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Comparative Assessment in Young and Elderly Men of the Gonadotropin Response to Aromatase Inhibition

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Context: Aging in men is associated with a decline in serum testosterone (T) levels.

Objective: Our objective was to assess whether decreased T in aging might result from increased estradiol (E_2) negative feedback on gonadotropin secretion.

Design and Setting: We conducted a comparative intervention study (2004) in the Outpatient Endocrinology Clinic, Ghent University Hospital.

Participants: Participants included healthy young and elderly men (n = 10 *vs.* 10).

Interventions: We used placebo and letrozole (2.5 mg/d) for 28 d, separated by 2 wk washout.

Main Outcome Measures: We assessed changes in serum levels of free E_2, LH, and FSH, free T, SHBG, and gonadotropins response to an iv 2.5-μg GnRH bolus.

Results: As assessed after 28 d of treatment, letrozole lowered E_2 by 46% in the young men ($P = 0.002$) and 62% in the elderly men ($P < 0.001$). In both age groups, letrozole, but not placebo, significantly increased LH levels (339 and 323% in the young and the elderly, respectively) and T (146 and 99%, respectively) (P value of young *vs.* elderly was not significant). Under letrozole, peak LH response to GnRH was 152 and 52% increase from baseline in young and older men, respectively ($P = 0.01$).

Conclusions: Aromatase inhibition markedly increased basal LH and T levels and the LH response to GnRH in both young and elderly men. The observation of similar to greater LH responses in the young compared with the elderly does not support the hypothesis that increased restraining of LH secretion by endogenous estrogens is instrumental in age-related decline of Leydig cell function. **(*J Clin Endocrinol Metab* 90: 5717–5722, 2005)**

AGING IN MEN is accompanied by a gradual decline in androgens that becomes more apparent after the age of 50 yr. Between the ages of 25 and 75 a modest decline of mean serum testosterone (T) levels up to 20–30% can be seen. The fall of the biologically active free T (FT) and non-SHBG-bound, or so-called bioavailable, T in serum is, however, of greater magnitude, with a reduction by 50% over the same age range (1–7). The decline in T production is underlaid by testicular changes and altered neuroendocrine regulation of LH secretion (8) with blunted circadian rhythms (9) and increased responsiveness to sex steroid hormone feedback compared with young men (10–12). Estrogens contribute substantially to the negative feedback regulation of gonadotropin secretion (13, 14). As a result of increasing aromatase activity with age and the age-associated increase in fat mass (15, 16), the decrease in T levels is not paralleled by a similar decline of plasma estradiol (E_2) levels (4, 15), with a consequent age-related decrease of the plasma ratio of the T over E_2 levels. Pharmacological inhibition of aromatase activity results in increased levels of gonadotropin and T levels, both in young and elderly men (13, 17, 18).

In the present clinical study, the hypothesis is tested that decreased T in aging men might result from increased E_2 negative feedback. To this end, we compared in young and elderly men the effect on gonadotropin and T secretion of aromatase inhibition by administration of letrozole, a specific and potent fourth-generation aromatase inhibitor. Letrozole, currently indicated as a treatment for breast cancer, reduces systemic E_2 concentration in males by 30–50% (19). The premise was that if increased E_2 negative feedback were instrumental in the age-related decline of T levels, aromatase inhibition would result in a greater gonadotropin response in elderly men compared with young men.

Subjects and Methods

Subjects

Ten healthy young men [mean age, 25.9 ± 4.6 yr (range, 20–33 yr); mean body mass index (BMI), 24.2 ± 2.9 kg/m^2 (range, 19.4–28.1)] and 10 elderly men [mean age, 76.1 ± 5.0 yr (range, 68–81 yr); mean BMI, 24.5 ± 2.6 kg/m^2 (range, 18.8–27.5)] gave their written informed consent to participate in this study, which was conducted according to the principles of the Declaration of Helsinki and approved by the Ethical Review Board of the University Hospital Ghent. Medical history, physical examination (male habitus, virilization, and testis size), biochemical measures of hematological, hepatic, renal, and metabolic function and fasting concentrations of T_4, TSH, T, E_2, prolactin, LH, and FSH were within the normal range at screening. Exclusion criteria included active

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Abbreviations: E_1, Estrone; E_2, estradiol; FE_2, free E_2; FT, free T; T, testosterone.

smoking, excessive alcohol or substance abuse, major psychiatric disease and dementia, exposure to psychotropic or neuroactive drugs, use of glucocorticoids or sex hormones, history of sleep apnea, significant cardiopulmonary disease, recent weight loss or gain, transmeridian travel, shift work, untreated prostatic disease or prostate-specific antigen greater than 4 ng/ml, and unwillingness to provide written informed consent.

