-obese boys, the stage of puberty and testosterone level appear to be even stronger determinants of adiponectin level than is BMI or the waist-to-hip ratio in adolescent males (164). Thus, increasing endogenous androgen concentrations down-regulate adiponectin secretion which, in turn, may contribute to the development of insulin resistance of puberty in males.

Models of sex steroid deficiency

Findings in men with estrogen deficiency (1,4,74) or estrogen resistance (5) suggest that congenital loss of estrogen action is associated with various degrees of insulin resistance. All the affected men were obese, had a high testosterone-to-estradiol ratio with clearly supraphysiological levels of testosterone in two of the cases, and all but one exhibited insulin resistance as evaluated by basal insulin level, the homeostasis model assessment of insulin resistance (HOMA-IR), or a glucose tolerance test (1,4,5,74). In addition, a man with estrogen deficiency had steatohepatitis, which improved by estrogen treatment (74). In these men, the impaired glucose metabolism may have been a direct consequence of estrogen deficiency or possibly a result of obesity and high testosterone concentrations. Treatment with estrogen improves insulin sensitivity in men with aromatase deficiency, but also simultaneously reduces testosterone secretion. Type 2 diabetes induced by testosterone treatment in a man with estrogen deficiency suggest that high concentrations of androgens in the absence of estrogen effects may impair glucose metabolism (74).

In terms of body composition and glucose homeostasis, findings in aromatase-deficient ArKO and ERαKO male mouse models correspond to findings in men with estrogen deficiency or resistance. These mice are obese and show significant hyperandrogenism, increased intra-abdominal adiposity, hepatic steatosis, and insulin resistance, all of which could be reversed with estrogen treatment in those with estrogen deficiency (126,127,165). Interestingly, male mice with disrupted androgen receptor and
normal estrogen levels similarly show increased total body and visceral fat, while their insulin sensitivity is normal and levels of adiponectin are increased (166). This observation suggests that androgens negatively influence insulin sensitivity through the suppression of adiponectin production. Taken together, data from human and mouse models demonstrate that the loss of estrogen effects in males leads to insulin resistance, which could be explained by increased androgen effects, the absence of estrogen effects, altered lipid accumulation or partitioning in the body, liver steatosis, or a combination of these factors. The concentrations of adiponectin in men or mice with estrogen deficiency have thus far not been reported.
6. AIMS OF THE STUDY

The aim of the current study was to investigate the efficacy and safety of suppressing estrogen biosynthesis with an aromatase inhibitor in boys with idiopathic short stature (I, III, IV) and constitutional delay of puberty (II). Specifically, we explored the effects of the aromatase inhibitor letrozole on:

1. Growth velocity, bone maturation, and predicted adult height (I);
2. Final adult height (II);
3. Lipids, lipoproteins, insulin sensitivity, and adiponectin (III);
4. Bone health, as evaluated by DEXA, markers of bone turnover, vertebral morphology, and metacarpal index (I, IV).
7. PATIENTS AND METHODS

7.1 PATIENTS AND STUDY PROTOCOL

7.1.1 Boys with idiopathic short stature
The study population was collected by systematically reviewing growth charts and medical records of boys examined and followed up for short stature at the outpatient clinic for pediatric endocrinology at the Hospital for Children and Adolescents, University of Helsinki, Finland. Those with no signs of chronic or endocrine illness in their medical history, in clinical examination, and in routine laboratory tests were considered potential candidates for recruitment. The inclusion criteria were: Calendar age of 9.0-14.5 years and height at least 2 SD below the mean for age, or height at least 2 SD below the mid-parental target height. Those with bone age of more than 14 years were excluded. If growth hormone deficiency was suspected on the basis of slow growth velocity, subnormal serum IGF-I, or subnormal IGFBP-3 concentrations, it was excluded with a growth hormone stimulation test.

Between May 2001 and May 2002, 40 boys were examined, and after initial assessment and provision of information, 31 boys with ISS were enrolled (Figure 1). Apart from seasonal or continuous inhaled corticosteroid treatment for asthma in four boys receiving letrozole and in two boys receiving placebo, none of the boys received any medication known to affect growth or bone maturation. Employing a computer-generated randomisation list, the boys were randomised in a double-blind manner to receive either letrozole (Femar®, Novartis AG, Stein, Switzerland), 2.5 mg orally once daily, or placebo orally once daily for 24 months. At baseline, no differences in baseline clinical characteristics were found between the treatment groups (Table 2).

The boys were examined at entry, every 6 months thereafter for two years, and finally one year after the cessation of treatments, at 36 months (Figure 2). The follow-up visits included a physical examination, a venous blood sample, measurement of BMD, and assessment of bone age. All patients, except for one placebo-treated boy diagnosed with diabetes mellitus after six months of treatment, completed the initial follow-up of 24 months, and 25 of the 31 boys initially recruited completed the follow-up of 36 months (Figure 1). The authors and the subjects were blind to treatment assignment throughout the follow-up period of 24 months, and the randomisation code was revealed to the researchers only after entering the data into the computer.
Figure 1. Trial profile for the ISS study.

Table 2. Clinical characteristics of the boys with ISS at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Letrozole (n = 16)</th>
<th>Placebo (n = 14)</th>
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<tr>
<td>Age (years)</td>
<td>11.02 (1.7)</td>
<td>10.97 (1.5)</td>
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<tr>
<td>Height (cm)</td>
<td>128.49 (6.9)</td>
<td>127.67 (6.6)</td>
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<td>Height (SDS)</td>
<td>-2.28 (0.3)</td>
<td>-2.44 (0.4)</td>
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<tr>
<td>Weight (kg)</td>
<td>27.94 (5.3)</td>
<td>25.98 (4.2)</td>
</tr>
<tr>
<td>Bone age (years)</td>
<td>9.05 (2.3)</td>
<td>8.89 (1.8)</td>
</tr>
<tr>
<td>Height for bone age (SDS)</td>
<td>-0.67 (1.4)</td>
<td>-0.83 (0.7)</td>
</tr>
<tr>
<td>Predicted adult height (cm)</td>
<td>167.0 (9.4)</td>
<td>165.8 (3.5)</td>
</tr>
<tr>
<td>Stage of puberty (G)</td>
<td>1 (1–3)</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td>Stage of puberty (P)</td>
<td>1 (1–2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Testis volume (mL)</td>
<td>1.30 (1.1)</td>
<td>0.86 (0.5)</td>
</tr>
<tr>
<td>Mid-parental target height (SDS)</td>
<td>-0.52 (0.3)</td>
<td>-0.26 (0.5)</td>
</tr>
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Values are means (SD), except for Tanner pubertal stage, which is median (range). No statistical differences between the groups were found.
7.1.2 Boys with constitutional delay of puberty

The study population comprised participants of a recent double-blind, placebo-controlled, randomised trial investigating the growth-preserving effects of the aromatase inhibitor letrozole in boys with constitutional delay of puberty. The inclusion criteria and baseline characteristics of the 23 boys initially recruited have been reported (7). In short, diagnosis of constitutional delay of puberty was defined as a Tanner G or P stage observed at an older age than the mean +2 SD for healthy Finnish boys (167), or a testis volume of less than 4 ml after 13.5 years of age. Only boys with no signs of chronic illness in medical history, physical examination, and routine laboratory tests were included. Of the 23 boys initially recruited, 19 (9 on testosterone and letrozole, 10 on testosterone and placebo) completed the initial 18-month follow-up, of whom 17 (9 on testosterone and letrozole, 8 on testosterone and placebo) completed the follow-up until near-final height, and constitute the population of the current study. In this population, seven of the nine boys in the testosterone and letrozole group, and seven of the eight boys in the testosterone and placebo group, had a family history of delayed puberty. At baseline, no significant differences in clinical characteristics were evident (Table 3). The mean level of serum testosterone had increased in both groups, suggesting that some of the boys had already reached early or mid-puberty by the beginning of the study. No boy, however, showed accelerated growth velocity before the start of treatment.

The participants received testosterone at a dose of 1 mg/kg i.m. every 4 weeks for 6 months, in combination with letrozole at a dose of 2.5 mg/d orally for 12 months, or testosterone as above, and oral placebo for 12 months (Figure 3). During the study, the patients were followed up at 2, 5, 12, and 18 months after the start of treatment, and finally, at near-final height. Treatment with testosterone and letrozole induced a five-fold increase in serum testosterone concentrations with no change in serum estradiol, indicating the significant inhibition of estrogen biosynthesis, whereas testosterone and placebo increased both testosterone and estradiol concentrations (7). After 18 months of follow-up, due to the slower rate of bone maturation, the predicted adult height of the boys treated with testosterone and letrozole increased by 5.1 cm with no change in the respective measure for those receiving testosterone and placebo (7).

Bone age greater than or equal to 15.75 years was defined as the achievement of near-final height. According to the tables of Bailey and Pinneau (168), boys at this bone age boys with an average tempo of puberty have achieved 97.9% of their adult height.
Table 3. Baseline characteristics of the boys with constitutional delay of puberty treated with testosterone and placebo, or with testosterone and letrozole, during adolescence. Values are means (SD), except for Tanner genital (G) and pubic hair (P) stages of puberty, which are medians (range). Bone age delay was calculated as calendar age minus bone age.

<table>
<thead>
<tr>
<th></th>
<th>Testosterone and placebo ( n = 8 )</th>
<th>Testosterone and letrozole ( n = 9 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>14.8 (0.9)</td>
<td>15.2 (0.8)</td>
</tr>
<tr>
<td>Bone age delay (years)</td>
<td>2.3 (0.8)</td>
<td>2.2 (0.3)</td>
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<tr>
<td>Stage of puberty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>2 (2-3)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>P</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
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<tr>
<td>S-testosterone (nmol/L)</td>
<td>11.7 (9.3)</td>
<td>8.3 (11.1)</td>
</tr>
<tr>
<td>Height (SDS)</td>
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<td>–1.8 (0.7)</td>
</tr>
<tr>
<td>Predicted adult height (cm)</td>
<td>173.7 (6.5)</td>
<td>177.1 (5.4)</td>
</tr>
<tr>
<td>Mid-parental target height (cm)</td>
<td>173.9 (4.0)</td>
<td>177.1 (4.1)</td>
</tr>
</tbody>
</table>

Figure 3. Treatment regimen and points of follow-up in boys with delayed of puberty.

