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Effects of Toremifene, a Selective Estrogen Receptor Modulator, on Spontaneous and Stimulated GH Secretion, IGF-I, and IGF-Binding Proteins in Healthy Elderly Subjects

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Context: Estrogens amplify spontaneous and stimulated growth hormone (GH) secretion, whereas they diminish GH-dependent insulin-like growth factor (IGF)-I in a dose-dependent manner. Selective estrogen receptor modulators (SERMs), including tamoxifen and toremifene, are widely adjunctively used in breast and prostate cancer. Although some endocrine effects of tamoxifen are known, few data are available for toremifene.

Objective: To explore sex-dependent effects of toremifene on spontaneous 10-hour overnight GH secretion, followed by GH-releasing hormone–ghrelin stimulation. Additionally, effects on IGF-I, its binding proteins, and sex hormone–binding globulin (SHBG) were quantified.

Participants and Design: Twenty men and 20 women, within an allowable age range of 50 to 80 years, volunteered for this double-blind, placebo-controlled prospective crossover study. Ten-minute blood sampling was done for 10 hours overnight and then for 2 hours after combined GH-releasing hormone–ghrelin injection.

Main Outcome Measures: Pulsatile GH and stimulated GH secretion, and fasting levels of IGF-I, IGF-binding protein (IGFBP)1, IGFBP3, and SHBG.

Results: Toremifene did not enhance pulsatile or stimulated GH secretion, but decreased IGF-I by 20% in men and women. IGFBP3 was unchanged, whereas while IGFBP1 and SHBG increased in both sexes to a similar extent.

Conclusions: The expected rise in spontaneous and stimulated GH secretion under the diminished negative feedback restraint of powered IGF-I favors a central inhibitory antiestrogenic effect of toremifene. Estrogenic effects of toremifene on the liver were present, as evidenced by increased IGFBP1 and SHBG levels. Men and women responded to this SERM comparably.

Freeform/Key Words: breast cancer, elderly, growth hormone, men, SERM, toremifene

Abbreviations: ApEn, approximate entropy; ANOVA, analysis of variance; CV, coefficient of variation; FSH, follicle-stimulating hormone; GH, growth hormone; GHRH, GH-releasing hormone; GLM, generalized linear model; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; LH, luteinizing hormone; SERM, selective estrogen receptor modulators; SHGB, sex hormone–binding globulin.

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Pulsatile growth hormone (GH) secretion in humans is regulated by the time-dependent interplay between the stimulating neuropeptide GH-releasing hormone (GHRH) and the inhibitory neuropeptide somatostatin. Additionally, many other factors impact this system, including negative feedback by liver-derived circulating insulin-like growth factor (IGF)-I and GH, stimulating action by hypothalamic and gastric secretion of acylated ghrelin, the active form of ghrelin, and inhibition by free fatty acids [1]. Clinical studies indicate that estradiol regulates hypothalamic–pituitary responses to each of these peptides [2–4]. The highest GH levels in humans are present at birth and at puberty, whereafter serum GH levels decrease steadily, together with testosterone, estradiol, IGF-I, and IGF-binding protein (IGFBP)3 concentrations. Relative sex-steroid deprivation accentuates GH and IGF-I depletion, because testosterone stimulates GH and IGF-I in older men, hypogonadal males of all ages, and patients undergoing (genotypic female-to-male) gender reassignment [1, 5]. The estrogen receptor antagonist, tamoxifen, blocks this effect of testosterone, suggesting involvement of estradiol in the stimulation of GH at least in young men [6].

Selective estrogen receptor modulators (SERMs) are crucial for the treatment of metastasized breast carcinoma in postmenopausal women [7, 8]. In breast tissue, this class of nonsteroidal drugs is antiestrogenic, but in other tissues it may have estrogenic effects (*e.g.*, liver, uterus, and bone). The first SERM that became available for clinical use was tamoxifen; since then, other SERMs, including toremifene, have been developed with the expectation of a more favorable action profile. The chemical structure of toremifene is identical to that of tamoxifen except for a chlorine atom at position 4. SERMs are also used adjunctively for treatment of symptomatic gynecomastia in testosterone-depleted men with prostatic cancer, as well as for prevention of osteoporosis and bone metastases [9, 10].

The effects of SERMs on the endocrine system have not been studied in detail. Scattered reports mention decreased serum gonadotropin levels, increased sex hormone–binding globulin (SHBG), and increased IGFBP-1 levels [11, 12], but the GH/IGF-I axis has not been evaluated comprehensively in detail in spite of the changed estrogenic milieu [12, 13]. Antiestrogenic effects of toremifene could attenuate GH secretion and IGF-I production, thus favoring the development of the metabolic syndrome in patients treated long-term with SERMS.

