Impact of Growth Hormone and Dehydroepiandrosterone on Protein Metabolism in Glucocorticoid-Treated Patients

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Context: Chronic pharmacological glucocorticoid (GC) use causes substantial morbidity from protein wasting. GH and androgens are anabolic agents that may potentially reverse GC-induced protein loss.

Objective: Our objective was to assess the effect of GH and dehydroepiandrosterone (DHEA) on protein metabolism in subjects on long-term GC therapy.

Design: This was an open, stepwise GH dose-finding study (study 1), followed by a randomized cross-over intervention study (study 2).

Setting: The studies were performed at a clinical research facility.

Patients and Intervention: In study 1, six subjects (age 69 $\pm$ 4 yr) treated with long-term (>6 months) GCs (prednisone dose 8.3 $\pm$ 0.8 mg/d) were studied before and after two sequential GH doses (0.8 and 1.6 mg/d) for 2 wk each. In study 2, 10 women (age 71 $\pm$ 3 yr) treated with long-term GCs (prednisone dose 5.4 $\pm$ 0.5 mg/d) were studied at baseline and after 2-wk treatment with GH 0.8 mg/d, DHEA 50 mg/d, or GH and DHEA (combination treatment).

Main Outcome Measure: Changes in whole body protein metabolism were assessed using a 3-h primed constant infusion of 1-[^{13}C]leucine, from which rates of leucine appearance, leucine oxidation, and leucine incorporation into protein were estimated.

Results: In study 1, GH 0.8 and 1.6 mg/d significantly reduced leucine oxidation by 19% ($P = 0.03$) and 31% ($P = 0.02$), and increased leucine incorporation into protein by 10% ($P = 0.13$) and 19% ($P = 0.04$), respectively. The lower GH dose did not cause hyperglycemia, whereas GH 1.6 mg/d resulted in fasting hyperglycemia in two of six subjects. In study 2, DHEA did not significantly change leucine metabolism alone or when combined with GH. Blood glucose was not affected by DHEA.

Conclusion: GH, at a modest supraphysiological dose of 0.8 mg/d, induces protein anabolism in chronic GC users without causing diabetes. DHEA 50 mg/d does not enhance the effect of GH. GH may safely prevent or reverse protein loss induced by chronic GC therapy. **(*J Clin Endocrinol Metab* 93: 688–695, 2008)**

Catabolism from various disorders is a cause of substantial morbidity and increased mortality (1–3). Endogenous glucocorticoid (GC) overproduction in Cushing's syndrome causes a marked reduction in lean body mass (LBM) (4–6) and leads to skin thinning, muscle wasting, and weakness (7). Exogenous GCs, used long-term by 0.5–0.9% of the community to treat

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Abbreviations: AUC, Area under the curve; CV, coefficient of variation; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; Fox, fat oxidation; GC, glucocorticoid; HOMA-R, homeostasis model assessment for insulin resistance; IGI, insulinogenic index; ISI_{comp}, insulin sensitivity index; KIC, ketoisocaproic acid; LBM, lean body mass; leucine Ra, leucine appearance; LIP, leucine incorporation into protein; Lox, leucine oxidation; PMR, polymyalgia rheumatica; REE, resting energy expenditure.

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inflammatory and autoimmune disease (8, 9), also appear to cause substantial morbidity from protein loss.

The leucine turnover technique provides accurate, noninvasive estimates of whole body rates of protein breakdown, oxidation, and synthesis, the key components of protein metabolism. High-dose exogenous GCs acutely stimulate protein breakdown more than synthesis, thereby increasing irreversible loss of amino acids through oxidation (10–12). Protein oxidation is also acutely increased by therapeutic doses of GCs (*e.g.* prednisolone 5 mg/d), but returns to normal with prolonged use (13). Therefore, long-term therapeutic GC use is likely to cause a stable, but reduced, protein mass. GH (14, 15) and androgens (16, 17) are potent anabolic hormones that reduce protein oxidation and have the potential to reverse GC-induced protein loss. Because the combination of GH and androgen produces a greater reduction in protein oxidation than each agent alone (18), combination therapy may exert the greatest anabolic effect.

Very high doses of GH prevent the acute stimulation of protein oxidation by GCs but cause hyperglycemia in young adults (11, 19, 20). Elderly subjects, in whom GCs are most frequently prescribed (9), are less responsive to GH's anabolic action (21, 22) and more susceptible to the adverse effects of GH (23, 24). Whether lower GH doses can exert a beneficial effect on protein metabolism in this patient group without affecting glucose tolerance is uncertain. Combining GH and androgens has the potential to induce protein anabolism using lower GH doses with a lesser deterioration in blood glucose.

