Somatic and psychological effects of low-dose aromatase inhibition in men with obesity-related hypogonadotropic hypotestosteronemia.

European Journal of Endocrinology (2013) **169** 705–714 ISSN 0804-4643

CLINICAL STUDY

Somatic and psychological effects of low-dose aromatase inhibition in men with obesity-related hypogonadotropic hypotestosteronemia

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Abstract

Introduction: Reduced testosterone levels are frequently observed in obese men. Increased aromatase activity may be an etiological factor.

Objective: In this study, we evaluate the clinical effects of aromatase inhibition in obesity-related hypogonadotropic hypotestosteronemia (OrHH).

Methods: Double-blind, placebo-controlled, 6-month trial in 42 obese men with a BMI >35 kg/m^2, and a serum total testosterone <10 nmol/l. All patients started on one tablet of 2.5 mg/week, with subsequent dose escalation every month until a serum total testosterone of 20 nmol/l was reached.

Endpoints: Psychological function, body composition, exercise capacity, and glucose, lipid, and bone metabolism.

Results: Thirty-nine patients completed the study according to protocol. Letrozole decreased serum estradiol from 119.1 ± 10.1 to 59.2 ± 6.1 pmol/l ($P<0.001$), and increased serum LH from 3.3 ± 0.3 to 8.8 ± 0.9 U/l ($P<0.0001$) and serum total testosterone from 8.6 ± 0.7 to 21.5 ± 1.3 nmol/l ($P<0.0001$). Significant effects on the predefined endpoints were not observed.

Conclusion: Despite a marked rise in serum testosterone, low-dose aromatase inhibition had no somatic or psychological effects in men with OrHH.

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Introduction

Reduced serum total testosterone levels are frequently observed in obese men. Its prevalence increases in proportion to BMI (1, 2, 3). Serum total testosterone levels <10 nmol/l are found in about 60% of men with a BMI >40 kg/m^2, and reduced free-testosterone levels (cut-off 225 pmol/l) in about 40% of these cases. Obesity-related hypogonadotropic hypotestosteronemia (OrHH) is characterized by the combination of a low total testosterone, a marked decrease in sex hormone-binding globulin (SHBG), a relatively high serum estradiol (E_2) and luteinizing hormone (LH) levels within the normal range, but inappropriately low for the degree of hypotestosteronemia (1, 2). The decrease in total testosterone is explained by two mechanisms, i.e. a decrease in SHBG levels and reduced LH secretion (1, 2, 4). The latter gains in importance with increasing degrees of obesity and this ultimately leads to reduced levels of free testosterone in severely obese men (4). Obesity-related suppression of LH secretion has been related to excessive aromatase activity (5, 6). Increased conversion of androgens to E_2 and estrone (E_1) promotes estrogen-mediated suppression of pituitary LH and follicle-stimulating hormone (FSH) secretion and will cause a secondary decrease in testosterone production. Leptin- or cytokine-induced inhibition of LH secretion and hypothalamic–pituitary dysfunction caused by obstructive sleep apnea may also have a role in the development of OrHH (7, 8).

Androgen deficiency is associated with increased fat mass, decreased muscle and bone mass, diminished muscle strength, insulin resistance, dyslipidemia, and hypertension (9, 10). It may also affect brain function and can lead to fatigue, depressive moods, loss of initiative and drive, and docile behavior (11, 12, 13, 14). This suggests that normalization of serum testosterone in obese men might be a beneficial approach. However, testosterone supplementation alone is unlikely to normalize the gonadal status in OrHH. It may normalize serum testosterone levels, but it will also fuel estrogen synthesis and cause a major rise in serum E_2 levels that could have adverse effects. Partial inhibition of aromatase activity is a more attractive approach, at

DOI: 10.1530/EJE-13-0190
Online version via www.eje-online.org

least in theory. So far, two open-label, uncontrolled pilot studies have shown that the aromatase inhibitor letrozole can normalize serum testosterone and E_2 levels in OrHH (15). However, the clinical impact of this intervention is currently not known.

The aim of the present study was to examine whether normalization of serum androgens by aromatase inhibition might improve body composition, glucose, and lipid metabolism and induce favorable psychological changes.