7.2 METHODS

7.2.1 Auxological measurements and staging of puberty (I, II)

Heights were measured with a Harpenden stadiometer with 0.1 cm precision. Pubertal maturation was evaluated using the classification of Tanner\(^{(169)}\). Testis volumes (mL) were determined using the formula: Length (cm) × width (cm)\(^2\) × 0.52\(^{(170)}\), and are presented as means of the two testes measured. A testis volume of more than 2 mL was considered an indication of the onset of puberty in boys with ISS. Since aromatase inhibition influences the sex hormone milieu differentially in prepubertal and pubertal boys, the boys were retrospectively subdivided into two groups. Those boys with ISS with a testis volume of less than 2 mL at the end of the treatment were classified as prepubertal, and those with a testis volume of more than 2 mL at 18 months after the start of study.
were classified as pubertal. According to these criteria, 7 of 16 and 6 of 14 boys remained prepubertal, and 9 of 16 and 8 of 14 boys progressed in puberty in the letrozole and placebo groups, respectively.

7.2.2 Bone age (I, II)
Bone ages were determined by Matti Hero and Leo Dunkel, who were blind to treatment assignment at the time of the procedure. The bone age of each X-ray film was first evaluated with the method of Greulich and Pyle \[^{171}\]. All X-ray films were then ranked in successive order according to degree of maturation. After this, the bone age in each film was defined.

7.2.3 Adult height prediction (I)
Adult height predictions were calculated with the Bayley-Pinneau method \[^{168}\]. The method employs tables giving the percentage height attained in relation to adult height for each bone age in the range of 6.0 to 18.5 years. Two boys had a bone age of less than six years at the start of the study, and their adult height predictions were calculated by extrapolating data from the tables of Bailey and Pinneau.

7.2.4 Body composition (III, IV)
Weight was measured on a digital scale. The body mass index was calculated using the formula: Weight (kg) / height (m)\(^2\). The amount of fat mass relative to total body mass (the percentage of fat mass) was determined by six skinfold measurements during the treatment \[^{172}\]. At the post-treatment follow-up visit 12 months after the cessation of treatments, body composition was assessed with a Hologic Discovery A DEXA device (Hologic inc., Bedford, MA, USA) in the ISS study.

7.2.5 Laboratory analyses
Venous blood samples were obtained between 07:30 and 10:00 after an overnight fast. Blood counts, serum gonadotrophins, transaminases, IGF-I, inhibin B, and urine aminoterminal telopeptide of type I collagen (U-INTP) levels were measured directly after each visit; other laboratory parameters from sera stored at -20 or -70 °C until required.

Sex hormones (I, III, IV). Serum estradiol concentrations were measured using a modified RIA (Spectria E2, ORION Diagnostica, Espoo, Finland) with a detection limit of 4.5 pmol/L. The interassay CVs were 40% and 12% at concentrations of 4.2 and 22 pmol/L, respectively, and less than 16% at concentrations above 22 pmol/L. The intra-assay CVs were less than 14% at 4.6 to 130 pmol/L. Serum testosterone concentrations were quantified with a modified RIA (Spectria testosterone, ORION Diagnostica, Espoo, Finland). The sensitivity of the assay was 0.03 nmol/L. The interassay CV was 16% at 0.2 nmol/L and below 10% at concentrations above 0.8 nmol/L. Intra-assay CVs were 11% at 0.2 nmol/L and below 7% above 0.4 nmol/L.
**Gonadotrophins** (I). Serum FSH and LH levels were measured with ultrasensitive immunofluorometric assays (Wallac, Turku, Finland), with a detection limit of 0.05 IU/L. The interassay CVs for FSH were less than 3.3% at concentrations ranging from 6.6 to 35.1 IU/L, and for LH, less than 4.4% at concentrations ranging from 2.0 to 54.0 IU/L. The intra-assay CVs for FSH were less than 4.4% at concentrations ranging from 0.3 to 6.6 IU/L, and for LH, less than 4.1% at concentrations ranging from 0.3 to 8.7 IU/L.

**IGF-I** (I, III). Serum IGF-I concentrations were measured with RIA (DiaSorin, Stillwater, MN, USA). The interassay CVs were below 16% at concentrations ranging from 9 to 33 nmol/L.

**Markers of bone turnover** (IV). Second morning voided urine was collected for measurement of the bone resorption marker U-INTP. Analyses were performed with a luminoimmunological assay (Vitros ECi®, NTx Reagent Pack, Ortho-Clinical Diagnostics, New York, USA), and the results are expressed as nmol Bone Collagen Equivalents/mmol creatinine. The intra-assay CVs of the method range from 9.8 to 5.0% in concentrations of 60 to 400 nmol/L, and the inter-assay CVs, from 2.0 to 3.0% in concentrations ranging from 187 to 1872 nmol/L. Concentrations of the bone formation marker serum aminoterminal propeptide of type I collagen (S-PINP) were determined with RIA (UniQ PINP RIA®, Orion Diagnostica, Espoo, Finland). The method has a measurement range of 5 to 250 µg/L and a detection limit of 2 µg/L. Intra-assay CVs range from 6.5 to 10.2% (reported by the manufacturer), and inter-assay CVs from 3.9% (at concentration of 43.5 µg/L) to 4.5% (107.0 µg/L). Concentrations of serum alkaline phosphatase (S-ALP) were measured with Modular device (Hitachi Ltd, Tokyo, Japan). Intra-assay CVs were 0.5% (at concentrations of 143 and 329 U/L), and inter-assay CVs, 1.4 to 2.1% (at concentrations of 82 and 350 U/L).

**Lipids** (III). Serum total cholesterol and triglycerides were measured with enzymatic methods (Hoffman-La Roche kits 0722138 and 0715166). Serum HDL-cholesterol and subfractions were determined by phosphotungastic acid/magnesium chloride precipitation procedures (Hoffman-La Roche kit 0720674). Serum LDL-cholesterol was calculated by the Friedewald formula \(^{(173)}\). Concentrations of apolipoprotein AI, apolipoprotein AII and apolipoprotein B were measured using immunoturbidimetric methods with commercial kits (Boehringer-Mannheim, Mannheim, Germany) \(^{(174)}\). Lipoprotein (a) levels were determined with a turbidimetric immunoassay (Lp(a)-HA WAKO Chemicals GmbH, Neuss, Germany).

**Insulin sensitivity** (III). Blood glucose values were quantified with amperometric glucose oxidase method (EBIO Compact, Eppendorf, Hamburg, Germany). Serum insulin concentrations were measured with a fluoroimmunometric assay (AutoDelfia Insulin, Wallac, Turku, Finland) and serum adiponectin concentrations with a sandwich ELISA based assay (Human Adiponectin ELISA Kit, B-Bridge International, Inc., San Jose, CA, USA). A measure of insulin resistance, the homeostasis model assessment of insulin resistance (HOMA-IR), was calculated as follows: HOMA-IR = [fasting insulin (mU/L) x fasting glucose (mmol/L)] / 22.5 \(^{(175)}\).

**Inhibin B** (I). Serum inhibin B levels were measured with a commercially available immunoenzymometric assay (Serotec, Oxford, UK) with a detection limit of 15.6
ng/L. The interassay CVs at concentrations of 229, 85 and 42 ng/L were 6.9%, 10.1% and 12.0%, respectively. The intra-assay CV was less than 5%.

7.2.6 Bone mineral density measurements (I, IV)

During the treatment period of two years, BMDs of the lumbar spine and femoral neck were assessed every six months with DEXA using a Hologic QDR 4500W device (Hologic, Waltham, MA, USA). The total BMD CV was 1.0%. Areal BMD (g/m²) measured by DEXA normalises bone mineral content (BMC) values for the two-dimensional projected area, and does not account for differences in the depth of the bone in the region measured. Since the attenuation of a radiation beam also depends on the length of the path within bone, areal BMD values are confounded by differences in bone size. To correct for this, bone mineral apparent densities (BMAD, g/m³) of the lumbar spine were also calculated using the formula: BMC / (area projected)¹.₅ (176).

BMD measurements at post-treatment follow-up visits were carried out with a Hologic Discovery A DEXA device (Hologic inc., Bedford, MA, USA), with a total BMD CV of 1.0%. In addition to areal BMD, the device produces true volumetric BMDs of the lumbar spine after measuring the BMD of a second through the fourth lumbar vertebra from both antero-posterior and lateral views. To adjust for differences in age, whole body BMDs are also expressed as Z-scores.

7.2.7 Vertebral morphology evaluation (IV)

Vertebral morphology was evaluated 12 months after the cessation of treatments using the instant vertebral assessment (IVA) tool of the Hologic Discovery A DEXA device. Using a low radiation dose (< 10 μSv), IVA produces a lateral projection image of the thoracic and lumbar spine. In addition, plain radiographs of the thoracic and lumbar spine, both antero-posterior and lateral projections, were obtained 1.5 to 2.5 years after the cessation of treatments in boys who had received letrozole.

Both IVA images and radiographs of the spine were blindly evaluated by a pediatric endocrinologist and a radiologist. First, the investigators independently examined the shape and size of all vertebral bodies and classified them as normal, wedged, or compressed. Second, wedged and compressed vertebrae were further graded as 2a, 2b, 3a, or 3b deformities using a paediatric vertebral body morphology classification (177). In that classification, an anterior wedge deformity is characterised by decreased middle and anterior vertebral height. A grade 2a anterior wedge deformity (mild wedging) is defined as a reduction in anterior vertebral body height of more than 20%, but less than 50%, in comparison with posterior vertebral body height. A grade 2b (severe wedging) is determined by a more than 50% reduction in vertebral anterior height. In a compression deformity, anterior, middle, and posterior vertebral heights are decreased compared to the adjacent normal vertebrae. A grade 3a compression deformity (mild deformity) is defined as a 20 to 30% decrease in vertebral middle height as compared with the adjacent normal vertebrae. In grade 3b (severe deformity), the vertebral middle height reduction exceeds
30%. Finally, the investigators reviewed discordant readings together, and the final grades were based on a consensus decision.