Study design

Figure 1 depicts the overall design of the study. The study design was a randomized, double-blind, placebo-controlled crossover intervention. Patients were first screened on d 14 before participation in the protocol. Placebo or letrozole (2.5 mg daily, Femara; Novartis AG, Stein, Switzerland) orally taken on awakening were administered in random order each day for a 28-d period separated by a 14-d treatment-free washout period (start of treatment on d 1 and 43, respectively). In both age cohorts, the same number of subjects (n = 5) started with the aromatase inhibitor and placebo, respectively. Blood sampling in the first phase was at d 1 (before dosing) and d 28 and in the second phase at d 43 (before dosing) and d 70. At the conclusion of the visits on d 1 and 43, sufficient letrozole or placebo was provided to last until the following visit 4 wk later; compliance was assessed by pill counting.

Sampling procedure

An iv catheter was placed in the antecubital vein of supine subjects after an overnight fast and 10 min of bed rest between 0800 and 1000 h. Blood samples for assay of serum T, E_2, SHBG, LH, and FSH were withdrawn at 0 and 20 min. At d 28 and 70, an iv 2.5-μg GnRH (Relefact, Aventis Pharma BV, Hoevelaken, The Netherlands) bolus was injected. Earlier studies of iv bolus injection of GnRH in small physiological doses have shown consistent responsiveness of the gonadotropins to this dose in both young and elderly men (20). Blood samples for serum LH and FSH determination were taken via the iv indwelling catheter at 40, 20, and 0 min before (mean of the three samples taken as baseline for calculation of response to GnRH) as well as at 10, 20, and 40 min after the bolus injection.

Hormone assays

Serum was stored at −80 C until assay; all samples from the same subject were assayed in a single assay run. Commercial immunoassays were used to determine the serum concentrations of E_2 (Incstar, Stillwater, MN) (adapted protocol with use of double amount of serum), estrone (E_1) (Bio Line, Brussels, Belgium), T and SHBG (Orion Diagnostica, Espoo, Finland), and LH and FSH (Elecsys LH and FSH immunoassay; Roche, Mannheim, Germany). Intra- and interassay coefficients of variation for the E_2 assay were 3 and 9% with a detection limit of 2 pg/ml, respectively; intra- and interassay coefficients of variation for all other assays were less than 10 and 15%, respectively. The T/E_2 ratio was used as an indirect indicator of aromatase activity. Serum FT and free E_2 (FE_2) were calculated from the total serum hormone concentrations, serum SHBG, and serum albumin using a validated equation derived from the mass action law (21, 22). For all considered hormonal variables, basal values for each sampling day are the mean of the result for two samples obtained at a 20-min interval.

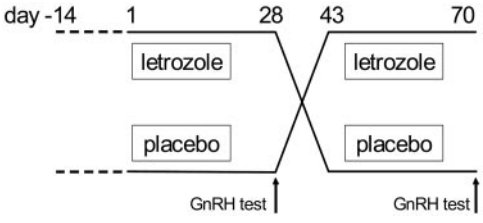

FIG. 1. Schematic representation of the study design. After the baseline visit, both young (n = 10) and elderly (n = 10) subjects were randomized into one of two groups, beginning with either placebo or letrozole for 28 d, followed by the alternative treatment after a 2-wk washout.

Data analysis

The primary end points of this study were between-age group differences in changes of biochemical hormonal values. Baseline characteristics of the groups were compared according to Student's *t* test. LH and FSH were ln-transformed to meet the model requirements. To examine the absolute changes from baseline for each hormone, the statistical significance of the Pearson correlation coefficients was evaluated using a Student's *t* test. Data are expressed as mean ± SD. A level of 0.05 was used to indicate statistical significance. All analyses were done using SPSS software (version 12.0).

