Gender Difference in the Neuroendocrine Regulation of Growth Hormone Axis by Selective Estrogen Receptor Modulators

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Context: In men, GH secretion is stimulated by estradiol derived locally from aromatization of testosterone. Recently, we showed that local estrogen also plays a major role in the central regulation of GH secretion in women. Tamoxifen and raloxifene are selective estrogen receptor modulators (SERMs), drugs that block central estrogen action but exert estrogen-like effects in the liver, inhibiting hepatic IGF-I production. The relative impact of SERMs on the GH-IGF-I axis in men and women has not been investigated.

Objective: The aim of the study was to determine whether there is a gender difference in the impact of SERMs on the GH-IGF-I axis.

Design: We conducted a comparative, randomized, open-label, crossover study of tamoxifen and raloxifene.

Patients and Intervention: Ten healthy postmenopausal women and 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 washout of 2 wk between treatments.

Main Outcome Measures: The GH response to arginine, IGF-I, testosterone, and SHBG was measured.

Results: In women, but not in men, tamoxifen significantly attenuated the GH response to arginine. The GH response was not significantly blunted by raloxifene in both sexes. Both SERMs significantly reduced mean IGF-I levels to a similar degree in men and women. In men, both SERMs significantly increased LH and testosterone levels.

Conclusions: In summary, GH secretion was blunted by tamoxifen in women in the face of reduced IGF-I feedback inhibition but not in men in whom the gonadal axis was stimulated. We conclude that potential blunting of GH secretion in men by SERMs was counteracted by concomitant central stimulation of GH secretion by testosterone. In therapeutic doses, tamoxifen may induce detrimental metabolic effects in women, but not men. **(*J Clin Endocrinol Metab* 97: E521–E527, 2012)**

Estrogen regulates the secretion and action of GH in both men and women. GH secretion is stimulated by estradiol derived locally from aromatization of testosterone. In hypogonadal men, testosterone treatment results in a significant increase in GH levels (1). This effect is blocked by central estrogen receptor antagonist tamoxifen, which indicates that testosterone stimulation of GH secretion requires prior aromatization to estradiol (1). Recently, we showed in women that local estrogen also stimulates GH secretion (2). Thus, estrogen plays a major role

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

in the central stimulation of GH secretion in both men and women.

In contrast to its central stimulatory effect, estrogen antagonizes the signaling of the GH receptor (3). When estrogen is administered orally, the liver is exposed to supraphysiological concentrations, resulting in a fall in circulating IGF-I levels (3, 4). Thus, estrogen exerts complex effects on the GH-IGF axis— central stimulatory and peripheral inhibitory effects.

Tamoxifen and raloxifene are selective estrogen receptor modulators (SERMs) in wide therapeutic use. Both antagonize central estrogen action but exert estrogen-like effects in the liver (5, 6). Therefore, these SERMs simultaneously block the central paracrine effects of estrogen while exerting a peripheral endocrine agonist effect. It can be predicted that by blocking central estrogen action, SERMs reduce GH secretion, and by exerting hepatic agonistic effects, they reduce IGF-I production. Indeed, we reported recently that in women tamoxifen reduced GH and IGF-I levels (2). However, the effects of other SERMs such as raloxifene have not been studied.

In men, estrogen plays a major role not only in the regulation of the GH-IGF-I axis, but also in the pituitary-gonadal axis. Testosterone-mediated negative feedback on LH secretion requires aromatization to estradiol (7). We recently reported that central estrogen blockade with SERMs results in stimulation of the pituitary-gonadal axis, enhancing LH secretion and increasing testosterone levels (8). The higher testosterone levels may in turn result in secondary stimulation of GH secretion. Therefore, it is conceivable that central estrogen blockade may result in an effect on the GH-IGF-I axis in men that is different from women.

The present investigation tests the hypothesis that the effects of estrogen receptor modulation on the GH-IGF-I axis differ between men and women. We have undertaken a prospective, randomized, two-phase crossover study comparing the effects of the two most commonly used SERMs, tamoxifen and raloxifene.