7.2.8 Metacarpal index (IV)
MCI, a measure of cortical bone thickness (178), was determined from a postero-anterior radiograph of the left hand at the beginning of the study, at 12 months, at 24 months, and at 36 months. MCI, which reflects the ratio of cortical width to total metacarpal width, was measured from the midpoint of the second metacarpal bone with the equation: Outer cortical diameter – inner cortical diameter / outer cortical diameter. MCIs were measured by a single trained examiner from a digitalised hand radiograph with a digital caliper. The examiner was unaware of the patients’ treatment allocation. Measurements of the outer and inner cortical width were repeated, and the mean of the two measurements was used to calculate the MCI. The coefficients of variation were 1.0% and 2.5% for outer and inner width, respectively.

7.2.9 Statistical analysis
Values are expressed as means (± standard deviation) unless otherwise stated. Analyses were conducted with SPSS statistical software, release 10.0 (SPSS, Inc., Chicago, IL, USA). All statistical tests were two-sided. A $P$-value of less than 0.05 was considered significant.

Population of boys with ISS
The primary end point of the study was the efficacy of letrozole in improving predicted adult height as evaluated by comparing changes in predicted adult height between the treatment groups after two years of treatment. Second, treatment-induced changes in surrogate markers of bone strength, in lipids and liporoteins, in insulin resistance, in body composition, in puberty progression, and in the GH-IGF-I axis in letrozole and placebo groups were compared.

To calculate the required sample size, we chose 5 cm as the smallest clinically significant treatment effect on predicted adult height. The standard deviation of the change in predicted adult height was set at 4.7 cm, based on the results of a previous study with letrozole (7). With a power of 80% and a significance level of 5%, the required sample size was then 28 subjects. Assuming a maximum dropout rate of 10%, we decided to recruit at least 31 boys.

Between-group differences in baseline characteristics were evaluated by the Mann-Whitney U-test (Tanner stages) and the $t$-test (others). Changes during treatment in growth velocity, bone age progression, height SDS for bone age, predicted adult height, testis volume, serum gonadotrophins, sex hormones, inhibin B and IGF-I concentrations, and BMD were analysed by paired $t$-tests. Differences in testis volume, growth velocity, bone age progression, height SDS for bone age, and predicted adult height between the groups were analysed with unpaired $t$-tests. Differences in testosterone, estradiol, inhibin B, and IGF-I between the groups during therapy were analysed with repeated measures
ANOVA using treatment as a between-subjects factor. Logarithmic transformation was applied when appropriate. When repeated measures ANOVA was employed, the default covariate structure of SPSS software (compound symmetry) was used. Sphericity was analysed by Mauchly’s test, and only Hyunh-Feldt corrected P-values are presented. In pubertal boys, the changes in FSH and LH during the treatment were compared between the treatment groups using the unpaired t-test. The changes in Tanner genital (G) and pubic hair (P) stages during the follow-up were evaluated with the Friedman test, followed by multiple comparison tests. Between-group differences in G and P stages were evaluated with the Mann–Whitney U test.

In pubertal boys, within-group changes in weight, in BMI, and in percentage of fat mass (FM) were analysed with repeated measures ANOVA using log-transformed data when appropriate. In prepubertal boys, the Wilcoxon signed ranks test was employed to analyse changes in lipids and body composition. Repeated measures ANOVA was used to analyse the interactions between the received treatment and lipids, the treatment and HOMA-IR, the stage of puberty and lipids, and the stage of puberty and HOMA-IR. The effects of BMI, percentage of FM, testosterone, estradiol, IGF-I, and adiponectin on HDL-cholesterol and HOMA-IR during the treatment were evaluated with repeated measures ANOVA with two-year changes in these variables as covariates.

Between-group differences in post-treatment BMD were analysed by the t-test. Fisher’s exact test was used for comparing the frequency of abnormal vertebral findings in the treatment groups. Concordance between readings in IVA scans and plain radiographs of the spine was assessed by Cohen’s kappa. In comparisons of MCI values and concentrations of bone turnover markers between the groups during the treatment period, we employed repeated measures ANOVA. Due to skewed distributions in concentrations of bone resorption and formation markers, log-transformation was applied. The associations of sex steroid and IGF-I concentrations with MCI and bone turnover markers were evaluated with repeated measures ANOVA, using the mean concentrations or mean testosterone-to-estradiol ratio as a covariate.

Pearson and Spearman correlation coefficients were used in the analysis of correlations within the total population and the treatment groups, respectively.

Population of boys with constitutional delay of puberty
We used the student’s t-test when appropriate. Statistical analyses concerning near-final heights were conducted after logarithmic transformation of the data, and the means of near-final heights refer to geometric means. The Pearson correlation coefficient was used for analysing the correlation between post-treatment predicted adult height and near-final height. Height discrepancy was calculated as near-final height minus mid-parental target height.

7.3. ETHICAL CONSIDERATIONS RELATED TO MANAGEMENT OF IDIOPATHIC SHORT STATURE
Since growth-promoting treatments of short stature are not free of risks, such treatment is justified only if sufficient evidence suggests that short stature is associated with
disadvantages in daily living, psychosocial functioning, or quality of life, and if the
treatment is expected to improve these disadvantages. Previous studies have suggested
that short stature is associated with negative stereotypes (179). Studies investigating the
relationship between short stature and psychosocial functioning, however, have reported
divergent results, with some reporting underachievement and behavioural problems (180-
182), and others normal psychosocial functioning (183). Clinic- and community-based studies
appear to differ (184).

The effects of growth-promoting treatment on psychosocial functioning,
behaviour, and quality of life have been poorly characterised. A recent prospective,
double-blind, placebo-controlled trial of GH treatment in a limited number of children and
adolescents with ISS found that the treatment was associated with a trend toward
improvement in problem behaviours (185). To improve clinical decision-making and
targeting growth-promoting treatments to those who will benefit most, a clear need exists
for further studies on the impact of such treatments on psychosocial status.

Some have also questioned whether children or adolescents with ISS should be
treated when no underlying disease has been identified, and whether their treatment
reinforces social forces that maintain negative stereotypes associated with short stature
(183). Children with ISS, however, may exhibit growth disturbance of a degree similar to
that of patients with an identified underlying condition. Therefore, it is difficult to limit
access to treatment only to those with an identified underlying cause based on currently
available methods, and to exclude those who do not without considering the severity of
height disturbance, predicted adult height, or associated psychosocial harm.

In the present study, the boys recruited had been examined and followed up for
their short stature, and after counseling and reassurance, still wished for treatment for their
condition. The boys and their guardians were provided information on the experimental
nature of the treatment, and written informed consent was obtained from the boy and his
guardian(s). The study protocol was approved by the Ethics Committee of the Hospital for
Children and Adolescents, and by the National Agency for Medicines.
8. RESULTS

8.1 EFFICACY OF LETROZOLE

8.1.1 Serum estradiol and the hypothalamic-pituitary-testicular axis (I)
In prepubertal boys treated with letrozole or placebo, the mean concentrations of serum estradiol were similar at the start (12.4 and 18.2 pmol/L, respectively) and at two years after the start of the treatments (9.6 and 11.5 pmol/L). Similarly, their mean values of serum testosterone showed no differences at the start (0.2 and 0.2 nmol/L) or at two years (0.6 and 0.7 nmol/L).

In contrast, the concentrations of gonadotrophins and sex hormones in pubertal boys changed differentially in the treatment groups (Figure 4). Serum concentrations of gonadotrophins increased already within six months in the letrozole-treated pubertal boys (LH from 1.0 to 4.5 IU/L, \( P = 0.002 \) and FSH from 2.5 to 5.8 IU/L, \( P = 0.007 \)), whereas in the placebo-treated pubertal boys, a smaller increase in gonadotrophins occurred during the two years of treatment (LH from 0.6 to 2.4 IU/L, \( P = 0.0002 \) and FSH 2.3 to 3.7 IU/L, \( P = 0.04 \)). Parallel to the gonadotrophin concentrations, serum testosterone increased rapidly in pubertal boys treated with letrozole, resulting in higher serum testosterone levels than in the placebo-treated (Figure 4). After two years of treatment, the mean testosterone concentration in pubertal boys treated with letrozole was 30.9 nmol/L (range 0.6 to 48.0 nmol/L), compared with 8.9 nmol/L (1.7 to 16.7 nmol/L) in the placebo-treated boys. Serum estradiol concentrations, in turn, remained at the pretreatment level in letrozole-treated pubertal boys throughout the treatment period, whereas in the placebo-treated boys, serum estradiol showed an increasing trend, which failed to reach significance (from 11.9 to 26.0 pmol/L, \( P = 0.06 \)) (Figure 4).

8.1.2 Growth velocity (I)
Letrozole- and placebo-treated boys grew at a similar velocity both during the first and second years of treatment (Figure 5 A). The treatment effect on pubertal growth velocity was evaluated by comparing the second year growth velocities of the eight letrozole-treated and five placebo-treated boys who had entered puberty by the 12-month time point. We observed no significant differences (6.7 vs. 7.4 cm/year, respectively, \( P = 0.58 \)). In addition, in the letrozole group during the second year of treatment, the growth velocity of pubertal boys exceeded that of the prepubertal boys (6.7 vs. 4.5 cm/year, \( P = 0.04 \)).
Figure 4. Serum concentrations of LH, FSH, testosterone, and estradiol in letrozole-treated (closed symbols, $n = 9$) and placebo-treated (open symbols, $n = 8$) boys, who entered puberty within the first 18 months of treatment. Values are means (± SEM). * ($P < 0.05$) and † ($P < 0.01$) refer to differences between the treatment groups (repeated measures ANOVA).