Results

Sex steroid levels

As shown in Table 1, at baseline, serum T, E_2, FE_2, LH, and FSH levels did not significantly differ between young and elderly subjects; SHBG levels were higher and FT concentrations lower in the elderly. No differences between subjects in the subgroups starting with placebo or letrozole were observed for either BMI or sex steroid levels. In subjects starting with active treatment, on d 43 at the start of the placebo phase, T concentrations were markedly higher compared with d 1 both in young men ($P = 0.006$) and elderly men ($P < 0.05$) (data not shown). Because of this unanticipated but prominent carryover effect from letrozole administration at d 43, additional results will summarize the data for n = 20 subjects (n = 10 young and n = 10 elderly men) who started with letrozole intake on d 1 or 43, and the results of n = 10 subjects (n = 5 young and n = 5 elderly men) who commenced the study with placebo on d 1. The data for the participants using placebo in the second phase of the study were excluded from statistical analysis.

Letrozole was well tolerated and lowered serum E_2 by 46% in the young men ($P = 0.002$) and by 62% in the elderly men ($P < 0.001$) (Table 2) and comparably over the two study periods; serum E_1 was lowered by 31% ($P = 0.01$) and by 50% ($P < 0.001$) in the young and the elderly, respectively. The decreases in E_2 and FE_2 were significantly greater in elderly men compared with the young ($P = 0.03$ and $P = 0.02$, respectively). LH, FSH, and FT concentrations increased significantly in young and elderly men, with a mean increase of serum LH, FSH, and T of 339, 204, and 146% (SD, 72%) in

TABLE 1. Hormonal characteristics of young and elderly men at screening

	Young men (n = 10)	Elderly men (n = 10)	Significance
SHBG (nmol/liter)	23.0 (9.1)	47.9 (12.9)	<0.001
T (ng/dl)	523.8 (157.3)	509.5 (82.3)	0.80
FT (ng/dl)	13.0 (3.3)	8.7 (2.0)	0.003
E_2 (pg/ml)	18.0 (4.4)	20.7 (4.4)	0.20
FE_2 (pg/ml)	0.340 (0.056)	0.342 (0.062)	0.93
E_1 (pg/ml)	32.4 (6.0)	34.9 (8.6)	0.46
LH (IU/liter)[a]	3.70 (1.61)	5.33 (1.79)	0.14
FSH (IU/liter)[a]	4.59 (1.74)	7.44 (2.00)	0.10
T/E_2[b]	2.96 (0.74)	2.52 (0.47)	0.13
FT/FE_2[b]	3.80 (0.57)	2.59 (0.62)	<0.001

All values are the mean (SD) for two samples obtained at a 20-min interval. Means of the two baseline blood samples (0 and 20 min) were analyzed. To convert E_2 to pmol/liter, multiply by 3.676, and to convert T to nmol/liter, multiply by 0.0347. Significance levels are according to Student's *t* test.

[a] For LH and FSH, geometric means are given.

[b] Divided by 100.