The primary endpoint of this study is spontaneous GH secretion, estimated by overnight blood sampling at 10-minute intervals and GH release induced by GH secretagogues, separately in men and women to test sex dependence. Secondary endpoints are circulating concentrations of liver-dependent proteins (*e.g.* IGF-I), the binding proteins IGFBP-1 and IGFBP-3 and SHBG, and the effects on basal levels of gonadotropins, prolactin, estrogens, and insulin resistance.

1. Material and Methods

A. Subjects

Twenty healthy, ambulatory community-dwelling older men (mean age, 65.5 years; range, 60 to 75 years) and 20 healthy postmenopausal women [mean age, 64 years; range, 53 to 73 years, and clinically defined by estradiol < 50 pg/mL, follicle-stimulating hormone (FSH) > 30 IU/L] participated in the overnight Clinical Research Unit–based study. The body mass index in men was 26.5 kg/m^2 (range, 23 to 33 kg/m^2) and in women it was 25.6 kg/m^2 (range, 18 to 36.7 kg/m^2). Volunteers were recruited by newspaper advertisements, local posters, the Clinical Trials Center Web page, and community (general and minority) bulletin boards. The key randomization in this double-blind, placebo-controlled prospective crossover study was oral placebo *vs* oral toremifene [60 mg taken once daily for 10 ($\pm$2) days, followed, after a 3-week washout interval, by the other agent]. Both men and women were studied in a

crossover design within sex. Each subject underwent two 12-hour overnight 10-minute blood sampling procedures from 2200 hours until 1000 hours in the Clinical Research Unit. A single combined injection of GHRH/ghrelin (both doses 0.3 μg/kg) was given by intravenous bolus at 0800 hours to test pituitary effects of the estrogen protocols. Overnight GH sampling is performed because >80% of total GH is secreted overnight.

To reduce nutritional confounds, volunteers were given a prescribed meal to ingest on the evening before the sampling session. Men received a standardized 10 kcal/kg meal and women an 8 kcal/kg meal (vegetarian or nonvegetarian) with a macronutrient composition of 20% protein, 50% carbohydrate, and 30% fat. Participants then remained fasting overnight (except for allowable intake of noncaloric and noncaffeinated liquids) until 1000 hours the next day. Subjects arrived in the Clinical Research Unit at or before 1800 hours to permit placement of bilateral forearm intravenous catheters. A blood sample was obtained at 0800 hours for baseline sex hormone and peptide measurements. Ambulation was allowed to the lavatory. The volunteer was allowed to sleep during the sampling window. Breakfast was offered after 1000 hours before discharge from the unit.

The protocol was approved by Mayo Institutional Review Board. Witnessed voluntary written consent was obtained before study enrollment. A complete medical history, physical examination, and screening tests of hematological, renal, hepatic, metabolic, and endocrine function were normal. Subjects underwent a single-slice computed tomography of the abdomen, level L3 to L4, as an exploratory measure to evaluate the impact of relative visceral obesity on GH responses. Existing data indicate that visceral fat mass is a key negative determinant of daily GH secretion, but the relative impact on specific secretagogue responses is unexplored. Visceral fat mass in men was 178 ± 17 cm^2 and was 95 ± 13 cm^2 in women (P = 0.001). In one female volunteer, 10-minute blood sampling during the placebo phase was incomplete due to technical problems.

B. Materials

Human GHRH was obtained from Bachem Americas (Torrance, CA). Ghrelin from C.S. Bio (Menlo Park, CA), and toremifene was from GTx (Memphis, TN).

C. Exclusion Criteria

Exclusion criteria were acute or chronic systemic diseases, HIV positivity by medical history, anemia, endocrine disorders (except hypothyroid subjects who were biochemically euthyroid on replacement), psychiatric illness, alcohol or drug abuse, history or suspicion of prostatic disease [elevated prostate-specific antigen (>4.0 ng/mL)], obstructive uropathy, deep venous or arterial thromboses, cancer of any type (except localized basal or squamous cell cancer of the skin treated surgically without recurrence), allergy to medications used in the study, significant recent weight change (loss/gain of 6 or more pounds during 6 weeks), transmeridian travel (exceeding three time zones within the preceding 3 weeks), systemic drugs, abnormal renal, hepatic, or hematologic function, concomitant sex hormone replacement, pregnancy or lactation, and unwillingness to provide written informed consent.

