CLINICAL STUDY

Short-term aromatase inhibition: effects on glucose metabolism and serum leptin levels in young and elderly men

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Abstract

Objective: To assess and compare the effects of short-term aromatase inhibition on glucose metabolism, lipid profile, and adipocytokine levels in young and elderly men.

Design and methods: Ten elderly and nine young healthy men were randomized to receive letrozole 2.5 mg daily or placebo for 28 days in a crossover design.

Results: Both in young and elderly men, active treatment significantly increased serum testosterone (+128 and +99%, respectively) and decreased estradiol levels (−41 and −62%, respectively). Fasting glucose and insulin levels decreased in young men after active intervention (−7 and −37%, respectively) compared with placebo. Leptin levels fell markedly in both age groups (−24 and −25%, respectively), while adiponectin levels were not affected by the intervention. Lipid profile was slightly impaired in both groups, with increasing low density lipoprotein-cholesterol levels (+14%) in the younger age group and 10% lower levels of APOA1 in the elderly. A decline in IGF1 levels (−15%) was observed in the younger age group. No changes in weight or body mass index were observed in either young or old men.

Conclusions: Short-term aromatase inhibition appears to affect glucose metabolism in young men, and lipid metabolism, including leptin secretion, in young and elderly men. Furthermore, the short period of exposure suggests that these changes might be mediated by direct effects of sex steroids rather than by changes in body composition.

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Introduction

Administration of aromatase inhibitors to men has recently been proposed as a potential therapeutic option in elderly men with low serum testosterone (T) levels (1), and in obesity-related male hypogonadism (2, 3). However, questions remain about the long-term efficacy and safety of these compounds (4), and presently little is known on the short-term effects of aromatase inhibition on glucose and lipid metabolism.

From epidemiologic data, we know that the ageing-related decline in T levels in men is often associated with an increase in (abdominal) fat mass, loss of muscle, and bone mass, impaired glucose tolerance, elevated lipid levels, and more prevalent atherosclerosis (5). Since all of these features are similar to those observed in patients with androgen deficiency (5), it has been hypothesized that the decrease in T levels contributes to at least some aspects of this detrimental metabolic profile. One hypothesis explaining this progressive hypogonadal–obesity–metabolic disease cycle is that increased aromatase activity caused by increasing fat mass accumulation leads to the depression of T production,

further enhancing the deposition of abdominal fat (6). From this point of view, administration of aromatase inhibitors to men with (obesity-related) hypogonadotropic hypogonadism may offer an alternative therapeutic option to restore the disrupted balance between sex steroid levels.

We have previously reported that oral administration for 28 days of an aromatase inhibitor markedly increased serum LH and T levels, while lowering estradiol (E₂) levels, in both young and elderly men (7). In these two age groups, we now examined and compared the effects of short-term aromatase inhibition on their metabolic profile by measuring serum levels of fasting insulin, glucose, lipids, leptin, insulin-like growth factor 1 (IGF1), and adiponectin. The rationale was that changes observed after short-term treatment are more likely to reflect direct effects of the hormonal changes induced by aromatase inhibition, whereas changes in metabolic profile after longer term treatment are more likely to reflect, at least for a substantial part, indirect effects resulting from changes in body composition.

Subjects and methods

Study subjects

Details of the subject selection and randomization have been described previously (7). In short, we recruited 10 healthy young (20–33 years, mean age 25.9 ± 4.6 years) and 10 healthy elderly (68–81 years, mean age 76.1 ± 5.0 years) men who gave their written informed consent to participate in this study, approved by the Ethical Review Board of the Ghent University Hospital. Subjects had to have a normal medical history, physical examination, and biochemical measures of hematological, hepatic, renal, gonadal, and metabolic function at screening. Smoking, excessive alcohol or substance abuse, and active disorders were considered as exclusion criteria. One of the participants of the younger age group had type 1 diabetes mellitus and was for this reason excluded from the present analysis.

Study design

The study design was a randomized, double-blind, placebo-controlled crossover intervention (7). Patients were first screened 2 weeks before the start of the intervention. Placebo or letrozole (2.5 mg daily, Femara®; Novartis AG, Stein, Switzerland) orally taken on awakening was administered in random order each day for a 28-day period, separated by a 14-day treatment-free washout period. In both age cohorts, the same number of subjects ($n=5$) started with the aromatase inhibitor. Compliance was assessed by pill counting. Venous blood sampling was obtained after overnight fasting and 10 min of bed rest between 0800 and 1000 h. Because of a prominent carry-over effect from letrozole administration at day 43, data of the placebo phase for participants using placebo in the second phase of the study were excluded from statistical analysis.

