PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 86 (2003) 345–356

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

Novel treatment of short stature with aromatase inhibitors☆

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Abstract

Estrogens have an essential role in the regulation of bone maturation and importantly in the closure of growth plates in both sexes. This prospective, randomized, placebo-controlled study was undertaken to evaluate whether suppression of estrogen synthesis in pubertal boys delays bone maturation and ultimately results in increased adult height.

A total of 23 boys with constitutional delay of puberty (CDP) received a conventional, low-dose testosterone treatment for inducing progression of puberty. Eleven of these 23 boys were randomized to receive a specific and potent P450-aromatase inhibitor, letrozole, for suppression of estrogen action, and 12 boys were randomized to receive placebo. Estradiol concentrations in the letrozole-treated boys remained at the pretreatment level during the administration of letrozole, whereas the concentrations increased during the treatment with testosterone alone and during spontaneous progression of puberty. Testosterone concentrations increased in all groups, but during the letrozole treatment, the increase was more than fivefold higher than in the group treated with testosterone alone.

The inhibition of estrogen synthesis delayed bone maturation. The slower bone maturation in the boys treated with testosterone and letrozole, despite higher androgen concentrations, than in the boys treated with testosterone indicate that estrogens are more important than androgens in regulation of bone maturation in pubertal boys. During the 18 months follow-up, an increase of 5.1 cm in predicted adult height was observed in the boys who received testosterone and letrozole, but no change was seen in the boys who received testosterone alone or in the untreated boys. This finding indicates that an increase in adult height can be attained in growing adolescent boys by inhibiting of estrogen action.

Keywords: Aromatase; Estrogens; Androgens; Growth; Puberty; Bone; Lipids; Insulin; Gonadotropins

1. Introduction

Increasing secretion of sex steroids in gonads during puberty induces acceleration of growth and development of secondary sexual characteristics. Androgens in boys and estrogens in girls have been generally assumed to be the primary sex steroids causing physical changes during puberty.

In 1994, the description of a man with inactivating mutation of the estrogen receptor (ER) revolutionized the traditional concept of the roles of sex steroids in male [1]. This 28-year-old man was 204 cm tall. He had a bone age of 15 years, open epiphyses of long bones, and consequently he was still growing. Moreover, he had no recollection of accelerated pubertal growth despite otherwise normal pubertal development. Soon thereafter, two males with similar phenotypes were described [2,3]. In these men, the effects of estrogens were suppressed due to mutations in the gene coding P450-aromatase enzyme, which converts androgens to estrogens. The administration of estrogen in these men closed the epiphyses and discontinued growth [3,4]. In all of these men, concentrations of androgens were normal or above normal. These case reports confirmed that estrogens are essential hormones for epiphyseal closure in males. Moreover, the reports suggest that estrogens do not participate in the regulation of linear growth, but induce growth acceleration during puberty.

Delayed puberty is defined as a lack of initial signs of puberty by an age that is more than 2 S.D. above the mean for the population (about 13.5 years in boys). In most instances, delay in pubertal development is not due to any underlying illness. Then it is an extreme end of the normal spectrum of pubertal timing and is defined as constitutional delay in puberty (CDP). Although being healthy, some boys with CDP do not exploit their genetic growth potential [5–8]. The delay in puberty and growth can be considerable psychological distress and in these situations medical intervention is justified. The boys have been treated with androgens, which induce development of secondary sexual characteristics and growth acceleration [9–11]. However, treatment with androgens does not increase adult height [7,9,12].

☆ Presented at the VI International Aromatase Conference: AROMATASE 2002, Kyoto, Japan, 26–30 October 2002.

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doi:10.1016/S0960-0760(03)00344-3

Since the role of estrogens in the regulation of bone maturation is unequivocal, we postulated that inhibition of estrogen synthesis in boys with CDP would delay maturation of growth plates and ultimately results in increased adult height. We hypothesized that this treatment would help the boys to achieve their genetic growth potential. The results presented in this review have been previously published [13–15].

2. Materials and methods

2.1. Patients and study protocol

A total of 33 boys were recruited for the study (Table 1). Diagnosis of constitutional delay of puberty was defined as a Tanner genital or pubic hair stage observed at an older age than the mean + 2 S.D. for healthy Finnish boys [16] or a testis volume of less than 4 ml after 13.5 years of age. At entry, none of the boys had had any pubertal increase in growth velocity. Boys whose target height was more than +1 S.D. were excluded from the study. Neither medical history, clinical examination, nor routine laboratory tests revealed any signs of chronic illnesses accounting for the delayed puberty in any of the boys. In all, 25 (76%) of the boys had a family history of delayed puberty. None of the boys had received any previous sex hormone treatment. Informed written consent was obtained from the patient and from his guardian. The protocol was approved by the Ethical Committee of the Hospital for Children and Adolescents, and the National Agency for Medicines.

Ten boys with a mean age of 15.0 ± 0.2 years (range, 14.4–16.8) decided to wait for spontaneous progression of puberty without medical intervention, and thus composed the untreated group. Twenty-three boys with a mean age of 15.1 ± 0.2 years (range, 13.5–16.1) desired medical intervention, and we randomly assigned them to receive one or other of the two treatments. The boys in the testosterone-plus-placebo-treated group (12 boys) received testosterone enanthate (Testoviron-Depot-250, Schering, Berlin, Germany) six times with a dose of 1 mg/kg intramuscularly every 4 weeks, and placebo orally once a day for 12 months. The testosterone-plus-letrozole-treated group (11 boys) received testosterone enanthate (as above) and, in addition, a specific and potent, fourth-generation aromatase inhibitor, letrozole (Femar, Novartis AG, Stein, Switzerland, commercially purchased from hospital pharmacy), 2.5 mg orally once a day for 12 months. The project was conducted as a randomized, double-blind, placebo-controlled study between the treated groups. The results of the non-treated control group are shown just for comparison.

The subjects were examined at the start, at 2 months (approximately 7 days after the third testosterone injection), at 5 months (approximately 7 days after the sixth testosterone injection), at 12 months and at 18 months.