Subjects and methods

Patients

Severely obese, but otherwise healthy men, 20–50 years of age, with a BMI 35–50 kg/m^2 were recruited by advertisement in local newspapers. All subjects underwent a general physical and psychological examination and laboratory screening, with blood samples taken after an overnight fast, between 0800 and 1000 h. OrHH was defined as a serum total testosterone level <10 nmol/l, associated with a serum LH level <9 U/l, and normal serum free thyroxine (FT_4), thyroid-stimulating hormone (TSH), prolactin, cortisol, adrenocorticotrophic hormone (ACTH), and insulin-like growth factor 1 (IGF1) levels. Additional inclusion criteria were normal pubertal development, normal testicular volume, intact sense of smell, and a stable body weight for at least 3 months preceding the study. Exclusion criteria were clinical or biochemical evidence of pituitary or hypothalamic disease, serum E_2 levels <40 pmol/l, type 2 diabetes mellitus requiring insulin to keep HbA1c below 7.0%, biochemical evidence of hemochromatosis (transferrin saturation >45% and serum ferritin >300 μg/l), symptomatic prostate disease or elevated serum PSA levels, unstable cardiac disease, liver disease, medication known to affect the gonadal axis, psychiatric disease, and men who had discontinued smoking within 6 months before the study.

The study protocol was approved by the Medical Ethical Committee and was registered at ClinicalTrials.gov as NCT00138710. All study subjects gave their written informed consent.

Study design

The patients were randomized to double-blind treatment with placebo or letrozole (tablets of 2.5 mg). All subjects started on one tablet per week. Each month, the dose was increased by one tablet a week until the total testosterone target level of 20 nmol/l was reached, side effects occurred, or the maximum dose of seven tablets (17.5 mg) per week was reached. The predefined dose schedule was as follows: 1st month, tablet on Monday; 2nd month, tablet on Monday and Friday; 3rd month, tablet on Monday, Wednesday, and Friday; 4th month, one tablet a day from Monday to Friday; 5th and 6th month, one tablet every day.

The dose was reduced by one tablet a week if total testosterone was >30 nmol/l or E_2 was <40 pmol/l for at least 4 weeks, or if severe side effects were reported that were considered to be related to treatment. If the decrease in dose did not result in normalization of serum total testosterone or serum E_2, the dose was further reduced by another tablet a week, and so on, until the total testosterone target of 20 nmol/l was reached or serum E_2 had increased to a value >40 pmol/l.

A mild hypocaloric diet was prescribed, consisting of 75% of the amount required to maintain ideal body weight. Patients were also advised to walk at least 30 min a day.

Outcome measures

Follow-up visits were scheduled every 4th week for 6 months. At each visit, body weight, waist circumference, and blood pressure were measured, and blood was collected between 0800 and 1000 h for measurement of serum LH, FSH, E_2, total testosterone, SHBG, albumin, and PSA. Body composition, bone density, exercise capacity, psychological characteristics, and an extensive laboratory investigation including whole blood count, serum creatinine, liver enzymes, androstenedione, E_1, α-subunits, FT_4, TSH, total triiodothyronine (T_3), IGF1, cortisol, ACTH, HbA1c, fasting glucose and C-peptide, glucose tolerance test (measurement of glucose and insulin levels at baseline, 30, 60, 90, and 120 min, after ingestion of 75 g glucose), fasting lipid profiles, and the bone markers N-terminal propeptide of type 1 procollagen (PINP) and C-terminal telopeptide of type 1 collagen (ICTP) were measured at baseline and after 6 months.

Laboratory assays

LH, FSH, and total testosterone were measured by solid-phase, two-site chemoluminescent immunometric assay (CLIA) on the Immulite 2500 analyzer (Siemens, Diagnostic Products Corporation, Los Angeles, CA, USA; male reference ranges: LH 1–8 IU/l, FSH 1–11 IU/l, and total testosterone 10–30 nmol/l). Total testosterone had an analytical sensitivity of 0.5 nmol/l, validated against a commercial calibrator (Siemens, Diagnostic Products Corporation).

E_2, SHBG, and C-peptide were measured with an ECLIA assay on an E170 analyzer (Roche Diagnostics GmbH, male reference ranges: E_2 40–160 pmol/l, SHBG 14.5–48.4 nmol/l, and C-peptide 0.27–1.28 nmol/l). E_2 had a detection limit of 40 pmol/l and an analytical sensitivity of 18.4 pmol/l, validated against isotopic dilution gas chromatography–mass spectrometry. E_1 was measured with a competitive RIA (Immunotech, Beckman Coulter (Prague, Czech Republic), male reference range: 10–80 ng/l). Androstenedione was

measured with a competitive radiometric immunoassay (Siemens, Diagnostic Products Corporation, male reference range: 2.8–10.5 nmol/l). IGF1 was measured with a CLIA on an Immulite 2000 analyzer (Siemens, Diagnostic Products Corporation, reference range age dependent). Intact PINP and ICTP were measured by RIA (Orion Diagnostica (Espoo, Finland), male reference range: 22–87 and 2.1–5.0 μg/l respectively). Free testosterone was calculated according to the equation of Vermeulen *et al*. (16). Insulin sensitivity was calculated using the homeostasis model assessment index (17).