Subjects and Methods

Subjects

Ten healthy postmenopausal women and 10 healthy men (aged 51–80 yr) were recruited from the community through advertisements. Study participants were in good general health and had normal hematological tests and renal and hepatic function. Exclusion criteria included body mass index of at least 30 kg/m^2, 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. 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 tamoxifen (Genox, Alphapharm Pty., Sydney, Australia) and raloxifene (Evista, Eili Lilly Co., Indianapolist, IN). Each of the phases consisted of two doses of tamoxifen or raloxifene given for consecutive 2 wk each (Fig. 1). The dose of tamoxifen was 10 mg/d for the first 2 wk and 20 mg/d for another 2 wk. The dose of raloxifene was 60 mg/d for the first 2 wk and 120 mg/d for another 2 wk. The washout between the two SERMs treatments was 2 wk.

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 underwent measurements at baseline and after each of the tamoxifen and raloxifene treatment doses. On each visit, assessment of GH status using the arginine stimulation test was performed, and circulating IGF-I, LH, 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. Data on tamoxifen effect in women and in men have been previously published (2, 8).

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) were infused over a 30-min period. Blood samples for GH level measurements were taken at baseline and at 30, 60, 90, and 120 min after commencement of arginine infusion. In healthy subjects, the arginine stimulation test has been shown to have good intraindividual reproducibility (9).

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 a sensitivity of less than 0.1 mIU/liter. The coefficients of variation (CV) for GH were 4.4% at 6.5 mIU/liter and 3.5% at 17.6 mIU/

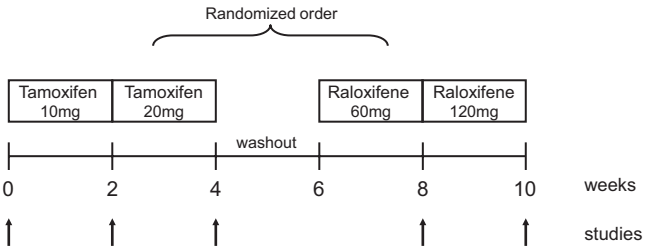

FIG. 1. Study design. Ten healthy postmenopausal women and 10 healthy men were randomized to 2-wk sequential treatment with tamoxifen (10 and 20 mg/d) and raloxifene (60 and 120 mg/d) with a washout of 2 wk in between. Studies were undertaken at baseline and after each of the treatment periods.

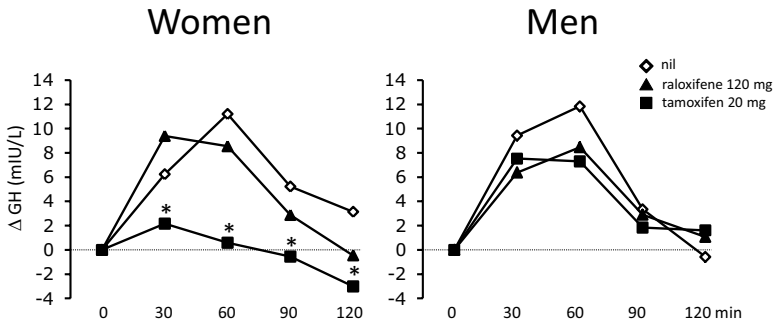

FIG. 2. GH response to arginine stimulation at baseline (*diamond*) and during high-dose tamoxifen (20 mg; *square*) and raloxifene (120 mg; *triangle*) treatment in healthy women and men. Data are presented as mean increment in serum GH after arginine infusion over prestimulation GH levels. *, $P < 0.05$ compared with nil. Conversion factor, 1 mIU/liter = 0.33 μg/liter.

liter. Serum IGF-I was measured by RIA after acid ethanol extraction as previously described (10–12). The CV 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 commercial assay (Immulite 2000; Siemens Medical Solution Diagnostics, Los Angeles, CA). The CV for SHBG at 5.3 and 86.2 nmol/liter were 5.0 and 7.5%, respectively. Serum testosterone was measured by RIA using commercial assay (Immulite 2000; Siemens Medical Solution Diagnostics). The interassay CV for testosterone at 3.6 and 23 nmol/liter were 9.3 and 9.0%, respectively. Serum LH was measured by ELISA calibrated against the WHO 2nd IS 80/552 (Diagnostics Biochem Canada Inc., London, Ontario, Canada). The CV 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 displayed 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, and testosterone were assessed using paired *t* tests with Bonferroni's correction and comparison between treatments analyzed by factorial ANOVA where appropriate. Results were expressed as mean with 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 was 63.6 ± 2.2 yr for the women and 64.8 ± 3 yr for the men. The mean body mass index was 24.9 ± 1.3 kg/m^2 for the women and 26.3 ± 1.2 kg/m^2 for the men. Median baseline GH concentrations were 2.1 and 0.7 mIU/liter for women and men, respectively. In men, mean baseline concentrations were 14.8 ± 1 nmol/liter for testosterone and 1.3 ± 0.1 IU/liter for LH.

