Neuroendocrine Regulation of Growth Hormone and Androgen Axes by Selective Estrogen Receptor Modulators in Healthy Men

Vita Birzniece, Akira Sata, Surya Sutanto, and Ken K. Y. Ho

Garvan Institute of Medical Research and Department of Endocrinology (V.B., A.S., S.S., K.K.Y.H.), St. Vincent's Hospital, Sydney, New South Wales 2010, Australia; and The University of New South Wales (V.B., K.K.Y.H.), Sydney, New South Wales 2052, Australia

Context: In men, the stimulation of GH and inhibition of LH secretion by testosterone requires aromatization to estradiol. Tamoxifen, a selective estrogen receptor modulator (SERM), possesses central estrogen antagonistic effect but peripheral hepatic agonist effect, lowering IGF-I. Thus, tamoxifen is likely to perturb the neuroendocrine regulation of GH and gonadal axes. Raloxifene, a SERM, is used for therapy of osteoporosis in both sexes. Its neuroendocrine effects in men are poorly understood.

Objective: The aim was to compare the impact of raloxifene and tamoxifen on GH-IGF-I and gonadal axes in healthy men.

Design: We conducted a randomized, open-label crossover study.

Patients and Intervention: Ten healthy men were randomized to 2-wk sequential treatment with tamoxifen (10 and 20 mg/d) and raloxifene (60 and 120 mg/d), with a 2-wk intervening washout period.

Main Outcome Measures: We measured the GH response to arginine and circulating levels of IGF-I, LH, FSH, testosterone, and SHBG.

Results: Tamoxifen, but not raloxifene, significantly reduced IGF-I levels by 25 $\pm$ 6% ($P < 0.01$) and increased SHBG levels by 20 $\pm$ 7% ($P < 0.05$) at the higher therapeutic dose. There was a nonstatistically significant trend toward a reduction in the GH response to arginine with both SERMs. Both drugs significantly increased LH, FSH, and testosterone concentrations. The mean increase in testosterone (40 *vs.* 25%; $P < 0.05$) and LH (70 *vs.* 30%; $P < 0.01$) was significantly greater with tamoxifen than with raloxifene treatment.

Conclusions: Tamoxifen, but not raloxifene, reduces IGF-I levels. Both SERMs stimulate the gonadal axis, with tamoxifen imparting a greater effect. We conclude that in therapeutic doses, raloxifene perturbs the GH and gonadal axes to a lesser degree than tamoxifen. **(*J Clin Endocrinol Metab* 95: 5443–5448, 2010)**

GH and androgens, the main anabolic hormones in men, interact to exert full anabolic effect (1, 2). In hypogonadal men, testosterone replacement stimulates GH secretion, which in turn increases IGF-I levels (3). However, this may not be associated with direct androgen receptor activation because nonaromatizable androgens do not affect GH secretion (4). The stimulation of GH secretion by testosterone requires prior aromatization to estradiol, an effect blocked by central estrogen antagonism with tamoxifen (3). This is also supported by a recent study of healthy older men in whom treatment with aromatase inhibitor reduced testosterone-stimulated GH secretion (5). In addition, inhibition of LH secretion by testosterone requires prior aromatization to estradiol (6). Therefore, central

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-1477 Received June 29, 2010. Accepted August 19, 2010.
First Published Online September 15, 2010

Abbreviations: CV, Coefficient of variation; SERM, selective estrogen receptor modulator.

estrogen antagonism is likely to perturb neuroendocrine regulation of the GH and gonadal axes in men.

Selective estrogen receptor modulators (SERMs) are synthetic estrogen-like compounds that possess estrogen agonistic or antagonistic effects in a tissue-specific manner. Tamoxifen, a SERM, is used as an adjuvant treatment of breast cancer because of its estrogen antagonistic effects in the breast. It exerts estrogen-like effects in the liver, as indicated by an increase in SHBG and reduction in IGF-I levels (7, 8). However, tamoxifen blocks estrogen effects centrally in the brain (8). Thus, the pharmacological effects of tamoxifen are unique because it simultaneously probes peripheral endocrine and central paracrine effects of estrogen. The central estrogen antagonism of tamoxifen is well established, especially on the regulation of the gonadal axis in men (9, 10). Raloxifene, a newer generation SERM, is widely used for the treatment of osteoporosis in both men and women because of its estrogen-like effects on bone (11). Raloxifene effectively prevents GnRH-induced bone loss in men with prostate cancer (12). However, its impact on the regulation of gonadal and GH-IGF-I axes in men at therapeutic doses is not known. To determine raloxifene's central estrogen antagonistic and peripheral hepatic agonistic effect, we have undertaken a comparative study with tamoxifen to investigate the impact on neuroendocrine regulation of GH and gonadal axes in healthy men.