Calorie intake

A mild hypocaloric diet, consisting of 75% of the amount needed to maintain ideal body weight, was prescribed in both groups. The calculation was based on the Harris–Benedict formula: $((66, 473)+(13.7516\times BW)+(5\times S)-(6.755\times A))\times 4.2$ kJ, where BW, ideal body weight (kg); *S*, stature (cm); and *A*, age (years). 1 kJ = 0.2390 kcal: 25% was added for physical activity (18).

Body composition and bone density

Body composition was measured by the deuterium dilution method according to the technique described by Westerterp *et al*. (19). Bone density of the lumbar spine and both femoral necks was measured by dual-energy X-ray absorptiometry (Lunar DXA, GE Healthcare, Madison, WI, USA).

Exercise capacity

The excessive body weights precluded the use of a standard bicycle test. Therefore, a staircase test was more feasible. All patients were asked to climb a 60-step staircase (equivalent to three levels) as fast as they could. Time, pulse rate, blood pressure, and oxygen saturation were measured at baseline, upon completion of climbing the staircase, and after a 5-min rest.

Psychometrics

Psychological testing was carried out with tests validated for the Dutch population: Symptom Checklist-90 (SCL-90), Groninger Intelligence Test (GIT), and the Dutch Personality Questionaire (DPQ). The SCL-90 measures anxiety, depression, somatization, insufficiency, distrust, hostility, and sleep disorders (20). The DPQ measures inadequacy, social inadequacy, rigidity, grievance, self-satisfaction, dominance, and self-esteem (21). The GIT measures overall intelligence and is included to investigate the patients' ability to understand and perform the psychometric tests (22). The reported scores for a general Dutch population were used as reference values.

Safety monitoring

Serum PSA levels were measured every 4 weeks. In case of a rise in serum PSA or symptoms of obstructive prostate disease, the study medication was discontinued and the patient was referred to the urologist. Serum E_2 levels were measured every 4 weeks. Dose reduction was carried out if serum E_2 decreased to a level <40 pmol/l.

Statistical analyses

A power analysis was performed to assess the number of participating subjects to be able detect a 10 kg difference in change of body weight between the two groups. With $\alpha=0.01$ and $\beta=0.05$, and a S.D. of 6 kg (23), 13 subjects in each group are required. Results of the study are shown as mean values and S.E.M. In this proof-of-concept study only patients who completed the 6-month study according to protocol were included in the analysis. After confirmation of normal distribution, differences in baseline characteristics between the placebo and letrozole group were examined by *t*-test. The results of treatment were analyzed by paired or unpaired *t*-tests, where appropriate. Mann–Whitney or Wilcoxon matched pair tests were used for variables that did not follow a normal distribution. Fisher's exact test was used to compare categorical variables between groups. A *P* value <0.05 was considered to represent statistical significance. E_2 levels that fell below the detection limit of 40 pmol/l were given a value of 20 pmol/l for statistical purposes.

Results

Baseline characteristics

Forty-two men were included in the study. Three men did not complete treatment for personal reasons not related to the study. Thirty-nine men completed the study according to protocol, 18 received letrozole and 21 were treated with placebo.

Age at inclusion was 44.6 ± 1.6 years (range 29.8–56.4 years), with a BMI of 40.8 ± 1.1 kg/m^2 (range 33.7–53.0 kg/m^2). Serum total testosterone ranged from 4.6–9.9 nmol/l, total E_2 from 51–210 pmol/l, and serum LH from 1–8.4 U/l. At baseline, the two groups were well matched for all outcome measures, except for a higher ICTP level in the letrozole group (Tables 1 and 2). In the placebo group, one man was treated with tolbutamide 250 mg once daily and one used metformin 500 mg once daily. In the letrozole group, four men used metformin in a stable dose throughout the study (mean daily dose 1200 ± 374 mg). Decreased libido tended to occur more frequently in the letrozole group (nine vs four men, $P=0.09$). Erectile dysfunction was reported equally in both groups (four vs three men, $P=0.68$). We did not use a validated questionnaire.