2.2. Auxological measurements and staging in puberty

Heights were measured on a Harpenden stadiometer with 0.1 cm precision. Pubertal stages were assessed according to Tanner [17]. Testis volumes were calculated from the formula, length × width^2 × 0.52 [18] and are presented as means of the two testes. Bone ages were determined blindly by the method of Greulich and Pyle [19]. Bone age X-ray films of each time-point were first ranked in successive order according to maturation, after which the bone age for each film was determined. Adult height predictions were calculated by the Bayley–Pinneau method [20]; the table for boys with average skeletal maturity was used, as the bone ages in most of the boys exceeded the range of bone ages reported for boys with retarded skeletal maturity. The body mass index (BMI) was calculated from the formula: weight (kg)/height^2 (m^2).

The bone mineral density (BMD) of the first through fourth lumbar spines and the femoral neck were determined by dual-energy X-ray absorptiometry (Hologic QDR-1000, Hologic Inc., Waltham, MA, USA).

All of the venous blood samples were drawn between 7:30 and 10:15 h, The pre-sampling fasting was at least 10 h. Serum 17β-estradiol concentrations were determined by a modified radio-immunoassay (RIA) using coated tube technology (Spectria estradiol, Orion Diagnostica, Espoo, Finland) after diethyl ether extraction (700 μl serum plus

Table 1
Clinical characteristics of the boys at the start of the follow-up

Characteristic	No treatment	Testosterone + placebo	Testosterone + letrozole
Chronological age (years)	15.0 ± 0.2	15.0 ± 0.2	15.2 ± 0.2
Bone age (years)[a]	12.7 ± 0.3	12.6 ± 0.4	13.1 ± 0.2
Height (cm)	154.3 ± 1.4	151.9 ± 2.4	155.3 ± 2.1
Pubertal stage[b]	G2(2–3) P2(1–2)	G2(2–3) P1(1–2)	G2(2–3) P1(1–2)
Testis volume (ml)[c]	5.9 ± 0.9	6.9 ± 1.2	5.5 ± 0.6
Predicted adult height (cm)[d]	178.3 ± 1.4	174.9 ± 2.4	176.5 ± 1.7

Mean ± S.E.M. or median (range).
[a] By Greulich and Pyles' method [19].
[b] According to Tanner [17].
[c] From the formula length × width^2 × 0.52 [18].
[d] By the Bayley–Pinneau method [20].

5 ml diethyl ether) [21]. The sensitivity of the assay was 6 pmol/l. Serum testosterone and DHT concentrations were measured by RIA after separation of steroid fractions on a Lipidex-5000 microcolumn (Packard-Becker, B.V. Chemical Operations, Groningen, The Netherlands) [22]. The concentrations of IGF-I and IGF-binding protein 3 (IGFBP3) were determined by RIAs (DiaSorin, Stillwater, Minnesota, USA and Nichols Institute Diagnostics, San Juan Capistrano, CA, USA, respectively). Serum IGF-I and IGF-binding protein 3 (IGFBP3) concentrations have been shown to reflect GH secretion in healthy children [23]. Concentrations of serum total cholesterol, HDL-cholesterol and triglycerides were determined by enzymatic colorimetric tests (Roche Diagnostics GmbH, Mannheim, Germany). Serum LDL-cholesterol concentrations were calculated using the equation of Friedewald [24]. Serum insulin concentrations were determined by RIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Serum inhibin B concentrations were determined by enzyme-linked immunosorbent assay (Serotec, Oxford, UK). Serum LH and FSH concentrations were measured by ultra-sensitive immunofluorometric assay (Wallac, Turku, Finland) [25]. The sensitivity of the assay for LH and FSH was 0.05 IU/l, as defined by the mean + 2 S.D. of 20 (LH) or 96 (FSH) replicates of a zero sample. Nocturnal gonadotropin pulses were studied at the start and at 5 months in five boys treated with testosterone and placebo and in five boys treated with testosterone and letrozole. For determination of nocturnal gonadotropin pulses, serum LH and FSH concentrations were determined every 15 min. The serum LH and FSH concentrations from each individual at a given time-point were analyzed in the same assay. LH and FSH pulsations were analyzed by a computerized pulse analysis program, Munro (Zaristow Software, East Lothian, Scotland).

2.3. Statistical analysis

Values are expressed as mean ± S.E.M. unless otherwise reported. Student's paired *t*-test or the Wilcoxon matched pairs signed rank-sum test were used for analyses of the changes within groups during the follow-up. For analysis of serial measurements, the summary measures, the differences from the start, were calculated for each subject, and these values were treated as raw data for the appropriate statistical analysis. One-way analyses of variance, Student's unpaired *t*-test, Kruskal–Wallis non-parametric analyses of variance, or Mann–Whitney *U* tests were used as appropriate. The Pearson's correlation coefficient was used to investigate the relationship between growth velocity and hormone concentrations. Pearson's or Spearman's correlation were used to assess the relationships between changes in insulin concentrations and changes in BMIs, concentrations of 17β-estradiol, testosterone, IGF-I, and IGFBP3, and the relationships between changes in concentrations of HDL-cholesterol and changes in BMIs, concentrations of 17β-estradiol, testosterone, IGF-I, and IGFBP3. All statistical tests were two-sided. A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Safety of letrozole

For detecting possible side-effects of letrozole, the concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and transaminases, the leukocyte count, and the BMD were determined during the follow-up. No changes, sufficient to indicate discontinuation of the treatment were observed in any of these parameters in any of the boys. Letrozole was well tolerated; no side-effects were observed.

3.2. 17β-Estradiol reflecting treatment effect

Letrozole inhibited estrogen synthesis effectively (Fig. 1). During the treatment with testosterone and placebo, the 17β-estradiol concentration increased, and an increase was also observed in the untreated group during the follow-up. In contrast, during the treatment with testosterone and letrozole, the concentration remained at the pretreatment level. After the discontinuation of letrozole treatment, the 17β-estradiol concentration increased in the testosterone-plus-letrozole-treated group also, and at 18 months, i.e. 6 months after discontinuation of the treatments, concentrations in all groups were similar.