Subjects and Methods

Subjects

Ten healthy men (aged 51–77 yr) were recruited from the community through advertisements. Subjects were in good general health and had normal hematological tests and renal and hepatic function. Exclusion criteria included body mass index of 30 kg/m^2 or greater, hypothalamic or pituitary disorders, diabetes mellitus, and chronic renal or hepatic illnesses. Subjects were not taking any medications known to interfere with endocrine systems. Throughout the study, subjects were instructed to follow their usual diet and physical activity and continue on their usual medications or supplements. St. Vincent's Hospital Human Research Ethics Committee approved the study, which was conducted in accordance with the principles of the Declaration of Helsinki. All study participants gave written informed consent. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12607000586415).

Study design

This was an open-labeled, randomized, two-phase crossover study of raloxifene (Evista; Eli Lilly Co., Indianapolis, IN) and tamoxifen (Genox; Alphapharm Pty., Sydney, Australia). Each of the phases consisted of two doses of raloxifene or tamoxifen each given for consecutive 2-wk periods (Fig. 1). The dose of raloxifene was 60 mg/d for the first 2 wk and 120 mg/d for another 2 wk. The dose of tamoxifen was 10 mg/d for the first 2 wk and 20 mg/d for another 2 wk. The washout between the two treatments was 2 wk. The washout period was designed such that there is no carryover effect because the elimination half-life of tamoxifen and raloxifene is around 1 wk and 30 h, respectively (13, 14).

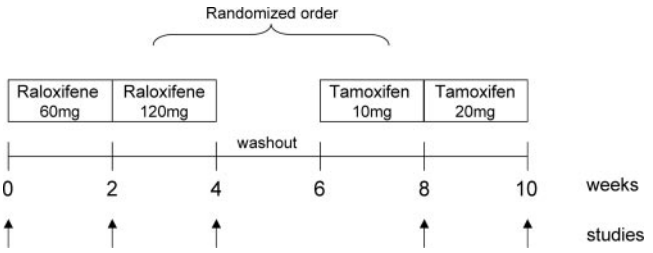

FIG. 1. Study design. Ten healthy men were randomized to 2-wk sequential treatment with raloxifene (60 and 120 mg/d) and tamoxifen (10 and 20 mg/d) with washout of 2 wk in between. Studies were undertaken at baseline and after each of the treatment periods.

Subjects were studied in the Clinical Research Facility, Garvan Institute of Medical Research. All participants were asked to fast the night before each visit. Participants first underwent measurements at baseline, which were repeated after each of the raloxifene and tamoxifen treatment doses. On each visit, assessment of GH status using the arginine stimulation test was performed, and circulating IGF-I, LH, FSH, testosterone, and SHBG levels were measured. We measured circulating LH levels in five samples taken every 30 min over a 2-h period during the arginine stimulation test. Study bloods were collected, and serum samples were obtained by centrifugation and stored at −20 C until analysis.

Arginine stimulation test

Subjects rested on a bed for at least 30 min before the baseline blood samples were taken. Thirty grams of L-arginine hydrochloride (Phebra Pty Ltd., Sydney, Australia) was infused over a 30-min period. Blood samples for GH level measurements were taken at baseline and 30, 60, 90, and 120 min after commencement of arginine infusion. No serious side effects or complications were reported during the test. In healthy subjects, the arginine stimulation test has been shown to have good intraindividual reproducibility (15).

