Modulatory Effect of Raloxifene and Estrogen on the Metabolic Action of Growth Hormone in Hypopituitary Women

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Context: The metabolic action of GH is attenuated by estrogens administered via the oral route. Selective estrogen receptor modulators lower IGF-I to a lesser degree than 17β-estradiol in GH-deficient women, and their effect on fat and protein metabolism is unknown.

Objective: The aim of the study was to compare the modulatory effects of 17β-estradiol and raloxifene, a selective estrogen receptor modulator, on the metabolic action of GH.

Design: We conducted an open-label, two-group, randomized, two-period crossover study.

Patients and Intervention: Ten hypopituitary women received GH therapy alone (0.5 mg/d) and GH plus 17β-estradiol (E_2; 2 mg/d). Eleven hypopituitary women received GH therapy alone and GH plus raloxifene (R; 60 mg/d). The treatment duration was 1 month, with a 4-wk washout period.

Main Outcome Measures: IGF-I, IGFBP-3, resting energy expenditure, and fat oxidation were quantified by indirect calorimetry. We measured whole body leucine turnover from which leucine rate of appearance and leucine incorporation into protein were estimated.

Results: GH significantly stimulated all outcome measures. During GH treatment, addition of R significantly reduced mean IGF-I but not IGFBP-3, whereas E_2 reduced both IGF-I and IGFBP-3 levels. Cotreatment with R but not E_2 significantly attenuated the stimulatory effects of GH on fat oxidation. There was a strong trend ($P = 0.08$) toward a greater reduction in leucine incorporation into protein after R compared to E_2 cotreatment.

Conclusions: The modulatory effects of E_2 and R at therapeutic doses on GH action are different. R during GH therapy exerts a greater inhibitory effect on lipid oxidation and protein anabolism compared to E_2. **(*J Clin Endocrinol Metab* 95: 2099–2106, 2010)**

GH deficiency in adults causes an increase in fat mass and a reduction in muscle and bone mass (1, 2). GH replacement reduces fat mass by stimulating fat oxidation (Fox), increases lean body mass by stimulating protein synthesis, and increases bone mass by stimulating bone turnover (3–7). Gonadal steroids modify the biological action of GH. The liver is a major GH-responsive metabolic organ and the major source of circulating IGF-I, which mediates the anabolic action of GH. In healthy and GH-deficient (GHD) women, oral administration of estrogen reduces circulating IGF-I levels, suppresses whole body Fox and protein synthesis, resulting in a significant increase in fat mass and reduc-

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Abbreviations: CTX, Carboxy-terminal telopeptide of type I collagen; CV, coefficient of variation; Fox, fat oxidation; GHD, GH-deficient; ICTP, carboxy-terminal propeptide of type I procollagen; IGFBP-3, IGF binding protein-3; KIC, α-ketoisocaproic acid; LIP, leucine incorporation into protein; LRa, leucine rate of appearance; PINP, amino-terminal propeptide of type I procollagen; REE, resting energy expenditure; SERM, selective estrogen receptor modulator.

TABLE 1. Clinical characteristics of study participants

Subject no.	Age (yr)	BMI (kg/m^2)	Diagnosis	Hormone replacement
1	45	33.6	Childhood onset hypopituitarism	A, T, G
2	59	48.3	Pituitary macroadenoma	G
3	52	32.2	Prolactinoma (S)	A, T
4	49	29.4	Prolactinoma (X)	T, G
5	23	24.3	Childhood onset hypopituitarism	T, G
6	59	33.6	Sheehan's postpartum necrosis	A, T, G
7	44	32.6	Prolactinoma (S, X)	A, T
8	61	34.7	Pituitary adenoma (S)	A, T
9	58	20.5	Pituitary adenoma	G
10	39	26.9	Cushing's disease (S, X)	A, T, G
11	53	34	Pituitary macroadenoma (S)	T, G
12	49	30.2	Empty sella syndrome	A, T, G, D
13	19	21.1	Hypopituitarism, unknown cause	A, T, G
14	55	33	Pituitary adenoma	A, T
15	22	27.9	Childhood onset hypopituitarism	G
16	44	33.1	Craniopharyngioma (S)	A, T, G, D
17	31	20.6	Head trauma (TBI)	A, T, G

BMI, Body mass index; S, surgery; X, irradiation; TBI, traumatic brain injury; A, adrenal replacement; T, thyroid replacement; G, gonadal replacement; D, desmopressin.

tion in lean body mass (3, 8, 9). Inhibition of GH action does not occur when the sex steroid is administered by the transdermal route (9). The route dependency suggests a first-pass effect of estrogen inhibiting the effects of GH on the liver. Thus, administration of estrogen by the oral route may worsen the metabolic sequelae of hypopituitarism and also attenuate the beneficial effects of GH therapy.