3.3. Testosterone and DHT

The testosterone concentrations increased in all three groups, but during the treatment with testosterone and letrozole, the increase from the start was more than five-fold higher than during the treatment with testosterone and placebo (Fig. 2). In the testosterone-plus-letrozole-treated group, the high concentration was sustained until discon-

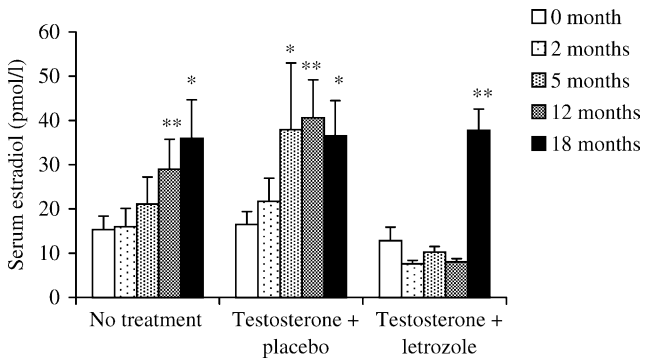

Fig. 1. Mean±S.E.M. serum 17β-estradiol concentration. Asterisks denote the significant changes from the start within the group: (∗) $P < 0.05$; (∗∗) $P < 0.01$.

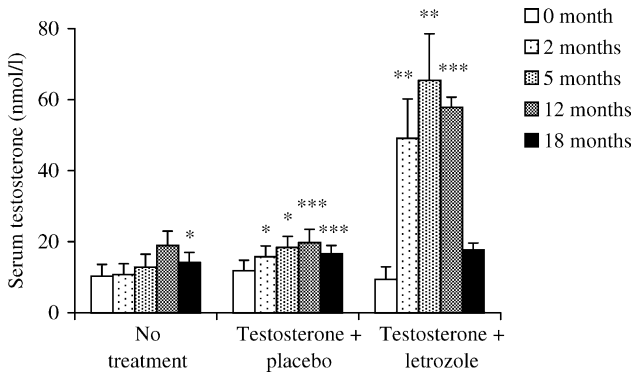

Fig. 2. Mean ± S.E.M. serum testosterone concentration. Asterisks denote the significant changes from the start within the group: (∗) $P < 0.05$; (∗∗) $P < 0.01$; (∗∗∗) $P < 0.001$.

tinuation of the letrozole treatment, after which the concentration decreased to a level comparable with those of the other groups. During the follow-up, the DHT concentrations had similar pattern of changes to the testosterone concentrations, although the magnitude of the changes was smaller.

3.4. IGF-I and IGFBP3

To assess GH secretion, the concentrations of IFG-I and IGFBP3 were determined. The IGF-I and IGFBP3 concentrations changed differently in the two treated groups during the treatments (Table 2). During the treatment with testosterone and placebo, both concentrations increased immediately after the start of the treatment, but during the treatment with testosterone and letrozole, the concentrations remained at the pretreatment level.

Table 2
Serum IGF-I and IGFBP3 concentrations

	No treatment	Testosterone + placebo	Testosterone + letrozole	*P*-value[a]
IGF-I (nmol/l)				
0 month	27.4 ± 3.8	28.3 ± 2.7	30.3 ± 3.4	
2 months	28.7 ± 2.9	34.0 ± 2.4[b]	25.6 ± 1.5	0.01
5 months	25.9 ± 2.0	34.5 ± 2.3[b]	25.2 ± 1.6	0.01
12 months	29.3 ± 3.3	34.3 ± 2.9[b]	27.4 ± 1.0	0.06
18 months	27.9 ± 2.6	31.9 ± 2.6	34.1 ± 1.2	0.9
IGFBP3 (mg/l)				
0 month	3.7 ± 0.2	3.8 ± 0.1	3.7 ± 0.2	
2 months	3.7 ± 0.3	4.1 ± 0.2[b]	3.6 ± 0.2	0.02
5 months	3.8 ± 0.2	4.3 ± 0.2[c]	3.4 ± 0.2	0.0004
12 months	3.9 ± 0.2	4.3 ± 0.1[d]	3.5 ± 0.2	0.008
18 months	4.5 ± 0.2[b]	4.7 ± 0.2[c]	4.4 ± 0.2[c]	0.8

Mean ± S.E.M.
[a] *P*-value refers to the difference between the treatment groups regarding changes in value from the start to the time-point indicated by *P*-value. Change within group from the start to indicated time-point.
[b] $P < 0.05$.
[c] $P < 0.001$.
[d] $P < 0.01$.

3.5. Growth, bone age, and adult height prediction

From the start to 5 months of treatment, the boys treated with testosterone and placebo grew slightly faster than the boys treated with testosterone and letrozole (9.9 ± 0.5 cm versus 7.3 ± 0.9 cm, respectively, $P = 0.02$). After 5 months, no statistically significant differences in growth velocity was observed between the two treated groups, although a borderline higher growth velocity after the discontinuation of treatments was observed in the testosterone-plus-letrozole-treated than in the testosterone-plus-placebo-treated group ($P = 0.06$). No correlation was found between the height velocity during the first 5 months and the serum IGF-I, IGFBP3, 17β-estradiol, testosterone, or DHT concentration at 5 months.

Inhibition of estrogen synthesis by letrozole delayed bone maturation. Within the follow-up period of 18 months, the bone age increased 1.7 ± 0.3 years in the testosterone-plus-placebo-treated group, but only 0.9 ± 0.2 years in the testosterone-plus-letrozole-treated group (significance of the difference between the treatment groups, $P = 0.03$; Fig. 3). In the untreated group, the respective increment was 1.1 ± 0.3 years (Fig. 3).

Letrozole treatment was associated with the increase in predicted adult height. In the testosterone-plus-placebo-treated group or in the untreated group, the predicted adult height did not change from the start to 18 months (Fig. 4). In contrast, in the testosterone-plus-letrozole-treated group, an increase of 5.1 ± 1.2 cm ($P = 0.004$) in predicted adult height was seen; in one patient the predicted adult height decreased by 3.5 cm, the increases in the other boys ranged from 2.5 to 8.8 cm. The difference between the treatment groups regarding the change in predicted adult height was significant ($P = 0.04$).