D. Assays

The 10-minute serum samples were assayed in a single batch in each subject by chemiluminescence GH assay, performed using a Beckman Coulter robotics Access ultrasensitive human GH assay (Beckman Instruments, Chaska, MN). Within-assay precision was 3.8% to 6.5% (range of 100 runs), sensitivity 0.010 μg/L, and specificity 97% 22-kDa GH. The following hormones and peptides were measured in the 0800 hours fasting blood specimen. Estrone, estradiol, and high-precision testosterone were measured with liquid chromatography–tandem mass spectrometry (Agilent Technologies, Santa Clara, CA). Interassay coefficients of variation (CVs) were: estrone, 12% at 0.25 pg/mL and 7.4% at 30 pg/mL; estradiol, 10.8% at 0.29 pg/mL and 5.1% at 32 pg/mL; testosterone, 8.9% at 0.69 ng/dL, 4.0% at 45 ng/dL, and 3.5%

at 841 ng/dL. IGF-I, IGFBP3, and SHBG were quantified by solid-phase chemiluminescent assay on the Siemens Immulite 2000 automated immunoassay system (Siemens Healthcare Diagnostics, Deerfield, IL). Interassay CVs for IGF-I are 4.9% at 37 ng/mL and 5% at 225 μg/L; for IGFBP3, 4% and 3.9% at 1.0 and 4.3 mg/L, respectively; and for SHBG, 4.0% at 5.4 nmol/L and 5.9% at 74 nmol/L. IGFBP1 was determined by a two-site immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX). Interassay CVs are 10.2% at 0.49 μg/L and 6.7% at 4.5 μg/L.

Insulin was measured by a two-site immunoenzymatic sandwich assay on the Roche e411 (Roche Diagnostics, Indianapolis, IN). Interassay CVs are 3.3%, 2.8%, and 2.5% at 18, 61, and 172 mU/L. Prolactin, FSH, and luteinizing hormone (LH) were measured by two-site chemiluminescent sandwich immunoassays on a DXL 800 automated immunoassay system (Beckman Instruments, Chaska, MN). For prolactin, the interassay CVs are 3.7%, 2,1%, and 4.8% at 6.1, 16.4, and 34.5 μg/L, respectively; for FSH, the interassay CVs are 3.6%, 3.2%, and 4.7% at 6.5, 16.7, and 58.0 IU/L, respectively; and for LH, interassay CVs are 9.3%, 6.0%, and 6.0% at 1.4, 15.6, and 48.8 IU/L, respectively. Free testosterone levels were calculated on the basis of SHBG and albumin concentrations [14].

E. Secondary Analyses

E-1. Deconvolution of 10-minute GH concentration profiles

Variable-waveform deconvolution analysis was used to reconstruct secretion into underlying trains of secretory bursts, superimposed upon basal (time-invariant) secretion, allowing biexponential elimination (fixed fast half-life for rapid diffusion and advection, and estimated slow half-life for delayed metabolic elimination). The analysis implements a MATLAB-based, adaptive-mesh, multiparameter-search algorithm cross-validated earlier [15].

E-2. Approximate entropy

Secretory regularity of GH secretion was appraised via the approximate entropy (ApEn) statistic. This metric provides a sensitive (>90%) and specific (>90%) model-free and scale-invariant measure of relative randomness, due to loss of feedback control within a network [16, 17].

F. Statistical Analysis

The primary endpoints were pulsatile GH secretion determined from the 10-hour serum concentration profiles before and the 2-hour profiles after combined GHRH/ghrelin injection. Secondary outcomes were effects on serum levels of IGF-I and its binding proteins.

Data were analyzed using a generalized linear model (GLM) comprising two-way analysis of covariance with repeated measures. The categorical variables were sex (two factors), toremifene or placebo administration (two factors), and age, visceral fat, mean 10-hour GH concentration, and, where indicated, serum insulin and estrone and estradiol concentrations as covariates. Linear regression analysis was used to identify significant predictors of the liver proteins during placebo and toremifene treatment. Calculations were performed with Systat 13 (Systat Software, San Jose, CA) and MATLAB 8.6 (MathWorks, Natick, MA). A P value < 0.01 was construed as significant for the overall study.

2. Results

The 10-hour GH concentration profiles in men and women during placebo and toremifene administration are shown in Fig. 1. The upper two panels illustrate GH secretion before the injection of GHRH/ghrelin, and the lower two panels reflect GH release after intravenous administration of the GH secretagogues. The results of the deconvolution analyses, separately for men and women, are shown in Table 1. GH levels tended to be higher in women than in men, especially after GHRH/ghrelin administration.

Pulsatile GH secretion is the major mediator of biological effects, and therefore any effect on its secretion by the SERM toremifene is clinically relevant. In the GLM there were no drug or drug–sex interaction effects [$P = 0.29$ and 0.46, analysis of variance (ANOVA) $P = 0.19$]. In general, age and visceral fat are negative determinants of (pulsatile) GH secretion. Therefore, in the full GLM, both variables were used as covariates, wherein age ranged from 53 to 75 years, whereas visceral fat mass ranged from 13 to 330 cm^2. Therein, toremifene did not alter GH secretion ($P = 0.87$) or interact with sex, age, or visceral fat ($P = 0.30$, 0.73, and 0.41, respectively). Comparable results were obtained for basal GH secretion, total (pulsatile plus basal) GH secretion, mean GH pulse mass, and mean 10-hour GH concentration (detailed statistical results are not shown).