Figure 5. Growth velocity (A) and rate of bone age progression (B) during the treatment period. Values are means (± SEM). * ($P < 0.05$) and † ($P < 0.01$) refer to differences between the treatment groups. BA = bone age, CA = calendar age.
8.1.3 Bone age and predicted adult height (I)
In boys treated with letrozole, bone age progressed by 1.24 years during the two years of treatment, whereas in the placebo-treated boys, bone age progressed by 2.05 years during the same period. The ratio of change in bone age and in calendar age during the treatment was 0.62 in boys treated with letrozole and 1.02 in placebo-treated boys (Figure 5 B, \( P = 0.04 \)). As a result of slower bone age advancement, height SDS for bone age increased in the letrozole-treated boys by 0.7 SDS, with no change in the placebo-treated boys (Figure 6 A). In a similar fashion, predicted adult height increased by 5.9 cm during the treatment in boys receiving letrozole, with no change in the placebo-treated boys (Figure 6 B). After two years of treatment, the predicted adult heights of boys treated with letrozole exceeded those of the placebo-treated boys (172.8 vs. 166.9 cm, \( P = 0.03 \)).

The efficacy of letrozole did not appear to be associated with pubertal maturation, since the increase in predicted adult height during the treatment did not differ between the prepubertal and pubertal boys (7.2 vs. 4.8 cm, respectively, \( P = 0.17 \)). Bone age at the start of the study did not correlate with the change in predicted adult height (\( r = -0.06, P = 0.83 \)).

8.1.4 Near-final height (II)
At near-final height, the mean ages of boys treated with testosterone and letrozole or with testosterone and placebo during adolescence were 19.2 (range 17.7-20.2) and 18.2 (17.1-19.1) years. Their respective mean bone ages were 16.9 (15.75-17.5) and 16.7 (15.75-18.0) years. Thus, while bone ages were still delayed in both groups at near-final height,
the mean difference between chronological age and bone age was greater in boys treated with testosterone and letrozole (2.2 vs. 1.5 years, \( P = 0.04 \)). The boys treated with testosterone and letrozole reached a near-final height of 175.8 cm while the respective mean of boys treated with testosterone and placebo was 169.1 cm (\( P = 0.04 \)). The near-final height of boys treated with testosterone and letrozole did not differ from their mid-parental target height (175.8 vs. 177.1 cm, respectively; \( P = 0.38 \); Figure 7 A), whereas in boys treated with testosterone and placebo, their near-final height was lower than their mid-parental target height (169.1 vs. 173.9 cm, respectively; \( P = 0.007 \)). The mean difference between near-final height and mid-parental target height, calculated as near-final height minus mid-parental target height, was smaller in boys treated with testosterone and letrozole, although the difference between the treatment groups failed to reach statistical significance (-1.2 vs. -4.8 cm, \( P = 0.06 \)). In keeping with delayed puberty and growth, the near-final height SDS was higher than the pretreatment height SDS in all patients (Figure 7 B). However, at near-final height, the gain in height SDS over the pretreatment height SDS was greater in boys treated with testosterone and letrozole than in boys who received testosterone and placebo (+1.4 vs. +0.8 SDS, \( P = 0.03 \); Figure 7 B).

To evaluate the reliability of adult height predictions calculated 18 months after the start of treatments, we studied their correlation to achieved near-final heights. We found that predicted adult heights correlated strongly with near-final heights (\( r = 0.91, P < 0.0001 \)). The Bailey-Pinneau prediction method, however, seemed to produce a slight overestimation, since the predicted heights were greater than near-final heights in both groups.

Figure 7. Near-final height (A) and gain in height standard deviation score (SDS) over the pretreatment height SDS (B) in boys with constitutional delay of puberty. Gain in height SDS was calculated as height SDS at near-final height minus pretreatment height SDS. Black arrows refer to the mean mid-parental target height; black bars denote means.
8.2 SAFETY OF LETROZOLE

8.2.1 The GH-IGF-I axis (I)
In pubertal boys with ISS, the concentrations of IGF-I changed differentially in the treatment groups (Figure 8). This resulted in lower serum IGF-I concentrations in pubertal letrozole-treated boys at 18 (19.2 vs. 28.5 nmol/L, \( P < 0.001 \)) and 24 months (18.2 vs. 28.6 nmol/L, \( P < 0.05 \)) after the beginning of the study.

![Figure 8. Serum concentrations of IGF-I in pubertal letrozole (Lz)- and placebo (Pl)-treated boys during the treatment. The marked P-value refers to difference between the groups as analysed by repeated measured ANOVA. Values are mean (SEM).](image)

8.2.2 Progression of puberty (I)
At the beginning of the study, 13 of the 16 (81%) boys treated with letrozole, and 13 of the 14 (93%) boys treated with placebo, were prepubertal. In the former group, those who had entered puberty had testis volumes of 2.4, 3.5, and 4.3 mL, and in the latter group, the respective volume for the only pubertal boy was 2.2 mL, indicating that they all were in the early stages of puberty. After two years of treatment in the letrozole and placebo groups, 7 of the 16 (44%) and 6 of the 14 (43%) boys remained prepubertal, while others had progressed to various stages of puberty. In those who entered puberty within 18 months after the study began, no significant differences in Tanner genital (G) stages appeared between the groups (Table 5). In keeping with stimulated testosterone secretion, however, pubertal boys treated with letrozole had reached a higher Tanner P stage at 12 and 18 months after the start of treatment (Table 5). In addition, their testis volumes increased more rapidly (Table 5). Indeed, after two years of treatment, all but one of the pubertal letrozole-treated boys had a testis volume of 10 mL or more, with all placebo-treated boys having testis volumes under 10 ml.
Table 5. Progression of puberty in the letrozole- and placebo-treated boys entering puberty within 18 months after the onset of treatment. Values are means (SD) for testis volume, and medians (range) for pubertal stages. Marked P-values refer to difference between the groups. * P < 0.05; † P < 0.01 compared with the baseline value.

<table>
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<th>Letrozole (n = 9)</th>
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<td>0 months</td>
<td>1 (1–3)</td>
<td>1 (1–2)</td>
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<td>6 months</td>
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<tr>
<td>12 months</td>
<td>3 (1–4)*</td>
<td>2 (1–3)</td>
<td>ns</td>
</tr>
<tr>
<td>18 months</td>
<td>3 (2–4)†</td>
<td>2.5 (2–3)*</td>
<td>ns</td>
</tr>
<tr>
<td>24 months</td>
<td>4 (2–5)†</td>
<td>3 (2–4)†</td>
<td>ns</td>
</tr>
<tr>
<td>Pubertal stage (P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 months</td>
<td>1 (1–2)</td>
<td>1 (1)</td>
<td>ns</td>
</tr>
<tr>
<td>6 months</td>
<td>1 (1–4)</td>
<td>1 (1–2)</td>
<td>ns</td>
</tr>
<tr>
<td>12 months</td>
<td>3 (1–4)</td>
<td>1 (1–2)</td>
<td>0.04</td>
</tr>
<tr>
<td>18 months</td>
<td>3 (1–4)*</td>
<td>1 (1–2)</td>
<td>0.01</td>
</tr>
<tr>
<td>24 months</td>
<td>4 (1–5)†</td>
<td>2 (1–4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Testis volume (mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 months</td>
<td>1.8 (1.3)</td>
<td>1.0 (0.5)</td>
<td>ns</td>
</tr>
<tr>
<td>6 months</td>
<td>5.0 (4.2)*</td>
<td>1.7 (1.1)*</td>
<td>0.05</td>
</tr>
<tr>
<td>12 months</td>
<td>7.9 (5.2)†</td>
<td>2.6 (1.5)†</td>
<td>0.02</td>
</tr>
<tr>
<td>18 months</td>
<td>9.9 (4.5)‡</td>
<td>4.1 (1.6)‡</td>
<td>0.005</td>
</tr>
<tr>
<td>24 months</td>
<td>11.6 (4.0)‡</td>
<td>5.1 (1.5)‡</td>
<td>0.001</td>
</tr>
</tbody>
</table>

8.2.3 Bone Strength (I, IV)

Bone mineral density

Areal BMD of the lumbar spine and femoral neck increased similarly during the study in letrozole and placebo groups (Figure 9 A, B). BMAD of the lumbar spine increased in the former (median increase 4.3%, P = 0.009), but not in the latter group (median increase 0.5%, P = 0.21; Figure 9 C). BMC of the lumbar spine increased similarly during treatment, by 4.1 and 4.9 g (P = 0.53), in the letrozole and placebo groups, respectively. An increase in lumbar spine BMD was apparent in all but one boy in the former group,
and in all boys of the latter group, during the two-year treatment. Femoral neck BMD, as well as BMAD of the lumbar spine, increased in all but one boy in both groups.

In post-treatment DEXA-scans obtained 12 months after cessation of treatments, no differences between the groups were evident in areal BMD of the lumbar spine or femoral neck, direct volumetric BMD of the lumbar spine, whole body BMD, whole body BMC, or in the ratio of BMC-to-lean body mass (Table 6).

Figure 9. Changes in bone mineral density in lumbar spine (A), femoral neck (B), and in bone mineral apparent density of the lumbar spine (C) in letrozole and placebo groups. Boxes represent interquartile ranges (50% of values) with medians, whiskers denote total ranges of values, excluding outliers (circles). * (P < 0.05), † (P < 0.01) and ‡ (P < 0.001) refer to changes from the baseline value.
Table 6. Bone mineral density 12 months after the cessation of treatment. Values are means (SD). vBMD, direct volumetric bone mineral density; BMC, bone mineral content.