Analytical methods

All samples for any individual were measured in the same assay run for each analyte. Serum GH was measured by ELISA calibrated against the World Health Organization (WHO) IS 80/505 (Bioclone Australia Pty Ltd., Sydney, Australia) with sensitivity of less than 0.1 mIU/liter. The coefficients of variation (CVs) for GH were 4.4% at 6.5 mIU/liter and 3.5% at 17.6 mIU/liter. Serum IGF-I was measured by RIA after acid ethanol extraction as previously described (2, 16, 17). The CVs for IGF-I were 8.3% at 14.7 nmol/liter and 7.4% at 28.6 nmol/liter. Serum SHBG was measured by RIA using a commercial assay (Immulite 2000; Siemens Medical Solution Diagnostics, Los Angeles, CA). The CVs for SHBG at 5.3 and 86.2 nmol/liter were 5.0 and 7.5%, respectively. The biological variability within individuals is around 20% for IGF-I (18) and 2% for SHBG (19). Serum testosterone was measured by RIA using a commercial assay (Immulite 2000). The interassay CVs for testosterone at 3.6 and 23 nmol/liter were 9.3 and 9.0%, respectively. Serum FSH was measured by two-site immunoassay using chemiluminometric detection on the Advia Centaur analyzer (Siemens Diagnostics, Sydney, Australia) calibrated against the WHO 2nd IRP 94/632. The CVs for FSH were 4.5% at 8.5 IU/liter and 4.7% at 14 IU/liter.

Serum LH was measured by ELISA calibrated against the WHO 2nd IS 80/552 (Diagnostics Biochem Canada Inc., London, Ontario, Canada). The CVs for LH were 5.1% at 5.15 IU/liter and 8.1% at 17.4 mIU/liter.

Statistical analysis

The GH response to arginine had large intersubject variation and was not normally distributed, and therefore data were logarithmically transformed for the analysis. The GH response to arginine and changes in circulating LH levels were analyzed by repeated measures ANOVA. Peak GH levels were calculated as maximum increment over prestimulated GH concentration. Treatment effects on peak GH levels and on circulating IGF-I, SHBG, FSH, testosterone, and free testosterone index, a ratio of total testosterone over SHBG, were assessed using paired *t* tests with Bonferroni's correction, and comparison between treatments was analyzed by factorial ANOVA where appropriate. Results were expressed as mean (SEM), and a *P* value of less than 0.05 was considered to be significant. Statistical analysis was undertaken using the statistical software package Statview 4.5 PPC (Abacus Concepts, Inc., Berkeley, CA).

Results

Mean age of the subjects was 64.8 ± 3 yr, with mean body mass index of 26.3 ± 1.2 kg/m^2. Mean baseline concentrations were 14.8 ± 1 nmol/liter for testosterone and 1.3 ± 0.1 IU/liter for LH. Mean baseline GH concentration was 4.1 ± 3.3 mIU/liter.

Growth hormone

GH response to arginine was not significantly affected by raloxifene or tamoxifen treatments, although there was a trend toward a reduction with both treatments (Fig. 2A and Table 1). There was no significant difference between raloxifene and tamoxifen treatments in GH response to stimulation.

IGF-I levels

Mean IGF-I levels were not significantly affected by either a 60- or 120-mg dose of raloxifene (Fig. 2B and Table 1). Mean circulating IGF-I levels fell significantly (24.8 ± 6.1%; $P < 0.01$) with the 20-mg, but not the 10-mg, dose of tamoxifen administration (Fig. 2B and Table 1). The overall treatment effect on circulating IGF-I levels with tamoxifen was significantly greater than with raloxifene treatment ($P < 0.05$).

SHBG levels

Mean circulating SHBG levels did not change significantly during raloxifene treatment (Table 1). By contrast, mean SHBG levels significantly increased during 10-mg and 20-mg tamoxifen treatments by 23.1 ± 5.4 and 20.1 ± 6.9%, respectively ($P < 0.05$; Table 1). The overall treatment effect on serum SHBG levels with tamoxifen was significantly greater than that with raloxifene treatment ($P < 0.01$).

