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Effects of growth hormone and/or sex steroid administration on whole-body protein turnover in healthy aged women and men☆

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Abstract

Aging is associated with reduced activities of the growth hormone (GH), insulin-like growth factor I (IGF-I), and sex steroid axes, and with decreased lean body mass and protein synthesis. Using a randomized, double-blinded, placebo-controlled design, we studied the effects of 6 months of administration of GH alone, sex hormone alone (hormone replacement therapy in women, testosterone enanthate [T] in men), or GH plus sex hormone on protein turnover in healthy men (n = 60) and women (n = 43), aged 65 to 88 years (mean, 71 ± 4.4 years). Growth hormone administration significantly increased IGF-I levels in both sexes, more markedly in men. Sex steroid administration increased the levels of estrogen and testosterone in women and men, respectively ($P = .05$). Protein turnover was measured before and after the 26-week treatment period by means of a primed, constant L-[1-^{13}C]leucine infusion. In men, GH plus T administration increased leucine flux from 80.2 ± 2.8 to 93.6 ± 4.2 μmol · h^{-1} · kg^{-1} ($P = .02$). Leucine oxidation did not change significantly after hormone treatment in either sex. Growth hormone treatment led to nonsignificant upward trends in nonoxidative leucine disposal in men (9.1 ± 5.2 mol · h^{-1} · kg^{-1}) and women (7.6 ± 7.1 mol · h^{-1} · kg^{-1}). Among all groups combined, changes in nonoxidative leucine disposal were directly related to those of serum IGF-I level ($r = 0.248$, $P < .02$). Whole-body protein turnover increased in GH plus T–treated men (0.6 ± 0.2 g protein · kg^{-1} · d^{-1}; $P < .01$). These data suggest that low-dose GH administration increases protein synthesis in healthy aged women and men, and that the coadministration of testosterone plus GH enhances this effect in elderly men.
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1. Introduction

Aging affects body composition by decreasing skeletal and bone mass and increasing fat mass relative to total body weight [1,2]. Lean body mass declines at an average rate of 5% per decade in men and at 2.5% per decade in women [1]. Muscle mass in 75-year-old men, estimated by 24-hour urinary creatinine excretion, is only half of that in men aged 30 years [1,3,4].

It has been proposed that age-related alterations in body composition in humans result in part from the diminished activity of the growth hormone (GH)–insulin-like growth factor I (IGF-I) axis [1,5,6]. In pathological states of GH deficiency, the reduction of lean body mass is reflected in decreased sizes of muscle, kidney, liver, and digestive tract. In these organs, GH, acting mainly via circulating and/or tissue-derived IGF-I, stimulates amino acid uptake and augments the synthesis of DNA, RNA, and protein [7], resulting in an increased rate of cell division. Growth hormone also reduces lipogenesis and promotes lipolysis within adipocytes, effects opposite to those of insulin [8].

The effects of GH on body composition have been well documented in GH-deficient children who exhibit reduced muscle mass and excessive body fat for both age and height. Treatment of these children with GH reverses or attenuates

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these abnormalities [9,10]. Similarly, GH treatment of GH-deficient young or middle-aged adults increases lean body mass and decreases fat mass [11-15].

Because GH administration increases lean body mass and nitrogen retention in healthy and GH-deficient adults, it is reasonable to suggest that the age-related diminution in GH secretion may play an important role in the lean mass depletion observed in older individuals.

Aging is also associated with decreased gonadal steroid secretion in men [16] and women [17]. In hypogonadal nonelderly men, replacement of androgens leads to nitrogen retention, increased muscle mass, and loss of adipose tissue [18]. Similar findings have been reported in elderly men [19]. In contrast, oral estrogen treatment inhibits hepatic IGF-I production, leading to increased GH secretion in postmenopausal women, as reported by Ho and Weissberger [20].

We conducted the present study to investigate the interactions of GH and sex steroid replacement on whole-body protein turnover, an index of skeletal muscle protein balance, in healthy aged women and men.