GHRH/ghrelin evoked an acute rise in GH concentrations, and this effect was larger in women than men (Fig. 1; Table 1). Toremifene did not alter this response, whether sexes were tested combined or separately. In the full GLM procedure toremifene administration also did not alter GH secretion ($P = 0.70$) or interact with other factors (sex $P = 0.65$, visceral fat $P = 0.86$, age $P = 0.77$). Also, other procedures to estimate the GH response after the administration of the GH secretagogues, that is, the difference between the 12-hour and 10-hour total and pulsatile secretion, resulted in the same outcome (data not shown).

The effect of toremifene on IGFBPs is listed in Table 2. IGF-I concentrations decreased by ~20% in men and women during the administration of the oral SERM toremifene. In the GLM procedure, treatment with toremifene was a significant factor ($P = 0.01$) without interactions by age, sex, and visceral fat mass. IGFBP1 concentrations increased by 1.5-fold in men and by 2.9-fold in women ($P < 0.0001$), and with a significant interaction with visceral fat ($P = 0.001$). In contrast, the small increase (4%) in IGFBP3 levels was borderline significant in men and women ($P = 0.032$). Sex hormone–binding globulin increased by 23% and 20% in men and women, respectively ($P < 0.01$). Estrone, estradiol, prolactin, insulin levels, and homeostatic model assessment of insulin resistance remained statistically unchanged under toremifene administration. There was a decrease in gonadotropin levels (14% to 17%) in women only (Table 2).

Regression analyses were done to test whether other factors influenced IGF-I and IGFBP1 during placebo and verum treatment (Fig. 2). Significant predictors of IGF-I during placebo were age ($\beta = -2.75 \pm 0.87$, $P = 0.004$) and mean 10-hour GH concentration ($\beta = 50.6 \pm 12.4$, $P < 0.0001$) and borderline for visceral fat ($\beta = 0.169 \pm 0.063$, $P = 0.01$) (overall ANOVA $P < 0.0001$). Regressions were not significant for IGF-I or estrone and estradiol. During toremifene treatment, only the mean GH concentration was a significant predictor of IGF-I ($\beta = 54.4 \pm 13.7$, $P = 0.001$) (ANOVA $P = 0.001$). Age was a weak predictor ($P = 0.04$), but visceral fat mass was not ($P = 0.06$). A strong predictor of IGFBP1 during the placebo phase was the fasting insulin level ($\beta = -0.366 \pm 0.100$, $P = 0.001$), whereas age was a weak predictor ($\beta = -0.138 \pm 0.64$, $P = 0.037$) (ANOVA $P = 0.002$). Visceral fat mass was also a predictor ($P = 0.005$), and its interaction with insulin was highly significant ($P = 0.007$), owing to a positive correlation between these variables ($R = 0.65$, $P < 0.0001$). During toremifene treatment, insulin was still a strongly negative predictor ($\beta = -1.151 \pm 0.289$, $P < 0.0001$), along with age ($\beta = 0.206 \pm 0.075$, $P = 0.01$) and visceral fat ($\beta = -0.051 \pm 0.013$, $P < 0.001$). Indeed, the interaction factor insulin times visceral fat was highly significant ($P = 0.005$), owing to positive correlation between these two variables ($R = 0.51$, ANOVA $P = 0.001$). Finally, the factors impacting the change in IGF-I and IGFBP1 levels were also analyzed in regression models. In the regression analyses, the change in IGF-I levels during placebo and toremifene treatment, expressed as an algebraic increase (Δ) or as their ratio, for visceral fat mass was a borderline and nonsignificant predictor ($P = 0.035$ and $P = 0.15$, respectively). Age, mean GH concentration, estrone, and estradiol levels were nonsignificant. For ΔIGFBP1 only insulin was a predictor ($P < 0.0001$) but not for the ratio.

Because of the decrease in IGF-I levels during toremifene treatment, feedback is diminished, and therefore an increase in GH ApEn was expected. However, ApEn was unchanged between placebo (0.791 ± 0.041) and toremifene treatment (0.758 ± 0.043). In the GLM procedure, treatment, sex, and age were all nonsignificant predictors for ApEn.