Growth hormone

In women, when compared with the baseline study, the GH response to arginine was significantly attenuated by a 20-mg ($P < 0.05$) but not 10-mg dose of tamoxifen. The overall reduction in GH response by the higher dose of tamoxifen was 88% (Fig. 2). The peak GH response to stimulation was also significantly reduced by a 20-mg dose of tamoxifen (Table 1). In men, the GH response to arginine was not significantly reduced by tamoxifen, although a trend was evident (Fig. 2). There was a strong trend toward a greater reduction in GH response to 20 mg tamoxifen in women, with the difference approaching sta-

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

		Tamoxifen		Raloxifene	
	Baseline	10 mg	20 mg	60 mg	120 mg
Women					
Peak GH (mIU/liter)	13.6 ± 4.4	9.4 ± 2.8	4.2 ± 1.4[a]	9.1 ± 2.8	13.7 ± 5.6
IGF-I (nM/liter)	16 ± 1.6	14.5 ± 1.6	12.3 ± 1.5[a,b]	14.1 ± 1.4	13.9 ± 1.8[a]
SHBG (nM/liter)	48.3 ± 7.9	60 ± 9.4[a]	61.9 ± 9.9[a,c]	58.9 ± 8.9[a,c]	57.4 ± 8.1[a,d]
Men					
Peak GH (mIU/liter)	13.8 ± 5.2	13.6 ± 5.9	9.1 ± 2.9	7.4 ± 2.6	9.8 ± 2.5
IGF-I (nM/liter)	18.9 ± 1.6	18.5 ± 2.1	14 ± 1.6[a,b]	20 ± 1.9	17.4 ± 1.9
SHBG (nM/liter)	33.5 ± 3.4	40.9 ± 4.1[a]	38.9 ± 3.5[a,b]	36.4 ± 3.4	34.4 ± 3.1
LH (IU/liter)	1.3 ± 0.1	1.7 ± 0.2[a]	2.3 ± 0.2[a,b]	1.6 ± 0.3	1.6 ± 0.2[a]
Testosterone (nM/liter)	14.8 ± 1	18.6 ± 1.7	22.6 ± 2[a,b]	18.6 ± 1.5[a]	18 ± 1.5[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.

[c] $P = 0.05$ *vs.* men.

[d] $P < 0.05$ *vs.* men.

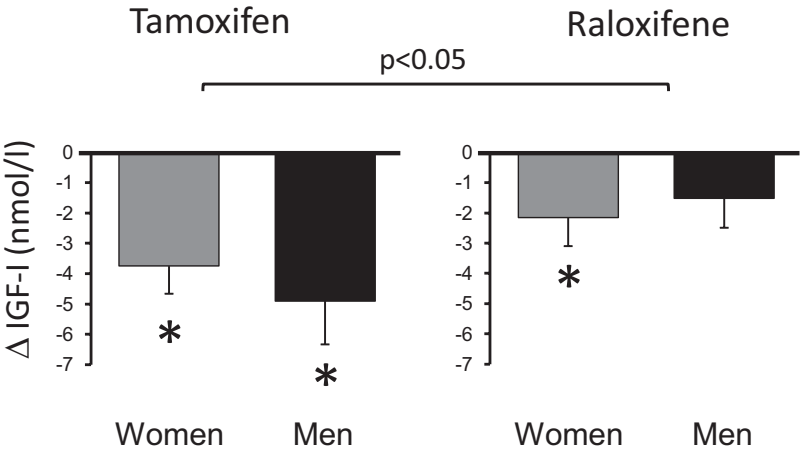

FIG. 3. Changes in serum IGF-I levels in women and men during 20 mg tamoxifen and 120 mg raloxifene treatments. Data are expressed as means ± SEM. *, $P < 0.05$ compared with nil.

tistical significance ($P = 0.06$; Fig. 2). Raloxifene did not significantly reduce the GH response to stimulation in either women or men.