2. Materials and methods

2.1. Subjects

One hundred three subjects (43 women and 60 men) 65 years and older (range, 65-88 years) were recruited from the community by mailed advertisements. Subjects were selected to be moderately active by self-report and to be performing usual household activities. Persons engaged in regular strenuous or athletic exercise training were excluded. All subjects were screened by history and physical examination, routine blood studies, urinalysis, and graded treadmill electrocardiogram. None of the women had taken any estrogen or progestogen for at least 3 months before the study. Subjects were excluded for history of malignancy, current depression, diabetes mellitus, untreated thyroid disease, pituitary or adrenal disease, severe dyslipidemia, or taking medications that could potentially affect the GH–IGF-I axis (eg, clonidine, L-dopa, arginine-containing dietary supplements). Subjects were nonsmokers (defined as subjects who stopped smoking for at least 1 year before entering this study) and consumed less than 2 fl oz of alcohol per day by self-report. This study was approved by the institutional review boards of the Johns Hopkins Bayview Medical Center/Gerontology Research Center and of the Johns Hopkins Hospital. Written informed consent was obtained from all subjects.

2.2. Study design

This study was a randomized, double-blind, 2 × 2 factorial, noncrossover design, with treatment given for a total period of 26 weeks. Subjects were assigned to 3 active hormone groups and 1 full placebo group as follows: recombinant human GH plus placebo sex steroid (GH group), sex steroid plus placebo GH (hormone replacement therapy [HRT] group for women, testosterone enanthate [T] group for men), GH plus sex steroids (GH + HRT for women, GH + T for men), or placebo GH plus placebo sex steroids (placebo group).

Human recombinant GH was given by subcutaneous self-injection at an average dose of 0.025 mg/kg, 3 times per week (after dose adjustments for adverse effects). The female sex hormone (HRT) regimen was a combination of an Estraderm (estradiol patch; Novartis, Basel, Switzerland) (100 μg/d) plus medroxyprogesterone (2.5 mg/d) taken orally for the first 10 days of each month. The male sex hormone regimen consisted of intramuscular injections of 100 mg of T in oil every 2 weeks. The placebos consisted of saline injections and/or skin patches containing no hormone.

2.3. Protein turnover studies

Whole-body protein turnover was assessed by means of a primed, constant intravenous infusion of [1-^{13}C]leucine. Each subject was studied on admission and again after 26 weeks of treatment.

The study was performed early in the morning, after an overnight fast. A 1.5-in indwelling catheter was inserted into a forearm vein for isotope tracer infusion, and a second one was placed in a retrograde fashion in a dorsal hand vein for blood sampling. This hand was kept inside a warming box at a constant temperature. After baseline blood and breath air samples were taken, priming doses of $NaH^{13}CO_3$ (0.09 mg/kg) and L-[1-^{13}C]leucine (4.5 μmol/kg) were given over a period of 2 minutes. An infusion of the leucine tracer was then started at a constant rate of 0.05 μmol · kg^{-1} · min^{-1} using screw-type infusion pumps (Harvard Apparatus, South Natick, Mass) and maintained for 4 hours. Blood and breath air samples were taken at 180, 185, 210, 225, and 240 minutes. Total carbon dioxide production was determined by continuous indirect calorimetry using a ventilated canopy system (Deltatrac; SensorMedics, Yorba Linda, Calif).

Table 1
Baseline characteristics of the subjects

Treatment groups	Women				Men			
	HRT	GH	GH + HRT	Placebo	T	GH	GH + T	Placebo
n	12	10	10	9	13	13	16	13
Age (y)	71.2 ± 3.00	70.1 ± 3.9	70.7 ± 4.8	71.7 ± 4.5	70.4 ± 3.3	71.3 ± 5.2	73.5 ± 6.3	70.8 ± 4.5
BMI (kg/m^2)	25.5 ± 2.6	26.3 ± 3.4	24.6 ± 3.6	26.1 ± 2.3	26.6 ± 3.4	27.4 ± 2.3	27.1 ± 3.1	27.2 ± 1.7

Table 2
Serum IGF-I level before and after treatment

	Women				Men			
	HRT	GH	GH + HRT	Placebo	T	GH	GH + T	Placebo
Baseline	116.7 ± 10.7	106.8 ± 11.5	132.3 ± 11.7	102.7 ± 10.6	126.4 ± 10.4	147.2 ± 11.5	115.8 ± 9.3	141.4 ± 11.6
After treatment	102.2 ± 8.6	191.2 ± 13.1*	165.5 ± 16.7*	100.6 ± 13.1	143.3 ± 7.9	250.3 ± 23.7*	226.8 ± 13.6*	123.3 ± 8.8

Values are expressed as nanograms per milliliter.
* $P < .05$ compared with baseline.

