CLINICAL STUDY

Ethinylestradiol and testosterone have divergent effects on circulating IGF system components in adolescents with constitutional tall stature

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Abstract

Objective: Pharmacological doses of estrogens or testosterone are used to limit the final height of girls or boys with constitutional tall stature but the mechanism behind this growth inhibition is still debated. We therefore studied the changes in the circulating components of the insulin-like growth factor (IGF) system during high dose sex steroid therapy.

Design and methods: Twenty three girls and twenty boys with constitutional tall stature were treated with 100 µg ethinylestradiol per day or 250 mg testosterone ester every 14 days respectively. In 19 girls and 18 boys, the levels of IGF-I, free IGF-I, IGF-II, acid-labile subunit (ALS) and IGF binding proteins (IGFBP)-2 to -6 were measured before and 3–6 months after the start of therapy (group 1). In 18 girls and 11 boys, samples were collected at the end of therapy and 3 to 6 months afterwards (group 2). Fourteen girls and nine boys belonged to both groups. All parameters were measured by radioimmunoassay or ELISA.

Results: Levels of IGF-I were decreased significantly by estrogen treatment but remained unchanged during testosterone treatment. Free IGF-I decreased during estrogen treatment but increased during testosterone therapy. Estrogens increased IGF-II and testosterone reduced it. The important reduction of IGFBP-2 during estrogen therapy is not reproduced by androgen therapy, neither is the stimulation by estrogens of IGFBP-4. IGFBP-3 is not modulated by either sex steroid. We found that IGFBP-6 is up-regulated by testosterone but not by estrogens; the reverse is true for ALS, which increased during estrogen treatment but remained unchanged during testosterone treatment.

Conclusions: Our findings demonstrate that androgens and estrogens exert differential effects on the circulating levels of several IGF components.

European Journal of Endocrinology 152 597–604

Introduction

Pharmacological doses of estrogens or testosterone are used to limit the final height of girls or boys with constitutional tall stature (1). The mechanism behind this growth inhibition is still debated and may involve an interaction with the circulating insulin-like growth factor (IGF) system.

This system is composed of two ligands, IGF-I and IGF-II, which are bound in the circulation to at least 6 different high-affinity IGF-binding proteins (IGFBPs) (2–4). Since there is a 50% molar excess of binding proteins over the IGFs in serum, only a minute fraction of IGF is in the free, unbound form. Approximately 75% of IGF-I is bound to IGFBP-3 and this IGFBP-3–IGF-I complex is itself associated to an acid-labile subunit

(ALS) to form a ternary complex that is unable to cross the vascular endothelium. The IGF-binding proteins therefore determine the pharmacokinetics and tissue distribution of the IGFs and hence their biological activity (3).

We previously reported that total IGF-I, free IGF-I and IGFBP-2 decreased and IGFBP-4 increased during high dose estrogen therapy (5). High doses of testosterone are used to limit adult height in boys with tall stature and we wondered whether testosterone induces similar changes in the circulating IGF components. In the present study we report the change in the circulating components of the IGF system during a high dose testosterone treatment in tall boys and compared these changes to the effects of high dose estrogen therapy in tall girls.

Patients and methods

Forty-three constitutionally tall children (23 girls and 20 boys) were included in this study, approved by the ethical review board of the Antwerp University Hospital. Informed consent was obtained from patients and parents.

The diagnosis of constitutional tall stature was based on (a) familial occurrence of tall stature (height of one or both parents > 2 standard deviations (s.d.) above the mean), according to the standards of Freeman *et al.* (6), (b) predicted adult height of more than 178 cm for girls and 195 cm for boys and (c) exclusion of endocrine and other disorders that may explain their tall stature.

The girls received 0.1 mg ethinylestradiol every day at bedtime. This treatment was supplemented with 10 mg medroxyprogesterone acetate for the first 12 days of each month. Tall boys were treated with 250 mg testosterone enanthate intramuscularly every 2 weeks.

