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Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women

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Wolthers, Troels, David M. Hoffman, Ailish G. Nugent, Mark W Duncan, Margot Umpleby, and Ken K. Y. Ho. Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. *Am J Physiol Endocrinol Metab* 281: E1191–E1196, 2001.—We have determined whether oral estrogen reduces the biological effects of growth hormone (GH) in GH-deficient (GHD) women compared with transdermal estrogen treatment. In two separate studies, eight GHD women randomly received either oral or transdermal estrogen for 8 wk before crossing over to the alternate route of administration. The first study assessed the effects of incremental doses of GH (0.5, 1.0, 2.0 IU/day for 1 wk each) on insulin-like growth factor I (IGF-I) levels during each estrogen treatment phase. The second study assessed the effects of GH (2 IU/day) on lipid oxidation and on protein metabolism using the whole body leucine turnover technique. Mean IGF-I level was significantly lower during oral estrogen treatment ($P < 0.05$) and rose dose dependently during GH administration by a lesser magnitude ($P < 0.05$) compared with transdermal treatment. Postprandial lipid oxidation was significantly lower with oral estrogen treatment, both before ($P < 0.05$) and during ($P < 0.05$) GH administration, compared with transdermal treatment. Protein synthesis was lower during oral estrogen both before and during GH administration ($P < 0.05$). Oral estrogen antagonizes several of the metabolic actions of GH. It may aggravate body composition abnormalities already present in GHD women and attenuate the beneficial effects of GH therapy. Estrogen replacement in GHD women should be administered by a nonoral route.

insulin-like growth factor I; lipid oxidation; protein turnover

THE ROUTE OF ESTROGEN replacement therapy in postmenopausal women has a major impact on the growth hormone (GH)-insulin-like growth factor I (IGF-I) axis (15, 31). Estrogen administration by the oral, but not the transdermal, route reduces serum IGF-I and increases circulating GH and GH-binding protein. We recently reported the metabolic consequences of these perturbations (21). In postmenopausal women, oral estrogen suppressed lipid oxidation and IGF-I and resulted in an increase in fat mass and a reduction of

lean body mass (LBM), respectively. The metabolic and body composition changes induced by the oral route are opposite those observed during GH replacement therapy (10, 14, 26) and indicate that oral estrogen therapy may antagonize GH action.

GH has recently been approved for replacement treatment in adults in several countries. GH plays an important role in regulating body composition and physical and psychological well-being in adult life (2, 10, 14, 26). There is limited information on the interaction of GH with other hormones during replacement therapy. The observations in postmenopausal women raised the question as to whether the traditional oral route of estrogen replacement reduces the biological effects of GH. We have undertaken two studies comparing the effects of oral and transdermal estrogen administration on the biological actions of GH. Doses employed are those routinely used in the therapy of women with hypopituitarism. The first study investigated IGF-I responses to three different doses of GH (dose-response study). The second study investigated metabolic effects of GH on lipid oxidation and whole body protein metabolism (metabolic study).

METHODS

Subjects

Ten hypopituitary GH-deficient women with hypogonadism were recruited from the Endocrine Outpatient Clinic at St. Vincent's Hospital (Sydney, Australia). Written informed consent was obtained from all subjects. The clinical characteristics of these GH-deficient subjects are shown in Table 1. They were randomized into two separate studies, which were separated by at least 3 mo. Six subjects participated in both studies (*patients 1, 3, 4, 5, 8, and 10*). GH deficiency was confirmed by a peak GH response of < 3 ng/ml during an insulin tolerance test (9). The duration of hypopituitarism was at least 1 yr, and no subjects had received GH before. All hypopituitary subjects were receiving stable hormone replacement for other deficiencies, except for one subject of postmenopausal age who did not receive sex steroid replace-

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Table 1. Characteristics of patients with GH deficiency

Subject No.	Age, yr	Cause of GH Deficiency	Treatment	Hormone Replacement
1	55	Pituitary macroadenoma	S/X	A, T, G
		Idiopathic		
2	52	hypopituitarism		A, G
3	47	Craniopharyngioma	S	A, T, G
4	70	Pituitary macroadenoma	S/X	A, T
5	29	Pituitary macroadenoma	S/X	A, T, G
6	41	Craniopharyngioma	S	A, T, G
		Idiopathic		
7	39	hypopituitarism		A, T, G
8	34	Lymphocytic hypophysitis	S	A, T, G
9	53	Pituitary microadenoma	S	A, T, G, D
10	55	Pituitary macroadenoma	S	A, T, G

GH, growth hormone; S, surgery; X, irradiation; A, adrenal replacement; T, thyroid replacement; G, gonadal replacement; D, desmopressin.

ment. The study was approved by the Research Ethics Committee of St. Vincent's Hospital.