3.6. Progression of puberty

Puberty advanced in all groups during the follow-up. The Tanner stages of puberty progressed in similar fashion in the two treatment groups. The increase in testis volume was

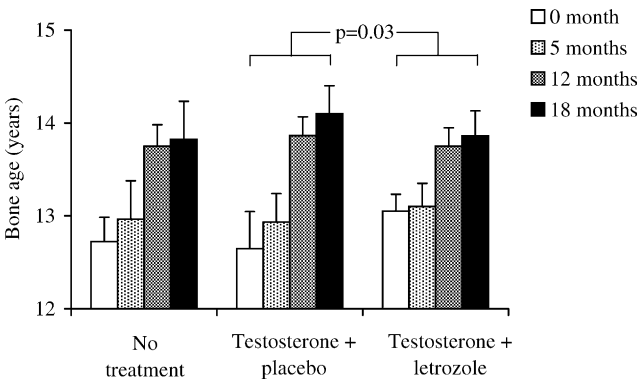

Fig. 3. Mean ± S.E.M. bone age. *P*-value refers to the difference between the treatment groups regarding changes in bone age in 18 months.

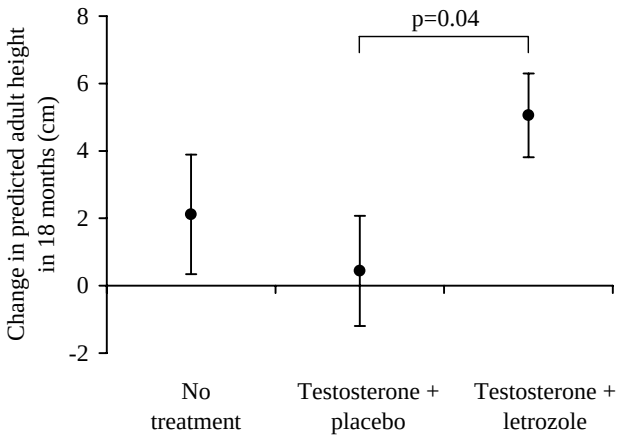

Fig. 4. Mean ± S.E.M. change in predicted adult height from the start to 18 months.

greater during the treatment with testosterone and letrozole than during the treatment with testosterone and placebo (Table 3). Gynecomastia was found in two boys in the testosterone-plus-placebo-treated group, in two boys in the testosterone-plus-letrozole-treated group, and in none of the boys in the untreated group.

3.7. Bone mineral density

The increase in the BMD of lumbar spine from the start was observed in both treatment groups at 12 and 18 months. An increase from the start in the femoral neck BMD was observed in the testosterone-plus-placebo-treated group at 12 and 18 months, but no change was seen in the testosterone-plus-letrozole-treated group. However, when the changes from the start in the lumbar spine and the femoral neck BMD were compared between the treated groups, no statistically significant differences were observed.

Table 3
Testis volume and serum inhibin B concentration

	No treatment	Testosterone + placebo	Testosterone + letrozole	*P*-value[a]
Testis volumes (ml)[b]				
0 month	5.9 ± 0.9	6.9 ± 1.2	5.5 ± 0.6	
5 months	8.1 ± 1.4	8.9 ± 1.5	11.5 ± 1.6	0.01
12 months	12.0 ± 1.8	13.4 ± 1.7	16.8 ± 1.2	0.0005
18 months	14.7 ± 2.3	18.6 ± 2.0	19.1 ± 1.1	0.2
Inhibin B (ng/l)				
0 month	153.7 ± 12.1	176.1 ± 12.5	161.2 ± 16.2	
5 months	186.4 ± 18.1	155.5 ± 21.8	200.5 ± 18.8	0.01
12 months	184.6 ± 10.3[c]	186.6 ± 19.0	219.8 ± 15.9[d]	0.1
18 months	180.6 ± 11.4[d]	216.8 ± 19.1	203.1 ± 16.6	0.8

Mean ± S.E.M.
[a] *P*-value refers to the difference between the treatment groups regarding changes in value from the start to the time-point indicated by *P*-value.
[b] From the formula length × width^2 × 0.52 [18]. Change within group from the start to indicated time-point.
[c] $P < 0.01$.
[d] $P < 0.05$.

Table 4
Serum concentrations of HDL-cholesterol, LDL-cholesterol, triglycerides, and insulin

	No treatment	Testosterone + placebo	Testosterone + letrozole	*P*-value[a]
HDL-cholesterol (mmol/l)				
0 month	1.8 ± 0.1	1.6 ± 0.09	1.6 ± 0.1	
5 months	1.6 ± 0.1	1.5 ± 0.1	1.2 ± 0.09[b]	0.005
12 months	1.5 ± 0.1	1.6 ± 0.1	1.4 ± 0.1[c]	0.06
18 months	1.4 ± 0.09[d]	1.4 ± 0.1	1.3 ± 0.09[c]	0.3
LDL-cholesterol (mmol/l)				
0 month	2.2 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	
5 months	2.7 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	0.5
12 months	2.5 ± 0.2	2.4 ± 0.2	2.6 ± 0.2	0.4
18 months	2.3 ± 0.2	2.4 ± 0.2	2.3 ±0.2	0.9
Triglycerides (mmol/l)				
0 month	0.58 ± 0.06	0.91 ± 0.1	0.89 ± 0.1	
5 months	0.62 ± 0.08	0.86 ± 0.1	0.96 ± 0.1	0.6
12 months	0.70 ± 0.2	0.89 ± 0.1	0.85 ± 0.1	0.6
18 months	0.69 ± 0.1	0.85 ± 0.1	1.1 ± 0.2	0.3
Insulin (mU/l)				
0 month	5.7 ± 0.8	7.1 ± 0.7	9.2 ± 1.3	
5 months	6.2 ± 1.2	6.8 ± 0.7	6.2 ± 0.9	0.2
12 months	6.6 ± 1.4	9.1 ± 1.2	6.5 ± 0.8[d]	0.02
18 months	7.6 ± 1.7	8.8 ± 1.2	9.4 ± 1.3	0.3

Mean ± S.E.M.
[a] *P*-value refers to the difference between the treatment groups regarding changes in value from the start to the time-point indicated by *P*-value. Change within group from the start to indicated time-point.
[b] $P < 0.01$.
[c] $P < 0.02$.
[d] $P < 0.05$.