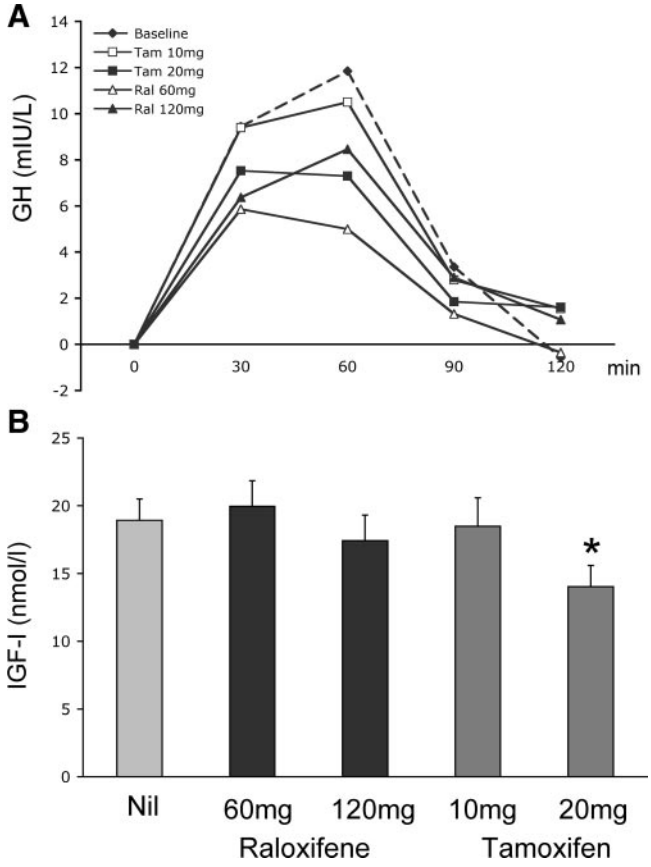

FIG. 2. A, GH response to arginine stimulation at baseline and during low (60-mg) and high (120-mg) dose raloxifene (Ral) treatment and during low (10-mg) and high (20-mg) dose tamoxifen (Tam) treatment in healthy men. Data are presented as mean increment in serum GH after arginine infusion over prestimulation GH levels. Conversion factor: 1 mIU/liter = 0.33 μg/liter. B, Mean serum IGF-I levels at baseline (nil) and during 60- and 120-mg raloxifene treatment and 10- and 20-mg tamoxifen treatment. Data are expressed as means ± SEM. *, $P < 0.05$ compared with nil.

LH levels

Repeated measures ANOVA revealed statistically significant higher mean LH concentrations with both raloxifene and tamoxifen treatments (Fig. 3A and Table 1). Only the 120-mg dose of raloxifene significantly increased mean LH levels (34.9 ± 18.7%; $P < 0.05$). The 10- and 20-mg doses of tamoxifen significantly increased circulating LH levels by 44.2 ± 18.5 and 97.3 ± 23.1% ($P < 0.01$ and $P < 0.001$), respectively. The mean treatment effect on LH during tamoxifen was significantly higher than with raloxifene treatment ($P < 0.01$).

Testosterone

Mean circulating testosterone levels significantly ($P < 0.05$) increased with both doses of raloxifene treatment by 27.3 ± 9.5 and 23.2 ± 8.1%, respectively (Fig. 3B and

TABLE 1. Serum peak GH response to arginine stimulation and mean serum IGF-I, LH, FSH, testosterone, and SHBG levels, and free testosterone index measured at baseline and during treatments with raloxifene (60 and 120 mg/d) and tamoxifen (10 and 20 mg/d)

		Raloxifene		Tamoxifen	
	Baseline	60 mg	120 mg	10 mg	20 mg
Peak GH (mIU/liter)	13.8 ± 5.2	7.4 ± 2.6	9.8 ± 2.5	13.6 ± 5.9	9.1 ± 2.9
IGF-I (nM/liter)	18.9 ± 1.6	20 ± 1.9	17.4 ± 1.9	18.5 ± 2.1	14 ± 1.6[a,b]
LH (IU/liter)	1.3 ± 0.1	1.6 ± 0.3	1.6 ± 0.2[a]	1.7 ± 0.2[a]	2.3 ± 0.2[a,b]
FSH (IU/liter)	4.3 ± 0.7	5.7 ± 0.9[a]	5.3 ± 0.9[a]	5.5 ± 1.0[a]	5.7 ± 0.9[a]
Testosterone (nM/liter)	14.8 ± 1	18.6 ± 1.5[a]	18 ± 1.5[a]	18.6 ± 1.7	22.6 ± 2[a,b]
SHBG (nM/liter)	33.5 ± 3.4	36.4 ± 3.4	34.4 ± 3.1	40.9 ± 4.1[a]	38.9 ± 3.5[a,b]
Free testosterone index	48.8 ± 6.3	54.4 ± 5.5	55.1 ± 4.8	48.8 ± 5.6	60 ± 4.8[a]

Data are expressed as means ± SEM. Conversion factor for GH: 1 mIU/liter = 0.33 μg/liter.

[a] $P < 0.05$ compared to baseline.

[b] $P < 0.05$ compared to treatment with raloxifene.

Table 1). Mean testosterone levels increased with 10 mg (by 28.6 ± 12.8%; $P = 0.05$) and 20 mg (by 52 ± 14.2%; $P < 0.01$) of tamoxifen. The overall treatment effect on testosterone levels during tamoxifen was greater than with raloxifene treatment ($P < 0.05$).