<table>
<thead>
<tr>
<th></th>
<th>Letrozole (n =13)</th>
<th>Placebo (n =11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Areal BMD, lumbar spine (g/cm²)</td>
<td>0.64 (0.16)</td>
<td>0.64 (0.14)</td>
</tr>
<tr>
<td>vBMD, lumbar spine (g/cm³)</td>
<td>0.19 (0.04)</td>
<td>0.20 (0.03)</td>
</tr>
<tr>
<td>Areal BMD, femoral neck (g/cm²)</td>
<td>0.72 (0.12)</td>
<td>0.73 (0.11)</td>
</tr>
<tr>
<td>Whole body BMD Z-score (SDS)</td>
<td>-0.1 (0.9)</td>
<td>-0.3 (0.9)</td>
</tr>
<tr>
<td>Whole body BMC (g)</td>
<td>1337 (353)</td>
<td>1369 (354)</td>
</tr>
<tr>
<td>BMC/lean body mass</td>
<td>0.045 (0.006)</td>
<td>0.044 (0.003)</td>
</tr>
</tbody>
</table>

**Bone turnover**

In the placebo group, concentrations of all markers of bone turnover increased significantly (P = 0.02-0.0004) during the treatment period (Figure 10). In contrast, in boys treated with letrozole, concentrations of the bone resorption marker U-INTP increased by 44 nmol/mmol krea (P = 0.06) during the first six months, and thereafter slowly declined. Their concentrations of S-PINP, a marker of bone formation, remained at the pretreatment level, and concentrations of S-ALP increased by 38 U/L (P = 0.002). Statistically significant between-group differences in concentrations of markers of bone turnover were evident in U-INTP and S-PINP (Figure 10).

As expected, significant interactions between S-PINP and growth velocity (P = 0.0006), and between S-ALP and growth velocity (P = 0.001), were evident in the placebo group during the treatment period, whereas U-INTP showed no interaction with growth velocity (P = 0.59). By contrast, in the letrozole-treated boys, we found no interactions between growth velocity and markers of bone turnover (P = 0.31-0.68). In the placebo group, serum hormone concentrations were associated with the levels of bone turnover markers; 24-month changes in both serum testosterone and estradiol showed an interaction with S-PINP (P = 0.0001 and P = 0.01, respectively) and S-ALP (P = 0.002 and P = 0.02). In addition, the change in IGF-I showed an interaction with S-PINP (P = 0.008). In contrast, in the letrozole group, we found no apparent interactions between concentrations of testosterone, estradiol, or IGF-I, and markers of bone turnover, except between serum testosterone and S-PINP (P = 0.02). In both treatment groups, all observed associations between measured hormones and bone turnover markers were positive, with Spearman correlation coefficients ranging from 0.67 to 0.89 (P = 0.02-0.00002).
Figure 10. Markers of bone turnover during treatment (0-24 months), and 12 months after the cessation of treatments (at 36 months). Values are geometric means. P-values refer to differences between the treatment groups as analysed by repeated measures ANOVA.

**Metacarpal index**

MCI increased by 0.06 and 0.03 (16 and 10%) in the letrozole and placebo groups during the two years of treatment (P = 0.22 between the groups). The treatment effect on MCI was further evaluated in boys with a rise in serum testosterone concentration above the
upper limit of prepubertal range (> 1.1 nmol/L) during the treatment period, leaving nine boys in both groups. MCI increased significantly more in boys receiving letrozole than in those receiving placebo [0.10 (25%) vs. 0.03 (9%), Figure 11]. Twelve months after the cessation of treatments, we found no difference in MCI between the groups (Figure 11).

A significant interaction was observed between MCI and the mean testosterone-to-estradiol ratio during treatment (P = 0.02). The association was positive, as evidenced by the positive correlation between the change in MCI during treatment and the mean testosterone-to-estradiol ratio during treatment (r = 0.59, P = 0.02). Interaction between MCI and mean testosterone failed to reach significance (P = 0.07), and we found no interaction between MCI and mean estradiol or between MCI and mean IGF-I.

Figure 11. Metacarpal index in letrozole- and placebo-treated boys (n = 9 in both groups) with serum testosterone above the prepubertal range during the treatment period. Boxes represent interquartile ranges (50% of values) with medians. Whiskers represent total ranges, excluding outliers (open circles). The P-value refers to difference between the treatment groups in the metacarpal index during the 24 months of treatment.

Vertebral morphology

Vertebral morphology was first assessed 12 months after the cessation of treatments by obtaining lateral IVA scans of the spine. IVA scans revealed vertebral deformities in six of the 13 and four of the 11 boys examined in the letrozole and placebo groups, respectively
(Table 7). All observed deformities were either class 2a (mild wedging) or 3a (mild compression deformity). No difference between the groups was evident in the number of vertebral deformities (Table 7). The deformities observed were located in the lower half of the thoracic spine, between vertebrae TH6 and TH10. As assessed by a post-treatment questionnaire, all letrozole-treated patients with vertebral deformity were asymptomatic, whereas two placebo-treated boys with vertebral deformity were symptomatic. Back pain reported by these two boys, however, had been mild, transient, and did not limit normal daily activities.

Table 7. Vertebral morphology in boys treated with letrozole or placebo. Differences between the groups were evaluated with the Fisher test.

<table>
<thead>
<tr>
<th></th>
<th>Letrozole</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXA IVA-scans (n = 13)</td>
<td></td>
<td>(n = 11)</td>
<td></td>
</tr>
<tr>
<td>Patients with vertebral deformity (n)</td>
<td>6 (46%)</td>
<td>4 (36%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Symptomatic (n)</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Deformed vertebrae (n)</td>
<td>14</td>
<td>9</td>
<td>0.53</td>
</tr>
<tr>
<td>Grade 2a</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Grade 2b</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 3a</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Grade 3b</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Plain radiographs (n = 13)</td>
<td></td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Patients with vertebral deformity (n)</td>
<td>6 (46%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic (n)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deformed vertebrae (n)</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2a</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 2b</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3a</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3b</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vertebral morphology was further evaluated 18 to 30 months after the cessation of treatment in those treated with letrozole by obtaining radiographs of the spine. Altogether 18 class 2a or 3a vertebral deformities were detected in 6 of the 13 (46%) boys examined (Table 7). All vertebrae with deformities were located in the thoracic spine, in the region between vertebrae TH6 and TH12, except grade 2a anterior wedging of vertebra L1 in one boy. Agreement between readings in IVA scans and plain radiographs of the spine was poor (Cohen’s kappa 0.32).
Risk factors for vertebral deformity were evaluated by comparing letrozole-treated deformity-positive and deformity-negative boys. During the treatment, those with vertebral deformity in plain radiographs showed similar gains in BMD of the lumbar spine (0.035 vs. 0.049 g/cm², \( P = 0.72 \)), BMAD of the lumbar spine (0.008 vs. 0.0004 g/cm³, \( P = 0.17 \)), and similar post-treatment BMD of the lumbar spine compared with those with normal vertebral morphology (Table 8). After two years of treatment, at 24 months, no significant differences in serum estradiol or IGF-I were evident between deformity-positive and deformity-negative boys, whereas serum testosterone was lower in the former (Table 8). Indeed, in five of the six boys with vertebral deformity, serum testosterone had not exceeded the upper limit of the prepubertal range (> 1.1 nmol/L) during treatment, whereas in those without deformity, six of the seven boys exhibited testosterone levels above the prepubertal range by the end of treatment.

Table 8. Characteristics of letrozole-treated boys with and without vertebral deformity in spinal radiographs obtained 12 months after the cessation of treatments (at 36 months). Values are medians (range). \( P \)-value refers to difference between the groups as evaluated with the Mann-Whitney U-test.

<table>
<thead>
<tr>
<th></th>
<th>Deformity + (n = 6)</th>
<th>Deformity – (n = 6 or 7)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone age at 24 months (years)</td>
<td>7.5 (6.0-13.0)</td>
<td>11.6 (7.0-14.0)</td>
<td>0.07</td>
</tr>
<tr>
<td>Testis volume at 24 months (mL)</td>
<td>1.8 (0.9-16.0)</td>
<td>10.5 (0.9-11.3)</td>
<td>0.37</td>
</tr>
<tr>
<td>S-testosterone at 24 months (nmol/L)</td>
<td>0.5 (0.2-37.3)</td>
<td>35.8 (0.8-48.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>S-estradiol at 24 months (pmol/L)</td>
<td>10.1 (5.5-26.5)</td>
<td>10.3 (7.0-15.2)</td>
<td>0.94</td>
</tr>
<tr>
<td>S-IGF-I at 24 months (nmol/L)</td>
<td>17.5 (15.0-24.0)</td>
<td>21.0 (18.0-34.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>Lumbar spine areal BMD at 36 months (g/cm²)</td>
<td>0.58 (0.50-1.05)</td>
<td>0.62 (0.49-0.85)</td>
<td>0.70</td>
</tr>
<tr>
<td>Lumbar spine vBMD at 36 months (g/cm³)</td>
<td>0.186 (0.15-0.28)</td>
<td>0.186 (0.15-0.23)</td>
<td>0.93</td>
</tr>
<tr>
<td>Whole body BMD Z-score at 36 months (SDS)</td>
<td>0.15 (-1.6-0.7)</td>
<td>-0.35 (-0.8-0.4)</td>
<td>0.18</td>
</tr>
<tr>
<td>BMC/lean body mass at 36 months</td>
<td>0.051 (0.04-0.05)</td>
<td>0.039 (0.04-0.05)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

8.2.4 Lipid metabolism (III)

In boys treated with letrozole, HDL2-cholesterol (from 0.50 to 0.32 mmol/L, \( P < 0.01 \)), Apo AI (from 140 to 116 mg/dL, \( P < 0.001 \)), and Apo AII (from 40.1 to 32.0 mg/dL, \( P < 0.01 \)) decreased during the two-year treatment. No significant changes in other lipids were
apparent. In the placebo group, Apo AI (from 146 to 129 mg/dL, \( P < 0.01 \)) and Apo AII (from 39.7 to 31.4 mg/dL, \( P < 0.001 \)) decreased as well, while the reduction in HDL-cholesterol failed to reach significance (from 0.62 to 0.50, \( P = 0.09 \)). In the analysis of associations between lipids and their predictors, no significant interactions between treatment and lipids or lipoproteins were apparent. However, we did detect a significant interaction between HDL-cholesterol and puberty (\( P < 0.05 \)), and between lipoprotein (a) and puberty (\( P < 0.05 \)).