3.8. Lipids

The HDL-cholesterol concentration decreased more during the treatment with testosterone and letrozole than during the treatment with testosterone and placebo. In the testosterone-plus-placebo-treated group, no significant change in the HDL-cholesterol concentration was observed during the follow-up ($P = 0.2$; Table 4). In the testosterone-plus-letrozole-treated group, the concentration decreased from the start to the lowest level at 5 months ($P = 0.002$), but no subsequent decrease was observed thereafter (Table 4). In the untreated group, the concentration decreased to the lowest value at 18 months ($P = 0.047$). At 18 months, i.e. 6 months after discontinuation of all treatments, the concentrations were similar in all three groups. The concentrations of LDL-cholesterol or triglycerides did not change during the follow-up in any of the three groups (Table 4).

3.9. Insulin

In the testosterone-plus-placebo-treated group, the insulin concentration did not change (Table 4). In contrast, in the testosterone-plus-letrozole-treated group, the insulin concentration decreased during letrozole treatment ($P = 0.04$,

from the start to 12 months), and after discontinuation of letrozole treatment, increased to the pretreatment level (Table 4). The changes in insulin concentration from the start to 12 months in the two treatment groups were different ($P = 0.02$).

To assess the relationship between insulin concentrations and BMIs, sex steroids, and growth factors, the changes from the start in insulin concentrations and the changes from the start in BMIs, concentrations of 17β-estradiol, testosterone, IGF-I, and IGFBP3 were determined; the data only contain the boys in the two treatment groups, omitting the boys in the untreated group.

The changes in serum insulin and IGF-I concentrations from the start to 12 and 18 months were correlated ($r = 0.5$, $P = 0.04$ for both). No associations between other variables were observed.

3.10. Gonadotropin secretion

In the testosterone-plus-letrozole-treated group, during treatment for 5 months, simultaneously with an increase of 606% in testosterone concentration ($P = 0.0005$) and unchanged concentrations of 17β-estradiol ($P = 0.2$), the basal LH concentration increased by 208% ($P = 0.001$; Fig. 5A), the basal FSH concentration by 167% ($P = 0.0005$; Fig. 5B), and the GnRH-induced LH response by 73% ($P = 0.0005$; data not shown), but the GnRH-induced FSH response did not change significantly ($P = 0.08$; Fig. 6). At 12 months, during the treatment with letrozole, the concentrations and GnRH-induced responses were at similar level than at 5 months.

3.11. Gonadotropin pulses

The gonadotropin pulses of a representative boy of the both treatment groups are shown in Fig. 6. Before the treatment, nocturnal elevation in gonadotropin concentrations, characteristic for early- and midpubertal boys, was seen in all the boys. During the treatment with testosterone and placebo, the nocturnal LH concentrations were lower

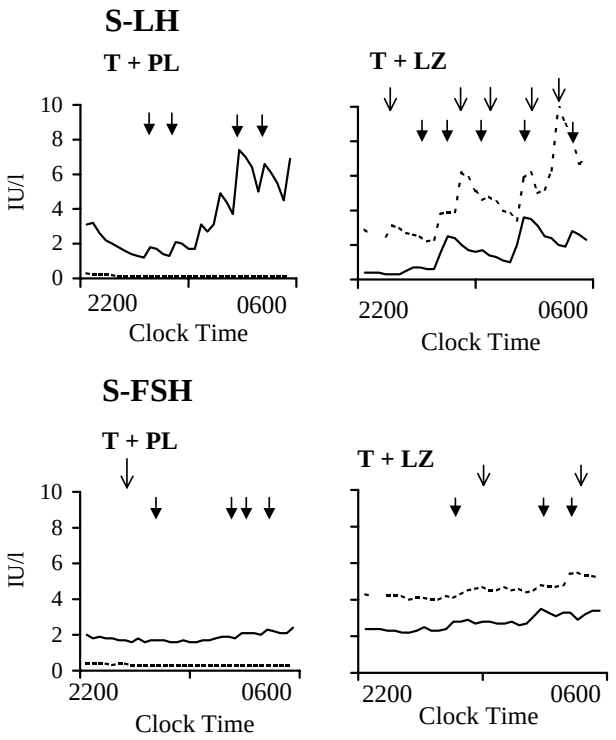

Fig. 6. The serum LH (upper panel) and FSH (lower panel) concentrations of a representative boy of both treatment groups. The concentrations have been determined every 15 min between 22:00 and 6:00 h before (solid line), and after (dashed line) 5 months of treatment. Arrowheads denote significant pulses before (closed), and after (open) 5 months of treatment. Boys in the t + pl group received testosterone and placebo, and boys in the t + lz group received testosterone and letrozole.

than at the start of the treatment in four of the five boys, and the FSH concentrations in all the boys. In three of the boys in the testosterone-plus-placebo-treated group, the LH concentrations decreased to or below the detection limit, and therefore no pulses could be determined, but in contrast, in other two boys, gonadotropin pulses and nocturnal increases in concentrations were also observed during the treatment. Since pulses could be observed in only two of five boys during the treatment with testosterone

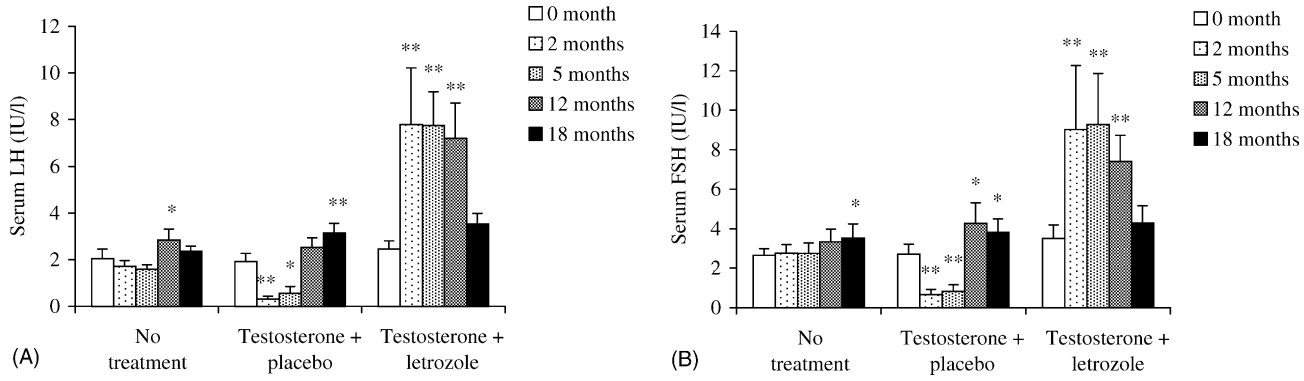

Fig. 5. Mean ± S.E.M. serum basal LH (A) and FSH (B) concentrations. Asterisks denote the significant changes from the start within the group: (∗) $P < 0.05$; (∗∗) $P < 0.01$.

and placebo, the changes in the pulse variables were not calculated.