Thus, tamoxifen significantly attenuated GH response to stimulation in women but not in men. Raloxifene failed to exert a significant reduction in both women and men.

IGF-I levels

In both women and men, mean IGF-I levels did not change significantly during treatment with 10 mg of tamoxifen. In both women and men, the mean IGF-I level significantly fell during treatment with 20 mg tamoxifen ($P < 0.01$; Fig. 3 and Table 1); however, the effect between women and men was not significantly different. In women, mean IGF-I levels fell significantly with a 120-mg dose of raloxifene ($P < 0.05$; Fig. 3 and Table 1). In men, IGF-I levels did not fall significantly with this dose, although a trend was evident. The reduction in IGF-I levels was significantly greater with tamoxifen than raloxifene treatments in both men and women ($P < 0.05$). The effect of both SERMs on circulating IGF-I levels was not significantly different between men and women.

SHBG levels

In women, SHBG rose significantly by 24.0 ± 5.6 and 29.5 ± 6.4% during treatment with 10 and 20 mg tamoxifen, respectively ($P < 0.01$; Fig. 4 and Table 1). In men, the mean SHBG level also rose significantly during 10 and 20 mg tamoxifen treatments by 23.1 ± 5.4 and 20.2 ± 6.9%, respectively ($P < 0.05$). The increase in SHBG during tamoxifen treatment was greater in women than in men. In women, mean circulating SHBG levels also increased significantly during treatment with 60 and 120 mg raloxifene by 23.7.0 ± 5.1 and 22.0 ± 4.0% ($P < 0.01$; Fig. 4 and Table 1). In men, raloxifene did not significantly increase mean circulating SHBG levels. The effect of raloxifene was significantly greater in women compared with that in men ($P < 0.05$). Thus, the treatment effect of both SERMs on circulating SHBG was significantly greater in women than in men.

LH levels

In men, mean LH concentrations rose significantly during tamoxifen and raloxifene treatments. Circulating LH levels were significantly increased during 10- and 20-mg tamoxifen treatment in a dose-dependent manner ($P < 0.01$ and $P < 0.001$, respectively; Table 1). Only the 120-mg dose of raloxifene significantly increased mean LH levels ($P < 0.05$; Table 1). The treatment effect of tamoxifen on LH was significantly greater than raloxifene ($P < 0.01$).

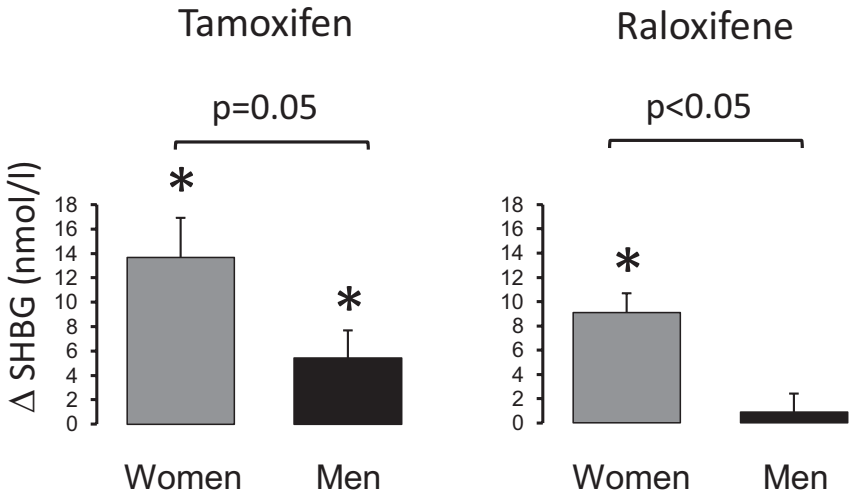

FIG. 4. Changes in serum SHBG levels in women and men during 20 mg tamoxifen and 120 mg raloxifene treatments. Data are expressed as means ± SEM. *, $P < 0.05$ compared with nil.

Testosterone

In men, mean testosterone levels increased during tamoxifen treatment in a dose-dependent manner ($P < 0.01$; Table 1). Mean circulating testosterone levels also increased significantly with both doses of raloxifene administration ($P < 0.05$; Table 1). The overall treatment effect on testosterone levels during tamoxifen was greater than with raloxifene treatment ($P < 0.05$).