Plasma [1-^{13}C]leucine was determined by chemical ionization selected ion monitoring in a Hewlett Packard (San Fernando, Calif) model 5790 GC-MS instrument [21,22]. ^{13}C enrichment in breath air carbon dioxide was measured in triplicate by isotope ratio mass spectrometry after double cryogenic extraction using a VG Isogas Sira 10 instrument (Middlewich, UK) [21,22].

Leucine plasma flux (Q_{leu}) was calculated based on a stochastic, steady-state kinetics model [21]. Steady-state was defined as a slope not significantly different from 0 from the regression of 5 plasma enrichment measurements during the last hour of the study. The average of these measurements was used for calculating plasma leucine flux as follows:

$$Q_{leu} = I_{leu} \cdot \left[\frac{EI_{leu}}{EP_{leu}} - 1\right],$$

where I_{leu} indicates isotope infusion rate (micromole per kilogram per hour); EI_{leu}, isotopic enrichment of the infusate; and EP_{leu}, isotopic enrichment of plasma at steady state (atom percent excess [APE]).

Leucine oxidation (O_{leu}) was calculated using data on total carbon dioxide production and breath $^{13}CO_2$ enrichments measured during this same period:

$$O_{leu} = F_{^{13}CO_2} \cdot \left[\frac{1}{E_p} - \frac{1}{E_i}\right] \cdot 100,$$

where O_{leu} indicates leucine oxidation; $F_{^{13}}CO_2$, fractional $^{13}CO_2$ production rate during steady state; E_p, leucine plasma isotopic enrichment at steady state; and E_i, leucine enrichment in the infusate (APE). Carbon fixation fraction was estimated at 0.75.

Whole-body protein turnover was calculated by multiplying the leucine parameters by the constant (24 h/d)/(590 μmol leucine/g of protein) to give values of grams per kilogram per day of protein. The 590 μmol leucine/g of protein factor was derived from average values for leucine content of protein in human tissues and other mammalian muscles listed in 2 different sources of the amino acid composition of food. The factor corresponds to a protein content of 7.8% [21].

3. Data analysis

Analysis of variance was used to compare net changes of serum IGF-I level, leucine flux, leucine oxidation, nonoxidative leucine disposal (NOLD), whole-body protein turnover, and lean body mass among groups. Paired t tests were used to compare changes after treatment in each of the groups.

4. Results

Of the 103 subjects enrolled, 96 completed the study with a distribution among study groups as shown in Table 1. There were no significant differences among groups in age or BMI (Table 1).

Serum IGF-I levels increased significantly ($P < .0001$) after treatment of women with GH (84.3 ± 18.0 ng/mL) or GH + HRT (33.2 ± 11.5 ng/mL), and treatment of men with

Table 3
Leucine flux and oxidation before and after treatment (mol · h^{-1} · kg^{-1})

	Women				Men			
	HRT	GH	GH + HRT	Placebo	T	GH	GH + T	Placebo
Leucine flux								
Baseline	81.8 ± 6.4	78.5 ± 5.8	79.5 ± 5.6	84.1 ± 4.4	92.3 ± 6.9	86.6 ± 3.9	80.2 ± 2.8	89.7 ± 5.5
After treatment	73.7 ± 4.0	84.8 ± 5.2	83.5 ± 10.1	86.0 ± 6.2	87.4 ± 5.3	98.2 ± 6.4	93.6 ± 4.2*	83.3 ± 3.5
Leucine oxidation								
Baseline	10.8 ± 0.8	12.0 ± 1.1	11.3 ± 1.0	11.4 ± 0.6	13.8 ± 1.0	13.0 ± 0.9	13.0 ± 0.8	14.1 ± 1.3
After treatment	9.9 ± 0.7	10.8 ± 0.7	10.8 ± 1.7	11.8 ± 0.9	13.8 ± 1.0	15.4 ± 1.8	13.6 ± 1.0	13.8 ± 1.1
NOLD								
Baseline	71.0 ± 5.9	66.5 ± 5.0	68.2 ± 4.9	72.7 ± 4.2	78.5 ± 6.5	73.6 ± 3.5	67.1 ± 2.4	75.6 ± 4.5
After treatment	63.8 ± 3.7	74.1 ± 4.8	72.7 ± 8.6	74.2 ± 5.8	73.6 ± 4.8	82.7 ± 5.2	80.0 ± 3.5*	69.5 ± 2.7

Values are expressed mean ± SEM.
* $P < .02$ compared with baseline.