Study Design

Eight subjects participated in each study. Both studies were of open-label, randomized, crossover design, allowing for differences in treatment effect (i.e., route of estrogen administration) to be compared during estrogen therapy. Each subject was randomized to 2 mg/day oral estradiol valerate (Progynova; Schering) or transdermal estrogen patches (Estraderm-TTS 100; Ciba-Geigy) delivering 100 µg of 17β-estradiol daily for 8 wk. The subjects then crossed over to the alternate estrogen treatment for a further 8 wk. The estrogen dosages used were based on data indicating equivalent biological activity, as measured by gonadotropin suppression and vaginal cytology (3, 15, 21, 25). Medroxyprogesterone acetate (10 mg daily, Provera; Pharmacia-Upjohn) was coadministered on the last 12 days of each 4-wk cycle of estrogen treatment to induce withdrawal bleeding (Fig. 1).

Dose-response study. GH (Genotropin; Pharmacia-Upjohn) was administered in a stepwise incremental regimen during the 2nd mo of each estrogen phase, at a dose of 0.5 IU/day (0.17 mg/day) for the 1st wk, 1 IU/day (0.33 mg/day) for the 2nd wk, and 2 IU/day (0.67 mg/day) for the 3rd wk. GH was administered daily by self-injection at 2000. Blood was withdrawn for IGF-I measurements before initiation of estrogen therapy, during estrogen phases immediately before initiation of GH administration, and again on the 7th day of each increment in GH.

Metabolic study. This study design was the same as in the dose-response study except that patients received a daily dose of GH from weeks 4 to 6 during each estrogen phase. On the basis of the IGF-I data obtained in the dose-response study, a GH dose of 2 IU (0.67 mg) was administered daily by self-injection at 2000. Serum IGF-I, lipid oxidation, and protein metabolism were measured just before GH was started and at the end of the 2nd wk of GH treatment during each estrogen phase. Lipid oxidation was estimated by indirect calorimetry before and after a standardized mixed meal (10, 21). Protein metabolism was measured by the whole body leucine turnover technique to derive the rates of protein breakdown, synthesis, and oxidation (8). In both studies, subjects were instructed to follow their usual diet and habitual activity. All subjects were studied at 0800 after an overnight fast.

Study Techniques

Whole body protein turnover and indirect calorimetry were performed sequentially over a 5-h period. Whole body protein turnover was undertaken using a primed constant infusion of L-[1-¹³C]leucine, as previously described (12). NaH¹³CO₃ (99% purity) was obtained from Cambridge Isotope Laboratories (Woburn, MA), and L-[1-¹³C]leucine (99% purity) was obtained from Mass Trace (Woburn, MA).

At 0800, cannulas were inserted in an antecubital vein of each arm, one for isotope infusion and one contralaterally for blood sampling. A priming dose of NaH¹³CO₃ (0.1 mg/kg) was followed immediately by L-[1-¹³C]leucine (prime, 0.5 mg/kg; infusion, 0.5 mg·kg⁻¹·h⁻¹). Blood and breath samples were collected at -10, 0, 160, 180, and 200 min from commencement of the infusion. CO₂ production rates were undertaken with an open-circuit, ventilated hood system (Deltatrac monitor; Datex Instrumentation, Helsinki, Finland).

Immediately after the completion of the protein turnover study (200 min), basal lipid oxidation was measured, as previously described (6, 24). A standardized mixed meal (14% protein, 31.5% fat, 54.5% carbohydrate; Ensure, Ross Laboratories, Columbus, OH) was then administered, and indirect calorimetry was performed during the 30- to 60-min and 90- to 120-min time intervals after the nutrient load. The mixed meal consisted of the caloric equivalent of 40% of the subject's basal energy expenditure. Rates of lipid oxidation are expressed as milligrams of fat oxidized and are based on a caloric equivalent of fat of 1 g = 9.3 kcal.

Rates of appearance of leucine (an index of proteolysis), leucine incorporation into protein (an index of protein synthesis), and leucine oxidation (an index of protein oxidation) were calculated using the reciprocal pool model, as previously described (12, 27). Because leucine represents 8% of total body protein, rates of protein turnover were estimated by using these constants (11, 18).