Discussion

We have investigated whether there is a gender difference in the perturbation of

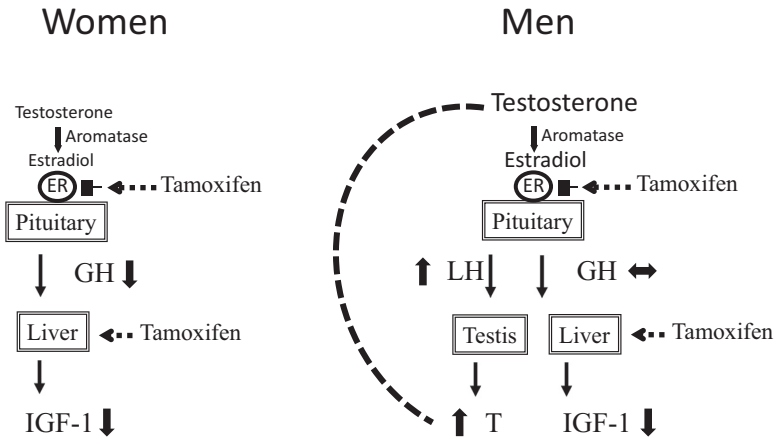

FIG. 5. In women, tamoxifen attenuates estrogen-stimulated GH secretion by blocking central estrogen receptors. In the liver, it exerts estrogen-like effect to further reduce IGF-I production. In men, tamoxifen treatment stimulates LH, which is followed by an increase in testosterone levels. The increase in testosterone will result in greater substrate availability for aromatization to estradiol, which counteracts the inhibitory effect of tamoxifen on GH secretion. As a consequence, there is no net effect on GH secretion, contrary to that in women. Tamoxifen, by exerting an estrogen-like effect on the liver, reduces IGF-I levels similar to that in women. ER, Estrogen receptor; T, testosterone.

the GH-IGF-I axis by two widely used estrogen receptor modulators. Tamoxifen significantly attenuated GH response to stimulation in women, but not in men. In women, both tamoxifen and raloxifene significantly reduced mean IGF-I and increased SHBG levels. In men, only tamoxifen significantly reduced circulating IGF-I and increased SHBG levels. The effect of both SERMs on circulating IGF-I levels was not significantly different between men and women, whereas their effect on SHBG was greater in women. In men, both drugs significantly increased LH and testosterone concentrations in a dose-dependent manner. Thus, these two SERMs exert a greater inhibitory effect on GH secretion in women than in men, but a similar degree in IGF-I reduction in both sexes.

Previous observations from our laboratory provide strong evidence that estrogens, derived from aromatization of testosterone rather than from the circulation, regulate central GH secretion in both women and men (1, 2). We have previously reported that tamoxifen increases circulating testosterone and estradiol in normal men (1). Thus, the observed increase in testosterone in men may result in higher circulating estradiol levels, which does not allow us to discriminate between putative effects of circulating and local estrogens. However, strong evidence for a dominant role of locally produced estrogens through aromatization comes from earlier studies. In peripubertal boys, nonaromatizable androgens fail to enhance GH secretion (13), in contrast to the inhibitory effects of aromatase inhibitors (14). Studies in rodents provide additional evidence of the importance of local estrogen in GH biology. In the aromatase knockout mice, somatotropes are hypoplastic, and GH levels are low (15). In men with inactivating mutations of aromatase gene, the GH response to stimulation is markedly blunted (16). Moreover, estradiol replacement in these men with aromatase deficiency does not restore GH secretion, further supporting evidence that GH secretion is stimulated by locally produced estrogen through aromatization (16). The central estrogen antagonistic properties of SERMs render these compounds invaluable pharmacological probes that provide unequivocal evidence for central paracrine control of GH secretion by estrogens. The corollary is that as therapeutic agents, they impair the GH system.

Our study revealed that this effect of SERMs on GH secretion was greater in women. What factors can explain this gender difference? We postulate that the effect in men is masked by concomitant activation of the gonadal axis, which in turn stimulates the GH axis. In men, estrogen also mediates the negative feedback of testosterone on LH secretion centrally via aromatization (7, 17). We have shown here and elsewhere that the central blockade of estrogen action by SERMs removes feedback inhibition, enhancing LH secretion leading to a secondary increase in testosterone production in men, an effect that does not occur in women (8). Higher testosterone levels in men increase substrate availability for aromatization to estradiol, enhancing central GH drive, and attenuating the tamoxifen-induced inhibitory effect observed in women as depicted in Fig. 5.