The aims were to determine: 1) a minimally effective dose of GH that induces protein anabolism in long-term users of pharmacological GC treatment, and 2) whether the addition of dehydroepiandrosterone (DHEA) to GH augments its anabolic action. To that end, two clinical studies were undertaken. First, a GH dose-finding study was undertaken to determine a dose of GH that reduced whole body protein oxidation to the same extent as replacement doses of GH in subjects with GH deficiency (25). This dose of GH was then used in a three-period randomized cross-over study to compare the effect of GH, DHEA, and the combination on whole body protein metabolism in women on long-term GCs.

Subjects and Methods

Subjects and study design

The Research Ethics Committee, St. Vincent's Hospital, approved the studies, and all subjects gave written informed consent.

GH dose-finding study (study 1)

Six subjects (three women) with polymyalgia rheumatica (PMR) or inflammatory arthritis aged 54–78 yr were recruited through the Department of Rheumatology, St. Vincent's Hospital. All subjects were receiving a chronic (>6 months) stable prednisone dose of 5–10 mg/d. Subjects were in remission from PMR or inflammatory arthritis based on resolution of clinical symptoms, and a normal erythrocyte sedimentation rate and C-reactive protein. Other medical therapies remained stable throughout the study period. Subjects were excluded if they had congestive heart failure, known diabetes mellitus, hepatic or renal disease or malignancy, or if they were receiving oral estrogen replacement therapy.

The study was an open-label, stepwise study of two incremental sc GH (Genotropin; Pfizer, West Ryde, Australia) doses, 0.8 and 1.6 mg/d, for 2 wk each. Nurses from the Clinical Research Facility instructed subjects in the administration of GH.

GH and DHEA in women on GCs (study 2)

Study 2 was initiated after the determination of a minimally effective dose of GH from study 1. Ten women treated with long-term GCs (prednisone 4–8 mg/d) for PMR or inflammatory arthritis aged 58–81 yr were recruited through the Department of Rheumatology, St. Vincent's Hospital, and by advertisement in local papers. Two women had also participated in the GH dose-finding study. However, a time period of more than 6 months elapsed between their participation in the two studies. The inclusion and exclusion criteria were the same as those for study 1.

The design was an open-label randomized cross-over study of GH (0.8 mg/d, Genotropin) for 2 wk, DHEA 50 mg/d for 2 wk, and the combination of GH 0.8 mg/d and DHEA 50 mg/d (combination treatment) for 2 wk, with a 2-wk washout period between each treatment. Micronized DHEA (99% grade) was obtained from Professional Compounding Chemists of America (Houston, TX) and compounded into capsules with a microcrystalline cellulose base by Fresh Therapeutics Pharmacy (Sydney, Australia).

Clinical protocol

Subjects attended the Clinical Research Facility, Garvan Institute of Medical Research, Sydney, Australia, at 0830 h after an overnight fast.

Assessment of whole body protein turnover

Whole body protein turnover was assessed using a primed constant infusion of 1-[^{13}C]leucine as previously described (26, 27). $NaH^{13}CO_3$ and 99% 1-[^{13}C]leucine were obtained from Cambridge Isotope Laboratories (Woburn, MA). Solutions were prepared under sterile conditions using 0.9% saline. A 0.1 mg/kg priming dose of $NaH^{13}CO_3$ was immediately followed by a 3-h primed constant infusion of 1-[^{13}C]leucine (prime, 0.5 mg/kg; infusion, 0.5 mg/kg·h), based on previous studies demonstrating that steady state was achieved during this time period (27). Blood and breath samples were collected before (−10, 0 min) and at the end of the infusion (140, 160, and 180 min). Blood was placed on ice, and plasma was separated and stored at −80 C until analysis. CO_2 production rates were measured with an open circuit ventilated hood system (Deltatrac metabolic monitor; Datex Instrumentation Corp., Helsinki, Finland), which was calibrated against standard gases before each study. Measurements of CO_2 production were collected during two 20-min periods. After an equilibration period of 5 min, the final 15-min recordings were averaged.

Calculation of whole body protein turnover

Whole body protein turnover was calculated using the reciprocal pool method (28). The method is based on the principle of steady-state kinetics, whereby the rate of appearance of a substrate equals its rate of disposal. For leucine there are two pathways of disposal (oxidation and reincorporation into protein), allowing calculation of rates of leucine appearance (leucine Ra) (an index of protein breakdown), leucine oxidation (Lox) (an index of oxidative loss of protein), and leucine incorporation into protein (LIP) (an index of protein synthesis). In the reciprocal pool method, α-ketoisocaproic acid (KIC), formed when leucine undergoes transamination, is used as a surrogate marker of intracellular leucine enrichment because it more accurately reflects the intracellular environment (28, 29). Leucine Ra is calculated as the leucine infusion rate divided by the change in isotopic enrichment of KIC with ^{13}C. Because the carboxyl group of leucine labeled with ^{13}C is removed in the first irreversible step in its oxidative degradation, changes in the isotopic enrichment of CO_2 with ^{13}C are used to estimate Lox. Therefore, Lox is calculated by multiplying leucine Ra by the fraction of isotope oxidized and dividing by a correction factor to account for the proportion of CO_2 that is excreted in breath and not fixed in other metabolic pathways, 71%