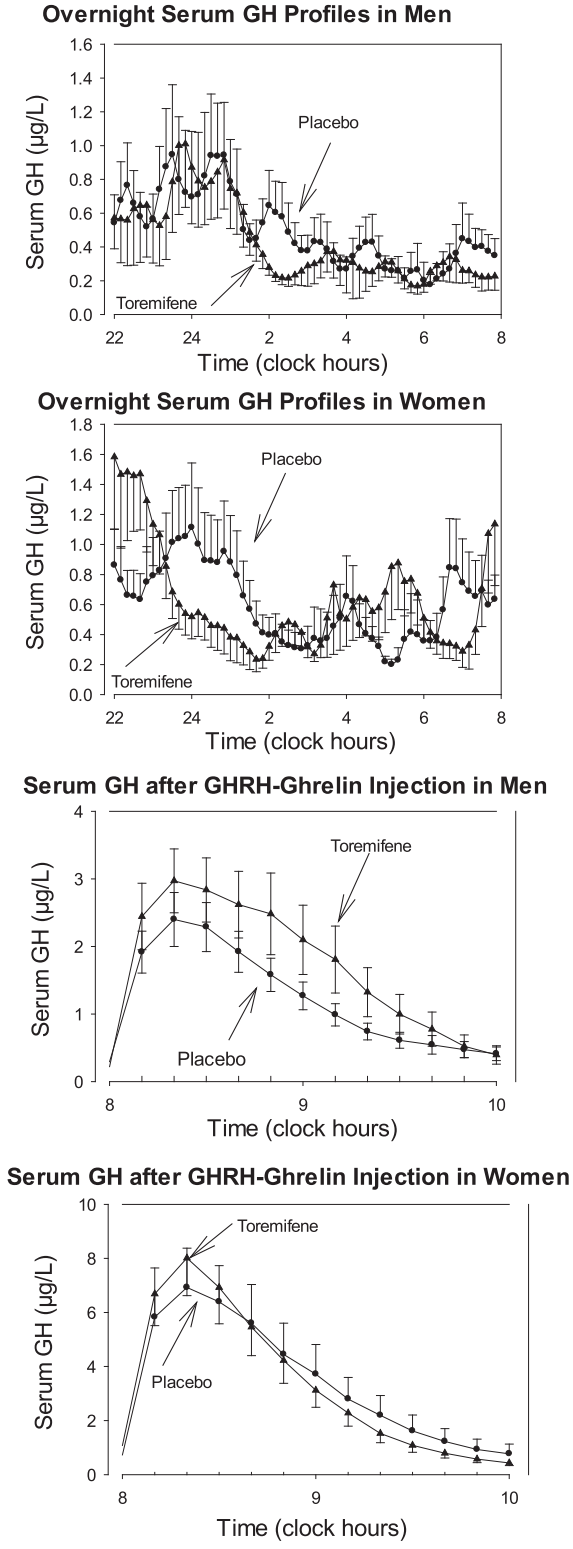

Figure 1. Measured GH time series in men and women (N = 40 total). Blood samples were drawn at 10-minute intervals, starting at 2200 hours until 0800 hours (upper two panels). At 0800 hours, the subjects received a bolus injection of 0.3 µg/kg of both GHRH and ghrelin and sampling was continued for another 2 hours. GH secretion under placebo and toremifene did differ significantly. Note differences in *y*-axis scales.

Table 1. GH Secretion Estimated by Deconvolution Analysis of 10-Hour GH Concentration Profiles

	Placebo		Toremifene	
	Men	Women	Men	Women
Basal secretion, μg/L/10 h	3.8 ± 0.6	5.6 ± 1.5	3.2 ± 0.5	3.0 ± 1.1
Pulsatile secretion, μg/L/10 h	15.9 ± 2.6	19.2 ± 3.4	13.0 ± 1.7	18.5 ± 2.6
Total secretion, μg/L/10 h	19.7 ± 3.1	24.9 ± 4.6	16.2 ± 2.0	23.5 ± 3.5
Mean pulse mass, μg/L	2.9 ± 0.5	3.7 ± 0.7	2.8 ± 0.5	3.4 ± 0.5
Half-life, min	14.9 ± 0.9	13.7 ± 1.1	14.1 ± 1.0	15.4 ± 1.2
Mode, min	15.4 ± 0.8	15.9 ± 1.0	16.2 ± 1.2	15.9 ± 0.8
GHRH/ghrelin-stimulated pulse mass, μg/L	8.4 ± 1.1	20.8 ± 4.2[a]	11.4 ± 2.2	20.9 ± 3.6[b]
Mean 10-hour concentration, μg/L	0.49 ± 0.08	0.60 ± 0.01	0.43 ± 0.07	0.63 ± 0.09[c]

Data are mean ± standard error of the mean. Statistical differences between men and women were with the unpaired two-tailed Student. *t* test.
[a] $P = 0.02$.
[b] $P = 0.01$.
[c] $P = 0.03$.