Selective estrogen receptor modulators (SERMs) are synthetic estrogen compounds that possess tissue-specific agonist and antagonist properties. Using therapeutic dosages, we have previously reported that raloxifene, a SERM, reduced circulatory IGF-I levels to a lesser degree than 17β-estradiol in normal and GHD women (8), suggesting that raloxifene may be less potent than 17β-estradiol as a GH antagonist in the liver.

The aim of this study is to compare the impact of 17β-estradiol and raloxifene at therapeutic doses on the metabolic effects of GH in hypopituitary women during GH replacement. We investigated the effects on liver GH action, bone turnover markers, energy expenditure, and substrate metabolism.

Subjects and Methods

Subjects

Seventeen GHD women were recruited from the Endocrine Outpatient Clinic, St. Vincent's Hospital, Sydney, Australia. The power calculations based on available prior data on the reduction in IGF-I and Fox by 20–30% in GHD women during estrogen and raloxifene treatments (8) resulted in a sample size of n = 10 in each treatment group, required to show a difference at the 0.05 level with a power of 80%.

Study subject clinical characteristics are shown in Table 1. All subjects had GH deficiency diagnosed for at least 1 yr before the study. GH deficiency was confirmed with insulin tolerance test by peak GH response to insulin-induced hypoglycemia of less than 3 ng/ml. Subjects were withdrawn from estrogen replacement for at least 2 months before commencement of the study. Before and throughout the study, subjects received standard thyroid hormone and cortisol replacement for thyroid and adrenal deficiencies, respectively. The doses of replacement were unchanged throughout the study. All subjects were instructed to follow their usual diet and physical activity as well as continuing their usual medications or supplements throughout the study.

The Human Research Ethics Committee of St. Vincent's Hospital approved the study, which was conducted in accordance with the principles of the Declaration of Helsinki. All subjects gave written informed consent. The study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12605000532606).

Study design

Seventeen subjects were randomized in an open-label, crossover study. The patients took part in one (n = 13) or both (n = 4) arms of a two-phase crossover study (Fig. 1). Overall, 10 subjects took part in a comparison of GH alone with GH plus 17β-estradiol (estrogen group), whereas 11 took part in a comparison of GH with GH plus raloxifene (raloxifene group). The order of GH therapy alone and combined treatment with 17β-estradiol or raloxifene was randomized. Thus, the study allowed a paired comparison of the modulatory effects of estrogen in 10 women and of raloxifene in 11 women and an unpaired cross-sectional comparison between estrogen and raloxifene effects in 17 women. The duration of treatment was 4 wk, followed by a 4-wk washout. GH was administered in a dose of 0.5 mg/d sc in the evening (Humatrope; Eli Lilly Australia, West Ryde, NSW, Australia), 17β-estradiol in a dose of 2 mg/d orally, and raloxifene (Evista; Eli Lilly Australia) in a dose of 60 mg/d orally. Medroxyprogesterone acetate (10 mg/d) was administered for 10 d after 17β-estradiol treatment to induce withdrawal bleeding.

The effects of GH on the following parameters were studied: 1) IGF-I and IGF binding protein-3 (IGFBP-3); 2) serum bone

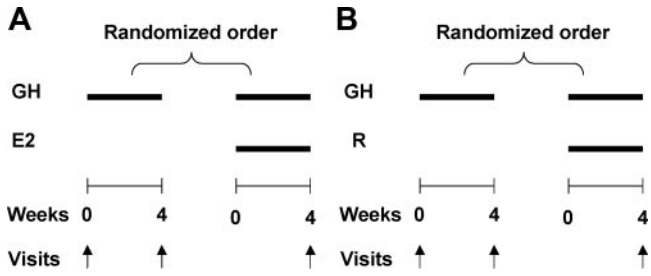

FIG. 1. Study design. Seventeen hypopituitary women were randomized to GH (0.5 mg/d) treatment alone or GH in combination with 17β-estradiol (E_2; 2 mg/d) (A) or raloxifene (R; 60 mg/d) (B). Six patients participated in study A, seven patients in study B, and four patients in both studies. Thus, overall, 10 subjects took part in a comparison of GH alone with GH plus 17β-estradiol, whereas 11 took part in a comparison of GH with GH plus raloxifene. The duration of treatment was 4 wk, followed by a 4-wk washout period. Metabolic studies were undertaken at baseline and at the end of each treatment period.