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in our laboratory (27). LIP is derived as the difference between leucine Ra and Lox. Because leucine represents 8% of total body protein, or 590 μmol leucine represents 1 g protein, rates of protein turnover may be estimated using these constants (30). The coefficients of variation (CVs) for leucine Ra, Lox, and LIP at our institution, based on seven subjects studied on two occasions, are 3.5, 6.1, and 3.5%, respectively (26).

Indirect calorimetry

Resting energy expenditure (REE) and substrate oxidation rates were calculated, as previously described (6), using the equations of Ferrannini (31). The mean day-to-day intrasubject CV for REE at our institute is approximately 4% (32, 33).

Glucose metabolism

Homeostasis model assessment for insulin resistance (HOMA-R) was calculated from measures of fasting glucose and insulin (34). In study 2, an oral glucose tolerance test was performed at the completion of whole body leucine turnover studies. Glucose and insulin area under the curve (AUC) during the oral glucose tolerance test were calculated as described by Tai (35). Estimates of insulin sensitivity index (ISI_{comp}) and β-cell function [insulinogenic index (IGI)] based on the oral glucose tolerance test were calculated as previously described (36, 37).

Analytical methods

KIC was extracted from plasma as described by Nissen *et al.* (38). KIC enrichment with ^{13}C was measured as the butyldimethylsilyl derivative by gas chromatography (model 5890; Hewlett-Packard Co., Palo Alto, CA)-mass spectrometry (MSD 5971A; Hewlett-Packard Co.), with selective monitoring of ions 301 and 302 (39). CO_2 enrichment in breath was measured at St. Thomas' Hospital, London, United Kingdom, on a SIRA Series II isotope ratio mass spectrometer (VG Isotech, Cheshire, UK).

Blood glucose measurements were performed using an immobilized glucose oxidase method on a glucose analyzer (model 23AM; Yellow Springs Instrument Inc., Yellow Springs, OH). DHEA-sulfate (DHEAS), testosterone, and SHBG were measured using a commercial assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). For DHEAS the limit of detection was 0.4 μmol/liter, and the CV was 7.3% at 2.1 μmol/liter and 7.6% at 4.4 μmol/liter. For the purpose of statistical analysis, samples with undetectable levels DHEAS were assigned a value of 0.4 μmol/liter. The CV for testosterone was 9.2% at 3.6 mmol/liter. The CVs for SHBG at 5.3 and 86.2 nmol/liter were 5.0 and 7.5%, respectively. IGF-I was measured using a two-site RIA after acid ethanol extraction, with a CV of 8.2% at 13.8 nmol/liter and 7.3% at 28.6 nmol/liter (40). Plasma insulin was measured by RIA using a commercial kit (LINCO Research, Inc., St. Charles, MO). The interassay CV for insulin was 4.2% at 42 μU/ml.

Statistical analysis

Statistical analysis was undertaken using the statistical software package Statview 4.5 PPC (Abacus Concepts, Inc., Berkeley, CA) and SPSS 15.0 (SPSS, Inc., Chicago, IL). Results are expressed as mean ± SE unless otherwise stated. In study 1, ANOVA with repeated measures was used to assess changes in variables across the three time points. *Post hoc* paired *t* tests were then used to assess the effect of the two GH doses. In study 2, paired *t* tests were performed to compare the effect of treatment to the baseline visit. Data that were not normally distributed were log transformed before statistical analysis to achieve a normal distribution but are presented in the text nontransformed. A *P* value less than 0.05 was considered statistically significant.

Results

GH dose-finding study (study 1)

Subjects

The subjects' mean age was 68.8 ± 3.5 yr, weight 77.1 ± 4.1 kg, body mass index 27.4 ± 0.6 kg/m^2, and prednisone dose 8.3 ± 0.8 mg/d. All subjects completed the study protocol, and on the basis of returned cartridges, complied with GH treatment.

Metabolic hormones and energy metabolism (Table 1)

GH significantly increased IGF-I, fasting glucose, and insulin and HOMA-R. The mean IGF-I of 39.5 ± 6.1 nmol/liter after GH 0.8 mg/d was within the reference range for young adults (17–42 nmol/liter), whereas GH 1.6 mg/d increased mean IGF-I to 60.0 ± 9.9 nmol/liter. Fasting glucose was less than 6 mmol/liter in all subjects at baseline and after GH 0.8 mg/d. However, two subjects developed diabetes (fasting glucose > 7 mmol/liter) on the higher GH dose. GH significantly increased REE and fat oxidation (Fox), with a greater increase on the higher GH dose.