The treatment effect on lipids and lipoproteins was then evaluated in the subgroup of pubertal boys in whom differences in concentrations of sex steroids developed during treatment (Figure 4). In pubertal boys treated with letrozole, the mean HDL-cholesterol concentration decreased by 0.47 mmol/L (\( P < 0.01 \)) in two years (Figure 12 A). The reduction was most apparent in the HDL2-subclass, whereas in the HDL3-subclass, no significant change appeared (Figure 12 B, C). Simultaneously, their mean Apo AI concentration decreased markedly (by 38 mg/dL, \( P < 0.01 \)), with a less marked reduction in Apo AII (10 mg/dL, \( P < 0.01 \)) (Figure 12 D, E). In contrast, in pubertal boys treated with placebo, no significant changes in concentrations of HDL-cholesterol, HDL2-cholesterol, HDL3-cholesterol, or Apo AI appeared during treatment, while their mean Apo AII concentration decreased by 9 mg/mL (\( P < 0.05 \)). In between-group comparisons, we found significant differences in changes of HDL-cholesterol concentrations (\( P < 0.05 \)) and Apo AI concentrations (\( P < 0.05 \)) during treatment.

Concentrations of total cholesterol, LDL-cholesterol, Apo B, and triglycerides did not change during the study period in pubertal boys of either group, whereas in the letrozole-treated boys, lipoprotein (a) concentrations slightly decreased (Table 9).

The changes in BMI, percentage of fat mass, serum testosterone, serum estradiol, and serum adiponectin were selected as potential predictors of lipid concentrations during treatment. These predictors were interrelated, as indicated by the negative correlation between the two-year changes in serum testosterone and in the percentage of fat mass (\( r = -0.76, P < 0.01 \)), the positive correlation between the changes in adiponectin and in the percentage of fat mass (\( r = 0.60, P < 0.05 \)), and the near-significant negative correlation between the changes in serum testosterone and in serum adiponectin (\( r = -0.47, P = 0.07 \)) in letrozole-treated boys. In this group of boys, significant interactions were evident between the change in percentage of fat mass and HDL-cholesterol (\( P < 0.01 \)) and between the change in testosterone concentration and HDL-cholesterol (\( P = 0.01 \)), while the change in adiponectin showed a borderline interaction with HDL-cholesterol (\( P = 0.05 \)). Analyses of correlations revealed that the association between testosterone and HDL-cholesterol was negative, whereas associations between the percentage of fat mass and HDL-cholesterol, and between adiponectin and HDL-cholesterol, were positive. The two-year changes in serum estradiol and BMI showed no significant association with the changes in HDL-cholesterol concentrations during the treatment.

No interactions between the LDL-cholesterol level and the selected predictors (two-year changes in BMI, percentage of fat mass, testosterone, estradiol, or adiponectin), or between triglyceride concentrations and the predictors were evident in the letrozole-
treated boys. The change in testosterone, however, correlated negatively with the change in lipoprotein (a) in this group of boys ($r = -0.86$, $P < 0.01$).

Figure 12. Response of HDL-cholesterol (HDL-C) and apolipoproteins in pubertal letrozole- ($n = 9$) and placebo-treated ($n = 8$) boys. Boxes represent interquartile ranges (50% of values) with medians. Whiskers represent total ranges, excluding outliers (circles). * ($P < 0.05$), † ($P < 0.01$) and ‡ ($P < 0.001$) refer to change from baseline evaluated by repeated measures ANOVA. HDL2-C, HDL2-cholesterol; HDL3-C, HDL3-cholesterol. White boxes represent 0 month values, light gray boxes 12 month values, and dark gray boxes 24 month values.
Table 9. Total cholesterol, LDL-cholesterol (LDL-C), triglycerides (TG), apolipoprotein B (Apo B), and lipoprotein (a) (Lp(a)) in pubertal boys during treatment with letrozole (Lz) or placebo. Values are means (SD), except for values of Apo B and Lp(a), which are geometric means due to skewed distributions. * P < 0.05 compared with baseline value as analysed by paired t-test after log-transformation.

<table>
<thead>
<tr>
<th></th>
<th>Lz (n = 9)</th>
<th>Placebo (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Months</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.6 (1.0)</td>
<td>4.3 (0.8)</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>4.4 (1.1)</td>
<td>4.3 (0.4)</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.6 (0.9)</td>
<td>2.7 (0.8)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.7 (0.2)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>Apo B (mg/dL)</td>
<td>70.8</td>
<td>75.9</td>
</tr>
<tr>
<td>Lp(a) (mg/L)</td>
<td>104.7</td>
<td>114.8</td>
</tr>
</tbody>
</table>

8.2.5 Insulin sensitivity (III)

In pubertal boys of both groups, HOMA-IR, fasting blood glucose, and serum insulin remained unchanged during the treatment (Table 10). We found no interaction between treatment or pubertal maturation and HOMA-IR. Blood glucose and serum insulin values were within the normal range in all patients at all points of follow-up. When the two-year changes in BMI, in the percentage of fat mass, in testosterone, in estradiol, in IGF-I, and in adiponectin were selected as covariates, no interactions with HOMA-IR were found (P = 0.16-0.62). Serum adiponectin concentrations decreased in a similar fashion in the letrozole- and placebo-treated pubertal boys by 2.9 mg/L and 3.3 mg/L, respectively, during the treatment.

8.2.6 Body composition (III, IV)

Including all boys in the analysis, the boys in the letrozole and placebo groups gained weight similarly (4.2 vs. 3.7 kg/year, respectively), had similar increases in BMI (1.8 and 1.5 kg/m²), and showed no significant changes in the percentage of fat mass, as evaluated by skin fold measurements, throughout the treatment. We detected no interaction between the treatment received and BMI, or between the treatment received and percentage of fat mass. The effect of the treatment effect on body composition was further analysed in pubertal boys. Weight and BMI increased similarly in letrozole- and placebo-treated pubertal subjects, whereas the percentage of fat mass decreased in those who received letrozole, but not in those who received placebo (Table 10).
Twelve months after the cessation of treatments, no differences between the letrozole and placebo groups were found in DEXA-assessed lean body mass (30 444 vs. 30 907 g, respectively, P = 0.90) or percentage of fat mass (21.7 vs. 17.5%, P = 0.15).

Table 10. Changes in body composition and markers of carbohydrate metabolism in pubertal boys treated with letrozole (Lz) or placebo. Values are mean (SD), except for values of weight, percentage of fat mass, serum insulin, and HOMA-IR, which are geometric means due to skewed distributions. * p < 0.05, ‡ p < 0.001 compared with baseline value (repeated measures ANOVA).

<table>
<thead>
<tr>
<th></th>
<th>Lz</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Months</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>30.9</td>
<td>34.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.8 (1.7)</td>
<td>17.5 (1.8)</td>
</tr>
<tr>
<td>Percentage of fat mass (%)</td>
<td>17.0</td>
<td>12.3</td>
</tr>
<tr>
<td>B-glucose (mmol/L)</td>
<td>4.7 (0.5)</td>
<td>4.7 (0.3)</td>
</tr>
<tr>
<td>S-insulin (mU/L)</td>
<td>4.7</td>
<td>4.1</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.98</td>
<td>0.85</td>
</tr>
<tr>
<td>S-adiponectin (mg/L)</td>
<td>10.9 (3.6)</td>
<td>8.6 (3.1)</td>
</tr>
</tbody>
</table>
9. DISCUSSION

9.1 EFFICACY OF LETROZOLE

9.1.1 Serum estradiol and the hypothalamic-pituitary-testicular axis (I)

In the letrozole-treated pubertal boys with ISS, serum estradiol concentrations remained at the pretreatment level despite the substantial increase in their mean serum testosterone concentration to 30.9 nmol/L and in their progression of puberty to median genital stage 4 during the therapy. Furthermore, in keeping with decreased estrogen-mediated central negative feedback (186), their gonadotropin concentrations increased significantly already within six months after the start of the treatment. These findings indicate that letrozole effectively inhibited the conversion of androgens to estrogens. Interestingly, the serum estradiol concentrations of prepubertal boys with ISS were somewhat higher than concentrations previously observed in a respective population of boys using the same method (187). The reason for this discrepancy is unknown, but if the measured concentrations in our study were higher than the true concentrations, this discrepancy may have masked possible differences in serum estradiol between the treatment groups.

Most probably due to decreased inhibition of gonadotropin secretion, letrozole treatment was associated with stimulated testosterone secretion and enhanced testicular growth in the pubertal boys. On average, the testis volumes increased twice as fast in the letrozole-treated pubertal boys as in the placebo-treated pubertal boys. Furthermore, the Tanner pubic hair stage of puberty was significantly higher in the letrozole-treated pubertal boys at 12 and 18 months after the start of treatment. These findings suggest that the increase in testosterone secretion associated with aromatase inhibition after the onset of puberty results in enhanced progression of puberty. However, a similar proportion of boys in the letrozole and placebo groups entered puberty during the two years of treatment. Thus, aromatase inhibition may have no effect on the timing of the onset of puberty.