Table 1 Baseline characteristics (B) and changes (Δ) in body composition and serum hormone levels after 6 months of treatment.

	Placebo ($n=21$)	**Letrozole** ($n=18$)	***P****
Body weight (kg)			
B	132.2±4.6	136.1±4.9	0.57
Δ	−5.1±1.9†	−5.1±1.5‡	0.99
BMI (kg/m^2)			
B	40.4±1.1	41.9±1.2	0.37
Δ	−1.6±0.6†	−1.6±0.5‡	0.49
Waist (cm)			
B	130.9±2.9	132.6±2.8	0.68
Δ	−4.5±1.6†	−4.1±0.8§	0.45
Fat mass (kg)			
B	52.9±3.3	56.7±3.2	0.41
Δ	−5.9±1.8‡	−7.0±1.5§	0.31
Fat-free mass (kg)			
B	80.3±2.9	79.7±1.9	0.86
Δ	+0.2±0.6	+1.3±0.9	0.32
LH (U/l)			
B	2.9±0.4	3.3±0.3	0.13
Δ	−0.1±0.3	+5.5±0.9§	<0.0001
FSH (U/l)			
B	4.8±0.5	5.1±0.6	0.72
Δ	−0.2±0.2	6.3±0.8§	<0.0001
α-Subunits (U/l)			
B	0.3±0.0	0.3±0.0	0.84
Δ	+0.1±0.1	+0.1±0.0‡	0.03
Total testosterone (nmol/l)			
B	8.8±0.5	8.6±0.7	0.80
Δ	+0.8±0.7	+12.9±0.8§	<0.0001
Free testosterone (pmol/l)			
B	244.3±14.1	243.7±19.4	0.98
Δ	+17.4±18.8	+447.3±24.4§	<0.0001
Androstenedione (ng/l)			
B	7.8±0.5	7.5±0.5	0.37
Δ	+0.1±0.4	+2.4±0.6‡	<0.001
Estradiol (pmol/l)			
B	124.3±9.3	119.1±10.1	0.70
Δ	−18.9±6.1‡	−59.8±12.2§	0.002
Estrone (ng/l)			
B	59.4±4.5	76.0±8.6	0.13
Δ	−2.9±3.8	−36.6±6.5§	<0.001
Albumin (g/l)			
B	41.2±0.6	41.6±0.5	0.59
Δ	−0.6±0.5	−0.9±0.6	0.45
SHBG (nmol/l)			
B	16.5±1.2	16.1±1.2	0.81
Δ	+1.3±0.7	−0.1±0.7	0.21
PSA (U/l)			
B	0.7±0.1	1.1±0.2	0.15
Δ	+0.1±0.1	+0.3±0.0§	0.03
Decreased libido			
B	4	9	0.09
Δ	3	8	0.09
Erectile dysfunction			
B	3	4	0.68
Δ	3	3	0.99
IGF1 (nmol/l)			
B	23.5±4.2	18.4±1.3	0.48
Δ	+2.3±1.1	−2.6±1.3	<0.01

*P significance level of differences between groups; †$P<0.05$, ‡$P<0.01$, and §$P<0.001$, significance level of changes within group. LH, luteinizing hormone; SHBG, sex hormone-binding globulin.

The baseline scores of the psychological items are summarized in Table 3. A higher score correlates with more severe psychological dysfunction. The scores were within the reference ranges and no statistical differences between the two groups were observed. The advised caloric intake was 1632±39 kcal/day in the placebo group and 1639±40 kcal/day in the letrozole group ($P=0.90$).

Table 2 Glucose homeostasis, lipid levels, bone markers, and bone density at baseline (B) and the changes (Δ) after 6 months of treatment.