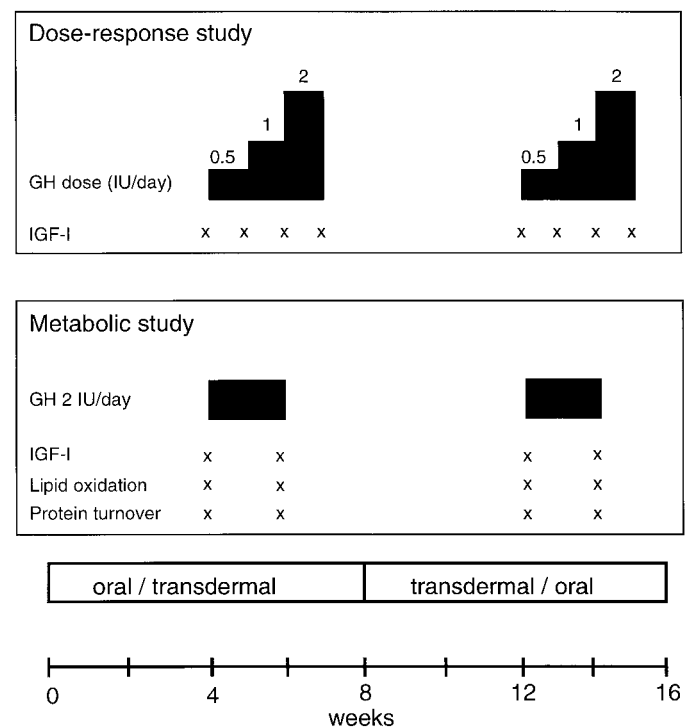


Fig. 1. Study design of dose-response and metabolic studies. See text for details. GH, growth hormone; IGF-I, insulin-like growth factor I; x, time of investigation.

Analytical Methods

Serum IGF-I was measured by RIA after acid-ethanol extraction (9). The intra-assay coefficients of variation were 9.4, 8.3, and 10.3% at 1.9, 10.1, and 60.4 nmol/l, respectively. α -Ketoisocaproic acid (KIC) was extracted by the method of Nissen (19). KIC enrichment was measured as the *t*-butyldimethylsilyl derivative by gas chromatography (model 5890; Hewlett-Packard, Palo Alto, CA)-mass spectrometry (MSD 5971A; Hewlett-Packard), with selective monitoring of ions at mass-to-charge ratios 301 and 302 at unit resolution (12). $^{13}\text{CO}_2$ enrichment in breath was measured on a SIRA Series II isotope ratio-mass spectrometer (VG Isotech, Cheshire, UK).

Statistics

The route-related treatment effects of estrogen and of GH on IGF-I, in the dose-response study and on substrate oxidation and protein turnover, were analyzed by two-way ANOVA with repeated measures and significance determined after Bonferroni's correction. Analysis of interactions between treatment and sequence was performed using the Grizzle method, an ANOVA in which the direct and residual effects of treatment can be measured separately in a cross-over study (22). No such interactions were found. Statistical significance was set at an α -level of 0.05. Results are expressed as means \pm SE.

RESULTS

Dose-Response Study

The mean IGF-I level fell significantly from baseline (11.2 ± 1.54 nmol/l) during oral ($P < 0.05$, 8.7 ± 1.3 nmol/l) but not transdermal (11.2 ± 1.6 nmol/l) treatment. GH administration significantly increased ($P < 0.01$, ANOVA) IGF-I levels in a stepwise, dose-dependent manner during both estrogen treatments; however, mean IGF-I levels were significantly lower during oral estrogen treatment ($P < 0.01$, ANOVA). The increment in IGF-I induced by GH was also less during the oral phase compared with the transdermal phase at each of the three different GH dosages ($P < 0.05$, ANOVA). The impact of the route of estrogen administration can be gauged by comparing mean IGF-I levels achieved across GH doses between estrogen

treatments. The mean IGF-I level in response to 1.0 IU GH during oral estrogen therapy was indistinguishable from that observed at baseline and during the transdermal phase before commencing GH. Administration of 2.0 IU GH during oral estrogen therapy resulted in a mean IGF-I level similar to that observed with one-half of this dose during the transdermal phase (Fig. 2). Comparable IGF-I levels between estrogen treatments were obtained at a GH dosage \sim 1.0 IU higher during oral estrogen treatment.