Whole body leucine turnover (Fig. 1)

GH treatment significantly (by ANOVA) reduced Lox ($P = 0.03$) and increased LIP ($P = 0.02$), whereas leucine Ra did not change significantly. Mean Lox decreased significantly from baseline by 19% with the 0.8 mg/d dose ($P = 0.03$) and by 31% with the higher GH dose ($P = 0.02$). Mean LIP increased by 10% with the lower dose and by 19% with the 1.6 mg/d GH dose.

The lower GH dose of 0.8 mg/d was chosen for study 2 because it exerted a significant anabolic effect without causing diabetes. Based on the 19% reduction in Lox with GH 0.8 mg/d, it was calculated that a sample size of 10 subjects would provide an 80% chance of finding a similar reduction in Lox at a significance level of 0.05.

TABLE 1. Metabolic hormones and energy metabolism in six subjects receiving long-term therapeutic GCs at baseline and after 2-wk treatment with GH 0.8 mg/d and a further 2 wk of GH 1.6 mg/d

	Baseline	GH 0.8 mg/d	GH 1.6 mg/d	*P* value
IGF-I (nmol/liter)	18.3 ± 1.3	39.5 ± 6.1[a]	60.0 ± 9.9[a,b]	0.0002
Glucose (mmol/liter)	4.6 ± 0.4	5.3 ± 0.3[a]	6.2 ± 0.6[a]	0.002
Insulin (μU/ml)	14.7 ± 1.4	25.3 ± 4.9	39.8 ± 11.2	0.03
HOMA-R	3.0 ± 0.4	6.1 ± 1.3	10.9 ± 3.3	0.02
REE (kcal/d)	1495 ± 102	1518 ± 112	1654 ± 97[a]	0.03
Fox (mg/min)	68.1 ± 6.6	74.5 ± 8.8	91.6 ± 7.4[a]	0.02

Values represent mean ± SE. *P* values relate to the overall effect of GH treatment, calculated using ANOVA with repeated measures.

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

[b] $P < 0.05$ *vs.* GH 0.8 mg/d.

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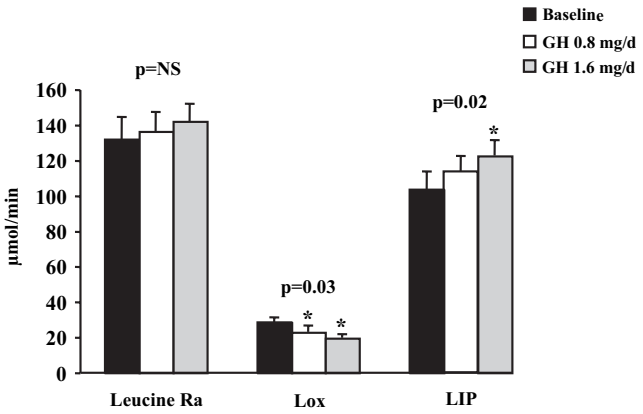

FIG. 1. Rates of leucine Ra, Lox, and LIP in six subjects receiving long-term therapeutic GCs at baseline (*black bars*) and after 2-wk treatment with GH 0.8 mg/d (*white bars*) and a further 2 wk of GH 1.6 mg/d (*gray bars*). *Bars* represent mean ± SE. *P* values relate to the overall effect of GH treatment, calculated using ANOVA with repeated measures. *, $P < 0.05$ *vs.* baseline. NS, Not significant.

GH and DHEA in women on GCs (study 2)

Subjects

The subjects' mean age was 70.8 ± 2.6 yr, weight 63.9 ± 2.5 kg, body mass index 25.5 ± 1.0 kg/m^2, and prednisone dose 5.4 ± 0.5 mg/d. All subjects completed the study protocol, and on the basis of returned cartridges and pill bottles, complied with GH and DHEA treatment.

Anabolic hormones (Fig. 2)

Serum testosterone increased during DHEA ($P = 0.004$) and combination treatment ($P = 0.0005$) but did not change significantly during GH treatment. SHBG was significantly reduced by DHEA ($P = 0.01$) and combination treatment ($P = 0.0008$), but not by GH alone. DHEAS increased during DHEA ($P < 0.0001$) and combination treatments ($P < 0.0001$) but did not change significantly with GH. IGF-I increased significantly during GH ($P < 0.0001$), DHEA ($P = 0.02$), and combination treatments ($P < 0.0001$). The increase in IGF-I with combination treatment was greater than that induced by GH alone ($P = 0.03$).