	Placebo ($n=21$)	Letrozole ($n=18$)	P^*
Fasting glucose (mmol/l)			
B	6.0±0.1	6.0±0.2	0.68
Δ	−0.0±0.2	−0.3±0.2	0.19
Fasting C-peptide (nmol/l)			
B	1.4±0.1	1.6±0.1	0.16
Δ	−0.2±0.1†	−0.3±0.1	0.84
HbA1c (%)			
B	5.7±0.1	6.0±0.2	0.99
Δ	+0.1±0.1	−0.2±0.1	0.18
AUC glucose$_{OGTT}$ (mmol×min/l)			
B	1068±49	1158±57	0.24
Δ	−33±45	−6±47	0.68
AUC insulin$_{OGTT}$ (mU×min/l)			
B	11 858±978	13 422±1353	0.24
Δ	−2844±828‡	−2473±1403	0.81
HOMA-IR			
B	1.1±0.4	0.9±0.2	0.45
Δ	−0.4±0.3	−0.0±0.1	0.28
HDL (mmol/l)			
B	1.1±0.0	1.2±0.0	0.64
Δ	−0.0±0.0	−0.1±0.0‡	0.04
LDL (mmol/l)			
B	3.3±0.2	3.1±0.2	0.57
Δ	−0.4±0.1†	−0.2±0.2	0.24
TG (mmol/l)			
B	1.7±0.2	2.2±0.3	0.16
Δ	−0.1±0.2	+0.1±0.2	0.45
PINP (μg/l)			
B	36.7±2.4	38.2±3.5	0.83
Δ	+7.2±4.7	+8.4±2.2‡	0.15
ICTP (μg/l)			
B	3.5±0.2	4.2±0.2	0.02
Δ	+0.2±0.3	+0.7±0.3†	0.09
Lumbar spine (*T*-score) (ΔBMC/g)			
B	0.4±0.3	−0.0±0.4	0.39
Δ	+0.1±0.6	−0.2±0.9	0.76
Left hip (*T*-score) (ΔBMC (g/cm^2))			
B	0.9±0.3	0.9±0.3	0.97
Δ	−0.3±0.3	+0.3±0.2	0.16
Right hip (*T*-score) (ΔBMC (g/cm^2))			
B	0.6±0.3	0.9±0.3	0.54
Δ	+0.1±0.3	+0.2±0.3	0.82

$^*P$ significance level of differences between groups. $^{\dagger}P<0.05$, $^{\ddagger}P<0.01$, significance level of changes within group. TG, triglycerides; HOMA-IR, homeostasis model assessment of insulin sensitivity; ΔBMC/g, change in bone mineral content.

Letrozole dose

At 6 months, all subjects in the placebo group reached a dose of seven tablets per week. In the letrozole group, the mean dose at 6 months was 1.9±0.4 tablets per week (4.7±1.0 mg/week). Ten men reached the total testosterone target level on one tablet per week, four men needed two tablets per week, and two men needed four tablets per week. Only one patient in the letrozole group received a dose of seven tablets per week. His total testosterone level did not rise above 15.5 nmol/l, despite a rise in serum LH from 3.3 to 9.4 U/l. One subject was treated with only 0.5 tablet per week because serum E_2 decreased below 40 pmol/l on a higher dose. After dose reduction, serum E_2 rose to 67 pmol/l with a total testosterone level of 29.6 nmol/l.

Changes in serum hormone levels

Letrozole therapy decreased serum E_2 from 119.1±10.1 to 59.2±6.1 pmol/l ($P=0.0001$), and E_1 levels from 76±8.5 to 31.9±2.9 ng/l ($P<0.0001$). After 6 months, serum E_2 levels were <40 pmol/l in three men in the letrozole group, and in one of the men of the placebo group (Fig. 1). Letrozole increased serum LH from 3.3±0.3 to 8.8±0.9 U/l ($P<0.0001$), FSH from 5.1±0.6 to 11.4±0.2 ($P<0.0001$), total testosterone from 8.6±0.7 to 21.5±1.3 nmol/l ($P<0.0001$), free testosterone from 244±19 to 691±39 nmol/l ($P<0.0001$), and androstenedione from 7.5±0.5 to 9.9±0.6 nmol/l ($P=0.0013$). Letrozole did not affect serum albumin or SHBG levels. Free testosterone rose to supra-physiological levels in 12 out of 18 men, with levels ranging from 636 to 1005 pmol/l (Fig. 1).

In the placebo group, with the exception of a slight but significant drop in serum E_2 level from 124±9.3 to 106±8.1 pmol/l ($P=0.006$), there were no significant changes in LH, FSH, total, or free testosterone, androstenedione, E_1, SHBG, or albumin levels (Table 1 and Fig. 2). Neither letrozole nor placebo had any effect on TSH, free T_4, T_3, prolactin, cortisol, or ACTH levels (data not shown). Erectile dysfunction or libido did not change significantly.

Table 3 Baseline psychological characteristics.

