

Effects of the Selective Estrogen Receptor Modulator, Raloxifene, on the Somatotropic Axis and Insulin-Glucose Homeostasis

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ABSTRACT

Raloxifene is the first selective estrogen receptor modulator registered for the prevention and treatment of postmenopausal osteoporosis. In addition to direct effects on bone cells, estrogen and raloxifene may act indirectly via changes in hormonal homeostasis. However, the menopause-related decrease in serum insulin-like growth factor I (IGF-I) and the increase in insulin or glucose are not always reversed by estrogen replacement. Especially orally administered estrogen was reported to decrease serum IGF-I levels. Understanding the effects of estrogens and raloxifene on the GH-IGF axis and insulin-glucose homeostasis are important because of their link to bone metabolism and cardiovascular health.

We investigated the effects of raloxifene on the GH-IGF-I axis and insulin-glucose homeostasis in a cross-sectional study in the third year of the Multiple Outcomes of Raloxifene Evaluation trial, a double blind, placebo-controlled, prospective study in postmenopausal women with osteoporosis (T-score of -2.5 or less or at least two moderate vertebral fractures). Patients with diabetes mellitus were excluded from this additional study. A fasting blood sample was obtained (0 h), and women received an sc injection of 0.05 mg recombinant human GH (Humatrope)/kg BW. The second blood sample was obtained 24 h later (24 h). GH, IGF-I, IGF-binding protein-3 (IGFBP-3), insulin, and glucose were measured. Group characteristics were tested by nonparametric ANOVA. The dose-response to raloxifene was tested by linear regression models, with age and body mass as covariates.

Seven women were taking placebo, 16 were taking raloxifene (60 mg/day), and 9 were taking raloxifene (120 mg/day). Patients from the 60 mg raloxifene group were the oldest (mean \pm SD, 64.4 ± 4.2 vs. 69.3 ± 6.9 and 63.3 ± 5.9 yr for placebo, 60 mg/day raloxifene, and 120 mg/day raloxifene, respectively; $P = 0.05$). Compared with placebo users, patients taking raloxifene had higher body mass index (24.7 ± 1.7 vs. 25.0 ± 3.1 and 28.8 ± 5.8 kg/m²; $P = 0.03$). At 0 h, raloxifene use was associated with lower IGF-I/IGFBP-3 ratio (4.3 ± 0.7 vs. 2.9 ± 0.7 and 3.0 ± 0.7 nmol/mg; $P = 0.001$) and insulin/glucose ratio (13.7 ± 5.2 vs. 11.9 ± 5.9 and 9.5 ± 2.3 pmol/mmol; $P = 0.04$). Similarly, raloxifene use was associated with lower IGF-I/IGFBP-3 and insulin/glucose ratios at 24 h ($P = 0.01$ and 0.07). Glucose, GH, and IGFBP-3 levels were similar among the groups ($0.12 < P < 0.67$).

In conclusion, raloxifene use is associated with decreased serum IGF levels and insulin/glucose ratio before and 24 h after one rhGH injection in nondiabetic postmenopausal women with osteoporosis. Therefore, raloxifene may decrease liver sensitivity to GH. Other explanations are increased clearance or increased tissue sensitivity to IGF-I or insulin. The raloxifene-induced increases in bone mineral density do not appear to be mediated by reversing the age- and menopause-related decreases in IGF-I levels. The results of this small cross-sectional study need confirmation by longitudinal studies. (*J Clin Endocrinol Metab* 86: 2763–2768, 2001)

RALOXIFENE IS the first selective estrogen receptor modulator that has been registered for the prevention and treatment of postmenopausal osteoporosis (1). In addition to direct effects on bone cells, estrogen and raloxifene may act indirectly via changes in hormonal homeostasis, contributing to overall risks and benefits. The effects of estrogens on the GH-insulin-like growth factor (GH-IGF) axis and insulin-glucose homeostasis have aroused interest for many years, mainly because of their link to bone metabolism and cardiovascular health (2–5).

IGF-I is abundant in the skeleton; it enhances osteoblast function and increases bone formation (6). In postmenopausal women, IGF-I is positively related to bone mineral

density (BMD) at the spine and hip (4, 5), whereas serum GH levels correlate positively with endogenous estradiol levels (7). GH stimulates bone growth and remodeling through interaction with specific GH-binding sites and indirectly via IGFs. Besides a direct action on the skeleton, GH effects on bone and mineral metabolism may also involve intestinal calcium absorption (8, 9), 1α -hydroxylation of 25-hydroxyvitamin D, and muscle strength (10). The IGFs in blood and other compartments are bound to specific binding proteins (IGFBPs) that modulate IGF-I action. IGFBP-3 is the most abundant IGFBP in the circulation (11).