3. Discussion

The salient outcome of this study is that toremifene, a widely used SERM in the treatment of breast cancer in postmenopausal women and for the treatment of symptomatic gynecomastia in androgen-deprived men with prostate carcinoma, does not augment or attenuate spontaneous and stimulated GH secretion. Estrogens play a major role in amplifying GH secretion in many species, including humans, via activating the α-type estrogen receptor of the somatotropes and GHRH-secreting neurons. The physiological role of 17β-estradiol is underscored by increasing GH secretion during the menstrual cycle, coupled to prevailing serum estradiol concentrations, the amplified GH secretion in girls with Turner syndrome treated with estrogen, and the GH-promoting effect in subjects undergoing male-to-female transition [18–20]. Furthermore, blocking the estrogen receptor with fulvestrant decreases GH secretion by one-third in estrogen-replete postmenopausal women, underscoring the vital role of estrogen receptors in the hypothalamus and pituitary gland [21]. However, the biological importance of estradiol is not restricted to women, but also applies to men, wherein

Table 2. Fasting Serum Peptide and Hormone Concentrations

	Women			Men		
Hormone/Peptide	Placebo	Toremifene	*P* Value	Placebo	Toremifene	*P* Value
Estrone, pg/mL	18.3 ± 2.1	19.0 ± 2.1	0.34	22.1 ± 1.4	23.9 ± 1.7	0.11
Estradiol, pg/mL	3.28 ± 0.33	3.37 ± 0.30	0.46	17.7 ± 1.1	19.3 ± 1.3	0.22
IGF-I, μg/L	105 ± 7.4	84 ± 7.5	<0.0001	108 ± 6.7	85 ± 5.1	<0.0001
BP3, mg/L	3.82 ± 0.19	3.91 ± 0.19	0.17	3.17 ± 0.13	3.31 ± 0.13	0.07
BP1, μg/L	3.69 ± 0.71	6.02 ± 0.89	<0.0001	2.80 ± 0.30	4.29 ± 0.48	<0.0001
Insulin, mU/L	6.38 ± 0.96	6.67 ± 0.87	0.61	7.30 ± 0.87	6.89 ± 0.80	0.51
Prolactin, μg/L	8.21 ± 0.86	7.59 ± 0.75	0.46	7.98 ± 0.73	6.73 ± 0.47	0.03
SHBG, nmol/L	59 ± 6.3	71 ± 6.8	<0.0001	43 ± 2.6	52 ± 3.9	<0.0001
LH, IU/L	22.5 ± 2.3	19.4 ± 1.4	0.026	5.6 ± 1.3	5.9 ± 0.9	0.82
FSH, IU/L	62.0 ± 5.2	52.0 ± 4.0	0.001	8.1 ± 1.8	9.0 ± 1.5	0.22
HOMA-IR	1.50 ± 0.25	1.52 ± 0.22	0.88	1.61 ± 0.20	1.58 ± 0.18	0.80
Total testosterone, ng/dL	18.7 ± 2.5	18.0 ± 2.4	0.90	402 ± 29	455 ± 28	0.012
Free testosterone, ng/dL	NC	NC		7.42 ± 0.53	7.61 ± 0.48	0.70
Bioavailable testosterone, ng/dL	NC	NC		153 ± 11	156 ± 10	0.77

Data are mean ±SEM. A Student two-tailed *t* test for paired observations was used. *P* values are for ANOVA.
Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; NC, not calculated.