	Reference range	Placebo ($n=21$)	Letrozole ($n=18$)	P^*
Anxiety	12–15	12.0±0.8	13.3±1.6	0.53
Depression	20–24	21.8±2.0	23.7±3.1	0.70
Somatization	15–19	17.0±1.4	17.6±1.3	0.76
Insufficient thinking	11–15	12.7±1.0	14.9±1.7	0.29
Distrust	22–27	23.2±1.2	24.9±2.2	0.82
Hostility	7–9	7.4±0.6	6.9±0.3	0.93
Sleeping disorder	4–6	5.3±0.6	5.7±0.7	0.45
Neuroticism	113–124	117.7±6.5	128.0±11.4	0.49
Inadequacy	7–14	6.6±1.8	9.6±1.9	0.05
Social inadequacy	7–13	6.9±1.5	8.3±1.7	0.53
Rigidity	24–32	22.6±1.9	24.7±1.7	0.44
Resentment	15–22	14.1±1.2	17.6±1.7	0.11
Self esteem	9–15	12.2±1.2	10.4±0.7	0.22
Dominance	13–20	18.9±1.5	18.5±2.0	0.88
Self sufficiency	25–32	31.1±1.2	27.3±1.4	0.048

$^*P$ significance level of differences between groups.

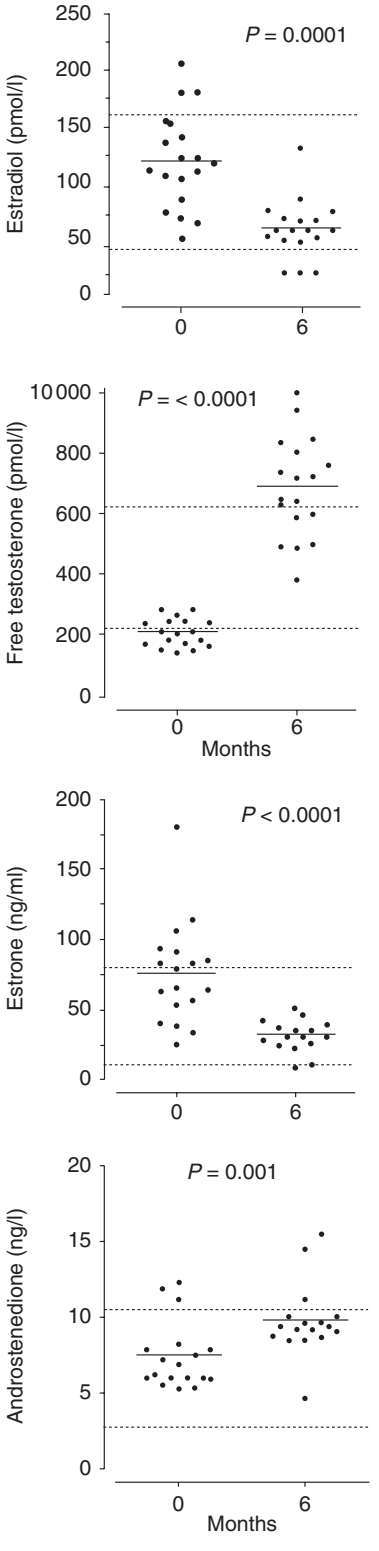

Figure 1 Changes in the levels of free serum total estradiol, estrone, testosterone, and androstenedione in the letrozole-treated patients before and after 6 months of treatment.

Changes in body composition and exercise capacity

Body weight decreased significantly in both groups, but the changes did not differ between the groups (letrozole vs placebo: -5.1 ± 1.5 vs -5.1 ± 1.9 kg, $P=0.99$). Similarly, waist circumference decreased in both groups but the changes did not differ between the groups (-4.1 ± 0.8 vs -4.5 ± 1.6 cm, $P=0.45$). Changes in fat mass and fat-free mass did not differ between groups (Table 1), and there also were no significant differences between groups in exercise performance (data not shown).

Changes in metabolic parameters

Compared with placebo, letrozole did not affect the fasting glucose levels, HbA1c, or insulin sensitivity (Table 2).

Safety

PSA levels increased in the letrozole group, but symptomatic prostate disease did not occur (letrozole, $+0.3\pm0.0$ U/l; placebo, $+0.1\pm0.0$ U/l, $P=0.03$). The letrozole group demonstrated a slight but significant increase in hemoglobin levels (letrozole, $+0.2\pm0.1$ mmol/l; placebo, -0.2 ± 0.1 mmol/l, $P=0.01$). IGF1 levels showed a small but significant decrease in the letrozole group (letrozole, -2.6 ± 1.3 nmol/l; placebo, $+2.3\pm1.1$ nmol/l, $P<0.01$). HDL levels decreased marginally in the letrozole group (letrozole, -0.1 ± 0.0 mmol/l; placebo, 0.0 ± 0.0 mmol/l, $P=0.04$).