Metabolic Study

IGF-I results obtained in this study confirmed the findings of the dose-response study. Thus mean baseline IGF-I concentration during oral estrogen was significantly lower than during transdermal estrogen (7.8 ± 0.5 vs. 9.8 ± 1.8 nmol/l, $P < 0.05$). GH administration increased IGF-I levels during both estrogen treatments ($P < 0.01$), with a mean IGF-I level lower during oral estrogen treatment ($P < 0.01$). Furthermore, the increase in IGF-I induced by GH was also of lower magnitude during the oral phase compared with the transdermal phase (change in IGF-I: 109 ± 43 vs. $135 \pm 41\%$, $P < 0.05$).

Lipid oxidation. Fasting lipid oxidation was not influenced by the route of estrogen administration. Ingestion of a standardized meal significantly suppressed lipid oxidation during both estrogen treatment phases ($P < 0.001$, ANOVA). However, when compared with the transdermal route, oral estrogen administration resulted in a greater suppression of lipid oxidation during the 1st h after ingestion of the standardized meal (690 ± 211 vs. $1,393 \pm 230$ mg lipid oxidized/30 min, $P < 0.05$; Fig. 3). The greater postmeal suppression of lipid oxidation during oral estrogen was transient, since the difference was no longer significant by the 2nd h.

Fasting and postmeal lipid oxidation was significantly increased ($P < 0.01$, ANOVA) by GH treatment, although the meal once again induced a decline in lipid oxidation during both estrogen treatments. However, postmeal lipid oxidation during GH treatment was

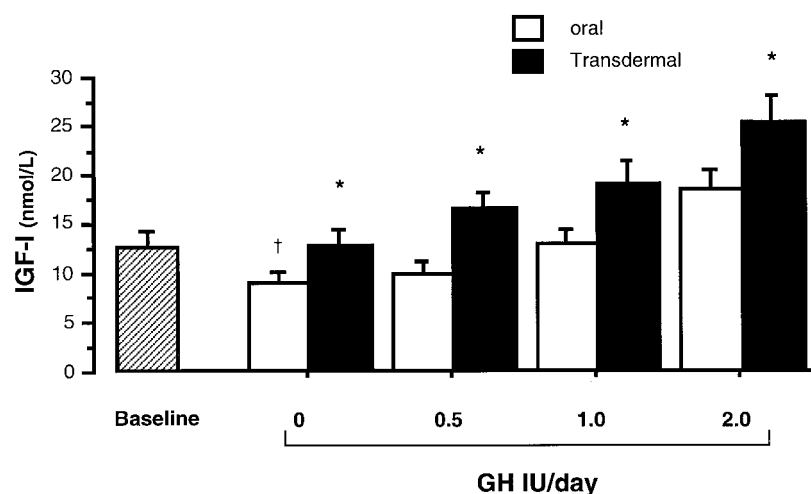


Fig. 2. Serum IGF-I concentrations (nmol/l, mean \pm SE) before and during incremental dosages of GH (0.5, 1.0, and 2.0 IU/day) during oral and transdermal estrogen therapy. For conversion of GH dose to mg, divide by 3. * $P < 0.05$ by ANOVA, oral vs. transdermal.

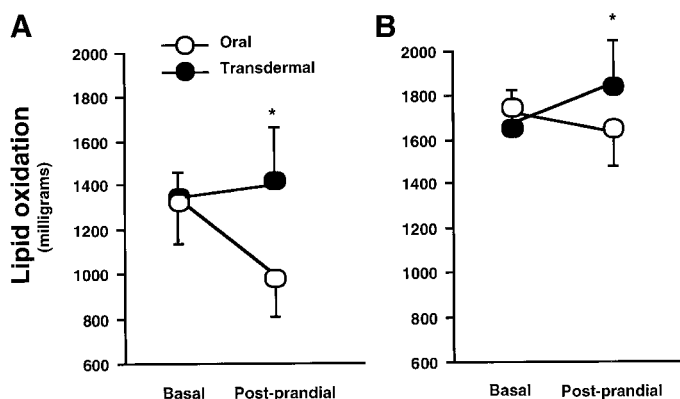


Fig. 3. Lipid oxidation (mg over 30 min, mean \pm SE) during fasting and 1 h after a standardized mixed meal (postprandial). Lipid oxidation is shown before (A) and after (B) GH replacement therapy (2 IU/day) during oral and transdermal estrogen administration. * $P < 0.05$, oral vs. transdermal.

again suppressed to a greater degree in the 1st h with oral treatment ($1,655 \pm 264$ vs. $1,915 \pm 284$ mg lipid/30 min, $P < 0.05$). The magnitude of GH-induced stimulation of lipid oxidation during fasting or in the post-meal state was not influenced by the route of estrogen. Thus oral estrogen reduces early postprandial lipid oxidation both before and during GH administration.