In the testosterone-plus-letrozole-treated group, the nocturnal LH and FSH concentrations increased in all the boys during the treatment; gonadotropin pulses and the diurnal profile of gonadotropin secretion were also observed. In the testosterone-plus-letrozole-treated group, the mean LH pulse amplitude, calculated from all the LH pulse amplitudes detected, increased during the treatment ($P = 0.01$); no significant changes were seen in the FSH pulse amplitude, gonadotropin pulse frequencies, or interpulse intervals.

3.12. Inhibin B

In the testosterone-plus-placebo-treated group, the inhibin B concentration did not change (Table 3). In contrast, during the treatment with testosterone and letrozole, the concentration was higher than at the start, but after discontinuation of the letrozole treatment, it did not differ from the pretreatment concentration (Table 3). The changes in inhibin B concentration from the start to 5 months were different in the two treatment groups ($P = 0.01$) which is consistent with the difference in increases in testis volume and the divergent pattern of change in gonadotropin concentration.

4. Discussion

This study was undertaken to evaluate whether suppression of estrogen synthesis by the P450-aromatase inhibitor, letrozole, in pubertal boys delays maturation of the growth plates and ultimately results in increased adult height.

4.1. Efficacy in inhibiting estrogen synthesis

We observed that letrozole is an effective inhibitor of estrogen synthesis in boys of pubertal age, as previously found in adult males [26]. During treatment of testosterone and letrozole, 17β-estradiol concentrations remained low, at the pretreatment level, whereas during the treatment of testosterone alone and in the untreated group, during spontaneous progression of puberty, the concentrations increased. These findings indicate that letrozole inhibited the increase in 17β-estradiol concentrations associated with testosterone administration. Furthermore, they indicate functional inhibition of endogenous estrogen synthesis during letrozole treatment.

4.2. Tolerability and safety

We found that letrozole was well tolerated. No such side-effects were observed in any of the boys during 1-year letrozole treatment which would have indicated discontinuation of the treatment. However, our findings cannot exclude minor disadvantageous effects on BMD or HDL-cholesterol metabolism. For that reason, we recommend close follow-up of bone metabolism and lipid concentrations during treatment with P450-aromatase inhibitors.

Estradiol has been shown to act as a germ cell survival factor in the human testis in vitro [27]. The role of estrogen in human spermatogenesis can further be assessed by the findings in men with a mutation in a gene for ERα [1] and P450-aromatase enzyme [3]. The man with a mutation in the ERα gene, had a testis volume of 20–25 ml, a normal sperm density (25×10^6 ml^{-1}), but a decreased sperm viability of 18% (normal, >50%) [1]. The aromatase-deficient male had a subnormal volume of testis (8 ml), a decreased sperm count ($\leq 1 \times 10^6$ ml^{-1}; normal, $>20 \times 10^6$ ml) with 100% immotile spermatozoa [3]. However, abnormal findings in semen analysis in the aromatase-deficient man may not be related to a suppression of estrogen action, since azoospermia and infertility were also reported in a brother of this man, who had a normal P450-aromatase gene [3]. Moreover, the results of semen analysis did not change during the treatment with transdermal estradiol [3] suggesting non-estrogen-dependent spermatogenic damage. In our study, neither the treatment with testosterone and placebo nor with testosterone and letrozole had an adverse effect on testis size or inhibin B concentration. These findings suggest that 1-year treatment with P450-aromatase inhibitors in early- and midpubertal boys did not interfere with spermatogenesis.

5. Effect of P450 aromatase inhibition

5.1. On growth and maturation

We found that the boys treated with testosterone and letrozole grew at a slower rate during the first 5 months of treatment than the boys treated with testosterone alone. This finding confirms the concept that testosterone accelerates growth via an estrogen-dependent mechanism. The growth-enhancing effect of estrogens may result, at least partly, from stimulation of GH secretion by estrogens [28]. Consistently, in our study, IGF-I and IGFBP3 concentrations increased in the testosterone-plus-placebo-treated group during treatment, but did not change in the testosterone-plus-letrozole-treated group. Our findings further suggest that other factors, in addition to estrogens, are involved in pubertal growth acceleration in males, for during letrozole treatment, the majority of the boys grew with normal pubertal growth velocity in spite of low 17β-estradiol concentrations. However, the normal pubertal growth velocity during letrozole treatment could also have resulted from activation of ER. If aromatization of androgens is inhibited, steroid biosynthesis will be directed to produce 5α-DHT and subsequently 3β-androstanediol. The latter is a weak estrogen; it has been demonstrated to bind to estrogen receptors [29] and may therefore have estrogenic effects.

Consistent with our original hypothesis, we observed that inhibition of estrogen synthesis delayed bone maturation. It is worth noting that in the boys treated with testosterone and letrozole bone maturation was slower, despite the considerably higher androgen concentrations, than in the boys treated with testosterone alone. This finding confirms the view that estrogens are more important than androgens in bone maturation in pubertal males and agrees with observations on males who lack estrogen action [1–3]. Furthermore, we noted that, even after discontinuation of all treatments, the progression of bone maturation was slower in the boys treated with testosterone plus letrozole than in the boys treated with testosterone alone, indicating that the effect of the treatment outlasts the period of treatment.