Plasma alanine and aspartate aminotransferase levels dropped slightly but significantly in both groups to a similar extent. Letrozole treatment did not change PINP and ICTP levels. Bone mineral content did not change in either group.

Changes in psychological parameters

Letrozole treatment had no effect on any psychological item that was tested (data not shown).

Discussion

The main finding of this study is that low-dose aromatase inhibition in men with OrHH can induce a major rise in serum total testosterone but does not induce beneficial effects on clinical endpoints. This lack of effect cannot be explained by an overall lack of power or poor matching. Although both groups contained several men with low normal instead of subnormal free testosterone levels, and this could have reduced the chance to detect an effect, it is unlikely to explain the negative findings. A *post hoc* analysis of men with

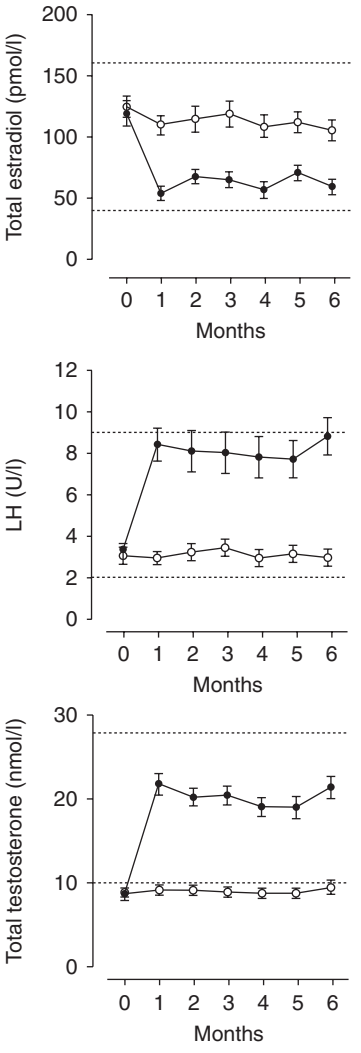

Figure 2 Mean changes in serum estradiol, serum LH, and testosterone levels during 6 months of treatment in patients treated with placebo (open dots) or letrozole (black dots). The dashed lines represent the lower and upper limits of normal.

baseline free testosterone levels <225 mol/l, with 90% power to detect a 10 kg difference in weight at $\alpha=0.05$, also did not reveal an effect. We therefore conclude that we are looking at a true lack of effect. To explain this unexpected finding, the following hypothesis is proposed: the clinical effects normally associated with testosterone replacement therapy in men are a result of both testosterone and E_2 receptor activation. In case of aromatase inhibition, these parallel and often synergistic effects of testosterone and E_2 are uncoupled, which could explain the lack of clinical effects. As this study was not primarily designed to test this role of estrogens in men, this hypothesis should be regarded as a retrospective explanation of the findings; however, an explanation that is also supported by direct and indirect evidence from the literature.

Previous experience with aromatase inhibition

The lack of effect of aromatase inhibition is in agreement with studies performed in other types of patients. Aromatase inhibitors have been used in boys with idiopathic short stature to promote linear growth (24, 25), in eugonadal young adults to study metabolic effects (26, 27), and in eugonadal and hypogonadal elderly to examine the effect on frailty (26, 28, 29). The only clinical effect shown thus far is an increase in body height of about 6 cm in boys with idiopathic short stature, i.e. an effect caused by the reduction of estrogen levels below the normal range. Other major effects were not observed.

Aromatase inhibition vs testosterone replacement

The hormonal changes induced by letrozole are fundamentally different from those seen during testosterone replacement therapy, and this may be the key to understand the differences in clinical efficacy. Testosterone replacement raises both serum testosterone and E_2, whereas aromatase inhibitors induce a rise in testosterone but a decrease in E_2 (15). It appears that the expected beneficial effects of a rise in testosterone are offset by the decrease in estrogens. It is likely that some of the effects of testosterone replacement are not caused by testosterone itself but are predominantly induced by the rise in E_2. The testosterone/E_2–GH interaction also needs to be considered. To some extent, the lack of anabolic and lipolytic effects may also have been caused by reduced GH/IGF1 secretion. Such a mechanism is suggested by the small but statistically significant decrease in serum IGF1 that was observed in the letrozole group. Testosterone is known to stimulate GH secretion, an effect that is mediated by estrogens (30, 31). Letrozole may have uncoupled this interaction.