Protein metabolism. When compared with the transdermal phase, leucine incorporation into protein was significantly lower during the oral phase ($P < 0.05$). Mean leucine turnover was lower during oral therapy, although the difference failed to reach statistical significance ($P = 0.058$). Leucine oxidation was not significantly different between estrogen treatments (Table 2 and Fig. 4).

GH administration did not significantly affect leucine turnover but induced a significant fall in leucine oxidation ($P < 0.05$). This was accompanied by a rise in leucine incorporation into protein, which increased significantly from 80.5 ± 0.8 to $84.1 \pm 1.0\%$ ($P < 0.05$) of leucine turnover. However, during GH treatment, leucine incorporation into protein remained significantly lower ($P < 0.05$) with oral estrogen compared with the transdermal route. When expressed as a fraction of leucine turnover, the stimulation of leucine incorporation into protein by GH tended to be less with oral estrogen (1.9 vs. 4.1%), as was the reduction of leucine oxidation (2.8 vs. 4.2%). No correlations

Table 2. Leucine kinetics during oral and transdermal estrogen therapy before and after GH administration in 8 GH-deficient women

	Estrogen		Estrogen Plus GH	
	Oral	Transdermal	Oral	Transdermal
Turnover	123.7 \pm 8.6	134.5 \pm 11.0	125.3 \pm 9.8	133.5 \pm 12.4
Incorporation into protein	99.8 \pm 7.6	108.9 \pm 9.6*	104.7 \pm 9.1†	113.6 \pm 11.0*†
Oxidation	23.9 \pm 1.7	25.6 \pm 2.3	20.7 \pm 2.1†	19.8 \pm 2.4†

Values are means \pm SE. Units are $\mu\text{mol}/\text{min}$. * $P < 0.05$, oral vs. transdermal. † $P < 0.05$ estrogen vs. estrogen + GH treatment.

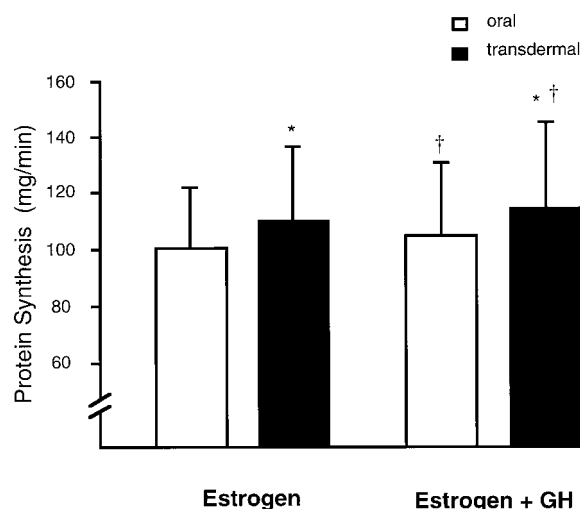


Fig. 4. Protein synthesis (mg/min, mean \pm SE) before and after GH replacement therapy (2 IU/day) during oral and transdermal estrogen administration. * $P < 0.05$, oral vs. transdermal. † $P < 0.05$, baseline vs. GH treatment.

between IGF-I levels and the components of protein turnover were observed (data not shown).

DISCUSSION

These two randomized, crossover studies show that oral estrogen replacement therapy suppresses the biological actions of GH in GH-deficient women. In the first study, mean IGF-I across all three GH doses was significantly lower, and the rise in IGF-I during oral estrogen was significantly less than that observed during transdermal therapy. In the metabolic study, postprandial lipid oxidation and leucine incorporation into protein were stimulated by GH treatment but remained significantly lower during the oral estrogen phase. Moreover, the route-dependent effects of estrogen on IGF-I, fat oxidation, and protein metabolism were evident even before GH administration. Thus, in GH-deficient, hypogonadal women, oral estrogen exhibits intrinsic metabolic actions that are opposite those of GH and are not overcome by replacement doses of GH currently used in clinical practice, indicating the physiological importance of these observations.