TABLE 2. Hormonal values before and after treatment

	Treatment					
	Letrozole			Placebo		
	d 1/42	d 28/70	Significance[a]	d 1	d 28	Significance[a]
Young men	n = 10	n = 10		n = 5	n = 5	
SHBG (nmol/liter)	21.1	17.5	0.003	18.7	19.5	0.61
T (ng/dl)	514.0	1198.8	<0.001	557.4	562.2	0.92
FT (ng/dl)	13.5	37.5	<0.001	14.6	14.8	0.88
E_2 (pg/ml)	18.9	10.2	0.002	17.5	18.9	0.54
FE_2 (pg/ml)	0.362	0.226	0.001	0.360	0.386	0.64
E_1 (pg/ml)	30.3	20.9	0.01	27.2	35.1	0.15
LH (IU/liter)[b]	4.12	16.40	<0.001	3.42	3.98	0.22
FSH (IU/liter)[b]	4.54	12.94	<0.001	3.29	3.43	0.55
T/E_2[c]	2.97	13.24	<0.001	3.13	3.05	0.75
FT/FE_2[c]	3.83	18.72	<0.001	4.04	4.00	0.88
Elderly men	n = 10	n = 10		n = 5	n = 5	
SHBG (nmol/liter)	47.2	40.8	<0.001	48.8	48.0	0.74
T (ng/dl)	512.7	973.5	<0.001	505.2	529.8	0.46
FT (ng/dl)	8.84	21.8	<0.001	8.46	9.06	0.52
E_2 (pg/ml)	20.4	7.8	<0.001	19.8	19.9	0.98
FE_2 (pg/ml)	0.341	0.148	<0.001	0.326	0.330	0.88
E_1 (pg/ml)	32.4	15.9	<0.001	32.2	30.9	0.43
LH (IU/liter)[b]	5.53	22.18	<0.001	5.75	4.72	0.01
FSH (IU/liter)[b]	7.16	19.75	<0.001	6.75	6.86	0.73
T/E_2[c]	2.53	12.83	<0.001	2.55	2.65	0.61
FT/FE_2[c]	2.64	14.81	<0.001	2.59	2.74	0.48

All values are the mean for two samples obtained at a 20-min interval.
[a] According to paired Student's *t* test.
[b] For LH and FSH, geometric means are given.
[c] Divided by 100.

young *vs.* 323, 182, and 99% (SD, 61%), respectively, in elderly men (*P* value for young *vs.* elderly was not significant). In both the young and elderly group, SHBG levels decreased during letrozole treatment ($P = 0.003$ and <0.001, respectively; *P* value for young *vs.* elderly was not significant). The ratios T/E_2 and FT/FE_2 were significantly higher after letrozole treatment in both groups ($P < 0.001$). In a multivariate analysis of differences in sex steroid levels and gonadotropins, the treatment effect was shown to be independent of age.

Response to GnRH administration

Under letrozole treatment, the peak LH response to stimulation by administration of 2.5 μg GnRH (after 20 min) was 152 and 52% increase from baseline in young and older men, respectively ($P = 0.01$) (Fig.2); under placebo, the increase was 221% in young men and 140% in elderly men ($P = 0.22$). As for FSH, peak response to GnRH stimulation under letrozole was 30 and 5% increase from baseline in young and older men, respectively ($P = 0.01$); the response under placebo was 13% in young men and 10% in elderly men ($P = 0.42$).

Discussion

In the present comparative study, aromatase inhibition with letrozole during 28 d induced a remarkable increase of gonadotropin and T serum levels both in young and older men. The observed similar response to aromatase inhibition in the young and the elderly men does not support the tested hypothesis that the altered gonadotropin secretion in the elderly (8) and its attendant decline of T production are the result of an increased restraining action of estrogens.

The major increase of gonadotropin levels under aromatase inhibition seen in the present study with letrozole, as well as in previous studies in men with the aromatase inhibitor anastrozole (17, 18, 23), illustrates the important contribution of estrogens to the sex steroid feedback inhibition of gonadotropin secretion in men. The restraining action of estrogens on gonadotropin secretion is also revealed by el-

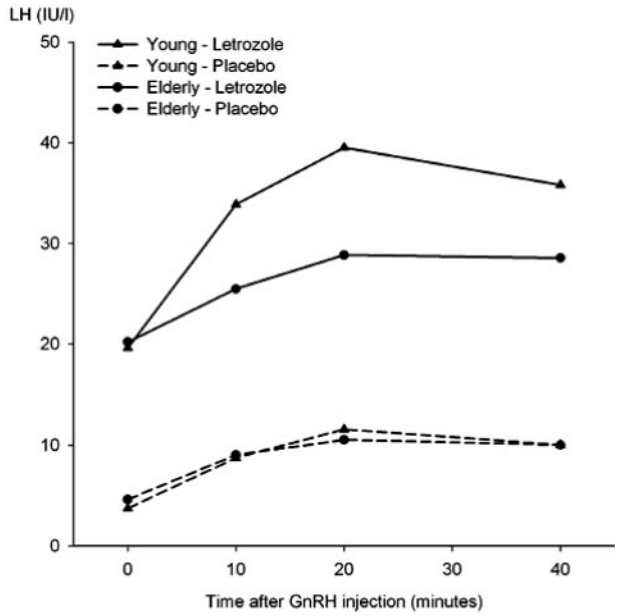

FIG. 2. LH response to stimulation with iv administration of 2.5 μg GnRH; baseline is the average for LH at 40, 20, and 0 min before GnRH injection.