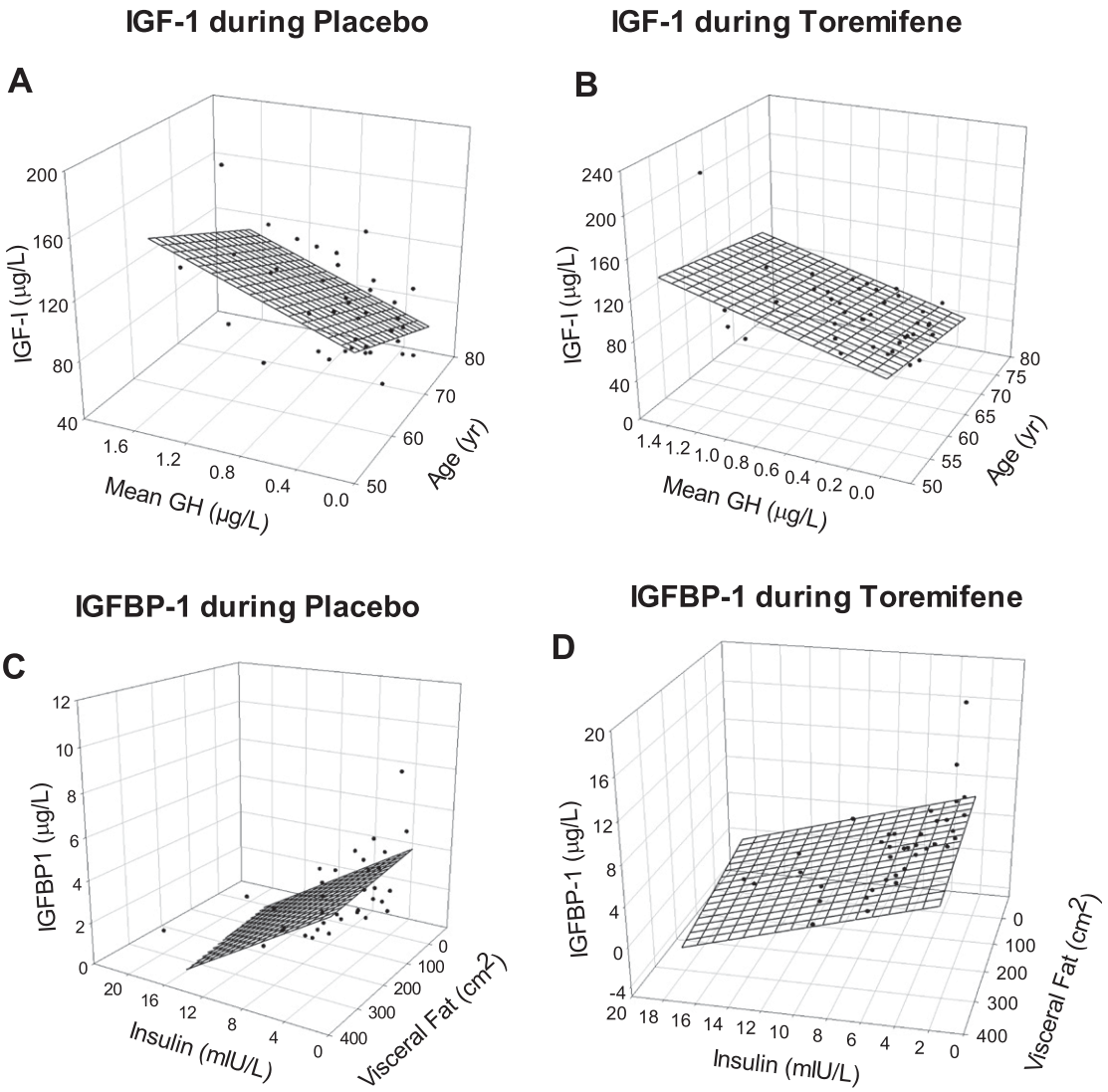

Figure 2. Serum IGF-I concentrations (vertical axis) plotted vs mean serum overnight GH concentration (mean of 60 samples) and age (horizontal and oblique axes) during placebo and toremifene treatment (upper two panels). The lower two panels show the regression between serum IGFBP1 concentration (vertical axis) and visceral fat and fasting insulin (horizontal and oblique axes) during placebo and toremifene treatment. Note differences in vertical axes scales.

testosterone is locally aromatized to 17β-estradiol in brain [6, 22]. Aromatase inhibition with anastrozole leads to diminished GH secretion in men, whereas administration of non-aromatizable androgens in hypogonadal men does not increase GH secretion [23]. Finally, the aromatase-knockout mouse has downregulated GH synthesis in somatotropes, which can be reversed by estradiol [24].

SERMs have partial agonistic and partial antagonistic effects, depending on the tissue involved, and probably also to the presence of α- and β-estrogen receptors. Nongenomic mechanisms may also play a role via the G protein–coupled estrogen receptor [25]. The endocrine effects of toremifene have not been studied well. In particular, no GH data could be found after extensive literature search, only a comment that fasting GH levels increase during toremifene treatment [12]. Nevertheless, limited GH data are available for tamoxifen, a SERM with similarity to toremifene, except for a chlorine atom at position 4 of the molecule. It seemed reasonable to assume that the pharmacokinetic profile of these two drugs would not differ greatly. In a small study comprising six postmenopausal women with breast carcinoma, chronic administration of tamoxifen numerically but nonsignificantly diminished the GH

response to a 50-μg GHRH bolus [26]. In other studies tamoxifen decreased GH secretion after arginine infusion in women, but not significantly in men [27, 28]. The sex difference in GH response was attributed to the 1.5-fold increase in serum total testosterone concentration in men on tamoxifen, providing more substrate for local conversion into estradiol [28]. In the present study, we also noted increased testosterone levels in men, but here the rise was fully attributable to the increased SHBG levels. Specifically, both bioavailable and free testosterone concentrations remained unchanged under toremifene in our study. Another study in 10 postmenopausal women after mastectomy for breast cancer reported diminished GHRH-stimulated GH secretion after 6 to 12 months treatment with 20 mg of tamoxifen [29]. In line with our study, another SERM, raloxifene, did not change the GH response to L-arginine infusion in men and women, but exhibited well-known estrogenic liver effects on SHBG and IGF-I [28]. Therefore, the hormonal effects of toremifene resemble those of raloxifene.