The present findings confirm recent reports that GH-deficient, hypogonadal women on oral estrogen therapy require a higher GH dose to obtain the same IGF-I level as that observed with transdermal estrogen therapy (4, 13). They demonstrate for the first time that the impact of oral estrogen extends beyond effects on circulating IGF-I levels in that GH-induced stimulation of fat oxidation and on protein metabolism are also affected. Although fat oxidation was stimulated by GH, it remained suppressed to a greater degree postprandially during the oral estrogen treatment. Similarly, although GH stimulated protein metabolism, leucine incorporation into protein was significantly lower during oral estrogen therapy. These latter metabolic changes cannot be assumed to be a sine qua non of changes in IGF-I levels. First, GH effects on fat



oxidation are not IGF-I mediated, and second, liver-specific knock-out studies have questioned the importance of liver-derived IGF-I in stimulating whole body anabolism (28, 32).

The differences in IGF-I and metabolic responses to GH therapy between oral and transdermal estrogen treatment are of similar magnitude to those observed in a recent study comparing the metabolic effects of oral and transdermal estrogen in postmenopausal women (21). In the present study, the IGF-I concentration was 20–30% lower on oral than on transdermal estrogen before and during GH administration; postprandial fat oxidation during a mixed meal was suppressed to a greater degree by ~30% (~700 mg fat) and 12% (~300 mg fat) before and during GH administration, respectively, with oral estrogen therapy. In the study of postmenopausal women, IGF-I was ~25% and postprandial lipid oxidation 20–30% (~900 mg fat) lower during oral estrogen treatment. Over a 6-mo period, these differences were associated with significant changes in body composition. This equated to a loss of >1 kg of LBM and an equivalent increase in fat mass in postmenopausal women during the oral phase (21).

These observations in postmenopausal women strongly suggest that similar detrimental changes may occur with conventional oral estrogen therapy in untreated GH-deficient women. This suggestion is underscored by the finding of a lower IGF-I level and lower rates of fat oxidation postprandially and of leucine incorporation into protein even before GH therapy. Moreover, the beneficial effects of GH treatment in reducing body fat and in increasing protein mass may be attenuated to a similar degree. This proposal is supported by the recent observation that GH-deficient men are more responsive to GH than GH-deficient women, most of whom were on conventional oral estrogen therapy (1, 5). The choice of estrogen formulation and the doses used for replacement therapy in hypopituitarism are highly variable. The dose selected for these studies is representative of an average for replacement needs. Because the metabolic effects of oral estrogen are dose dependent (23), a higher dose or a more potent estrogen is likely to induce a higher degree of GH antagonism when using IGF-I for dose titration, as is recommended by the Growth Hormone Research Society (7).

The mechanism by which oral estrogen antagonizes the action of GH is not known. Although dissimilar estrogen formulations were used for the oral and transdermal routes, it is unlikely that the contrasting effects arose from chemical differences, in the light of previous observations reporting that different estrogen formulations cause an equal dissociation of the GH-IGF-I axis (15). The presence of a highly reproducible route-dependent effect of estrogen strongly suggests an effect on hepatic function arising through exposure of the liver to high portal estrogen concentrations after intestinal absorption (15, 22, 31). We have also observed consistently that oral but not transdermal estrogen increases serum GH-binding protein (15, 31), which

has been shown to attenuate GH action in vitro (16, 17). Alternatively, the reductions in circulating IGF-I and in fat oxidation may have arisen from a direct estrogen inhibition of hepatic IGF-I production and fatty acid metabolism. Evidence for this is provided by in vitro studies showing that pharmacological concentrations of estrogen reduce fatty acid oxidation and increase fatty acid incorporation into triglycerides (20, 30) and by clinical observations that oral but not transdermal estrogen therapy stimulates hepatic triglyceride synthesis and increases triglyceride levels (29). Further studies at the cellular level are needed to elucidate the mechanisms behind these clinical observations.

In summary, estrogen at a therapeutic dose exerts significant route-dependent effects on GH action in women with organic GH deficiency. Compared with the transdermal route, oral estrogen aggravates metabolic abnormalities of GH deficiency and attenuates the metabolic effects of GH therapy. Thus oral estrogen may worsen the body composition abnormalities of GH deficiency and limit the benefits of GH replacement therapy in GH-deficient women. We conclude that the route of estrogen administration is an important consideration both before and during GH replacement therapy in hypogonadal GH-deficient women.

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