Hormonal and biochemical assays

Serum was stored at -80°C until analysis; all samples from the same subject were assayed in a single assay run. Fasting triglycerides, cholesterol fractions, glucose, and insulin serum concentrations were determined using standard laboratory assays (modular immunoassay, Roche Diagnostics). Intra- and interassay coefficients of variation (CV) for all parameters were less than 6 and 3% respectively. Apolipoproteins A1 (APOA1) and B (APOB) plasma concentrations were determined by nephelometry (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). Commercial immunoassays were used to determine serum E_2 (Incstar, Stillwater, MN, USA; adapted protocol with use of double amount of serum), T and sex hormone-binding globulin (SHBG) (Orion Diagnostica, Espoo, Finland), leptin (Linco Research, St Charles, MO, USA),

IGF1, and adiponectin (Diagnostic Systems Laboratories, Webster, TX, USA). Intra- and interassay CV were 6 and 5%, 1.5 and 3.7%, 5.6 and 7% for leptin, IGF1, and adiponectin respectively. Free T (FT) and free E_2 (FE_2) levels were calculated using a validated equation based on the mass action law (7). Normal reference ranges for FT (4–25 ng/dl) and FE_2 (0.19–1.19 pg/ml) are based on observations in 677 healthy young men, using the same methodology (8). For all considered hormonal variables, values for each sampling day are the mean of the result for two samples obtained at a 20-min interval.

Data analysis

All variables were checked for the normality of distribution by the Kolmogorov–Smirnov one-sample test for goodness-of-fit. Unless otherwise stated, all variables are expressed as mean $\pm$ s.d. Differences between age groups for baseline values were explored using an independent Student's *t*-test or Mann–Whitney *U* test in the case of non-Gaussian distribution. Student's paired *t*-test or the Wilcoxon matched pairs signed-rank sum test was used for analyses of changes from baseline for each biochemical parameter. All statistical procedures were performed using SPSS 12.0 software package (SPSS Inc., Chicago, IL, USA) and a *P* value of <0.05 was considered to indicate statistical significance; all *P* values were two-tailed.

Results

Baseline characteristics

Participants of both age groups had body mass indices within the normal range. No differences in weight, lipids, insulin, glucose, leptin, or adiponectin levels were observed at screening between young and elderly men (data not shown, all $P>0.1$), except for IGF1 levels, which were higher in the younger age group (462 vs 245 ng/ml, $P<0.001$). Mean triglyceride (TG) levels at baseline were in the low range for the general population. Baseline hormonal values have been published previously and were not different between both groups except for higher SHBG and lower FT levels in the elderly (7). No differences between subjects in the subgroups starting with placebo or letrozole were observed for either body mass index (BMI), sex steroid levels, or other variables (data not shown).

Changes after 28-days intervention by placebo versus letrozole

Changes after 28 days of letrozole and placebo treatment are listed in Table 1. During placebo administration, no changes in sex steroid or gonadotropin levels were found (7), although we did observe a

Table 1 Hormonal and biochemical changes after 28 days of letrozole and placebo treatment in the younger and elderly age groups.