The finding that IGF-I levels were reduced to the same extent by tamoxifen in both sexes was unexpected. The net effect of SERMs on circulating IGF-I reflects the sum from a central inhibitory component on GH secretion and a peripheral hepatic component. SERMs exert hepatic estrogen-like effects that inhibit GH action, reducing IGF-I production from the liver (18, 19). The greater inhibition of GH secretion by tamoxifen in women would be expected to result in a greater reduction in mean circulating IGF-I if the net hepatic contribution was equal. However, we observed the mean reduction in circulating IGF-I to be similar between men and women.

A possible explanation may be a gender difference in the hepatic metabolism of drugs. Studies in rodents have reported that hepatic expression of cytochrome P4503A, an enzyme involved in the degradation of SERMs, is lower in males than in females (20). If this is the case in humans, a higher effect of tamoxifen would be expected in men, which could result in a greater hepatic estrogen-like effect. Another explanation may be that the hepatic sensitivity to

the estrogen agonistic properties of SERMs is less in women than in men. We measured SHBG, a hepatic estrogen-responsive protein (21). We observed that circulating SHBG was increased by SERMs to a greater extent in women compared with that in men. Therefore, it is unlikely that the liver in women is less sensitive to estrogen than in men. However, it is possible that the regulatory effects of estrogen on IGF-I and SHBG are different and that assumption of a linear effect may not be valid. In summary, there is a discordance in the net effect of SERMs on IGF-I status attributable to their central GH inhibitory action, an observation suggesting an unequal gender-related peripheral hepatic effect of these compounds.

What are the metabolic consequences of tamoxifen and raloxifene therapy? The dual blunting of GH secretion and hepatic IGF-I production indicates that these synthetic estrogen compounds induce a condition of GH deficiency. There is evidence linking the use of SERMs to detrimental metabolic outcomes. Hepatic steatosis is a consequence of GH deficiency that can progress to severe liver disease (22, 23). In men with aromatase deficiency, hepatic steatosis develops in parallel with severely reduced GH secretion (16, 24). Hepatic steatosis is a frequent and reversible complication of tamoxifen treatment for breast cancer (25), the mechanism of which is poorly understood. We speculate that the development of severe hepatic steatosis is an iatrogenic consequence arising from combined induction of central GH deficiency and peripheral blunting of GH-driven hepatic fat oxidation (11, 26). Our study observations predict that women are more susceptible than men and that in therapeutic doses, tamoxifen confers a greater detrimental effect than raloxifene. Indeed, hepatic steatosis is not a recognized complication of long-term raloxifene therapy. Our results predict that men have a reduced risk compared with women, based on the degree of GH and IGF-I suppression and the compensatory activation of the gonadal axis. Because testosterone stimulates lipid oxidation and protein anabolism, effects that are enhanced by GH (12, 27), activation of the gonadal axis provides metabolic protection.

A weakness of this study relates to small sample size. We performed comparative analysis delineating gender difference in neuroendocrine regulation of GH-IGF-I axis by SERMs using a dataset previously published in part (2, 8). We did not measure IGF-I binding proteins. It is known that SERMs increase circulating IGF binding protein-3 levels (11, 28), which in the face of reduced IGF-I levels may result in further reduction in IGF-I bioavailability and which in turn result in an even greater loss of anabolism than is apparent from the change in circulating total IGF-I (28).

In summary, this study reveals that tamoxifen and raloxifene, two widely used SERMs, perturb components of the GH system via their unique possession of tissue-specific estrogen antagonistic and agonistic actions. They exert a dual effect of inhibiting GH secretion through central antagonism while simultaneously blunting peripheral hepatic GH action. Our findings indicate that the net metabolic outcomes are likely to be gender-, dose-, and SERMs type-dependent. We conclude that in therapeutic doses, SERMs exert greater inhibitory effects on the GH secretion in women compared with that in men and may therefore result in adverse effects on fat and protein metabolism, rendering greater metabolic derangements in women than in men.

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Disclosure Summary: All authors have nothing to declare.

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