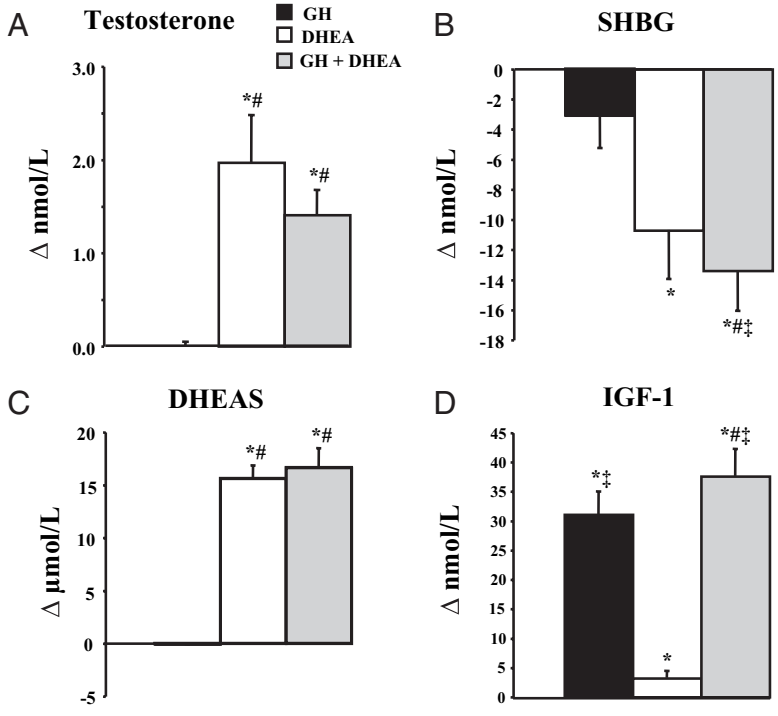

FIG. 2. Changes from baseline (Δ) in serum testosterone (A), SHBG (B), DHEAS (C), and IGF-I (D) in 10 women on long-term GCs treated with GH 0.8 mg/d (*black bars*), DHEA 50 mg/d (*white bars*), and GH 0.8 mg/d and DHEA 50 mg/d (*gray bars*) for 2 wk. *Bars* represent mean ± SE of change from baseline. *, $P < 0.05$ *vs.* baseline. #, $P < 0.05$ *vs.* GH. ‡, $P < 0.05$ *vs.* DHEA.

Glucose metabolism (Table 2)

Fasting glucose increased significantly after GH (+0.4 ± 0.2 mmol/liter; $P = 0.04$) and combination therapy (+0.5 ± 0.1 mmol/liter; $P < 0.0001$) but did not change with DHEA. Consistent with the findings of study 1, no patient developed fasting hyperglycemia (glucose > 7 mmol/liter) during GH treatment. Two-hour postglucose-load glucose levels did not change significantly after any treatment. The glucose AUC during an oral glucose tolerance test was increased by combination treatment (+60.3 ± 22.8 mmol/liter·min; $P = 0.03$), but not after individual treatment with GH and DHEA. Fasting and 2-h postglucose-load insulin and the insulin AUC during an oral glucose tolerance test were significantly increased by GH and combination treatment but were not significantly different after DHEA. There were no significant differences between the effects of GH and combination treatment on glucose and insulin levels.

The glucose and insulin levels recorded during the oral glucose tolerance test were used to calculate estimates of insulin sensitivity (Fig. 3A) and secretion (IGI) (Fig. 3B). Higher levels of ISI_{comp} and IGI reflect greater insulin sensitivity and secretion, respectively. ISI_{comp} was significantly reduced by GH ($P = 0.01$) and combination treatment ($P = 0.006$), and significantly increased by DHEA ($P = 0.04$). IGI increased significantly with GH ($P = 0.0002$) and combination treatments ($P = 0.01$) but was not affected by DHEA. There were no significant differences between the effect of GH and combination treatment on indices of insulin sensitivity and secretion.

Energy metabolism (Fig. 4)

REE was not significantly affected by GH or DHEA but increased significantly with combination treatment ($P = 0.03$). Fox increased slightly with GH, a change that did not reach statistical significance ($P = 0.08$). However, Fox increased significantly when DHEA was coadministered ($P = 0.01$). DHEA alone did not affect REE or Fox.