Free testosterone index

When compared with baseline, low- and high-dose raloxifene treatment did not significantly change the mean free testosterone index (Table 1). Free testosterone index significantly ($P < 0.05$) increased only with 20 mg of tamoxifen by 30.9 ± 9.6%. The treatment effect on free testosterone index showed a trend toward a greater effect with tamoxifen, although this did not reach statistical significance (Table 1).

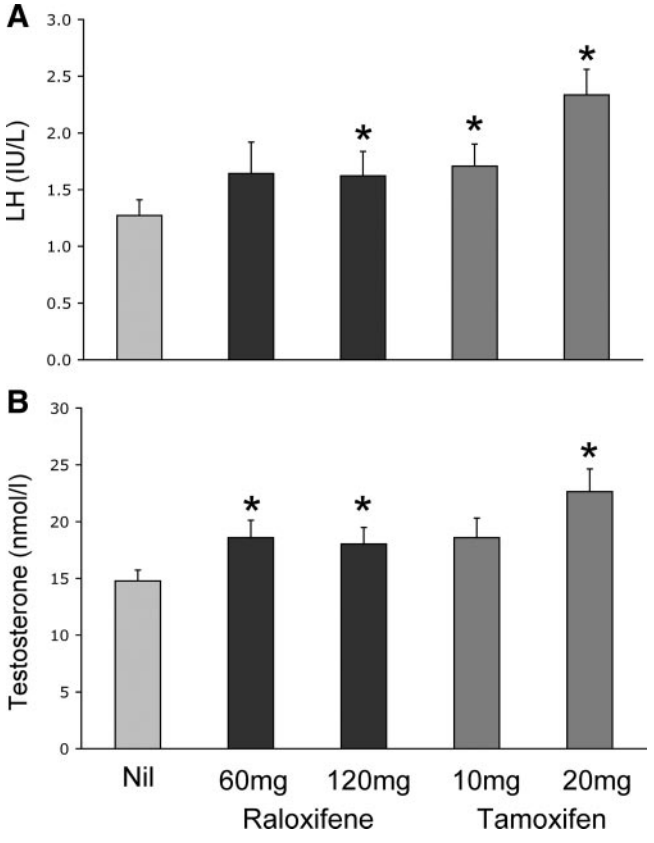

FIG. 3. Mean serum LH (A) and testosterone (B) levels at baseline (nil) and during 60- and 120-mg raloxifene treatment and 10- and 20-mg tamoxifen treatment. Data are expressed as means ± SEM. *, $P < 0.05$ compared with nil.

Discussion

We have investigated the effects of raloxifene on GH-IGF-I and gonadal axes in healthy men and compared these effects to those of tamoxifen. There was a nonstatistically significant trend toward a reduction in the GH response to arginine with both SERMs. Tamoxifen, but not raloxifene, reduced IGF-I levels and increased SHBG levels. Both drugs increased LH, FSH, and testosterone concentrations. The effects on IGF-I, LH, testosterone, and SHBG levels were significantly greater with tamoxifen than with raloxifene treatment. This is the first study showing that in therapeutic doses, both raloxifene and tamoxifen perturb gonadal axis, with tamoxifen imparting a greater effect.

There is strong evidence that estrogens produced locally in the brain play a major role in the neuroendocrine regulation of gonadal and GH axes in men. Studies in men with aromatase deficiency provided evidence that estrogens are required for the negative feedback on LH secretion (6). The evidence comes also from studies with aromatase inhibitors and tamoxifen, which, by reducing tissue estrogen availability and by blocking central estrogen receptors, reduce the negative feedback of estrogen derived from testosterone on gonadotropin secretion (9). In the current study, we found that tamoxifen stimulates LH, FSH, and testosterone production, responses expected from the abrogation of estrogen-mediated feedback inhibition (Fig. 4). Raloxifene also stimulated the gonadal axis; however, the effect was smaller, indicating that raloxifene, at the doses used, exerts a milder