The GH-IGF axis is strictly related to the insulin-glucose homeostasis. Hypoglycemia is a strong stimulator of GH secretion, and insulin is necessary for the GH-stimulated IGF-I production in the liver (12) that is responsible for 95% of circulatory IGF-I. In contrast, GH impairs insulin action on the liver and glucose incorporation by tissues (13). Finally, IGF-I down-regulates insulin (14, 15), glucagon (16), and GH secretion (negative feedback) (17).

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Loss of ovarian function is associated with lower serum GH and IGF-I and with higher insulin levels. The latter may be due to alterations in pancreatic secretion (18) and in the clearance of circulating insulin (18, 19). An increasing time interval after menopause is associated with a reduction in insulin sensitivity (20). Depending on chemical formulation, dose, and route of administration, estrogen may reverse some of those changes. Although transdermal estrogen might increase IGF-I levels (21–23) without having an effect on insulin (24, 25), orally administered estrogen was reported to decrease both serum IGF-I (21, 26–28) and insulin (29–31).

We studied the effects of the selective estrogen receptor modulator, raloxifene (60 and 120 mg/day), on GH-IGF and insulin-glucose homeostasis before and after a single sc injection of recombinant human GH (rhGH). This ancillary study was performed in the third year of the Multiple Outcomes of Raloxifene Evaluation (MORE) trial (1, 32), a double blind, placebo-controlled, prospective study of 7705 postmenopausal women with osteoporosis (T-score of -2.5 or less or at least 2 moderate vertebral fractures). We hypothesized that, similar to oral estrogen, raloxifene treatment is associated with decreased responsiveness to GH expressed by lower spontaneous IGF-I and insulin levels and with an attenuated response to rhGH injection.

Materials and Methods

At the baseline of the MORE trial, the women were at least 2 yr after menopause, or in case of hysterectomy, had serum FSH levels greater than 30 IU/L and serum estradiol levels less than 73 pmol/L. Patients with a history of metabolic bone disease, malignancy, or insulin-dependent diabetes mellitus were excluded. Their mean age was 66.5 yr, and their mean body mass index (BMI) was 25.2 kg/m². After enrolment, patients were randomly allocated to three treatment groups: placebo, 60 mg raloxifene/day, and 120 mg raloxifene/day.

The present additional study was performed in our study center in the third year of follow-up. Patients eligible for the main protocol and not using any glucose-lowering medication were asked to volunteer for this study. The minimal sample size was based on a cross-sectional study involving older women, off ($n = 7$) or on ($n = 6$) estrogen replacement, where the magnitude of the group difference in IGF-I at 0 and 24 h was estimated at, respectively, 3.4 and 5.7 SEM in the nontreated group (26). All volunteers participated in our study. During the recruitment and the test, the allocation to treatment groups was unknown because the study was still double-blinded. The study protocol was approved by the ethical review board of the Academic Hospital, Vrije Universiteit (Amsterdam, The Netherlands).

Thirty-two women were included after giving informed consent. At the time of this additional study the women were 66.6 ± 6.6 yr old, had a mean BMI of 26.0 ± 4.1 kg/m², and had fasting glucose levels of up to 6.7 mmol/L. After an overnight fast, a blood sample was obtained, and each patient received a single sc injection of recombinant human GH (rhGH; Humatrope, provided by Elli Lilly & Co., Indianapolis, IN; 0.05

mg/kg BW). After 24 h, following an overnight fast, a second blood sample was obtained. Serum samples were stored frozen in small aliquots and were only thawed once directly before assay.