turnover markers [amino-terminal propeptide of type I procollagen (PINP; a marker of bone formation), carboxy-terminal telopeptide of type I collagen (CTX; a marker of bone resorption), and carboxy-terminal propeptide of type I procollagen (ICTP; a marker of bone resorption); 3) whole body resting energy expenditure (REE) and Fox; and 4) whole body protein turnover.

Subjects were studied in the Clinical Research Facility, Garvan Institute of Medical Research. Metabolic studies were undertaken at baseline and at the end of each treatment period. At each visit, study bloods were collected and placed on ice, and plasma was separated and stored at −80 C until analysis.

Methods

Indirect calorimetry

REE and substrate metabolism (fat and carbohydrate oxidation) was quantified after overnight fast using indirect calorimetry. Subjects were rested on a bed for at least 30 min. A clear plastic hood was placed loosely over the subject's head for a 20-min period. Measurements were collected during two 20-min periods and averaged. Oxygen consumption and carbon dioxide production were measured using an open circuit ventilated hood system (Deltatrac Metabolic Monitor; Datex Instrumentarium Corp., Helsinki, Finland) and calibrated against standard gases before each study. REE and Fox were estimated using weight-based equations adjusted from Ferrannini (10). REE is expressed as kilocalories per day and Fox as milligrams per minute. The mean intrasubject coefficients of variation (CVs) for REE and Fox at the Garvan Institute are 4.2 and 4%, respectively (9).

Protein turnover

Whole body protein metabolism was measured using the leucine turnover technique, which provides an estimate of protein turnover, oxidation, and synthesis. The method is based on the principle of steady-state kinetics in which the rate of appearance of substrate equals its rate of disposal. For leucine, there are two pathways of disposal: oxidation and reincorporation into protein. Rates of leucine appearance (LRa, an index of protein turnover), leucine oxidation (an index of oxidative loss of protein), and leucine incorporation into protein (LIP, an index of protein synthesis) were calculated as previously described (11). α-Ketoisocaproic acid (KIC) is formed when leucine undergoes transamination, and it is used as a surrogate marker of leucine because it more accurately reflects the intracellular environment (12).

After an overnight fast, a 0.104 mg/kg priming dose of $NaH^{13}CO_3$ was followed by primed constant 3-h infusion of 1-[^{13}C]leucine (prime 0.5 mg/kg, infusion 0.5 mg/kg/h), as previously described (3, 13). $NaH^{13}CO_3$ and 99% 1-[^{13}C]leucine were obtained from Cambridge Isotope Laboratories (Woburn, MA) and prepared under sterile conditions using 0.9% saline. On each visit, blood and breath samples were collected before (−10 and 0 min) and during the leucine infusion (140, 160, and 180 min). Blood was placed on ice and plasma was separated and stored at −80 C. KIC was extracted from plasma as described by Nissen *et al.* (14). Plasma KIC enrichment with ^{13}C was measured by gas chromatography mass spectrometry (MSD 5971A, model 5890; Hewlett-Packard Co., Palo Alto, CA). CO_2 enrichment with ^{13}C in breath samples was measured at St. Thomas' Hospital (London, UK) on a SIRA Series II isotope ratio mass spectrometer (VG Isotech, Cheshire, UK). The CVs for LRa, leucine oxidation, and LIP at the Garvan Institute are 3.5, 6.1, and 3.5%, respectively.