Whole body leucine turnover (Fig. 5)

GH treatment significantly increased LIP ($P = 0.02$), whereas the corresponding changes in Lox and leucine Ra did not reach statistical significance. Addition of DHEA to GH did not result in significant changes in leucine metabolism compared with GH alone. However, with combined treatment the increase in leucine Ra and LIP and reduction in Lox were all significantly different from baseline. DHEA alone did not significantly affect any component of

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TABLE 2. Fasting (f) and 2-h post 75 g glucose-load (2 h) glucose and insulin levels and the glucose and insulin AUC during an oral glucose tolerance test in 10 women on long-term GC therapy at baseline and after 2-wk treatment with GH 0.8 mg/d, DHEA 50 mg/d, and the combination of GH and DHEA

	Baseline	GH	DHEA	GH+DHEA
Glucose (f) (mmol/liter)	4.8 ± 0.2	5.2 ± 0.3[a,b]	4.7 ± 0.2	5.3 ± 0.2[a,b]
Glucose (2 h) (mmol/liter)	10.9 ± 0.7	11.0 ± 0.9	10.8 ± 0.9	11.1 ± 0.9
Glucose (AUC) (mmol/liter·min)	1,084 ± 65	1,102 ± 73	1,057 ± 79	1,144 ± 72[a]
Insulin (f) (mU/liter)	14.0 ± 1.4	19.3 ± 2.3[a,b]	12.9 ± 1.0	18.5 ± 4.8[a,b]
Insulin (2 h) (mU/liter)	108 ± 12	148 ± 15[a,b]	92 ± 12	139 ± 16[a,b]
Insulin (AUC) (mU/liter·min)	8,258 ± 654	11,758 ± 3,991[a,b]	7,556 ± 581	10,902 ± 1,095[a,b]

Values represent mean ± SE.

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

[b] $P < 0.05$ *vs.* DHEA.

leucine metabolism, although there was a trend toward a reduction in Lox and a corresponding increase in LIP. A similar trend was observed when the effect of combined treatment was compared with that of GH alone.

Side effects of treatment

Treatment with GH and DHEA was well tolerated. Five subjects reported peripheral edema after GH, two subjects after DHEA, and three subjects with combination treatment. One subject reported increased oiliness of the skin with DHEA treatment. The erythrocyte sedimentation rate was significantly greater than baseline by 4.7 ± 1.7 mm/h after combination treatment ($P = 0.02$), but the change was not significant after GH and DHEA alone (data not shown). C-reactive protein and systolic and diastolic blood pressure were not significantly different from baseline after any treatment period (data not shown).

Discussion

The aims of this study were to determine a minimally effective anabolic dose of GH in chronic GC users and to investigate whether the addition of a weak androgen could augment the anabolic action of GH in women. GH dose estimates were based on the expectation that doses higher than daily production rates

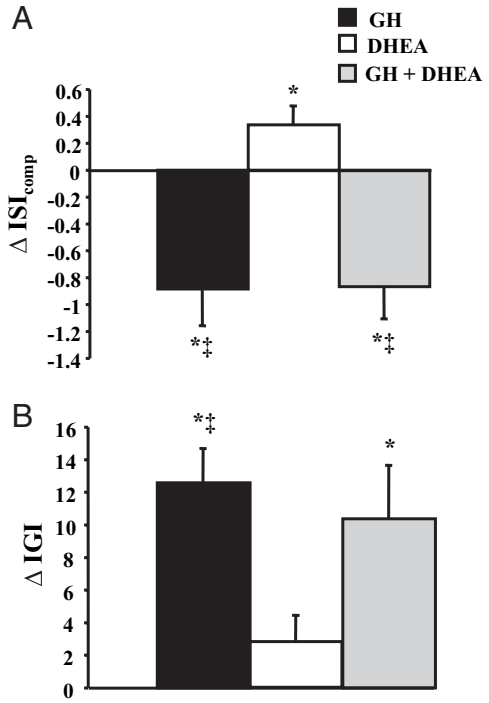

FIG. 3. Changes from baseline (Δ) in ISI_{comp} index (A) and IGI (B) after 2-wk treatment with GH 0.8 mg/d (*black bars*), DHEA 50 mg/d (*white bars*), and the combination of GH and DHEA (*gray bars*) in 10 women on long-term GC therapy. *Bars* represent mean ± SE of change from baseline. *, $P < 0.05$ *vs.* baseline. ‡, $P < 0.05$ *vs.* DHEA.