Plasma glucose (millimoles per L) was assessed colorimetrically according to the hexokinase principle from heparinized blood using a Hitachi 747 apparatus (Roche, Tokyo, Japan). The measurements of serum GH, IGF-I, IGFBP-3, and insulin were performed using commercially available immunoassays. GH (milliunits per L) was measured by immunocolorimetric assay (Sorin Biomedica, Sallugia, Italy). IGFBP-3 (milligrams per L) and IGF-I (nanomoles per L) were measured by immunoradiometric assay (Diagnostic Systems Laboratories, Inc., Webster, TX), the latter after acid extraction to remove binding protein. Insulin (picomoles per L) was assessed in nonhemolytic samples using a highly specific immunoradiometric assay (Biosource Technologies, Inc., Fleurus, Belgium). The intraassay coefficients of variation were: GH, 4% or less; IGF-I, 4% or less; IGFBP-3, 4% or less; and insulin, 5% or less. The interassay coefficients of variation were: GH, 16% or less; IGF-I, 11% or less; IGFBP-3, 12% or less; and insulin, 7% or less. All specimens from a single participant were run in the same assay batch.

Statistics was determined using SAS 6.12 software (SAS Institute, Inc., Cary, NC). The group differences in patient characteristics were tested using nonparametric ANOVA (Kruskal-Wallis test). The effectiveness of rhGH injection was determined by paired *t* test. After running the preliminary Spearman correlation analysis, lower serum IGF-I levels at 0 and 24 h after rhGH injection correlated with higher age ($P = 0.003$), as expected. There was a borderline significant positive correlation between age and serum insulin levels ($P = 0.10$). BMI correlated at 0 h with IGF-I and insulin levels as well as with several responses to rhGH injection, expressed as a percentage of the spontaneous value (GH, IGF-I/IGFBP-3 ratio, glucose, and insulin/glucose ratio: $P \leq 0.10$).

Based on these results, to determine whether there was an association between raloxifene use and study variables, linear regression models were used in which raloxifene treatment was a continuous main predictor, and age and BMI were covariates. This was done for 0 and 24 h measurements as well as for the difference between 0 and 24 h (Δ). The relationships were considered significant at $P < 0.05$ and borderline significant at $P < 0.10$. The *P* values are reported without adjustment for multiple comparisons. All data are expressed as the mean \pm SD.

Results

Patients from the 60 mg raloxifene group were the oldest ($P = 0.05$; Table 1). Interestingly, patients using 120 mg/day raloxifene tended to be heavier than placebo users ($P = 0.06$), but this also was true at randomization ($P = 0.06$). The BMI and glucose changes from randomization to the additional study were not different between patients using raloxifene and those using placebo ($P = 0.25$ and 0.24).

The rhGH injection resulted in suppression of endogenous GH ($P = 0.002$; Fig. 1). The treatment groups did not differ with regard to GH levels before and 24 h after rhGH injection ($P = 0.54$ and 0.59; Table 2). As expected, the injection of rhGH resulted at 24 h in significant increases in IGF-I ($P < 0.001$), IGFBP-3 ($P < 0.001$), and IGF-I/IGFBP-3 ratio ($P < 0.001$). Before and 24 h after rhGH injection, IGF-I levels were

TABLE 1. Group characteristics in the third year of raloxifene (RLX) or placebo (PBO) treatment

	PBO	RLX 60	RLX 120	<i>P</i>
No.	7	16	9	
Age (yr)	64.4 \pm 4.2	69.3 \pm 6.9	63.3 \pm 5.9	0.05
BMI (baseline MORE; kg/m ²)	25.5 \pm 2.6	24.6 \pm 2.7	28.8 \pm 5.1	0.06
BMI (3rd yr; kg/m ²)	24.7 \pm 1.7	25.0 \pm 3.1	28.8 \pm 5.8	0.06
Δ BMI (3rd yr–0; kg/m ²)	–0.8 \pm 1.3	0.5 \pm 1.8	0.04 \pm 1.3	0.25
Glucose (baseline MORE; mmol/L)	5.2 \pm 0.4	5.3 \pm 0.6	5.4 \pm 0.7	0.95
Glucose (3rd yr; mmol/L)	5.0 \pm 0.3	5.2 \pm 0.4	5.1 \pm 0.6	0.24
Δ Glucose (3rd yr–0; mmol/L)	0.3 \pm 0.3	0.1 \pm 0.5	0.3 \pm 0.4	0.55

Δ , The change from randomization to the beginning of the ancillary study. Data are expressed as the mean and SD. Differences between groups were tested using nonparametric ANOVA (Kruskal-Wallis).