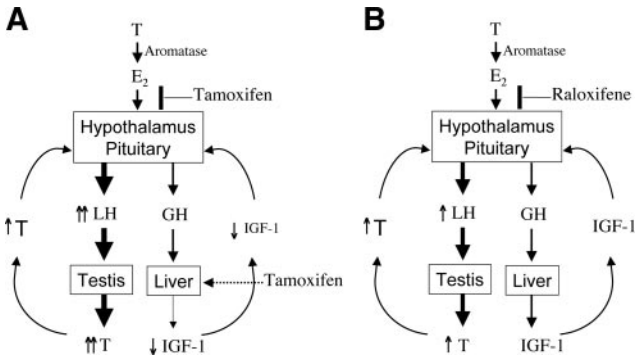

FIG. 4. A, Tamoxifen administered via the oral route acts on the liver to reduce IGF-I production. The reduction in IGF-I lessens negative feedback to the hypothalamus and pituitary gland; however, GH secretion is not stimulated. The finding of an unchanged GH response to stimulation despite reduced IGF-I feedback inhibition indicates central suppression of GH output by tamoxifen. Tamoxifen stimulates LH and testosterone production. The lack of negative feedback of estrogen derived from testosterone on LH secretion indicates potent central estrogen receptor antagonism by tamoxifen. B, Raloxifene treatment, at the doses used, does not change GH and IGF-I levels, whereas it stimulates LH and testosterone production. Raloxifene-mediated stimulation of gonadal axis indicates a mild central estrogen receptor antagonistic effect. T, Testosterone; E_2, estradiol.

degree of central estrogen receptor antagonism (Fig. 4). Thus, both SERMs significantly perturbed the gonadal axis, but tamoxifen imparted a greater effect.

Local estrogen derived from testosterone also plays a major role in the neuroendocrine regulation of GH secretion. We recently showed that local estrogen regulates GH secretion in women (20). In hypogonadal men, testosterone replacement stimulates GH secretion, an effect that is abolished by central estrogen antagonism of tamoxifen (3). The role of local estrogen is supported by the observation that treatment with aromatase inhibitor attenuates testosterone-induced increase in GH levels (5), and that nonaromatizable androgens do not stimulate GH secretion in peripubertal boys (4). In addition, animal studies show that androgen receptor expression is absent in the GHRH hypothalamic neurones (21). These findings provide strong evidence for the important role of local estrogen in the neuroregulation of gonadal and GH-IGF-I axes in men. Collectively, the effects of tamoxifen on gonadal and GH-IGF-I axes were greater than those of raloxifene.

In the present study, both raloxifene and tamoxifen increased circulating testosterone levels, which should have stimulated GH secretion, but this did not happen. IGF-I mediates negative feedback inhibition of GH secretion (22). Therefore, the fall in IGF-I level induced by tamoxifen should also have been accompanied by a stimulation of GH secretion; however, this was not observed (Fig. 4). What we observed was a strong trend toward an inhibition of GH response to arginine stimulation. The attenuation of GH response by tamoxifen and raloxifene in the face of increased positive testosterone and reduced IGF-I feedback inhibition provide strong evidence that in therapeutic doses these two SERMs exert sufficient central antagonistic effect to blunt the local effects of estrogen.

A fall in circulating IGF-I levels with tamoxifen is indicative of hepatic estrogenic effect, a finding in line with previous studies (23, 24). Another marker of hepatic estrogen action is circulating SHBG (25), which also increased with tamoxifen treatment. However, raloxifene failed to show significant change in mean SHBG and IGF-I levels. Thus, tamoxifen exerts greater hepatic estrogenic effect than raloxifene in the doses used. In women, raloxifene increased SHBG and reduced IGF-I by about 25% after 4 wk of treatment (17). Studies in men are sparse, with a reported 7% increase in SHBG and about a 20% reduction in IGF-I level after 3 months of raloxifene treatment (26, 27). The lack of a significant effect in our study and the absence of a clear dose-dependent effect for some measures may be due to the relatively small sample size. If extrapolated from the earlier publications in men, a sample size of 15–16 is required to detect significant change in IGF-I and SHBG levels. Nevertheless, these findings indicate that raloxifene at the doses used exerted modest hepatic estrogen agonist effect in healthy men.

These findings could have profound clinical significance, given the marked concurrent perturbation by SERMs on the GH-IGF-I and gonadal axes. Both IGF-I and testosterone are anabolic. Tamoxifen reduced IGF-I but increased testosterone concentration, imparting divergent effects on protein metabolism, the outcome of which is uncertain and deserves further study. The consequences from raloxifene treatment may favor anabolism because it exerted no significant effect on the GH-IGF-I axis while stimulating gonadal axis activity. Thus, future work into long-term effects of SERMs on the regulation of GH and gonadal axes and how the perturbation of that affects body composition is warranted.