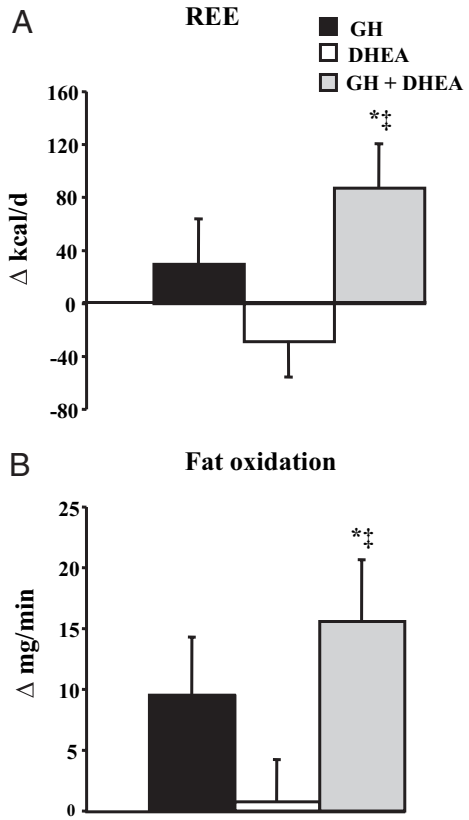

FIG. 4. Changes from baseline (Δ) in REE (A) and Fox (B) after 2-wk treatment with GH 0.8 mg/d (*black bars*), DHEA 50 mg/d (*white bars*), and the combination of GH and DHEA (*gray bars*) in 10 women on long-term GC therapy. *Bars* represent mean ± SE of change from baseline. *, $P < 0.05$ *vs.* baseline. ‡, $P < 0.05$ *vs.* DHEA.

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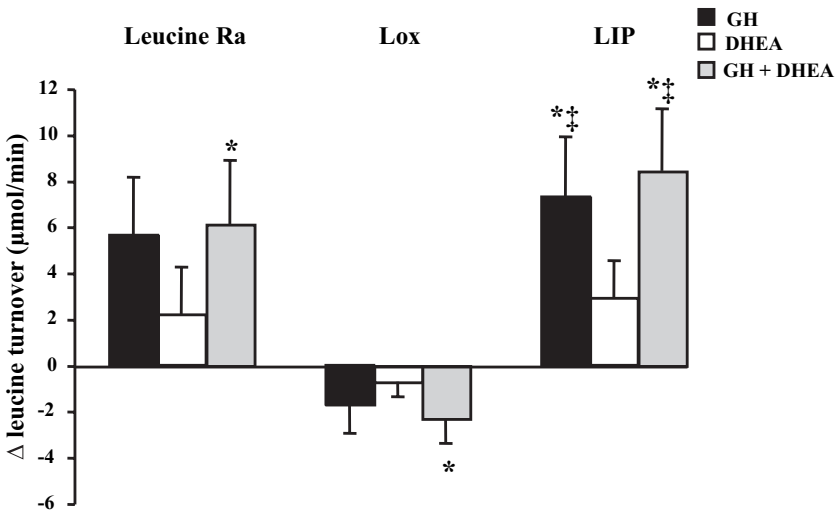

FIG. 5. Changes from baseline (Δ) in rates of leucine Ra, Lox, and LIP after 2-wk treatment with GH 0.8 mg/d (*black bars*), DHEA 50 mg/d (*white bars*), and the combination of GH and DHEA (*gray bars*) in 10 women on long-term GC therapy. *Bars* represent mean ± SE change from baseline. *, $P < 0.05$ *vs.* baseline. ‡, $P < 0.05$ *vs.* DHEA.

would be necessary to overcome the catabolic effects of GCs in these GH-sufficient subjects. Because Lox represents irreversible protein loss, a reduction in Lox was used as a metabolic indicator of anabolism. Study 1 was modeled to identify a dose of GH that induced a decrease in Lox approximating that observed in GH-deficient subjects treated with a replacement dose of GH that also increased LBM. The lower GH dose significantly reduced Lox without inducing fasting hyperglycemia. When evaluated in study 2, the effect of GH was not significantly enhanced by DHEA. DHEA alone did not induce significant changes in any aspect of leucine metabolism, although a modest trend toward an anabolic effect was apparent. Finally, the lower GH dose did not induce diabetes alone or when combined with DHEA.

Previous studies have reported that treatment with GH approximating seven to 40 times replacement doses in healthy young adults can attenuate the increase in protein oxidation induced by doses of prednisone eight to 10 times higher than those used in this study (11, 19, 20). However, this was accompanied by an increase in blood glucose concentration that would limit the therapeutic potential of GH in this context. In patients with Cushing's syndrome, GH at a dose approximating four times physiological production increased mean fasting glucose by 1.7 mmol/liter (41). The dose of GC needed long term to control inflammatory disease in the elderly is much less than in the aforementioned studies (8, 9). Although the anabolic-catabolic relationship between GH and GC dosage is unknown, it might be expected that lower doses of GH may achieve an anabolic effect in patients taking much lower doses of GC.