	Treatment					
	Letrozole			Placebo		
	d 1/42 (n=9)	d 28/70 (n=9)	P value	d 1 (n=4)	d 28 (n=4)	P value
Young men						
Weight (kg)	78.2±9.4	79.2±9.1	0.058	80.2±3.5	79.5±3.0	0.19
BMI (kg/m ²)	24.4±2.9	24.7±2.9	0.054	24.4±1.8	24.2±2.0	0.19
Glucose (mg/dl)	0.85±0.06	0.79±0.08	0.022	0.82±0.08	0.78±0.08	0.20
Insulin (IU/l)	8.8±2.5	5.5±2.0	0.001	6.4±0.5	5.4±2.1	0.47
Total cholesterol (mg/dl)	166±25	173±31	0.32	166±50	161±15	0.85
HDL-cholesterol (mg/dl)	53±13	50±13	0.24	56±13	51±12	0.008
LDL-cholesterol (mg/dl)	95±24	108±30	0.038	112±43	92±23	0.17
APOA1 (mg/dl)	151±20	144±26	0.40	180±48	149±12	0.20
APOB (mg/dl)*	69 (58–88)	73 (67–85)	0.11	81 (42–87)	59 (44–84)	0.47
Triglycerides (mg/dl)*	70 (56–164)	76 (47–154)	0.77	92 (71–107)	65 (55–113)	0.72
Leptin (ng/ml)*	5.0 (2.4–6.1)	3.4 (2.1–4.5)	0.015	4.6 (2.7–10.0)	5.3 (2.3–10.2)	0.72
IGF1 (ng/ml)	477±146	404±143	0.002	421±75	397±58	0.07
Adiponectin (µg/ml)	8.7±3.6	9.1±2.7	0.70	9.1±2.6	6.3±1.8	0.22
Elderly men						
Weight (kg)	72.3±10.4	71.9±11.3	0.57	66.6±13.1	67.6±13.4	0.17
BMI (kg/m ²)	24.9±2.4	24.8±2.8	0.87	23.4±3.2	23.7±3.2	0.14
Glucose (mg/dl)	0.99±0.27	0.94±0.19	0.16	1.14±0.37	1.04±0.25	0.40
Insulin (IU/l)	8.5±5.3	7.5±4.8	0.18	8.2±4.0	9.1±3.1	0.52
Total cholesterol (mg/dl)	197±50	190±49	0.30	196±56	199±69	0.79
HDL-cholesterol (mg/dl)	64±19	59±14	0.08	65±16	67±24	0.54
LDL-cholesterol (mg/dl)	116±41	116±45	0.96	110±51	115±51	0.25
APOA1 (mg/dl)	173±34	156±30	0.016	169±27	177±44	0.64
APOB (mg/dl)*	98 (73–106)	93 (66–110)	0.58	104 (58–116)	96 (52–122)	0.69
Triglycerides (mg/dl)*	84 (61–107)	70 (53–107)	0.083	116 (55–160)	91 (55–113)	0.28
Leptin (ng/ml)*	4.3 (3.2–6.3)	3.1 (2.5–5.0)	0.028	2.9 (1.7–7.5)	3.3 (2.2–8.2)	0.043
IGF1 (ng/ml)	241±81	257±87	0.28	218±68	223±73	0.49
Adiponectin (µg/ml)	11.9±6.0	11.7±7.1	0.68	10.2±5.5	10.2±6.0	0.99

Data are presented as mean ± s.d., unless *median (first–third quartile) in case of non-Gaussian distribution.

decrease in high density lipoprotein (HDL)-cholesterol levels in the younger and a slight increase in leptin levels in the elderly participants.

Letrozole administration decreased E₂ (−41 and −62% in the young and older age groups respectively) and SHBG levels (−18 and −15% respectively). In both age groups, a similar increase in T (+128 and +99% respectively) and gonadotropin levels was observed (7). After letrozole treatment, all younger and three out of ten elderly participants had FT levels moderately above the upper reference value, whereas all younger and elderly participants had FE₂ levels below the lower reference values as observed in healthy young men.

In both age groups, no significant changes in weight or BMI were observed. Fasting plasma glucose levels decreased modestly (−7%) and, consistently, fasting insulin levels decreased by 37% in young men. However, it should be noted that a similar though non-significant decrease in glucose levels was observed during placebo treatment. By contrast, parameters of glucose metabolism were not significantly altered in the elderly. Lipid profile was impaired in the younger age group with a 14% increase in low density lipoprotein (LDL)-cholesterol levels after intervention, which was reflected by a non-significant increase of APOB, the main protein

constituent of LDL-cholesterol. In the elderly, a 10% decrease in APOA1 levels was observed, together with a trend toward lower serum levels of HDL-cholesterol. No significant changes in total cholesterol or TG levels were found.

Short-term aromatase inhibition led to a decline in IGF1 levels in the younger age group (−15%), whereas no changes were observed in the elderly. Regarding leptin levels, we observed marked decreases in both age groups: young men displayed 24% lower leptin levels after active treatment while leptin levels in elderly men were lowered by 25%. Adiponectin levels were not significantly altered by our intervention.

Discussion

This study shows that hormonal changes induced by aromatase inhibition have modest but significant short-term effects on parameters of glucose and lipid metabolism, with some apparent differences between young and elderly men.

In young men, aromatase inhibition resulted in presumably lower fasting glucose levels, and markedly lower insulin and IGF1 levels. These findings

corroborate with those of Wickman *et al.* who reported decreased insulin levels, correlating with changes in IGF1 levels, in boys treated long-term with T plus letrozole (9). By contrast, 2 years administration of letrozole to younger peripubertal boys did not improve insulin sensitivity (HOMA) (10). In line with our results in elderly men, Dougherty *et al.* found no changes in insulin sensitivity (HOMA) in elderly men with mild hypogonadism after 12 weeks of aromatase inhibition (anastrozole 1 mg daily or 2 mg weekly) (11).

Although Wickman *et al.* (9) and Hero *et al.* (10) reported decreased HDL-cholesterol levels in their patients, no changes in LDL-cholesterol levels were observed and no changes in lipid levels were found by Dougherty *et al.* (11). In another study, 6-week administration of testolactone (4×250 mg daily) to adult men decreased HDL and APOA1 concentrations (12), which seems in line with our observations in elderly men.