In 19 girls and 18 boys, EDTA plasma and serum samples were collected between 0900 and 1200 h, at the start of therapy and after 2 to 4 months of treatment (group 1). In 18 girls and 11 boys, samples were collected at the end of therapy, and 3 to 6 months afterwards (group 2). Fourteen girls and nine boys belonged to both groups.

Total IGF-I was measured in EDTA plasma by radioimmunoassay (RIA) (Biosource Europe, Nivelles, Belgium) after acid-ethanol extraction (7). Interassay variability was less than 10%. The concentration of IGF-II was determined after Sep-Pak C18 column extraction by a specific RIA (8). IGFBP-2 was analyzed by an RIA (DSL 7100) developed by Diagnostic Systems Laboratories (Webster, Texas, USA) using a rabbit polyclonal IGFBP-2 antiserum. IGFBP-3 was measured in a solid phase immunoradiometric assay (BC 1040; Biocode, Liège, Belgium) using a mouse anti-IGFBP-3 monoclonal antibody. Serum IGFBP-4 levels were determined by an RIA using recombinant human IGFBP-4 as tracer and standard, and antibodies against recombinant human IGFBP-4 raised in guinea pigs (9). Serum IGFBP-5 levels were also determined by an RIA using recombinant human IGFBP-5 as tracer and standard, and antibodies against recombinant human IGFBP-5 raised in guinea pigs (10). IGFBP-6 was measured by radioimmunoassay using a rabbit polyclonal antiserum

raised against a synthetic fragment containing residues 90–118 that was also used as a tracer. Technical details and data on reproducibility and stability after storage have been described elsewhere (11).

Easy dissociable or 'free' IGF-I was measured by a two-site immunoradiometric assay (DSL 9400; Diagnostic Systems Laboratories).

The acid-labile subunit was determined by ELISA (DSL 10-8200; Diagnostic Systems Laboratories). Due to the large interassay variability, the samples of a particular patient were always measured in the same assay. For the patients in group 1, the concentrations of ALS found in the samples obtained during treatment were expressed as a fraction of those taken before treatment which was set at 100%. Similarly, in group 2 the results for the samples taken after treatment were expressed as a fraction of those obtained at the end of treatment.

All results are reported as medians and their range is indicated in brackets. The statistical analysis of the results was performed by Wilcoxon signed ranks test (SPSS version 11; SPSS, Chicago, IL, USA).

Results

Patient characteristics (Table 1)

The girls had a median age of 13.7 years and a median bone age of 13.5 years at the start of treatment. At the end of 1 to 2.5 years of treatment (group 2), the girls had a median bone age of 15.5 years. Treatment of the boys was started at a median age of 14.6 and a bone age of 14.25 years when their testicular volume was at least 8 ml. After a median 1.5 years of treatment (group 2), the boys had a median bone age of 17.5 years.

Changes in total IGF-I, IGF-II and free-dissociable IGF-I (Fig. 1)

Total plasma IGF-I decreased significantly from 530 (270–660) ng/ml to 350 (210–440) ng/ml during estrogen treatment and rose again from 258 (220–400) ng/ml to 450 (170–680) ng/ml after cessation of therapy. In contrast, total IGF-I did not change during testosterone treatment in boys.

During the first 3 months of estrogen therapy, IGF-II transiently increased from 407 (186–612) ng/ml to 471 (365–693) ng/ml. The IGF-II levels at the end of

Table 1 Characteristics of boys and girls at the start (group 1) and at the end (group 2) of treatment. The results are shown as medians and ranges.