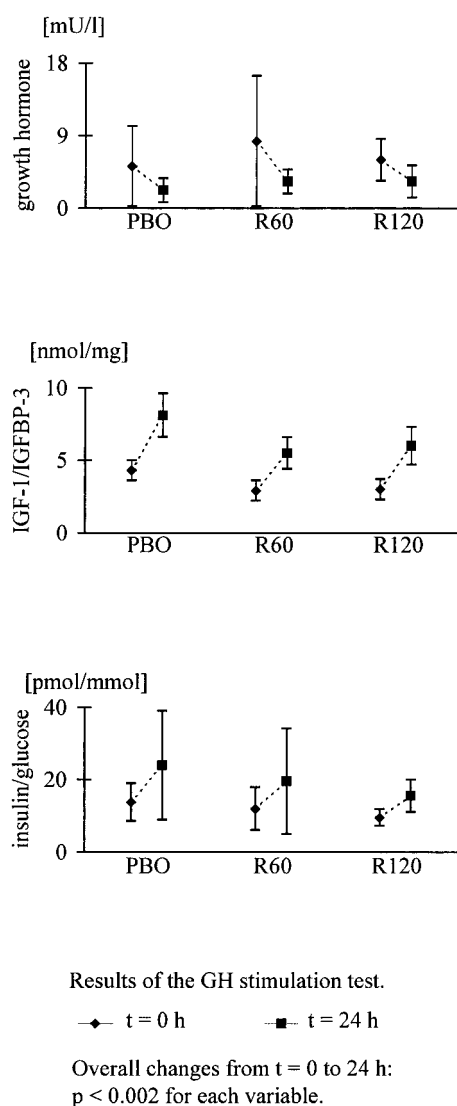


FIG. 1. GH stimulation test in the third year of treatment with placebo (PBO; $n = 7$), 60 mg raloxifene (R60; $n = 16$), or 120 mg raloxifene (R120; $n = 9$) during the MORE study. Before the test, a fasting blood sample was obtained (0 h), and the women received an sc injection of 0.05 mg rhGH (Humatrope)/kg BW. The second blood sample was obtained 24 h later (24 h). The results are expressed as the mean and SD. The trend between raloxifene dose and outcome variables was tested in linear regression models with age and BMI as covariates (see Tables 2 and 3). Overall changes were determined by t test for paired samples, with no adjustments for treatment.

progressively lower with increasing raloxifene dose ($P = 0.001$ and 0.002). Also, the absolute IGF-I change (Δ) from 0 to 24 h was lower in the raloxifene groups compared with that in placebo users ($P = 0.020$). The treatment groups were similar with regard to IGFBP-3 (0 h, 24 h, and Δ : $P > 0.66$). Consequently, the IGF-I/IGFBP-3-ratio at 0 and 24 h and the change in IGF-I/IGFBP-3 ratio were lower in raloxifene than in placebo users, confirming findings for IGF-I ($P = 0.001$, 0.002 , and 0.022 , respectively).

The data for insulin-glucose homeostasis are presented in Table 3. As expected, the injection of rhGH resulted in significant increases in insulin ($P < 0.001$), glucose ($P < 0.001$), and insulin/glucose ratio ($P < 0.001$). The insulin/glucose

ratio at 0 h was lower in patients using raloxifene than in placebo users ($P = 0.036$). The treatment groups were similar with regard to blood glucose at 0 and 24 h and to blood glucose change from 0 to 24 h ($P = 0.58$, 0.50 , and 0.12). In final linear regression models, the associations between age, BMI, and insulin or IGF remained unchanged.

Discussion

To our knowledge, this is the first study reporting the effects of raloxifene on responsiveness to GH. Compared with patients using placebo, those using raloxifene had lower serum IGF-I, IGF-I/IGFBP-3 ratio, insulin, and insulin/glucose ratio both before (0 h) and 24 h after (24 h) an injection of rhGH. This was also true for the difference from 0 to 24 h. Concerning IGF-I, similar findings were reported for oral estrogen (21, 26–28), transdermal estrogen given at high dose (33), or oral tamoxifen (34, 35). In contrast, transdermal estrogen given at low dose may increase IGF-I levels (21–23) and thus reverse menopause-related changes (4, 36). The observed relations between raloxifene treatment and fasting serum levels of insulin and glucose place raloxifene in between the oral and transdermal regimens. Regardless of effects on insulin sensitivity, most oral estrogen regimens decrease both insulin and glucose levels (29–31), whereas transdermal estrogen does not induce significant changes (24–25).