Assays

All samples for any individual were measured in the same assay run for each analyte. IGF-I, IGFBP-3, PINP, CTX, and ICTP were measured. Serum IGF-I levels were measured by RIA after acid ethanol extraction as previously described (3, 8, 15). The CVs for IGF-I were 8.3% at 14.7 nmol/liter and 7.4% at 28.6 nmol/liter. Serum IGFBP-3 levels were measured by RIA using antiserum R-100 in an in-house assay as previously described (16). PINP concentration in serum was determined using RIA (Orion Diagnostica, Espoo, Finland) with intraassay and interassay CVs less than 9% and less than 12%, respectively. Serum levels of CTX were determined using an automated immunoassay (Elecsys 170; Roche Diagnostics, Indianapolis, IN) with intraassay CV of approximately 2.0%. Serum ICTP levels were measured by RIA (Orion Diagnostica) with intraassay and interassay CVs less than 10%.

Statistical analysis

Within-group treatment effects of GH alone and GH combined with raloxifene or 17β-estradiol were assessed using paired *t* tests with Bonferroni's correction. Between-group differences from comparing the effects of estrogen and raloxifene during GH therapy were analyzed using the unpaired *t* test. Data on bone turnover markers were not normally distributed and therefore were logarithmically transformed for further analysis. Nonparametric analysis was used where appropriate. Results were expressed as mean with SE values (SEM), unless otherwise stated, 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

One subject from the raloxifene group was excluded from analysis due to noncompliance, as indicated by no change in IGF-I levels during GH therapy and return of unused

TABLE 2. Clinical, biochemical, and metabolic parameters in two groups of GHD women randomized to 1-month treatment with human GH (0.5 mg/d) alone and in combination with 17β-estradiol (GH + E_2; 2 mg/d) or raloxifene (GH + R; 60 mg/d)

	Estrogen group			Raloxifene group		
	Baseline	GH	GH + E_2	Baseline	GH	GH + R
Weight (kg)	90.4 ± 7.1	91.2 ± 7.1	92.1 ± 7.2	84.7 ± 7.9	85.7 ± 8[a]	85.3 ± 8.1
GH action markers						
IGF-I (nmol/liter)	8.9 ± 1.6	26.8 ± 3.5[b]	16.6 ± 1.9[d]	9.2 ± 1.8	30.3 ± 4.3[b]	20.7 ± 2.5[d]
IGFBP-3 (nmol/liter)	75.1 ± 9	110.8 ± 9[b]	81.4 ± 7.6[d]	65.6 ± 10.1	104.5 ± 8.6[b]	111.3 ± 10.1
Bone turnover markers						
PINP (μg/liter)	27.2	48.4[a]	46.1	30.6	74.6[b]	54.3
ICTP (μg/liter)	4.6	6.3[a]	5.4	4.2	9.1[b]	6.8
CTX (μg/liter)	0.14	0.21[a]	0.21	0.18	0.38[b]	0.37
Metabolic parameters						
REE (kcal/d)	1629 ± 111	1698 ± 121	1738 ± 109	1519 ± 140	1681 ± 131[a]	1614 ± 128
Fox (mg/min)	73.8 ± 6.3	83.5 ± 10.2	80.5 ± 8.5	65.3 ± 8	90.9 ± 9.4[b]	72.8 ± 8.7[c]
LRa (μmol/min)	127.4 ± 9.1	138.4 ± 7.5	145.7 ± 9	113.4 ± 11.8	125.6 ± 8.9	122.4 ± 10.8
LIP (μmol/min)	100.4 ± 7.2	113.5 ± 6.3	118.7 ± 7.7	86.5 ± 9.4	101.8 ± 7.3[a]	95 ± 8.3

Data are shown as mean ± SEM, except for the PINP, ICTP, and CTX where data are shown as median.

[a] $P < 0.05$ *vs.* baseline.

[b] $P < 0.01$ *vs.* baseline.

[c] $P < 0.05$ *vs.* GH.

[d] $P < 0.01$ *vs.* GH.

GH cartridges. The mean age of the subjects was 44.8 ± 3.3 yr, with mean body mass index of 30.7 ± 1.8 kg/m^2. Subject characteristics are shown in Table 1. There was no significant difference in variables between estrogen and raloxifene treatment groups at baseline (Table 2). No carryover effects were seen for any of the parameters during different treatment regimes when data were analyzed according to randomization sequence.

GH markers

In the estrogen group, GH treatment alone significantly increased mean circulating levels of IGF-I by 242 ± 48% ($P < 0.01$), IGFBP-3 by 62 ± 15% ($P < 0.001$), and the IGF-I/IGFBP-3 molar ratio by 109 ± 13% ($P < 0.001$; Table 2). In the raloxifene group, GH treatment significantly increased the mean circulating levels of IGF-I by 341 ± 94% ($P < 0.01$), IGFBP-3 by 84 ± 20% ($P < 0.001$), and IGF-I/IGFBP-3 by 142 ± 44% ($P < 0.001$). The between-group difference for each of the measures was not statistically significant.