In summary, neither raloxifene nor tamoxifen significantly affected the GH response to stimulation. Tamoxifen, but not raloxifene, reduced IGF-I and increased SHBG levels. The effect on IGF-I and SHBG levels with tamoxifen was significantly greater than that observed with raloxifene treatment. Both drugs significantly increased LH, FSH, and testosterone concentrations. The increases in testosterone and LH were significantly greater with tamoxifen than with raloxifene treatment. We conclude that in therapeutic doses, tamoxifen perturbs the GH and gonadal axes to a greater extent than raloxifene.

Acknowledgments

We thank Alphapharm for providing Genox. We gratefully thank the research nurses, particularly Jen Hansen, for clinical assistance; and Selina Sutton and the Endocrinology Lab, Royal

Prince Alfred Hospital, Sydney, Australia, for providing laboratory assistance.

Address all correspondence and requests for reprints to: Prof. Ken K. Y. Ho, Pituitary Research Unit, Garvan Institute of Medical Research, Darlinghurst, New South Wales 2010, Australia. E-mail: k.ho@garvan.org.au.

This work was supported by the National Health and Medical Research Council of Australia.

The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12607000586415).

Disclosure Summary: All authors have nothing to declare.

References

1. **Meinhardt U, Nelson AE, Hansen JL, Birzniece V, Clifford D, Leung KC, Graham K, Ho KK** 2010 The effects of growth hormone on body composition and physical performance in recreational athletes: a randomized placebo-controlled trial. Ann Intern Med 152:568–577
2. **Gibney J, Wolthers T, Johannsson G, Umpleby AM, Ho KK** 2005 Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. Am J Physiol Endocrinol Metab 289:E266–E271
3. **Weissberger AJ, Ho KK** 1993 Activation of the somatotropic axis by testosterone in adult males: evidence for the role of aromatization. J Clin Endocrinol Metab 76:1407–1412
4. **Veldhuis JD, Metzger DL, Martha Jr PM, Mauras N, Kerrigan JR, Keenan B, Rogol AD, Pincus SM** 1997 Estrogen and testosterone, but not a nonaromatizable androgen, direct network integration of the hypothalamo-somatotrope (growth hormone)-insulin-like growth factor I axis in the human: evidence from pubertal pathophysiology and sex-steroid hormone replacement. J Clin Endocrinol Metab 82:3414–3420
5. **Veldhuis JD, Mielke KL, Cosma M, Soares-Welch C, Paulo R, Miles JM, Bowers CY** 2009 Aromatase and 5α-reductase inhibition during an exogenous testosterone clamp unveils selective sex steroid modulation of somatostatin and growth hormone secretagogue actions in healthy older men. J Clin Endocrinol Metab 94:973–981
6. **Rochira V, Zirilli L, Genazzani AD, Balestrieri A, Aranda C, Fabre B, Antunez P, Diazzi C, Carani C, Maffei L** 2006 Hypothalamic-pituitary-gonadal axis in two men with aromatase deficiency: evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback. Eur J Endocrinol 155:513–522
7. **Löfgren L, Wallberg B, Wilking N, Fornander T, Rutqvist LE, Carlström K, von Schoultz B, von Schoultz E** 2004 Tamoxifen and megestrol acetate for postmenopausal breast cancer: diverging effects on liver proteins, androgens, and glucocorticoids. Med Oncol 21:309–318
8. **Riggs BL, Hartmann LC** 2003 Selective estrogen-receptor modulators—mechanisms of action and application to clinical practice. N Engl J Med 348:618–629
9. **Gooren LJ, van der Veen EA, van Kessel H, Harmsen-Louman W** 1984 Estrogens in the feedback regulation of gonadotropin secretion in men: effects of administration of estrogen to agonadal subjects and the antiestrogen tamoxifen and the aromatase inhibitor delta'-testolactone to eugonadal subjects. Andrologia 16:568–577
10. **Stahl F, Schnorr D, Rohde W, Poppe I, Geier T, Dörner G** 1983 Effects of tamoxifen on the levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin (PRL), 17 β-oestradiol (E2), total and free testosterone (T) and total and free dihydrotestosterone (DHT) in blood of patients with benign prostatic hyperplasia. Exp Clin Endocrinol 82:21–28