If real, the observed association between raloxifene and lower serum IGF-I could be the result of a decreased hepatic sensitivity to GH (26, 27, 37). Other explanations are that raloxifene might alter the clearance of IGF-I or that raloxifene might act at the tissue level, for example by enhancing the expression of IGF-I receptors (38, 39). Also, the reduction in serum IGF-I may not parallel the local production of IGF-I in bone or elsewhere. This is conceivable when, besides the low IGF-I levels, increased GH levels are observed. All estrogen replacement regimens that decrease serum IGF-I are associated with increases in serum GH (22, 27, 40). It is generally believed that this increased GH secretion is the result of diminished feedback by IGF-I (41). However, estrogen interaction with GH secretion at other levels, such as the hypothalamus, cannot be excluded (42–44). In our study the difference in IGF-I levels between raloxifene and placebo users was not accompanied by any significant difference in GH levels, which may be due to the small sample size and differences in BMI (45).

What the decreased liver sensitivity to GH might mean with regard to insulin sensitivity is uncertain. IGF-I attenuates the induction of insulin resistance by GH, so the decreased hepatic sensitivity to GH will result in a decreased rather than in an increased insulin sensitivity. However, although oral estrogen is believed to decrease liver sensitivity to GH, the insulin sensitivity was impaired only when treatment with alkylated estrogens or conjugated equine estrogens at 1.25 mg/day was studied (20, 29–31, 46–48).

We observed lower fasting serum insulin and insulin/glucose ratio in patients using raloxifene compared with placebo users. This finding cannot be attributed to a high BMI in patients using 120 mg raloxifene, because obesity is a risk factor for impaired insulin sensitivity and higher serum in-

TABLE 2. Somatotrophic axis in postmenopausal women with osteoporosis after treatment for 2 yr with raloxifene (RLX) or placebo (PBO)

	PBO	RLX 60	RLX 120	<i>P</i>
No.	7	16	9	
GH (0 h; mU/L)	5.2 ± 5.0	8.3 ± 8.1	6.0 ± 2.6	0.541
GH (24 h)				
mU/L	2.2 ± 3.3	3.3 ± 1.5	3.3 ± 2.0	0.585
Δ GH mU/L	-3.0 ± 5.0	-5.1 ± 8.2	-2.8 ± 3.2	0.459
IGF-I (0 h; nmol/L)	16.4 ± 4.2	11.5 ± 2.8	10.8 ± 2.4	0.001
IGF-I (24 h)				
nmol/L	35.1 ± 9.3	24.2 ± 5.0	24.8 ± 5.5	0.002
Δ IGF-I (nmol/L)	18.8 ± 6.1	12.7 ± 3.6	13.9 ± 3.8	0.020
IGFBP-3 (0 h; mg/L)	3.8 ± 0.5	4.0 ± 0.5	3.7 ± 3.7	0.860
IGFBP-3 (24 h)				
mg/L	4.3 ± 0.5	4.4 ± 0.5	4.2 ± 0.5	0.970
Δ IGFBP-3 (mg/L)	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.660
IGF-I/IGFBP-3 (0 h; nmol/mg)	4.3 ± 0.7	2.9 ± 0.7	3.0 ± 0.7	0.001
IGF-I/IGFBP-3 (24 h)				
nmol/mg	8.1 ± 1.5	5.5 ± 1.1	6.0 ± 1.3	0.002
Δ IGF-I/IGFBP-3 (nmol/mg)	3.8 ± 1.0	2.6 ± 0.8	3.0 ± 0.8	0.022

The results are expressed as the mean and SD. Fasting values before (0 h) and 24 h after (24 h) a single sc rhGH injection. The change from 0 to 24 h (Δ) was significant for each variable ($P \leq 0.002$, by paired *t* test). The association between study variables and raloxifene was tested in linear regression models in which raloxifene treatment was a continuous main predictor, and age and BMI were covariates (*P* is the value for trend).

TABLE 3. Insulin and glucose homeostasis in postmenopausal women with osteoporosis during the third year of treatment with raloxifene (RLX) or placebo (PBO)