In the estrogen group, addition of estrogen to GH treatment reduced the mean IGF-I level significantly by 35 ± 6% ($P < 0.01$). In the raloxifene group, addition of raloxifene to GH reduced the mean IGF-I level by 27 ± 7% ($P < 0.01$; Fig. 2A and Table 2). The changes in serum IGF-I levels between estrogen and raloxifene groups during GH treatment were not significantly different (Table 3). In the estrogen group, addition of estrogen to GH treatment resulted in a mean IGFBP-3 level that was 25 ± 5% lower than with GH treatment alone ($P < 0.01$). However, in the raloxifene group, addition of raloxifene to GH did not significantly change IGFBP-3 levels compared with GH treatment alone (Fig. 2B and Table 2). The changes in serum IGFBP-3 levels between estrogen and raloxifene groups during GH treatment were significantly different ($P < 0.001$), with levels being higher with raloxifene treatment (Table 3). Cotreatment with estrogen did not significantly change the IGF-I/IGFBP-3 molar ratio compared with GH treatment alone (data not shown). In contrast, cotreatment with raloxifene significantly reduced the mean IGF-I/IGFBP-3 molar ratio by 30 ± 7% ($P < 0.05$). During concurrent GH treatment, the changes in IGF-I/IGFBP-3 ratio were different between the two groups, with a lower ratio achieved in the raloxifene group that approached statistical significance ($P = 0.08$).

Thus, during GH treatment, cotreatment with both estrogen and raloxifene significantly reduced IGF-I levels, with only estrogen significantly reducing IGFBP-3 and raloxifene reducing the IGF-I/IGFBP-3 molar ratio.

Bone turnover markers

In the estrogen group, GH treatment alone significantly increased the median level of PINP by 51% ($P < 0.01$), ICTP by 42% ($P < 0.01$), and CTX by 37% ($P < 0.01$; Table 2). In the raloxifene group, GH treatment significantly increased the median circulating level of PINP by 182% ($P < 0.001$), ICTP by 102% ($P < 0.001$), and CTX by 113% ($P < 0.01$).

Neither raloxifene nor estrogen significantly changed the concentration of PNIP, CTX, and ICTP during GH

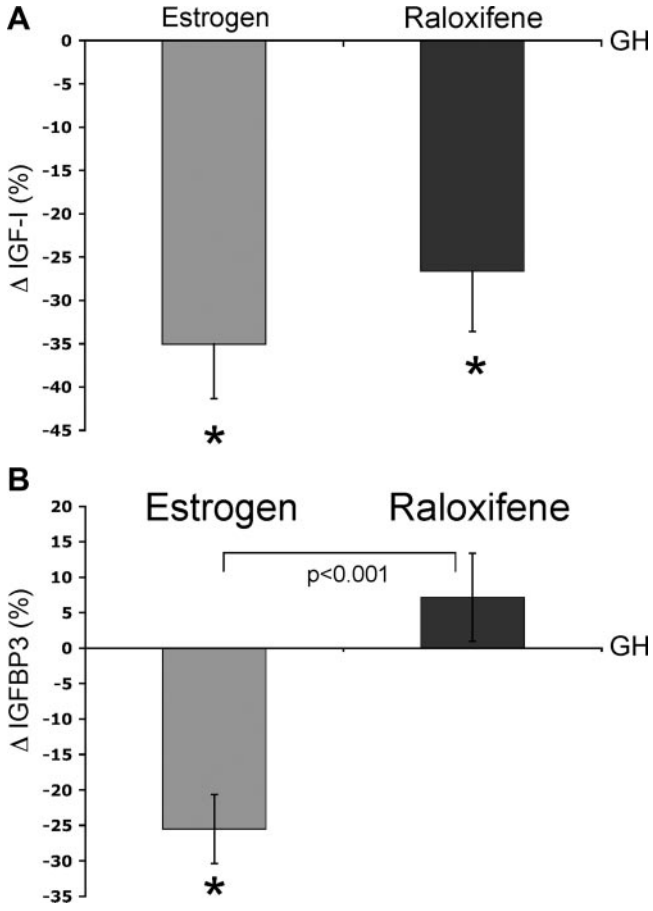

FIG. 2. Changes in serum IGF-I levels (A) and IGFBP-3 levels (B) in hypopituitary women after 4 wk of GH (0.5 mg/d) cotreatment with 17β-estradiol (2 mg/d) and raloxifene (60 mg/d). Data are presented as percentage change from GH treatment alone and expressed as means ± SEM. *, $P < 0.01$ compared with GH treatment alone using paired comparison. Between-group differences were analyzed using unpaired comparison.