In contrast to all other earlier studies evaluating the effect of SERMs on GH, we did not rely exclusively on GH stimulation to infer conclusions about overall GH production. Instead, the present study estimated GH secretion overnight, when most (>85%) of the 24-hour secretion takes place. One elegant study has shown that the administration of different types of estrogens (ethinyl estradiol, conjugated equine estrogen, and estradiol valerate) causes diminished IGF-I levels in healthy postmenopausal women and that the IGF-I decrease is correlated with the increase in the mean 24-hour serum GH concentration [30]. The observed 20% decrease in serum IGF-I concentrations found in our study was accompanied by unchanged IGFBP3 and increased IGFBP1 levels. These changes are expected to decrease free and biologically available IGF-I. Diminished IGF-I feedback should have led to increased GH secretion in our male and female volunteers. Therefore, the absence of any GH amplification during overnight blood sampling and after injection of GHRH/ghrelin as a strong stimulus suggests a central antagonistic estrogenic effect of toremifene. This might also explain the unchanged approximate entropy of GH profiles, which should have increased under diminished IGF-I feedback, as recognized in fasting and after pegvisomant injection [31]. Additional *in vitro* and animal studies are required to localize this hypothesized effect, that is, the somatotroph and/or GHRH and ghrelin or somatostatin neurons. Thus, toremifene does not change spontaneous and dual peptidyl-stimulated GH secretion in either sex, but prevents GH amplification under putatively diminished IGF-I feedback restraint.

Depending on the route of administration, estrogens affect the synthesis of various proteins in the liver, including GH-dependent IGF-I, two of its binding proteins, IGFBP1 and IGFBP3, and SHBG. Other routes of administration of estrogens, that is, transdermal or intravaginal, avoid first-pass liver effects, and they generally have less or no effect on these measures, unless the dose is increased. In our study, toremifene resulted in a 20% decrease in serum IGF-I concentrations in men and women with only a weak negative effect of visceral fat. An IGF-I–lowering effect has been noted in a few other SERM studies. Tamoxifen in a 20-mg dose lowers serum IGF-I in men and women, but raloxifene seems to have a lesser action (14% vs 20% to 25% in tamoxifen reports) and only at a high dose in women, but not in men [28, 32]. A drawback of these studies is that they are rather small (about 10 subjects), and hence negative results might be caused by lack of statistical power.

In regression analyses, separately done for the placebo and toremifene treatments, IGF-I levels related positively to visceral fat and mean 10-hour GH concentrations and negatively to age, as predicted from other studies on estrogen on IGF-I [1]. IGFBP3 is the major IGF-I–binding protein that associates together with acid labile subunit to form a ternary complex, regulated by GH. Most studies indicate that estrogens do not influence circulating IGFBP3 levels [33–35], with one exception [36]. Studies reporting effects of SERMs on IGFBP3 levels are rare. One study mentions that raloxifene increased IGFBP3 levels in postmenopausal women, whereas estradiol diminished IGFBP3 [32]. Our findings of unchanged IGFBP3 levels during toremifene administration are thus in line with reported absent effects of 17β-estradiol on the concentration of this binding protein. The other important IGF-binding protein is IGFBP1, which is regulated reciprocally by insulin, thereby raising levels of free IGF-I postprandially. Higher free IGF-I confers anabolic effects. Estrogens increase serum IGFBP1 concentration [34,

37, 38]. However, no data on the effect of SERMs on this binding protein have been reported previously. In this study, toremifene increased IGFBP1 levels by 1.4- and 1.5-fold in men and women, respectively, during treatment with toremifene. A strong negative predictor of IGFBP1 levels during placebo and toremifene treatment was the fasting insulin concentration, in agreement with literature data [39]. There were no evident factors that modulated the increase of IGFBP1 by toremifene. Whether diminished bioavailable IGF-I, as inferred from this study, is clinically relevant is not known. However, diminished free IGF-I during toremifene treatment might restrict tumor growth in breast and prostate cancer. Moreover, IGF-I might also promote the emergence of the metabolic syndrome after hormone deprivation.

Estradiol and other estrogens, including tamoxifen, elevate SHBG. For unclear reasons raloxifene appears to lack this stimulating effect [32], but no independent studies are available. In our study, we found an estrogen-like effect of toremifene to raise SHBG in men and women to a similar extent. In the GLM procedure visceral fat was a significant interaction factor, corroborated by the finding that the increase in SHBG depended negatively on visceral fat, although its magnitude was rather restricted.

Caveats of this study are the relative short time of drug administration and the fixed 60-mg dose. Thus, different effects are possible on a higher dose and/or during prolonged treatment. Alternatively, GH secretion was accurately quantified by the intensive blood sampling procedure, and maximal GH release was explored by the combined injection of GHRH and ghrelin. Importantly, note that single GH measurements do not reflect daily GH secretion. Moreover, at present there are no data on the relationship between stimulated GH release (by any test) and spontaneous daily GH secretion [40].

4. Summary and Conclusions

This study uncovers the central antiestrogenic effects of toremifene. The precise site of this action, either the somatotropes and/or hypothalamic neurons, is not known. Conversely, toremifene induced several well-known effects of estradiol and tamoxifen on liver proteins. From the perspective of cancer treatment, the 20% IGF-I decrease could be a favorable effect, while unchanged GH secretion will most likely protect tissues such as brain, bone, and muscle.

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