Little information was available as to what represented a minimally effective anabolic dose of GH in patients on chronic GC therapy. A GH dose of 0.8 mg/d, which is approximately two times physiological GH production, reduced protein oxidation in a small study of four GC users (42). However, the subjects were much younger than in the current study. Because the anabolic action of GH may be attenuated in older subjects, a higher GH dose of 1.6 mg/d was also studied. Study 1 demonstrated that a GH dose of 0.8 mg/d induces protein anabolism in elderly patients receiving long-term GC therapy. Using the constants described by Matthews *et al.* (30), the 19% reduction in Lox in study 1 would result in a gain in protein mass of approximately 1.2 kg over 3 months.

We were concerned that the combined use of GH and GC in the elderly may be problematic because of their greater susceptibility to GH-induced hyperglycemia (23, 24). In study 2, a GH dose of 0.8 mg/d, alone and in combination with DHEA, increased mean fasting glucose by less than 0.5 mmol/liter. Because one study reported that acute GC and GH administration exerted a greater effect on postprandial than fasting glucose (11), oral glucose tolerance tests were performed in study 2. There was no significant increase in postglucose-load glucose level or in the glucose AUC with GH during oral glucose tolerance testing. GH exerted a greater effect on insulin sensitivity, manifested by a doubling of HOMA-R in study 1 and a reduction in ISI_{comp} in study 2. However, the early deterioration in insulin sensitivity with GH therapy may diminish during long-term GH treatment as abdominal adiposity reduces (43). In summary, in elderly patients on long-term low-dose prednisone therapy approximating 5–10 mg/d, a GH dose of 0.8 mg/d is likely to induce an anabolic effect, at the cost of reduced insulin sensitivity but only a minor increase in blood glucose level.

This study was designed to define the GH dose response in GC users and whether DHEA enhances GH action, and does not provide insight into the metabolic mechanisms of GH action. The anabolic action of GH is predominantly mediated by GH-induced IGF-I production (44). An important component of the protein-conserving effect of GH is stimulation of lipolysis and Fox because this generates free fatty acids and ketone bodies that can be used as substrate for protein synthesis (45). The extent to which changes in insulin secretion and action and mobilization of glucose and amino acids contribute to the anabolic action of GH, and whether GH exerts an anabolic effect in all tissues, remains to be fully clarified (46).

DHEA alone did not significantly change leucine turnover, a finding consistent with a previous study (47). The addition of DHEA to GH also did not significantly enhance the anabolic effect of GH. However, there was evidence of a modest effect of DHEA on Lox, REE, and Fox when combined with GH because GH-induced changes only reached statistical significance in study 2 when GH and DHEA were coadministered. Any metabolic effects of DHEA could be direct or indirect occurring from stimulation of IGF-I production. This study was powered to assess the reduction in Lox with GH compared with baseline. Because the anabolic effect of DHEA in the dose used is, at best, modest and much weaker than GH, the failure to find a significant change is likely due to a type II error. Based on our data, the predicted increase in protein mass over 3 months with DHEA is 150 g and of uncertain clinical significance. A larger study is

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required to determine whether this dose of DHEA exerts a significant anabolic effect in GC users.

Treatment with DHEA has improved insulin sensitivity in some (48, 49), but not all (50), studies. If DHEA improved insulin sensitivity in subjects receiving long-term GCs, it could potentially offset the diabetogenic effect of GH. In this study, DHEA did not alter fasting glucose and insulin *per se* but significantly improved insulin sensitivity as estimated by ISI_{comp}. However, combined treatment with DHEA and GH increased glucose and insulin levels to the same extent as GH alone. This suggests that the addition of DHEA does not attenuate GH-induced insulin resistance.

Strengths of this study include that the study group was relatively homogenous and representative of typical patients receiving commonly prescribed chronic GC doses. Limitations of the study include that the sample size was small, which could have resulted in underestimation of the effect of treatment and that the results of study 2 cannot be extrapolated to men. This was a proof-of-concept study assessing a short duration of anabolic therapy and, as such, was not designed to assess changes in body composition, functional end-points, or adverse consequences that could develop over a longer treatment period. However, the utility of a reduction in Lox, which represents a change in the balance between protein breakdown and synthesis, as a marker of anabolic efficacy was underscored by our recent findings that the 2-wk reduction in Lox during GH treatment was significantly correlated with LBM accrual (25).

In conclusion, GH has the potential to safely reverse protein catabolism induced by low-dose GC treatment in elderly patients. A GH dose approximating two times daily production represents an effective anabolic dose that does not induce diabetes. The anabolic effect of DHEA 50 mg/d in this patient group is, at best, modest and of uncertain clinical significance. A higher dose merits further study as does an evaluation of the separate and combined effects of GH and testosterone supplementation in men on long-term GC therapy. There is a need for a randomized placebo-controlled study assessing the effect of GH alone and in combination with androgens on body composition, physical function, and quality of life in subjects receiving therapeutic GCs.

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