11. **MacLean C, Newberry S, Maglione M, McMahon M, Ranganath V, Suttorp M, Mojica W, Timmer M, Alexander A, McNamara M, Desai SB, Zhou A, Chen S, Carter J, Tringale C, Valentine D, Johnsen B, Grossman J** 2008 Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. Ann Intern Med 148:197–213
12. **Smith MR, Fallon MA, Lee H, Finkelstein JS** 2004 Raloxifene to prevent gonadotropin-releasing hormone agonist-induced bone loss in men with prostate cancer: a randomized controlled trial. J Clin Endocrinol Metab 89:3841–3846
13. **de Vos D, Slee PH, Stevenson D, Briggs RJ** 1992 Serum elimination half-life of tamoxifen and its metabolites in patients with advanced breast cancer. Cancer Chemother Pharmacol 31:76–78
14. **Czock D, Keller F, Heringa M, Rasche FM** 2005 Raloxifene pharmacokinetics in males with normal and impaired renal function. Br J Clin Pharmacol 59:479–482
15. **Fideleff HL, Frigeri AE, Sobrado PG, Llano MN, Ruibal GF, Boquete HR** 1999 Reproducibility and variability of the arginine test in normal adults. Comparison between sexes. Medicina (B Aires) 59:249–253
16. **Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, Ho KK** 2001 Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. Am J Physiol Endocrinol Metab 281:E1191–E1196
17. **Gibney J, Johannsson G, Leung KC, Ho KK** 2005 Comparison of the metabolic effects of raloxifene and oral estrogen in postmenopausal and growth hormone-deficient women. J Clin Endocrinol Metab 90:3897–3903
18. **Nguyen TV, Nelson AE, Howe CJ, Seibel MJ, Baxter RC, Handelsman DJ, Kazlauskas R, Ho KK** 2008 Within-subject variability and analytic imprecision of insulin-like growth factor axis and collagen markers: implications for clinical diagnosis and doping tests. Clin Chem 54:1268–1276
19. **Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL** 2003 The biological variation of testosterone and sex hormone-binding globulin (SHBG) in polycystic ovarian syndrome: implications for SHBG as a surrogate marker of insulin resistance. J Clin Endocrinol Metab 88:1528–1533
20. **Birzniece V, Sata A, Sutanto S, Ho KK** 2010 Paracrine regulation of growth hormone secretion by estrogen in women. J Clin Endocrinol Metab 95:3771–3776
21. **Fodor M, Oudejans CB, Delemarre-van de Waal HA** 2001 Absence of androgen receptor in the growth hormone releasing hormone-containing neurones in the rat mediobasal hypothalamus. J Neuroendocrinol 13:724–727
22. **Chapman IM, Hartman ML, Pieper KS, Skiles EH, Pezzoli SS, Hintz RL, Thorner MO** 1998 Recovery of growth hormone release from suppression by exogenous insulin-like growth factor I (IGF-I): evidence for a suppressive action of free rather than bound IGF-I. J Clin Endocrinol Metab 83:2836–2842
23. **Corsello SM, Rota CA, Putignano P, Della Casa S, Barnabei A, Migneco MG, Vangeli V, Barini A, Mandalà M, Barone C, Barbarino A** 1998 Effect of acute and chronic administration of tamoxifen on GH response to GHRH and on IGF-I serum levels in women with breast cancer. Eur J Endocrinol 139:309–313
24. **Mandalà M, Moro C, Ferretti G, Calabro MG, Nolè F, Rocca A, Munzone E, Castro A, Curigliano G** 2001 Effect of tamoxifen on GH and IGF-1 serum level in stage I-II breast cancer patients. Anticancer Res 21:585–588
25. **Kelly JJ, Rajkovic IA, O'Sullivan AJ, Sernia C, Ho KK** 1993 Effects of different oral oestrogen formulations on insulin-like growth factor-I, growth hormone and growth hormone binding protein in postmenopausal women. Clin Endocrinol (Oxf) 39:561–567
26. **Duschek EJ, Gooren LJ, Netelenbos C** 2004 Effects of raloxifene on gonadotrophins, sex hormones, bone turnover and lipids in healthy elderly men. Eur J Endocrinol 150:539–546
27. **Duschek EJ, Gooren LJ, Netelenbos C** 2005 Comparison of effects of the rise in serum testosterone by raloxifene and oral testosterone on serum insulin-like growth factor-1 and insulin-like growth factor binding protein-3. Maturitas 51:286–293