	Boys		Girls	
	Group 1 (start) (n = 18)	Group 2 (stop) (n = 11)	Group 1 (start) (n = 19)	Group 2 (stop) (n = 18)
Age (years)	14.6 (13.4–18.4)	16.05 (14.4–19.3)	13.7 (11.5–5.7)	15.7 (12.7–17.2)
Bone age (years)	14.25 (12.75–15.50)	17.50 (15.5–18.0)	13.50 (12.25–15.00)	15.50 (13.50–17.00)
Height (cm)	186.5 (180.0–201.0)	191.9 (187.9–197)	176.4 (172.6–181.9)	180.8 (178.3–182.7)
Predicted height (cm)	199.7 (195.2–214.1)	—	182.3 (178.4–189.3)	—

Figure 1 Changes in total IGF-I, free IGF-I and IGF-II before (before), after 3 months (on), at the end (end) and 3–6 months after estrogen or testosterone therapy (after) of constitutionally tall children. The box represents the median and the 25th and 75th centiles; the whiskers show the 10th and 90th centile. The plus signs represent outliers. a, $P \leq 0.05$ (Wilcoxon signed ranks test); ns, not statistically significant.

therapy (group 2) however were not different from the values found at the start of treatment (group 1). In contrast, testosterone reduced IGF-II concentration from 415 (341–620) ng/ml to 365 (262–511) ng/ml. After the cessation of treatment, the levels recovered to pretreatment values. Both steroids also exerted an

opposite effect on free IGF-I which fell significantly from 2.3 (0.9–5.4) ng/ml to 0.6 (0.2–1.5) ng/ml during treatment with estrogen but rose during testosterone therapy from 2.6 (0.9–4.8) ng/ml to 3.1 (1.7–7.8) ng/ml after 3 months and to 4.0 (1.7–7.8) ng/ml at the end of treatment.

Modulation of IGFBP-2 to -6 (Fig. 2)

Estrogen treatment reduced circulating IGFBP-2 levels from 482 (185–1120) ng/ml to 208 (69–437) ng/ml. The IGFBP-2 concentrations rose again after the end of therapy. In contrast, testosterone treatment increased IGFBP-2 from 578 (240–879) ng/ml to 717 (223–1260) ng/ml. Serum IGFBP-2 concentrations at the end of treatment in group 2 were comparable to the levels before treatment in group 1 and remained unchanged 3 to 6 months after the end of treatment.

The circulating IGFBP-3 concentrations decreased slightly after 3 months of testosterone treatment but not during estrogen treatment. In both cases, the levels at the end of treatment in group 2 were lower than at the start of treatment in group 1. No further changes were seen 3–6 months after the end of therapy.

Serum IGFBP-4 levels increased during estrogen treatment and decreased afterwards while testosterone had no effect on this binding protein. IGFBP-5 remained unaffected by either steroid.

The IGFBP-6 levels in the circulation increased during testosterone administration and decreased afterwards. Treatment with estrogen tended to induce an opposite effect.

In testosterone-treated boys the ALS in serum decreased to $97 \pm 11\%$ of pretreatment values and rose again to $104 \pm 5\%$ of the values at the end of treatment. In girls, serum ALS rose significantly to $119 \pm 15\%$ during estrogen treatment. Three to six months after the end of therapy, the ALS concentration decreased to $86 \pm 12\%$ of the levels obtained at the end of treatment.

Discussion

High dose estrogen treatment reduced the levels of total IGF-I as reported previously in a smaller group of tall girls (5). Similarly in adults, estrogens invariably decrease plasma IGF-I levels whether given in low doses as an oral contraceptive in young adult women (12, 13) or as hormonal replacement therapy in postmenopausal women (14, 15) or in high doses to human volunteers (16), acromegalics (17) or male to female transsexuals (18). This effect is probably explained by a direct effect of estrogens on IGF-I gene transcription (19, 20). Although the administration of testosterone to pubertal tall boys yields a similar degree of growth inhibition, we did not observe any effect of testosterone on their total IGF-I levels. It has been reported that plasma IGF-I increases after administering testosterone to normal men (21), hypogonadal men (22, 23), boys with delayed puberty (24–30) or female to male transsexuals (31). However, when testosterone was given to men with growth hormone (GH) deficiency total and free IGF-I remained unchanged (24, 32, 33). This implies that the increase in IGF-I by substitutive doses of testosterone are

mediated by GH. We speculate that testosterone did not increase total IGF-I in these tall pubertal boys because GH secretion is already maximally stimulated by the endogenous testosterone production.