	PBO	RLX 60	RLX 120	<i>P</i>
No.	7	16	9	
Insulin (0 h; pmol/L)	69.3 ± 29.9	62.2 ± 32.0	48.9 ± 15.5	0.058
Insulin (24 h)				
pmol/L	150 ± 114	114 ± 95	89 ± 30	0.063
Δ Insulin (pmol/L)	80.3 ± 101.3	52.1 ± 65.6	39.7 ± 18.5	0.098
Glucose (0 h; mmol/L)	5.0 ± 0.3	5.2 ± 0.4	5.1 ± 0.6	0.579
Glucose (24 h)				
mmol/L	6.0 ± 0.8	5.7 ± 0.5	5.7 ± 0.9	0.503
Δ Glucose (mmol/L)	0.9 ± 0.7	0.5 ± 0.4	0.6 ± 0.4	0.121
Insulin/glucose (t = 0) pmol/mmol	13.7 ± 5.2	11.9 ± 5.9	9.5 ± 2.3	0.036
Insulin/glucose (24 h)				
pmol/mmol	23.9 ± 15.1	19.5 ± 14.6	15.5 ± 4.5	0.073
Δ Insulin/glucose (pmol/mmol)	10.2 ± 12.5	7.5 ± 9.1	6.0 ± 3.3	0.160

The results are expressed as the mean and SD. Fasting values before (0 h) and 24 h after (24 h) a single sc rhGH injection. The change from 0 to 24 h (Δ) was significant for each variable ($P \leq 0.002$, by paired *t* test). The association between study variables and raloxifene was tested in linear regression models in which raloxifene treatment was a continuous main predictor, and age and BMI were covariates (*P* is the *P* value for trend).

ulin levels (49). Although fasting insulin has been postulated as a reliable marker of insulin resistance (50), functional studies should be performed to demonstrate improvement or deterioration of insulin action. The euglycemic insulin-glucose clamp technique is considered the most representative method of assessing insulin sensitivity in humans. Recently, it was reported that the insulin/glucose ratio was not correlated to the measurement of insulin sensitivity during the euglycemic insulin clamp (51). Besides possible effects on insulin action, the estrogen-related changes in insulin levels are a net effect of increased hepatic clearance of insulin (47), enhanced pancreatic insulin secretion in response to glucose (47), and, especially in the case of alkylated estrogens, changes in glucagon and cortisol levels (20).

The present study has some limitations. Age and BMI were unequally distributed, as the study drug allocation was blinded at the time of rhGH injection, and preliminary matching of the groups for age and BMI was not performed. Although the study subjects were randomized at baseline in the MORE study, this additional study is not randomized.

Because the rhGH dose was based on body weight (26, 52), the observed effects could be biased by obesity. For this reason the statistical testing was performed after simultaneous adjustment for age and BMI. On the other hand, high BMI would result in rhGH overdose and thus enhanced response, but the response of the 120 mg raloxifene group with the highest BMI was similar to that of the 60 mg raloxifene group. Also, one would expect higher insulin in the more obese patients of this group than in the placebo group (4). Moreover, a recent study suggests that IGF-I levels in postmenopausal women are related to age and not to body composition (41).

The results of our study cannot be extrapolated to premenopausal women or to women without osteoporosis. The fact that all women had osteoporosis may have induced a bias because of possible abnormalities in the GH-IGF-I axis in patients with osteoporosis.

The sample size of this additional study was based on the expected differences in IGF-I levels (26), and the resulting power was probably too low for demonstrating statistically

significant differences in other variables. The small sample size and the method of patient selection could also be responsible for the fact that the distribution over the treatment groups was not representative of the whole MORE population; the majority of patients had been assigned to 60 mg raloxifene. Although the number of patients in the placebo and 120 mg raloxifene groups were comparable, a drug-related reason for participation in the study must be considered. Repeating the measurements in a longitudinal setting could decrease the intersubject variance. Studies of integrated GH production or GH secretion pattern could provide more information about the raloxifene-related changes in GH secretion.

The IGF-I generation test has been postulated as a convenient method for assessing responsiveness to GH. The single rhGH injection was reported to elevate circulating IGF-I levels, with the peak concentration at 24 h after the injection (26). We extended this test to fasting insulin and glucose levels. The complaints of headache and dizziness described by others led us to use half of the reported dose (26). However, the test resulted in significant changes in all assessed parameters, suggesting that the injected dose was sufficient (52).

In summary, according to this small cross-sectional study in nondiabetic women with osteoporosis, patients using raloxifene, compared with those using placebo, had lower IGF-I levels and insulin/glucose ratios before and after a single rhGH injection. If real, such an association could be due to a decreased hepatic sensitivity to GH. Other mechanisms could be a change in the clearance of or the tissue sensitivity for IGF-I or insulin. The raloxifene-induced increases in BMD do not appear to be mediated by reversing the age- and menopause-related decreases in IGF-I levels. These observations need to be confirmed by further investigations.

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