TABLE 3. Between-group differences in clinical, biochemical, and metabolic parameters in two groups of GHD women after addition of 17β-estradiol (2 mg/d) or raloxifene (60 mg/d) during GH therapy (0.5 mg/d)

	Change from GH therapy		
	17β-Estradiol	Raloxifene	*P*
Weight (kg)	0.9 ± 0.5	−0.3 ± 0.5	0.11
(%)	1.0 ± 0.5	−0.5 ± 0.6	0.08
GH action markers			
IGF-I (nmol/liter)	−10.2 ± 2.6	−9.6 ± 2.8	0.86
(%)	−35.0 ± 6.3	−26.6 ± 6.9	0.38
IGFBP-3 (nmol/liter)	−0.8 ± 0.2	0.2 ± 0.2	<0.001
(%)	−25.5 ± 4.9	7.2 ± 6.2	<0.001
Bone turnover markers			
PINP (μg/liter)	2.8	−16.8	0.16
(%)	7.0	−29.9	0.22
ICTP (μg/liter)	−1.03	−2.02	0.33
(%)	−18.6	−19.2	0.65
CTX (μg/liter)	−0.05	−0.07	0.43
(%)	−18.8	−27.0	0.56
Metabolic parameters			
REE (kcal/d)	40.8 ± 48.5	−66.9 ± 36.7	0.1
(%)	3.4 ± 3.6	−3.8 ± 2.4	0.12
Fox (mg/min)	−3.0 ± 6.6	−18.0 ± 7.4	0.15
(%)	0.8 ± 7.7	−18.0 ± 8.1	0.11
LRa (μmol/min)	7.3 ± 7.3	−3.2 ± 5	0.26
(%)	6.2 ± 5.1	−2.8 ± 3.7	0.18
LIP (μmol/min)	5.2 ± 6.2	−6.8 ± 4.0	0.13
(%)	5.4 ± 5.2	−6.7 ± 3.8	0.08

Data are reported as absolute and relative changes from GH treatment and are shown as mean ± SEM, except for the bone turnover markers where data are shown as median.

therapy. However, there was a trend toward an inhibitory effect of raloxifene on bone resorption markers compared with estrogen, although the between-group differences were not statistically significant (Table 3). During GH treatment, addition of estrogen resulted in little effect on PINP; however, addition of raloxifene reduced median levels of PINP by 30% with the change approaching statistical significance ($P = 0.08$; Table 2). Between-group analysis revealed that the change in serum PINP with raloxifene treatment was not significantly different from that of estrogen during GH treatment (Table 3).

Thus, there was no significant change in markers of bone resorption and formation by estrogen or raloxifene treatments, although there was a trend toward a greater inhibitory effect on bone formation marker with raloxifene treatment.

Energy expenditure and substrate metabolism

In the estrogen group, GH treatment did not significantly increase REE or whole body Fox, although a positive trend was evident (increase by 5 ± 4%, $P = 0.3$; and by 11 ± 7%, $P = 0.1$, respectively). In the raloxifene group, GH treatment significantly increased REE by 12 ± 3% ($P < 0.01$) and Fox by 43 ± 7% ($P < 0.001$; Table 2). The effect of GH between the groups was not statistically different.

Addition of estrogen or raloxifene did not significantly change REE, and the between-group difference was not statistically significant (Tables 2 and 3). In the estrogen group, addition of estrogen to GH treatment did not significantly change Fox. However, addition of raloxifene resulted in whole body Fox that was 18 ± 8% lower than with GH treatment alone ($P < 0.05$; Fig. 3 and Table 2). Between-group analysis revealed a trend toward a lower rate of Fox in the raloxifene group compared with estrogen group during GH treatment; however, the difference between the groups did not reach statistical significance ($P = 0.1$; Table 3).