Delayed bone maturation simultaneously with good growth response resulted in the increase in predicted adult height in boys treated with testosterone and letrozole. This supports our primary hypothesis that inhibition of estrogen synthesis in growing adolescents increases adult height. Since some boys with CDP do not appear to exploit their genetic growth potential [5–8], we assume that these boys can achieve an adult height closer to their genetic growth potential if the estrogen actions are inhibited. The observations that the predicted adult height did not change either in the boys who received no treatment or in those who were treated with testosterone alone are consistent with previous studies, which have shown that androgen treatment does not increase adult height [7,9,12].

The two treatments advanced the appearance of secondary sexual characteristics similarly, despite the considerably higher androgen concentrations in the boys treated with testosterone plus letrozole. The reason why highly supraphysiological androgen concentrations did not advance puberty more rapidly is unclear. It is possible that the maximal effect at the cell level is attained with a certain concentration of testosterone, and the concentrations above this threshold level do not enhance the biological effect. The minor differences in rates of progression of puberty between the two treatment groups may also have remained undetected due to a small number of patients in the present study.

5.2. On developing peak bone mass

We were unable to show any significant differences in the changes in BMD of lumbar spine or proximal femur between the groups treated with testosterone and placebo or with testosterone and letrozole. This indicates that a treatment for 1 year with a new, specific, and potent P450-aromatase inhibitor in pubertal boys is unlikely to have a significantly harmful effect on BMD. The cases with an inactive ERα [1] or defective aromatase enzymes [2,3] showed that an action of estrogen is needed for optimal development of peak bone mass in males. Patients with androgen insensitivity syndrome also have decreased BMD even before attainment of peak bone mass [30,31], indicating the importance of endogenous androgens in the development of peak bone mass in males. The observation that no difference in the changes in BMD between the two treatment groups was observed, may be explained by the fact that during letrozole administration, high androgen concentrations have compensated the disadvantageous effects of suppressed estrogen action.

However, minor disadvantageous effects on developing peak bone mass during the treatment with P450-aromatase inhibitors cannot be excluded on the basis of our study. We found no statistically significant increase in the femoral neck BMD during suppression of estrogen action by letrozole, while this parameter showed an increase in the testosterone-plus-placebo-treated group with intact P450-aromatase activity. For that reason, we suggest a close follow-up of bone metabolism during treatment with aromatase inhibitors.

5.3. On serum lipid concentrations

The difference in changes in HDL-cholesterol concentration between the boys treated with testosterone and letrozole and with those treated with testosterone and placebo indicates that the letrozole treatment may have minor disadvantageous effects on serum HDL-cholesterol concentrations. This is in accord with the finding of two aromatase-deficient men who had subnormal HDL-cholesterol concentrations which increased during estrogen administration [2–4]. In the letrozole-treated group, the lowest level in the HDL-cholesterol concentration was observed at 5 months, and no subsequent decrease was observed thereafter despite the continued treatment with letrozole. Moreover, the HDL-cholesterol concentration in the letrozole-treated boys was at the similar level than in the boys of the other two groups after the discontinuation of all the treatments indicating that 1 year's treatment with letrozole in pubertal boys is unlikely to have a permanent harmful effect on HDL-cholesterol concentration. However, our findings emphasize the importance of following-up HDL-cholesterol regularly during administration of P450-aromatase inhibitors.

The more profound decrease in the HDL-cholesterol concentrations during the treatment with testosterone and letrozole than during the treatment with testosterone and placebo may be due to the greater increase in androgen concentrations during the former than during the latter treatment. This is supported by the finding that decreasing HDL-cholesterol concentrations associated strongly with increasing testosterone concentrations. The important action of androgens in the regulation of HDL-cholesterol metabolism in adolescent boys was indicated by previous findings of inverse association between HDL-cholesterol and testosterone concentration even when factors affecting lipid metabolism had been taken into account [32,33], and an association of testosterone [34,35] or DHT treatment [36,37] with the decrease in HDL-cholesterol concentration in boys with delayed puberty.

The concentrations of LDL-cholesterol and triglycerides did not change during either of the two treatments, suggesting that treatment with P450-aromatase inhibitors do not contribute significantly in the regulation of concentrations of LDL-cholesterol or triglycerides in early- and midpubertal boys. This finding of unchanged concentrations of LDL-cholesterol and triglycerides in both treatment groups despite great difference in changes in sex steroid concentrations indicates that sex steroids may not have important regulatory roles in the metabolism of LDL-cholesterol and triglycerides in boys during early- and midpuberty. However, previous studies have suggested that variation in lipoprotein concentrations in adolescent boys can be explained, to some extent, by the changes in body mass, testosterone, estrogen, and their interactions [38,39]. Since physiological concentrations of estrogens appear to have a favorable impact on concentrations of LDL-cholesterol and triglycerides in adult men [2–4], we recommend the close follow-up of the concentrations of lipids during administration of P450-aromatase inhibitors.

5.4. On insulin sensitivity

Decreasing insulin sensitivity during puberty simultaneously with rising concentrations of sex steroids is well-known phenomenon [40–44]. The role of sex steroids in the development of puberty-associated insulin resistance is, however, unclear.

Our findings suggest that inhibition of estrogen synthesis in early- and midpubertal boys do no have disadvantageous effects on insulin sensitivity. During the administration of letrozole, the insulin concentration decreased, suggesting rather an improvement in insulin sensitivity. It is worth noting that insulin concentrations decreased during letrozole treatment despite a more than fivefold higher increase in the mean testosterone concentration in this group than in the group treated with testosterone and placebo in which the insulin concentration did not change. This finding suggest that an increase in the concentration of androgens during puberty does not contribute significantly to the development of puberty-associated insulin resistance in boys which is also indicated in other studies [35,36]. Neither do estrogens appear to have a role in the regulation of insulin sensitivity in boys during puberty. The changes in 17β-estradiol concentrations did not correlate with changes in insulin concentrations in our study, nor did the changes in insulin sensitivity in boys during progression of puberty associate with changes in estradiol concentrations in another study [45]. Decreasing insulin sensitivity has been shown to associate with increasing BMI in adolescent boys [46]. The decrease in insulin concentration during letrozole treatment was obviously not a consequence of decreased body mass, since an increase in BMI was seen during letrozole treatment. The decrease in insulin concentrations during letrozole treatment may have been due to the suppression of GH secretion since no pubertal increases in IGF-I or IGFBP3 concentrations were observed during letrozole treatment. This assumption is further supported by the finding that the changes in insulin concentration correlated positively with the changes in IGF-I concentration. These observations are in accord with the previous findings which have indicated that a decrease in insulin sensitivity during puberty results from the increasing action of GH [40–43,47]. Since androgens can be converted estrogens which are able to augment GH secretion [28], the effects of GH on regulation of insulin sensitivity may also indirectly reflect the action of sex steroids.