Findings in animal studies

Animal studies support the hypothesis of testosterone and E_2 synergism in males. Both androgen receptors (AR) and estrogen receptors (ER) are omnipresent in male animals, and activation of both receptors is crucial to observe the full benefit of testosterone replacement therapy. Dihydrotestosterone, a non-aromatizable androgen that only activates the AR, does not change body composition in orchidectomized rats. In contrast, treatment with E_2 increases muscle mass, prevents the post-orchidectomy increase in fat mass, and is more effective in preventing bone loss (32). The importance of E_2 has also been shown in models of males with estrogen deficiency such as the ERα knockout (ERαKO) and the aromatase-KO mice. The ERαKO mice demonstrate adipocyte hypertrophy, glucose intolerance, insulin resistance, and reduced energy expenditure (33).

Aromatase-KO mice have increased intra-abdominal adipose tissue, decreased lean body mass, dyslipidemia, and insulin resistance, and administration of estrogen decreases their fat depots to the size of WT littermates (34). All these observations support the notion that E_2/ERα signaling is of major importance for the regulation of fat mass, lean body mass, and physical activity in male mice (33, 34). ERα activation alone is sufficient to reduce fat mass, but AR and ERα signaling are both required to achieve an optimal muscle mass (35). Observations in adult men with aromatase deficiency (ArD) confirm the importance of estrogens in men (36). ArD men have high LH levels, normal or elevated serum levels of testosterone and androstenedione, and low or undetectable serum E_2 and E_1 levels. Thus, they lack estrogen but not testosterone effects. They are very tall because of non-fusion of the epiphyses, and have excess abdominal fat, reduced bone mineral density, elevated triglyceride levels, low HDL, hepatic steatosis, and insulin resistance. E_2 treatment induces epiphysial fusion, increases bone density, and improves glucose and lipid metabolism in these men (37).

Potentially deleterious effects

Aromatase inhibitors can be expected to have deleterious effects in men when doses are used that cause estrogen deficiency. In this study with low-dose letrozole, we did not detect adverse effects. Apparently, the dose reduction performed when serum E_2 levels decreased below 40 pmol/l was sufficient to prevent that. Trials using high-dose aromatase inhibition reported deleterious effects. Mild vertebral deformities were detected in 45% of the letrozole-treated boys (38). In elderly men treated with anastrozole 1 mg daily for a year, spinal BMD was significantly decreased (39).

Limitations of this study

Power analysis to detect an effect was only based on changes in body weight, and therefore a type 2 error for other clinical endpoints cannot be definitely excluded. Addition of a testosterone-treated group as control might have served to better understand the study results by offering a background of combined testosterone and E_2 effects. Fear of excessive E_2 effects was the main reason to exclude this as an option.

Future research

The only potential indication for aromatase inhibition in adult men that has arisen so far is the treatment of obesity-related infertility. A relationship has been found between increased BMI and decreased sperm concentrations, sperm motility, abnormal morphology, and decreased fertility rates (40). Aromatase inhibition raises serum FSH levels in hypo- and eugonadal men, and this may have positive effects on fertility. Aromatase inhibition in overweight and obese men with oligozoospermic infertility and a high E_2:testosterone ratio as a result of increased aromatase activity improved semen quantity and quality and raised the frequency of pregnancies (40).

Advised approach in men with OrHH

A recent meta-analysis has convincingly shown that weight loss is associated with a substantial rise in total and free testosterone levels and a decline in estrogen levels (41). The decrease in BMI was the main determinant of the rise in testosterone levels. Therefore, induction of weight loss is recommended as the option of first choice in men with OrHH.

In conclusion, a 6-month course of low-dose letrozole-induced aromatase inhibition in men with OrHH did not have beneficial somatic or psychological effects. This lack of effect might be attributed to an uncoupling of testosterone and estrogen effects. For non-sexual functions in men, estrogens seem to be at least as important as androgens. If this point of view proves to be correct it may become necessary to include serum estrogen levels in the definition of male hypogonadism.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This investigator-initiated study was supported by Novartis (Basel, Switzerland). Novartis did not participate in the design, data analysis, or writing of the manuscript.

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Received 7 March 2013
Revised version received 12 July 2013
Accepted 15 August 2013