The opposite changes in the letrozole and placebo groups in serum IGF-I suggest that the puberty-associated stimulation of the GH-IGF-I axis was inhibited in those treated with letrozole. This is in line with the concept that estrogen induces the activation of the GH-IGF-I axis during puberty (149). Since increased estrogen secretion and augmentation of the GH-IGF-I axis are considered the most important regulators of the pubertal growth spurt in males (188,189), it is noteworthy that the growth rate of letrozole-treated pubertal boys was similar to that of pubertal placebo-treated boys, and faster than that of prepubertal boys. Thus, in the presence of prepubertal levels of IGF-I and estradiol, androgens appear to have the capacity to stimulate the growth rate above the prepubertal level. This view is also supported by findings in a previous study of letrozole treatment in boys with delayed puberty (7).
9.1.2 Predicted adult height (I)

In accord with the concept that estrogen is the principal regulator of skeletal maturation in males, letrozole effectively delayed bone maturation. Simultaneously, no major effect on growth velocity was apparent, and consequently, predicted adult height increased by 5.9 cm in those who received letrozole. Thus far, two other studies have reported data on the efficacy of letrozole in increasing predicted adult height, with corresponding efficacy. In the first randomised placebo-controlled study (7), a one-year treatment with letrozole in combination with low-dose testosterone increased predicted adult height by 5.1 cm in boys with delayed puberty. In another retrospective, uncontrolled study, a 5.5 cm increase in predicted adult height was observed in a mixed population of short adolescent boys after a mean treatment duration of 12 months (8). Thus, a relatively short duration of treatment with letrozole already appears to result in a clinically significant improvement in predicted adult height. Studies with older aromatase inhibitors (6,9,10,53) and another third generation aromatase inhibitor, anastrozole (31,190), have provided more heterogeneous results. Taken together, currently published studies of aromatase inhibitor treatment in growth indications lend support to the concept that selective inhibition of estrogen biosynthesis is effective in increasing predicted adult height. However, treatment response appears to be influenced by the potency of the compound used, the condition treated, and possibly by the duration of the treatment.

Interestingly, in boys with ISS, letrozole already appeared effective during prepuberty. This finding supports the view that (local) estrogen production may play a role in skeletal maturation already during prepuberty, when circulating estradiol levels are extremely low in boys (191).

9.1.3 Near-final height

Findings in boys with delayed puberty who received letrozole for one year during adolescence support the view that the achieved increase in predicted adult height also translates into taller adult height. First, at near-final height, boys treated with letrozole and testosterone were taller than boys treated with placebo and testosterone. Second, despite similar delays in bone maturation, boys on letrozole showed a greater height SDS increment from the pretreatment level. Third, boys on letrozole achieved their mid-parental target height, while boys on placebo failed to do so. However, this is the first report on the efficacy of aromatase inhibitor treatment as regards near-final or final height, and therefore more studies are needed to conclusively establish the final benefit of such treatment, and the size of the treatment effect. Nevertheless, these preliminary data suggest that relatively short-term treatment with letrozole may increase adult height in a similar fashion to longer-term treatment with GH or GnRH-agonist in relatively similar populations. In a recent report, a 0.5 SD greater adult height occurred in children with ISS treated peripubertally with GH for 4.4 years as compared to placebo (18). In another placebo-controlled study of GnRH-agonist therapy in short adolescent boys, a 0.6 SD increase in final adult height was noted after a mean treatment duration of 3.5 years (26).
9.2 SAFETY OF LETROZOLE

9.2.1 Bone health (I, IV)
In boys with ISS, no significant differences between the treatment groups were evident in areal BMD of the lumbar spine or femoral neck during the treatment period, whereas BMAD increased only in the letrozole-treated boys. In addition, DEXA-derived measures of BMD, including direct volumetric BMD of the lumbar spine, were similar in the treatment groups 12 months after the cessation of treatments. Thus, as evaluated by DEXA, a two-year treatment with letrozole showed no adverse influence on bone mass accrual. This is in line with findings in previous studies of aromatase inhibitor treatment in adolescent boys. A one-year treatment of boys with GH deficiency with anastrozole in combination with GH showed no influence on DEXA-measured BMD, as compared with placebo and GH \(^{(11)}\). Similarly, in the study of boys with delayed puberty, areal BMD of the lumbar spine and femoral neck increased in a similar fashion in the letrozole and placebo groups during treatment \(^{(12)}\). However, in that study, BMAD of the lumbar spine increased during treatment in those who received testosterone and placebo, but only after the cessation of treatments in those who received testosterone and letrozole. Taken together, the influence of aromatase inhibitor treatment for one or two years in boys appears to differ from that of total, long-term deprivation of estrogen effects, as indicated by severely reduced BMD in adult men with aromatase deficiency \(^{(1,2,4,74,75)}\).

Comparison of bone turnover markers between the letrozole- and placebo-treated boys with ISS suggests that aromatase inhibition influenced bone metabolism. Initially, within the first six months, letrozole appeared to increase bone resorption, as suggested by the transient increase in U-INTP level. Similar findings (increases in concentrations of bone resorption markers) have been reported in studies of short-term selective estrogen deprivation in adult males \(^{(81,82)}\). Thereafter, as suggested by the differences in U-INTP and S-PINP between the treatment groups, letrozole reduced the rate of bone turnover. As expected by the slight increase in growth velocity and advancement of puberty in some of the boys \(^{(192)}\), all markers of bone turnover increased in the placebo-treated group. In contrast, in the letrozole group only S-ALP concentrations increased, whereas the concentrations of U-INTP and S-PINP remained at the pretreatment level. These findings correspond to those in the study of boys with delayed puberty in whom concentrations of most markers of bone turnover did not change in those treated with testosterone and letrozole, whereas in those treated with testosterone and placebo, the concentrations of all markers of bone turnover increased during treatment \(^{(12)}\).

The reason for the lower bone turnover in the letrozole-treated is unclear. Although growth velocity and concentrations of markers of bone turnover are normally tightly coupled \(^{(192)}\), factor(s) other than differences in growth velocity clearly explain the difference in bone turnover between the groups. The uncoupling of the growth rate from the concentrations of bone turnover markers in the letrozole-treated boys also supports this view. Lower bone turnover in the letrozole-treated boys could be a result of stimulated testosterone secretion. In support of this view, androgens have been shown to inhibit osteoclast differentiation and bone resorption \textit{in vitro} \(^{(193,194)}\). Furthermore, they appear
independently to control bone resorption in males in vivo as well, as suggested by recent interventional studies employing sex hormone modulative treatments in men (83,195), and by a study of male mice with aromatase deficiency (196). However, in our study, no associations between sex hormones and bone turnover markers were found in the letrozole group, except for the positive association between serum testosterone and S-PINP.

The clinical significance of the lower bone turnover associated with the letrozole treatment is unclear. Similar increases in BMD in the treatment groups, and the greater increase in MCI in pubertal boys receiving letrozole, would suggest that bone mass accrual was not adversely affected. With the methods used, however, we cannot rule out the selective impairment of trabecular bone density or architecture in those treated with letrozole. Indeed, trabecular bone may be more sensitive to estrogen deficiency than cortical bone in males (90). Moreover, low bone turnover could, in theory, impair the repairing of microfractures.

The greater increase in MCI in the pubertal letrozole-treated boys suggests that aromatase inhibitor treatment is associated with stimulated cortical bone growth in males after the onset of puberty. This appears to result from increased exposure to androgen and from decreased exposure to estrogen, as suggested by the significant positive association between MCI and the mean testosterone-to-estradiol ratio. This finding supports the view that androgen increases and estrogen decreases periosteal bone formation (95,197,198). This finding is also supported by a recent large observational study of young Swedish men, in whom free testosterone was found to be a positive, and free estradiol a negative, predictor of cortical bone size, as evaluated by pqCT (90). However, optimal stimulation of cortical bone growth by androgens may require a certain level of estrogen receptor activation (199). As regards bone strength, the increase in MCI is a positive finding, since cortical bone size is an important predictor of bone strength (93,200). Furthermore, even after adjustment for DEXA-measured BMD, a negative association between MCI and the risk of wrist and forearm fractures in children has been reported (92).

Stimulated cortical bone growth in association with increased testosterone secretion may reflect a direct androgen effect on bone-forming osteoblasts through the androgen receptor (201,202). On the other hand, androgens may indirectly enhance cortical bone growth by increasing muscle mass and the mechanical loading of bones (203). Lending some support to the latter view, muscle mass probably increased more in the letrozole-treated than in the placebo-treated pubertal boys, as indicated by the greater decrease in the percentage of fat mass in the former group, and by similar increases in weight and BMI.

The prevalence of vertebral body deformities in post-treatment imaging studies was surprisingly high in both treatment groups, as evaluated by a paediatric classification for vertebral body morphology. In fact, the proportion of subjects with vertebral deformities was similar to or even greater than what has previously been observed with the same classification in children with chronic disease (177) or with solid organ transplantation (204). Thus, factors other than the treatment could explain the high rate of positive findings. Indeed, ISS is a condition with a heterogeneous background, and in some of the boys the observed deformities may reflect a defect in bone metabolism that
not only impairs bone growth, but also bone strength. The vertebral morphology of boys with ISS during childhood or adolescence has not been characterised before. The scientific validity of the high rate of vertebral body deformities observed in post-treatment spine radiographs of the letrozole-treated boys is compromised by its uncontrolled nature. Even so, there remains a possibility that aromatase inhibitor treatment impairs the strength of vertebral bodies in growing boys. If so, this would occur despite apparently normal accrual of bone mass, as evaluated by DEXA. Thus, aromatase inhibition would impair the quality rather than the quantity of bone in vertebral bodies. Since almost all vertebral body deformities in the letrozole-treated group were observed in boys who remained prepubertal throughout the treatment, the coinciding increase in testosterone concentration could then have a protective influence.

As regards vertebral imaging studies, several methodological issues deserve mention. First, using IVA scans and plain radiographs it is not possible to differentiate between fracture and vertebral body growth failure. Therefore, mild wedging and the appearance of compression in vertebral bodies may be a result of disturbed vertebral body growth rather than disturbed bone strength. Second, the small sample size and the retrospective nature of vertebral imaging studies must be taken into account when interpreting current findings. Finally, the classification of vertebral body morphology employed has been validated in a relatively small sample of healthy boys (177) Thus, the specificity of mild anterior wedge and compression deformities in diagnosing osteoporosis, particularly in boys with ISS, may be questionable. As regards back health in later life, the clinical significance of a mild anterior wedge or compression deformity in children and adolescents has not been characterised.