evated gonadotropin levels in men with congenital aromatase deficiency (24–26) or lack of functional estrogen receptor α (27). In agreement with the observations in the present study of stimulation by aromatase inhibition of both basal gonadotropin levels and the response to exogenous GnRH, the restraining action of estrogens on gonadotropin secretion in men has been shown to be exerted both at the pituitary and at the hypothalamic levels (13–14).

Few studies in men are available in which letrozole was chosen as aromatase inhibitor. One study in elderly men made use of letrozole, however, in combination with a long-acting GnRH agonist (28). In studies in early and midpubertal boys, letrozole 2.5 mg has been used in combination with T (29). Short-term letrozole treatment with a daily dose of 2.5 mg or 2.5 mg three times a week in severely obese men with hypogonadotropic hypogonadism normalized serum T levels in all participants (30). The decrease of serum estradiol by a mean of 46% in young men and 62% in elderly men after 28 d of treatment with 2.5 mg letrozole daily in the present study is on the same order of magnitude as previously reported decreases of 50% after 10 wk treatment with 1 mg anastrozole daily in young men (23), of 30% after 9 wk treatment with 2 mg anastrozole daily in eugonadal men over 65 yr (18), and of 40% after 12 wk treatment with a daily dose of 1 mg anastrozole in elderly men with initially low serum T (17). Observed decreases of serum E_1 levels in the present study with letrozole and in previous studies with anastrozole were somewhat greater but still on the same order of magnitude as for E_2. In studies with anastrozole in young and elderly men, treatment induced substantial increases in serum gonadotropin and T levels, but the observed increases were generally smaller than seen in the present study. An increase of serum total T by 58% after 10 wk of 1 mg anastrozole in young men was reported (23) compared with the increase of 146% in the young men in the present study; an increase of up to 50% was seen in elderly men treated for 9 wk with 2 mg anastrozole (18) or with 1 mg anastrozole daily for 12 wk (17) compared with an increase of 99% observed by us in the elderly men. In the present study, treatment with 2.5 mg letrozole daily for 28 d increased serum T levels in elderly men to or above the upper limit of the normal range for young men. This confirms the previous reports (17, 18) indicating that aromatase inhibition in elderly men can increase T serum levels with achieved serum values in the upper normal range for young men.

In accordance with previous observations (17, 18), aromatase inhibition results in a slight but significant decrease of SHBG serum levels. Nevertheless, the marked age-related difference in SHBG concentrations is maintained under aromatase inhibition. As a result of the decrease of SHBG, the increases of the bioavailable, non-SHBG-bound fractions of T under aromatase inhibition are even more marked than for total T.

The increase of T serum levels in men treated with aromatase inhibitors is explained by LH stimulation of T secretion with increase of both basal LH levels and LH response to GnRH. The increases in gonadotropin levels observed in the present study under 2.5 mg letrozole daily are generally of greater amplitude than previously described under anastrozole treatment at doses of 1 or 2 mg daily (17, 18, 23). The plasma terminal elimination half-life of letrozole is approximately 2 d (31). The powerful effect of aromatase inhibition with letrozole on gonadotropin and T levels is illustrated by the marked carryover effect we have seen in both young and older study subjects initially treated with letrozole, with still marked elevation of gonadotropin and T levels at the beginning of the placebo phase, 14 d after discontinuation of letrozole administration. It is remarkable that in the present as well as in previous studies with anastrozole, a marked increase of gonadotropin and T levels is being achieved even though the decrease in circulating estrogens is of rather modest amplitude. This might suggest that the restraining action of estrogens on gonadotropin secretion in men is at least in part dependent on local aromatization of T to estrogens in the hypothalamus and pituitary gland, as previously suggested by Winters *et al.* (32). However, the design of the present study does not allow differentiating between effects on gonadotropin secretion of blood-borne and locally produced estrogens. Also, one cannot exclude the possibility that technical limitations in terms of assay sensitivity and specificity may have resulted in an underestimation of the reduction of serum estradiol levels during aromatase inhibition in studies in men.