Thus, only raloxifene (but not estrogen) significantly reduced Fox during GH treatment.

Leucine turnover

In both the estrogen and raloxifene groups, GH treatment did not significantly change whole body LRa but

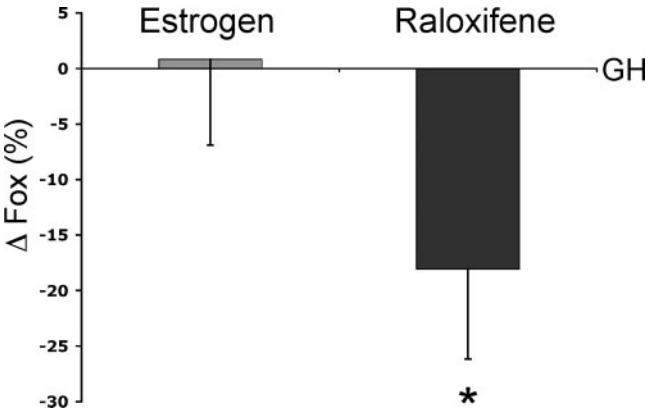

FIG. 3. Changes in Fox in hypopituitary women after 4 wk of GH (0.5 mg/d) cotreatment with 17β-estradiol (2 mg/d) and raloxifene (60 mg/d). Data are presented as percentage change from GH treatment alone and expressed as means ± SEM. *, $P < 0.05$ compared with GH treatment alone.

significantly increased LIP by 15 ± 5 and 22 ± 7%, respectively ($P < 0.05$; Table 2). When expressed as percentage of LRa, LIP increased by approximately 3.3% in the estrogen group and by 4.9% in the raloxifene group ($P < 0.001$). There was no statistically significant difference in increase in LIP between the two groups (Table 2).

During GH treatment, addition of estrogen did not significantly change LRa or LIP (Table 2). During GH treatment, addition of raloxifene did not significantly change LRa but was associated with a small nonstatistically significant reduction in LIP of 7 ± 4%. The difference between the effects of estrogen and raloxifene observed during GH therapy of 12% approached statistical significance ($P = 0.08$; Table 3).

Thus, in comparison to estrogen, there was a trend toward a reduction in LIP, a measure of protein synthesis, by raloxifene during GH therapy.

Discussion

The purpose of this study was to compare the modulatory effects of 17β-estradiol and raloxifene on the action of GH. In hypopituitary women, GH significantly stimulated IGF-I and IGFBP-3, bone turnover markers (PINP, ICTP, and CTX), REE, Fox, and LIP. Addition of 17β-estradiol or raloxifene significantly reduced IGF-I levels, whereas only 17β-estradiol lowered IGFBP-3 levels during GH treatment, such that mean IGF-I/IGFBP-3 ratio was lower in the raloxifene group. Raloxifene but not 17β-estradiol significantly reduced Fox during GH treatment. Although addition of raloxifene or 17β-estradiol during GH therapy did not significantly change many metabolic outcome measures within the same group, LIP during raloxifene treatment was lower than that during 17β-estradiol treatment, with the difference between groups approaching statistical significance.

This is the first study investigating the modulatory effect of raloxifene in GHD adults receiving GH replacement. Raloxifene is a SERM that exhibits estrogen-like effects in certain tissues such as bone and antiestrogen effects in other tissues such as breast (17). There is strong evidence that raloxifene exerts an estrogen agonist effect in liver, as shown by effects on cholesterol (18) and SHBG (19), and on IGF-I (8, 20–23) concentration in blood. Raloxifene reduces circulating IGF-I level in healthy postmenopausal women (23) and in GHD women (8), effects similar to those observed with oral administration of estrogens. We have previously reported that in healthy postmenopausal and GHD women, therapeutic doses of raloxifene induced a lesser IGF-I suppressive effect than that observed with 17β-estradiol (8). Therefore, we postulated that raloxifene might impart a lesser attenuating effect on fat and protein metabolism during GH treatment. Using similar doses, the current study showed that raloxifene reduced circulating IGF-I to a similar extent to estrogen. However, unexpectedly, there appeared to be a greater suppression of GH-stimulated Fox and protein synthesis by raloxifene compared with 17β-estradiol.