9.2.2 Lipids and lipoproteins (III)

Among pubertal boys with ISS, a significant decrease in HDL-cholesterol concentration occurred in those treated with letrozole, with no change in those treated with placebo. This finding is in line with previous findings on letrozole treatment in boys with delayed puberty (13). In both of these studies, letrozole reduced HDL-cholesterol in an inverse relationship with testosterone, suggesting that the reduction resulted from treatment-induced stimulation of testosterone secretion. In letrozole-treated pubertal boys with ISS, the decrease in HDL-cholesterol was particularly apparent in the HDL2-cholesterol subclass. Since large HDL2-cholesterol particles are preferred substrates of hepatic lipase (110,135), which is stimulated by testosterone (135), increased catabolism potentially explains the reduction in the HDL-cholesterol level. Additionally, a positive association of borderline significance was found between serum adiponectin and HDL-cholesterol. This suggests that adiponectin, the secretion of which is strongly influenced by testosterone and the degree of obesity (164,205), may contribute to regulation of the HDL-cholesterol level during puberty. Indeed, studies have reported a positive association between adiponectin and HDL-cholesterol in different populations, including obese children and adolescents (164,206,207).
Studies with anastrozole, another third-generation aromatase inhibitor, have reported divergent results. In healthy (91) and GH-deficient boys (11), anastrozole did not influence the lipid profile. The different response of lipids in adolescent boys treated with anastrozole could result from its somewhat lower potency compared to letrozole (50).

In line with the results of previous studies on aromatase inhibitor therapy in adolescent boys (11,13,91), the concentrations of LDL-cholesterol, Apo B, and triglycerides were unaffected by the letrozole treatment in pubertal boys. Considering the high testosterone and low estradiol concentrations in these boys at the cessation of treatment, this finding supports the view that neither androgen nor estrogen significantly contributes to regulation of lipid concentrations in males during early adolescence. The concentrations of lipoprotein (a), in turn, decreased in pubertal boys treated with letrozole, in an inverse relationship with serum testosterone. Thus, androgen may directly reduce lipoprotein (a) level. Indeed, in a previous report, testosterone treatment in adult men reduced lipoprotein (a) independently of estrogen (139). The falling lipoprotein (a) level may have clinical significance, since the lipoprotein (a) level is considered an independent risk factor for coronary heart disease (208).

9.2.3 Insulin sensitivity (III)
As evaluated by HOMA-IR, no significant changes in insulin sensitivity appeared in pubertal boys of either treatment group during the treatment period. In addition, we found no significant interaction between treatment and HOMA-IR. These findings suggest that letrozole had no major effect on insulin sensitivity. However, given that placebo-treated pubertal boys were still in the early stages of puberty at the cessation of treatment, significant differences between the groups in favour of the letrozole-treated boys could have appeared with longer follow-up and further progression of puberty. In support of this, the HOMA-IR of pubertal boys treated with letrozole remained unchanged throughout treatment despite the progression of puberty from median genital stage 1 to stage 4. Thus, our findings do not contradict previous findings on letrozole treatment in boys with delayed puberty, in whom the serum fasting insulin level decreased during treatment with letrozole, but not with placebo (13). Together these studies demonstrate that raising testosterone concentrations do not directly contribute to the development of insulin resistance during male puberty. Instead, the conversion of androgens to estrogens and the following stimulation of GH secretion may play a fundamental role. This concept is supported by the close connection between the rise and fall in insulin resistance, as assessed by the euglycemic hyperinsulinemic clamp technique, and the rise and fall in serum the IGF-I level during puberty (154). In our study, both IGF-I and HOMA-IR remained at pretreatment levels during the treatment period in the letrozole-treated pubertal subjects, but no correlations between the two measures emerged.

9.2.4 Other related safety issues
Several lines of evidence suggest that estrogen is implicated in the regulation of cognitive function. Supporting a role for estrogen, particularly in the modulation of verbal memory,
performance in some tasks of verbal ability and estrogen levels have been observed to change in parallel during the menstrual cycle in women (209). Moreover, in a cross-sectional study of women with breast cancer, the performance of women treated with the aromatase inhibitor anastrozole was poorer in tests of verbal memory function than was the performance of the control group (210). Only one study has addressed the impact of aromatase inhibition on cognitive function in males. In that study, the treatment of healthy older men with testosterone improved their spatial and verbal memory function, whereas those treated with testosterone and anastrozole showed improved spatial memory with no change in verbal memory function (211). Thus, estrogen may play a role in the regulation of verbal memory function in males. At present, the role of estrogen in the regulation of memory function in males during childhood and adolescence remains unknown.

Findings in knockout animal models have suggested that normal activation of the estrogen receptor α plays an important role in male fertility. Estrogen receptor α knockout male mice show progressive impairment of fertility due to loss of fluid reabsorption function in epithelial cells of efferent ductules, resulting in the swelling of rete testis and efferent ductules (212). Male estrogen receptor β knockout mice, in turn, are fully fertile (213). Male aromatase knockout mice have a different phenotype than do the estrogen receptor α knockout mice, but their fertility is also affected by impaired spermatogenesis at an older age (214). The finding that treatment with letrozole reduced sperm count and quality in monkeys further supports a physiological role for estrogen and aromatase in male testes (215). Indeed, in men, aromatase has been detected in ejaculated sperm, and estrogen produced locally in the sperm may influence the sperm fertilising capacity (216). However, in infertile men with a low serum testosterone-to-estradiol ratio, treatment with anastrozole effectively improved sperm count and quality (217). Moreover, treatment of GH-deficient boys with anastrozole during adolescence did not appear to influence their sperm parameters later in life (218). Thus, even though studies in adult and adolescent males have not reported impairment in sperm parameters in association with aromatase inhibitor treatment, estrogen may play a role in maturing spermatogenesis and sperm function, and therefore further studies on this issue are still needed.
10. CONCLUSIONS AND FUTURE RECOMMENDATIONS

1. The treatment of peripubertal boys with the aromatase inhibitor letrozole results in stimulated gonadotropin and testosterone secretion in those who advance in puberty. Despite high serum testosterone concentrations, serum estradiol concentrations remain at the pretreatment level, indicating efficient inhibition of androgen-to-estrogen conversion. In addition, the increase in IGF-I associated with puberty is blunted in letrozole-treated boys, probably due to the suppression of growth hormone secretion.

2. In boys with ISS, treatment with the aromatase inhibitor letrozole decreases the rate of bone maturation with no significant effect on growth velocity. This results in increased predicted adult height. In boys with constitutional delay of puberty treated with letrozole or placebo for one year during adolescence in combination with low-dose testosterone, those receiving letrozole exhibited greater near-adult height. Thus, the achieved increase in predicted adult height also appears to translate into taller adult height in letrozole-treated boys.

3. In pubertal boys with ISS, stimulated gonadotropin and testosterone secretion associated with letrozole treatment enhances testicular growth and advances pubertal maturation.

4. As evaluated by DEXA, treatment with letrozole does not interfere with normal accrual of bone mass. However, as suggested by differences in markers of bone turnover between the groups, the treatment is associated with an initial increase in bone resorption followed by a low bone turnover state. The impact of this finding on bone strength is currently unclear.

5. Treatment with letrozole was associated with increased MCI in pubertal boys with ISS. The change in MCI correlated positively with the testosterone-to-estradiol ratio, suggesting androgen-associated stimulation of cortical bone growth.

6. In post-treatment radiographic imaging studies, vertebral body deformities (mild anterior wedging and mild compression deformities) were observed at a high rate in both letrozole and placebo groups. All deformity-positive boys treated with letrozole were asymptomatic, while two of the placebo-treated boys had mild, transient back pain, which did not limit normal daily activities. The aetiology and clinical significance of this finding remains unclear. These findings indicate a need for the characterisation of vertebral body morphology in boys with ISS at different stages of pubertal maturation. Further, detailed studies on the impact of aromatase inhibition on bone geometrical properties, BMD in different bone compartments, and vertebral body morphology are warranted.

7. Treatment with letrozole decreases HDL-cholesterol after the onset of puberty. The decrease in HDL-cholesterol is most evident in the HDL2-cholesterol subclass. The
change in HDL-cholesterol correlated negatively with serum testosterone, suggesting that
the reduction in HDL-cholesterol is a result of treatment-induced stimulation of
testosterone secretion. No changes in LDL-cholesterol or serum triglycerides were evident
in either treatment group, supporting the view that sex hormones do not significantly
regulate their concentrations during male puberty.

8. As evaluated by HOMA-IR, no differences in insulin sensitivity were evident between
the treatment groups. However, the percentage of fat mass decreased in pubertal boys
treated with letrozole, but not in those who received placebo. The change in the percentage
of fat mass was negatively associated with serum testosterone, suggesting that the reduced
percentage of fat mass was a result of stimulated testosterone secretion.

Future recommendations
The findings of the present study support the view that aromatase inhibition is an effective
treatment for boys with short stature. However, as regards efficacy, several clinically
important issues remain to be addressed in future studies. For example, the efficacy of the
treatment in increasing adult height remains to be confirmed in larger studies. In addition,
the optimal time for beginning the therapy in relation to bone age and pubertal maturation
remains unknown. In addition, the efficacy of potent third generation aromatase inhibitors
in different conditions associated with growth disturbances remain to be characterised.

Due to the limited quantity of safety data currently available on aromatase
inhibitor treatment in children and adolescents, the use of such therapy outside the
research setting is not recommended. In particular, future studies should clarify the impact
of aromatase inhibition on BMD and bone quality in different compartments of bone, and
on vertebral body growth and strength. Other safety issues to be addressed include the
effects of the treatment on maturing spermatogenesis, cognitive function, vascular wall
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