Together with our data, these observations might suggest that short-term effects of aromatase inhibition on glucose and lipid metabolism could be due to effects on GH and IGF1 metabolism, as endogenous estrogens are known to stimulate secretion of these hormones (13) and effects of IGF1 on glucose metabolism are well established (14). This could also explain the discrepant effects of aromatase inhibition in young versus elderly men, since IGF1 metabolism is known to decrease with ageing (15). However, 10-week administration of anastrozole (1 mg/day) to eight healthy men did not affect insulin, glucose, or lipid levels, despite decreasing IGF1 concentrations (16), nor were lipid levels affected in another study (17). In this regard, possible differences between studies might be explained by the higher aromatase-inhibiting potency of letrozole as compared with anastrozole (18). However, age-specific effects cannot be excluded, since despite similar changes in both of our age groups after active intervention, younger men presented higher absolute levels of FT and FE_2 as compared with elderly men, both before and after treatment and in line with the well-known age-related changes in sex steroid levels. In addition, there is evidence for a decreased number of androgen receptors, and thus androgen sensitivity, in various tissues in elderly men (5). Taken together, age-specific effects may, at least partly, explain our divergent findings between young and elderly men.

Although direct effects of sex steroids on pancreatic β -cells could be suspected (19, 20), another possible explanation for our findings might be that aromatase inhibition in men alters the oxidative strategy of the body, favoring fat over glucose oxidation. This could be due to the inhibition of lipoprotein lipase activity, hereby inhibiting TG uptake and accelerating TG release from abdominal adipose tissue (21). Although this could explain the lower fasting glucose and insulin levels, we did not observe significant changes in fasting plasma TG levels.

In both young and elderly men, we observed a marked decline in leptin levels after aromatase inhibition. Although a single dose of the aromatase inhibitor MPV 2213ad showed no effects on serum leptin levels in young men (22), our findings are in line with the observations in hormonally treated male-to-female transsexual persons (23) and young men treated with T (24). Furthermore, notwithstanding cross-sectional associations between T and adiponectin levels (25) and changes in adiponectin levels after modulation of sex steroid levels (10, 26, 27) suggesting an influence of sex steroids on adiponectin production, no significant changes in adiponectin levels were observed in our study. However, since T appears to affect mainly the high molecular weight fraction of adiponectin (27), which was not specifically measured by our assay, it is possible we missed some changes. Another possible explanation for the divergent effects of aromatase inhibition on leptin versus adiponectin levels could be that sex steroids influence leptin secretion via central hypothalamic or β -adrenergic effects, whereas adiponectin secretion might be preferentially affected by the alterations in adipocyte size and/or number. Furthermore, leptin is mainly secreted from s.c. adipocytes (28), whereas adiponectin mainly results from visceral adipocytes (29), and divergent effects of sex steroids on different fat depots have been reported (21).

Although it cannot be concluded from our study that changing sex steroid milieu affects glucose, lipid, and leptin metabolism independently from changes in body composition, the relatively short duration of our intervention would not allow large changes in fat and/or muscle mass, and no significant changes in body weight or BMI were observed in either group. Unfortunately, this could not be confirmed by more precise measurements of body composition. In support, however, a trial by Mauras *et al.* reported no changes in body composition or whole-body anabolism after 10 weeks of aromatase inhibition (16), notwithstanding similar changes in sex steroid levels as observed in our study. Therefore, our results are suggestive for direct effects of sex steroids on glucose, lipid, and leptin metabolism. In addition, acute sex steroid withdrawal in hypogonadal men has been shown to reduce insulin sensitivity independent of changes in body composition (30).

Limitations of our study are obviously the small sample size and the prominent carry-over effect in the second placebo phase, which did not allow us to compare changes during letrozole versus placebo administration. Furthermore, since aromatase inhibition affects both E_2 and T levels, it is impossible to assess whether the observed changes are due to enhanced androgenic versus attenuated estrogenic action. Finally, some at random significant though probably spurious changes were observed during placebo treatment.

In summary, short-term aromatase inhibition with letrozole 2.5 mg daily affected glucose metabolism in

young, but not elderly men, and lowered leptin levels in both age groups. Since the short duration of our intervention, these effects appear independent from changes in body composition. However, further research is needed regarding whether these effects are indeed direct due to sex steroid action, modulated by age, replicable in different populations and by changes within the normal physiological range or after even shorter term intervention.

Declaration of interest

All authors have nothing to disclose, except for the generous gift of letrozole (Femara®) tablets from Novartis, Department of Oncology, Belgium.

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