Table 4
Whole-body protein turnover and synthesis before and after treatment

Treatment group	Whole-body protein turnover (g protein · kg^{-1} · d^{-1})			Whole-body protein synthesis (g protein · kg^{-1} · d^{-1})		
	Baseline	After	Change	Baseline	After	Change
Men						
Placebo	3.7 ± 0.2	3.4 ± 0.1	−0.3 ± 0.2	3.1 ± 0.2	2.8 ± 0.1	−0.2 ± 0.2
GH	3.5 ± 0.2	4.0 ± 0.3	0.5 ± 0.3	3.0 ± 0.1	3.4 ± 0.2	0.4 ± 0.2
T	3.8 ± 0.3	3.6 ± 0.2	−0.2 ± 0.2	3.2 ± 0.3	3.0 ± 0.2	−0.2 ± 0.2
GH + T	3.3 ± 0.1	3.8 ± 0.2	0.6 ± 0.2*	2.7 ± 0.1	3.2 ± 0.1	0.5 ± 0.2*
Women						
Placebo	3.4 ± 0.2	3.5 ± 0.2	0.1 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	0.1 ± 0.3
GH	3.2 ± 0.2	3.5 ± 0.2	0.3 ± 0.3	2.7 ± 0.2	3.0 ± 0.2	0.3 ± 0.3
HRT	3.3 ± 0.3	3.0 ± 0.2	−0.3 ± 0.2	2.9 ± 0.2	2.6 ± 0.1	−0.3 ± 0.2
GH + HRT	3.2 ± 0.2	3.4 ± 0.4	0.2 ± 0.5	2.8 ± 0.2	3.0 ± 0.4	0.2 ± 0.4

Values are expressed as mean ± SEM.
* $P < .01$.

GH (103.1 ± 19.4 ng/mL) or GH + T (110.9 ± 13.6 ng/mL). There were no significant increases in IGF-I levels after the administration of HRT alone, T alone, or placebo (Table 2).

Baseline leucine flux and oxidation did not differ significantly between sexes or among treatment groups. After treatment, leucine flux increased significantly only in men in the GH + T group ($P = .02$; Table 3). There were no changes in leucine oxidation (Table 3).

Nonoxidative leucine disposal was calculated as the difference between leucine flux and leucine oxidation. Routes of leucine disposal other than oxidation and incorporation into protein were assumed to be negligible. There was a significant increase of NOLD in men treated with GH + T. Increases were also seen with GH alone, but these did not achieve statistical significance (Table 3).

Whole-body protein turnover increased significantly in men in the GH + T group ($P < .01$), whereas women in the GH + HRT group and men and women in the GH groups exhibited only nonsignificant increases (Table 4).

There was a modest but statistically significant correlation between changes of serum IGF-I level and changes of NOLD ($R = 0.248$, $P < .02$) (Fig. 1).

Results on body composition and muscle strength were reported elsewhere [23,24].

5. Discussion

In this study of hormone administration in healthy, somatopausal, and gonadopausal men and women older than 65 years, we demonstrated that treatment with a combination of GH and T significantly increased leucine flux and whole-body protein turnover in men, whereas the administration of GH alone in men or GH without or with HRT in women was less effective.

We quantified plasma amino acid flux and whole-body protein turnover using the intravenous, primed, constant infusion tracer method. Use of the amino acid leucine labeled with carbon 13 in the carboxyl carbon permits the estimation