This study has identified a small but lesser rate of Fox and a trend toward a reduction in LIP with raloxifene compared with estrogen during GH therapy. These changes are small but could result over the long term in significant changes in body fat and in lean mass. In the current study, raloxifene but not estrogen reduced Fox by almost 20%, raising the possibility of a greater propensity of attenuating the GH-mediated reduction in fat mass than 17β-estradiol. In a study comparing the metabolic effects from the route of estrogen administration, we observed a small but greater transient postprandial suppression of Fox during the oral phase, which over 6 months was accompanied by a significant measurable gain in fat mass compared with that observed during the transdermal phase (9). In addition, we have previously reported that in GHD women during oral estrogen treatment, GH replacement results in a lower increase in protein synthesis than during the transdermal estrogen treatment phase (3). However, in the current study, addition of raloxifene but not estrogen reduced protein synthesis during GH treatment, and the difference between estrogen and raloxifene groups approached statistical significance. In a time course study of GHD subjects, Burt *et al.* (4) observed that the change in protein synthesis observed early during GH replacement was significantly correlated to a later change in lean body mass at 3 months. The possibility that the minor differences in substrate metabolism between raloxifene and estrogen effects translate to longer term changes

is supported by our observation at the end of a longitudinal 2-yr follow-up that a smaller reduction in fat mass and a lesser gain in lean body mass occurred in the raloxifene group during concurrent GH therapy (manuscript in preparation).

We cannot explain why the metabolic effect on GH action by raloxifene is opposite to that predicted from effects in untreated GHD women and in postmenopausal women. These earlier studies revealed a lesser effect of raloxifene than estrogen at equivalent therapeutic doses on IGF-I and similar inhibitory effect on postprandial Fox (8), which are both regulated by GH but not exclusively so. The regulation of IGF-I and Fox is complex and involves many other factors, including insulin, as well as intracellular processes that regulate IGF-I gene expression, fatty acid availability, and transport. That raloxifene treatment did not translate to a greater GH-sparing effect suggests that in the GHD state, non-GH-mediated mechanisms are less susceptible to the inhibitory effects of raloxifene than to oral estrogen, but the potency of GH inhibition by raloxifene is unmasked during GH therapy.

The effect of raloxifene on IGFBP-3 was significantly different from that of estrogen. This is supported by studies in postmenopausal and GHD women reporting that raloxifene increases IGFBP-3 levels in contrast to a lowering effect of estrogen (8, 23, 24). Because IGF-I was reduced to a similar extent by both estrogen and raloxifene, only raloxifene treatment led to a reduction in IGF-I/IGFBP-3 molar ratio. The reduced molar ratio of IGF-I to IGFBP-3 may be significant because of evidence that IGFBP-3 alters the bioavailability of IGF-I and inhibits IGF-I action (25–28). Our finding of a strong trend in the reduction of protein synthesis when raloxifene was added during GH treatment is consistent with an attenuation in GH anabolic effect.

A weakness of the study is the relatively small sample size in the setting of therapeutic rather than pharmacological interventions. This has resulted in trends rather than significant changes in many of the outcome measures. The sample size was derived from calculations based on our earlier study in GHD women in whom the effects of raloxifene and 17β-estradiol were compared. Another weakness was the two-group parallel design instead of the original three-period crossover study that would have increased the statistical strength. It was necessary to change from the original study design for logistical reasons. Because of this, we acknowledge that power calculations may not be valid. Nevertheless, the subsequent changes in fat mass and in lean body mass (our unpublished data) are consistent with the acute changes in fat and protein anabolic outcome measures between raloxifene and estrogen treatments.

In summary, we found significant differences in effect between estrogen and raloxifene during GH therapy. In the doses used, raloxifene but not estrogen reduced IGF-I/IGFBP-3 ratio and Fox and led to nonsignificant reduction in the levels of bone turnover markers and leucine incorporation into protein. Our unpublished observations over 2 yr on body composition in these patients suggest that small changes in the IGF-I system, in substrate metabolism, and in bone turnover markers may predict later changes in body composition.

Conclusions

We conclude that, in the doses used, raloxifene exerts a greater inhibitory effect than estrogen on the metabolic action of GH that may translate to a greater attenuation of the metabolic-body compositional benefits of GH. Raloxifene is unlikely to have a metabolic advantage over estrogen in GHD women receiving GH replacement.

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