Both sex steroids had minor but opposite effects on the total IGF-II concentrations in serum. In a previous study on a limited number of patients we reported an increase in IGF-II when estrogen treatment was discontinued (5) but this finding is not reproduced in this larger group. The effect of estrogens on circulating IGF-II levels remains controversial. Most authors found that oral contraception (13), and oral (34, 35) or transdermal (34) estrogen supplementation had little effect on IGF-II levels in postmenopausal women or in Turner patients (36). However, a significant decrease in IGF-II was found after 5 years in a longitudinal study of estrogen replacement in perimenopausal women (Danish Osteoporosis Prevention Study) (37) and estrogen withdrawal by a gonadotropin releasing hormone (GnRH) agonist in adult women with endometriosis resulted in a 40% increase in total IGF-II concentrations (38). IGF-II levels also decreased in postmenopausal women treated with high doses of diethylstilbestrol (DES) as anti-tumor therapy (39). There are no data in the literature on changes in IGF-II during testosterone therapy. One possible explanation for our findings is that IGF-II competes with IGF-I for the same binding sites on the IGF binding proteins and therefore rises when IGF-I decreases (estrogen effect) and falls when IGF-I increases. However, the possibility of a direct but opposite effect of both steroids on IGF-II gene transcription cannot be excluded.

Before any treatment, the girls had slightly lower levels of IGFBP-2 than the boys. Estrogens further reduced the IGFBP-2 serum concentration to less than 50% of pretreatment levels but testosterone increased serum IGFBP-2, at least during the first months of therapy. These opposite effects may explain why adolescent girls and young women have slightly lower levels of IGFBP-2 than men of the same age (40). The reduction of IGFBP-2 by estrogens was not seen in women taking oral contraceptives (13) nor in postmenopausal women or Turner patients on hormone replacement therapy (35, 36). In contrast, IGFBP-2 decreased in postmenopausal women treated with high doses of DES for metastatic breast cancer (38). These data suggest that serum IGFBP-2 levels decrease only at high doses of estrogens. The increase in serum IGFBP-2 caused by testosterone therapy has not been reported before but testosterone increased IGFBP-2 synthesis in human fetal osteoblasts *in vitro* (41).

Before any treatment the IGFBP-3 levels were similar in boys and girls. At the end of treatment serum IGFBP-3 was 15–20% lower in both sexes. The decrease in IGFBP-3 was already noticeable after 3 months of testosterone in the boys. In humans, concentrations of estrogens within the normal physiological range have

Figure 2 Levels of IGFBP-2 to -6 at the start (start), 3 months after the start (3mo), at the end (end) and 3-6 months after the cessation of high dose estrogen or testosterone therapy (after) in constitutionally tall adolescents. The box represents the median and the 25th and 75th centiles; the whiskers show the 10th and 90th centile. The plus signs represent outliers. a, $P \leq 0.05$ (Wilcoxon signed ranks test); ns, not statistically significant.

little or no effect on serum IGFBP-3 levels. For example, during the normal menstrual cycle IGFBP-3 levels remain constant (13, 42–44). In postmenopausal women, oral estrogen supplementation caused no change (34, 37, 44, 45) or a limited decrease (35, 47) in IGFBP-3 levels. Transdermal estrogen supplementation had no effect on IGFBP-3 either (34, 45, 46, 48). Circulating IGFBP-3 levels also remained unchanged in adult Turner women who started estrogen therapy. However, a 30% decrease in IGFBP-3 was also noted in postmenopausal women receiving high doses of DES for metastatic breast cancer (38). It is plausible that IGFBP-3 only decreases during prolonged exposure to high doses of estrogens. A low or high dose of testosterone did not change serum IGFBP-3 levels in normal men (21, 29, 49, 50), female to male transsexuals (31) or oophorectomized women (51).