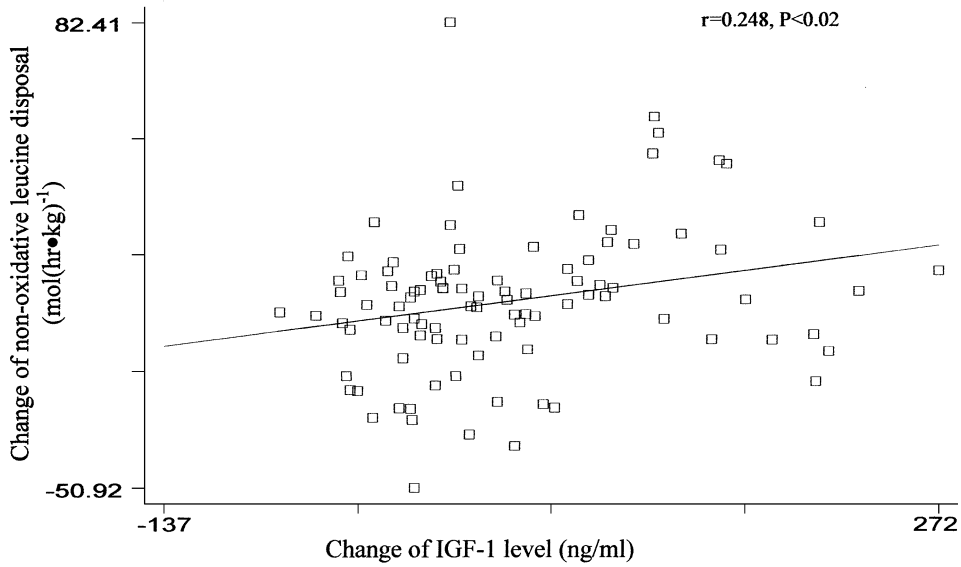

Fig. 1. Correlation between change of IGF-I level and change of NOLD.

of the intracellular amino acid pool available for protein synthesis, as well as the rate of amino acid disposal by oxidation [21,22]. Protein turnover can then be calculated, assuming an average leucine content of body proteins.

5.1. Insulin-like growth factor I level change after treatment

As expected, serum IGF-I levels increased significantly after GH treatment of men and women, with a somewhat greater effect observed in men. The increases in IGF-I levels were similar in the absence and presence of coadministered sex steroid. Neither T treatment in men nor HRT administration in women significantly affected IGF-I levels [25-27].

5.2. Effects on protein turnover

Baseline whole-body protein turnover rates were similar to those reported in other studies in comparable populations [28]. One important difference of our study compared with previous ones is the duration of 6 months of hormone administration. The effect of GH appears to be consistent, increasing NOLD and whole-body protein synthesis when administered to elderly women [29] or hypogonadal men [30]. One study, however, found no effect of GH on leucine oxidation [31,32]. The effects of GH on whole-body leucine kinetics are also consistent with skeletal muscle perfusion models, showing a net increase in protein synthesis [33]. These anabolic effects of GH on protein synthesis have been also documented in elderly persons recovering from hip fracture [34].

The current study demonstrates that healthy elderly individuals are able to increase whole-body protein synthesis in response to GH administration. The group showing the greatest net increase of NOLD (men treated with GH + T) also had the largest increase in circulating IGF-I level. The positive correlation between IGF-I and NOLD suggests a dose-response relationship between changes in IGF-I and changes in protein synthesis. Changes in groups receiving no GH were minimal. Because IGF-I is known to promote whole-body protein synthesis, as well as amino acid uptake in several tissues [33-36], our results suggest that GH replacement increases protein synthesis by increasing circulating IGF-I levels, although the relative roles of circulating vs tissue-generated, endogenous (autocrine and paracrine) IGF-I responses remain to be defined [7]. It is also possible that some or all of the observed effects on protein turnover were mediated by direct actions of GH, independent of IGF-I production.

The finding that men treated with GH + T exhibited the greatest increase in leucine flux and NOLD but no greater increase in circulating IGF-I than men treated with GH alone suggests that T and IGF-I may exert additive peripheral effects to increase protein synthesis.

Our study showed a nonsignificant decrease in IGF-I levels and NOLD in women treated with HRT, administered as conventional-dose transdermal estrogen plus oral progestogen. Estrogen replacement therapy has been shown to be beneficial in preventing osteoporosis and depression in menopausal women [37]. However, no convincing anabolic effect of estrogen or estrogen plus progestogen has been reported in prospective studies in older women [38]. Oral or high-dose transdermal estrogens given to postmenopausal women reduce basal IGF-I levels to varying degrees [39,40], and GH-deficient women required higher doses of GH when they are on estrogen replacement therapy [39,40]. Whether the suppression of circulating IGF-I levels by postmenopausal estrogen replacement exerts any clinical adverse effects remains to be determined.

Although there were no significant subgroup differences in IGF-I levels in either sex, IGF-I levels were lower in women vs men before ($P = .07$) and after treatment ($P = .0007$). Whether the known sexual dimorphism in baseline GH secretion and IGF-I levels contribute to sex differences in body composition remains to be determined [41]. In the current study, all subjects were given GH doses based on body weight, independent of sex. Thus, it is possible that we would have observed a greater increase of NOLD in women had we given a proportionately greater dose of GH to women vs men.

In summary, our study shows that the administration of GH increases whole-body protein synthesis in healthy, elderly, somatopausal, and gonadopausal men and women, and that this effect is augmented in men after treatment with GH + T. As reported elsewhere in detail [24], these changes were associated with increased muscle strength and fat-free body mass.

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