5.5. On gonadotropin secretion

Endogenous androgens appear to regulate negatively gonadotropin secretion in boys from early stages of puberty onward [48]. It has previously been demonstrated that supraphysiologic estradiol concentrations, attained by exogenously administered estradiol, decreased LH concentrations in early- and midpubertal boys [49] suggesting the existence of negative feedback between estrogen and gonadotropin secretion. We found that when the action of endogenous estrogens was suppressed by the administration of letrozole, LH and FSH concentrations increased despite very high androgen concentrations. These findings demonstrated that the negative feedback between endogenous estrogens and gonadotropin secretion, established in adult men [1–4,50], is already operative from early puberty onward. Furthermore, our observations suggest that androgens have a minor role compared with estrogens in regulating LH as well as FSH secretion in early- and midpubertal boys.

When the action of endogenous estrogens was suppressed by the testosterone plus letrozole treatment in early- and midpubertal boys, the LH pulse amplitude increased, but the LH pulse frequency, which is assumed to reflect the frequency of hypothalamic GnRH secretion [51,52], did not change. These observations suggest that low concentrations of endogenous estrogens in early- and midpubertal boys may not influence the GnRH pulse generator, and that, in boys during early- and midpuberty, the site of action of estrogens is the pituitary. Previous findings have, however, been contradictory. In early- and midpubertal boys, estradiol administration decreased LH concentrations and the LH pulse frequency, but had no effect on the GnRH-induced LH response or the LH pulse amplitude [49] suggesting the hypothalamic site of actions of estrogens in boys during early- and midpuberty. The discrepancy between our and the previous study concerning the site of action of estrogens in the hypothalamic-pituitary unit is unclear. One possibility is that supraphysiological concentrations of estrogens and endogenous estrogens act on gonadotropin secretion differently. In the previous study, supraphysiologic estradiol concentrations were attained by exogenous administration [49]. In contrast, in our study, in the group treated with testosterone plus letrozole, we demonstrated the effects of suppression of low, early-pubertal concentrations of estrogens. However, in adult men, endogenous estrogens have

been clearly demonstrated to act at the site of both pituitary and hypothalamus [50]. The evaluation of the precise sites of the negative feedback effects of gonadal steroids in hypophyseal-pituitary system is difficult in vivo. In humans, it is not possible to monitor hypothalamic GnRH secretion directly since GnRH is consumed in the hypophyseal-portal blood due to a short half-life of 2–4 min [53]. In animal models, exact temporal relationship between GnRH secretion and LH secretion has been observed [51,52], and thus monitoring LH pulse frequency in humans has been used in estimating the frequency of hypothalamic GnRH secretion. The results of indirect methods may not, however, demonstrate the true site of action at the hypothalamic-pituitary system. This, as well as different pulse detection programs which were used, may also partly explain the discrepancy in results between our and the previous study [49]. Thus, future studies with larger patient groups and longer periods of blood sampling are needed to confirm our conclusion of the pituitary site of action of endogenous estrogens and to clarify whether endogenous estrogens also act at the site of the hypothalamus in boys during early- and midpuberty.

The negative feedback regulation between FSH and endogenous estrogens has previously observed in adult males [25,50]. Our results suggest that this regulatory loop is already operative in early- and midpubertal boys. Although inhibin B participates in the regulation of FSH secretion from early- and midpuberty onward [54–58], the increase in FSH concentrations during letrozole treatment is probably not due to a diminished negative feedback signal from inhibin B, for inhibin B concentrations increased concomitantly with FSH concentrations.

The nocturnal augmentation of gonadotropin secretion, which is characteristic for early- and midpubertal boys [59,60], was demonstrated before the start of the treatments in all of the boys and also in all boys during the testosterone plus letrozole treatment. Thus, the suppression of the action of estrogens does not affect the diurnal profile of gonadotropin secretion, nor does the considerable increase in the concentrations of androgens. This observation is in accord with previous findings of a diurnal rhythm in gonadotropin secretion in children with gonadal dysgenesis [61,62] and indicates that the circadian rhythm of gonadotropin secretion is regulated by mechanisms mediated by the central nervous system.

6. Future prospects

The results of this study indicate that an increase in adult height may be attained in growing adolescent boys by inhibition of estrogen action. However, at the time of the completion of this study skeleton was relatively immature and growth was not decelerating in most of the boys. Therefore, it is important to confirm whether a P450-aromatase inhibitor treatment for 1 year in growing adolescent boys increases adult height by following-up the boys until attainment of final adult heights. Moreover, although our findings suggest that treatment for 1 year with a new P450-aromatase inhibitor in pubertal boys is unlikely to have a significantly harmful effect on developing peak bone mass, measuring BMD after the attainment of peak bone mass, at an age about 20–25 years, confirms this issue.

Since our results show that bone maturation is delayed by suppressing the action of estrogens in growing children, the treatment with fourth-generation P450-aromatase inhibitors may prove to be an efficient treatment in various growth disorders. Future studies are needed to establish if treatment with aromatase inhibitors can be used, e.g. in patients with precocious puberty or congenital adrenal hyperplasia with significantly advanced bone age. Moreover, they may be useful in some boys with delayed puberty and/or genetic short stature.

Acknowledgements

We gratefully acknowledge the help of Carina Ankarberg-Lindgren and Ensio Norjavaara, of Göteborg Pediatric Growth Research Center, Göteborg University, Sweden, for performing the 17β-estradiol assays, and of Ilkka Sipilä, of the Hospital for Children and Adolescents, for helping in determination of bone ages.

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