The changes in serum IGFBP-3 levels, observed in the present study, are not the consequence of changes in ALS, which remained unaffected by testosterone but was clearly increased by estrogen. The latter finding agrees with the reduction in ALS reported in untreated postmenopausal women (47).

We found an important increase in serum IGFBP-4 during estrogen treatment. Estrogens are known to stimulate IGFBP-4 mRNA synthesis in ovariectomized animals (52–54) and increase the amount of IGFBP-4 in the serum of postmenopausal women receiving estrogen substitution (55), of women on oral contraceptives (13) and of women receiving high doses of DES for breast cancer (39). Since IGFBP-4 is generally considered an inhibitor of IGF-I action (56, 57) it may mediate the growth inhibiting effect of estrogens. Serum IGFBP-4 did not change during the first months of testosterone therapy but the serum levels were higher in group 2 at the end of therapy than in group 1. One hypothesis is that some of the testosterone is aromatized to estrogens, causing an increase in IGFBP-4. This hypothesis, however, does not explain why the IGFBP-4 concentrations remain higher when all treatment is stopped.

Mohan *et al.* (10) reported a rise in IGFBP-5 during pubertal development in girls. Estrogens were therefore hypothesized to regulate IGFBP-5 synthesis. Our study shows that serum IGFBP-5 concentrations are not significantly influenced by either ethinylestradiol or testosterone. Therefore, the rise in IGFBP-5 during puberty in females is probably the result of an estrogen-dependent increase in GH secretion during puberty. There are no data on the effect of testosterone therapy on IGFBP-5 concentrations.

We confirmed that there is little difference in serum IGFBP-6 between boys and girls during puberty (11). High doses of estrogens and testosterone had opposite effects on IGFBP-6. Estrogens tended to decrease while testosterone significantly increased serum IGFBP-6 levels. We are not aware of any

data in the literature on the modulation of IGFBP-6 by sex steroids.

The opposite effects of estrogen and testosterone on IGF-I, free IGF-I and IGF-II are difficult to explain on the basis of changes in the IGFBPs. For example, IGFBP-2 and IGFBP-6 have a higher affinity for IGF-II than for IGF-I. Because testosterone increases both IGFBP-2 and IGFBP-6, one would expect an increase in IGF-II levels. However, the contrary is found. The concentrations of most IGFBPs, with the exception of IGFBP-3, rise during testosterone therapy, yet free IGF-I also rises. Other mechanisms must therefore be involved, such as a direct effect on gene expression or a change in affinity of the binding proteins for their ligands by limited proteolysis.

We have previously speculated that the growth inhibition caused by estrogens is partly explained by a dramatic fall in free IGF-I and the generation of inhibiting IGFBPs such as IGFBP-4 and an IGFBP-3 fragment (5). Unfortunately, a reliable assay to quantify this inhibitory IGFBP-3 fragment is currently unavailable. Boys treated with high doses of testosterone also have higher levels of estradiol due to aromatization of testosterone. This mechanism cannot, however, be invoked to explain the growth inhibition caused by testosterone. Our data indicate that any effects of increased levels of estradiol in these boys must be overruled by those of testosterone.

We did not provide any direct evidence that the changes in the circulating IGF system are important mediators in the growth inhibitory effects of high doses of sex steroids. Indeed, estrogens and androgens have pleiotropic effects on many biological processes that occur, for example, in the growth plate. Correlation studies between the degree of growth inhibition and the changes in the various IGF components may be a first step to find a causal link, but definitive answers should be provided by selectively adding or removing each of these components.

Acknowledgements

This study was supported by a grant from the Belgian Study Group for Pediatric Endocrinology (RR) and NIH grant AR31062 (SM).

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Received 21 September 2004

Accepted 27 December 2004