The primary focus of interest in the present study was the comparison of the effects of aromatase between young and elderly men. Notwithstanding a somewhat greater reduction of estrogen serum levels in the elderly compared with the young, in young men the increase of basal gonadotropin levels in response to aromatase inhibition was comparable to that in elderly men. The response to a challenge with low-dose GnRH under aromatase inhibition was greater in the young compared with the elderly. Thus, aromatase inhibition did not uncover a state of increased inhibitory tone by endogenous estrogens in the elderly compared with the young, which would have been expected to result in a greater gonadotropin response in the elderly. The greater response to GnRH under aromatase blockade in the young compared with the elderly could indicate a greater responsiveness of the gonadotropes and/or a lower frequency of endogenous GnRH stimulation with build up of a greater LH releasable pool in the young under these experimental conditions. The observation of Veldhuis and Iranmanesh (33) of a greater disorderliness of spontaneous LH secretion in the elderly compared with the young under treatment with an aromatase inhibitor might be relevant to this context. The responses for T and FT in the elderly, although not significantly different from those in young men, tended to be somewhat smaller. This is as expected in view of the known moderate decrease in responsiveness of the Leydig cells to LH in the elderly (34, 35).

We are aware of one other side-to-side comparison of the effects of aromatase inhibition on gonadotropin and T levels in young and elderly men. In the latter study by Veldhuis and Iranmanesh (33), administration of 10 mg anastrozole daily for 5 d increased 24-h mean LH concentrations significantly and equivalently in young and older men, whereas the T response in the elderly was only limited compared with that in young men. In the elderly, there was a diminished incremental LH pulse amplitude and area, failure to further accelerate LH pulse frequency, and a more disorderly secretory

pattern of LH compared with young men. Except for the limited T response in the elderly in this short-duration study compared with the marked T increase observed during longer-duration aromatase inhibition, the results of the latter study are in general agreement with the present study. Earlier, a comparable response of LH levels and a smaller increase of serum T in elderly, compared with young men, were observed after administration of the selective estrogen receptor modulator clomiphene citrate (36). Finally, also relevant to the present discussion are the observations by Winters *et al*. (11) that elderly men respond with a greater inhibition of gonadotropin secretion than young men to infusion of dihydrotestosterone and T, but not of estradiol, suggesting that gonadotropin secretion in the elderly is not more sensitive to suppression by E_2 in the young.

From the whole of these data it can be concluded that, whereas elderly men are more responsive to the inhibitory action of exogenously administered T on LH secretion (11, 12), the age-related decline in serum T levels is not the result of increased restraining activity by endogenous estrogens. Previously, it has been reported that these age-related changes in regulation of LH secretion that are situated at the hypothalamic level (20), with evidence of diminished GnRH secretion (37), are neither the consequence of an increased opioidergic tone (38) or from relative leptin deficiency (39). The exact mechanisms of the age-related changes in regulation of LH secretion in men are yet to be uncovered and may involve loss of GnRH neurons, intrinsic or regulatory functional changes in the GnRH neurons, and/or less effective coordinated recruitment of GnRH neurons needed for the intermittent release of an adequate bolus of GnRH into the pituitary portal circulation. Limitations of the present study are the relatively small number of subjects and the fact that the carryover effect seen after initial letrozole treatment did not allow for the benefits of an analysis according to a crossover design as was initially intended. However, this was compensated to a large extent by the consistent and robust responses observed during aromatase inhibition.

In conclusion, aromatase inhibition with 2.5 mg letrozole daily for 28 d produced a remarkable and comparable elevation of gonadotropin serum levels in young and elderly men, with also a marked T response in both groups. The results of this study, together with the results of a previous side-to-side comparison of the effects of aromatase inhibition (35), allow us to reject the tested hypothesis that increased restraining of LH secretion by endogenous estrogens is instrumental in the age-related decline of Leydig-cell function.

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