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Impact of Acute and Chronic Low-Dose Glucocorticoids on Protein Metabolism

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Context: High-dose glucocorticoids cause acute protein loss by increasing protein breakdown and oxidation. Whether lower glucocorticoid doses, typical of therapeutic use, induce sustained catabolism has not been studied.

Objective: Our objective was to assess the effect of acute and chronic therapeutic glucocorticoid doses on protein metabolism.

Design and Setting: We conducted an open longitudinal and a cross-sectional study at a clinical research facility.

Patients and Intervention: Ten healthy subjects were studied before and after a short course of prednisolone (5 and 10 mg/d sequentially for 7 d each). Twelve subjects with inactive polymyalgia rheumatica receiving chronic (>12 months) prednisone (mean = 5.0 ± 0.8 mg/d) were compared with 12 age- and gender-matched normal subjects.

Main Outcome Measure: Whole-body protein metabolism was assessed using a 3-h primed constant infusion of 1-[^{13}C]leucine, from which rates of leucine appearance (leucine Ra, an index of protein breakdown), leucine oxidation (Lox, index of protein oxidation) and leucine incorporation into protein (LIP, index of protein synthesis) were estimated.

Results: Prednisolone induced an acute significant increase in Lox ($P = 0.008$) and a fall in LIP ($P = 0.08$) but did not affect leucine Ra. There was no significant difference between the effects of the 5- and 10-mg prednisolone doses on leucine metabolism. In subjects receiving chronic prednisone therapy, leucine Ra, Lox, and LIP were not significantly different from normal subjects.

Conclusion: Glucocorticoids stimulate protein oxidation after acute but not chronic administration. This time-related change suggests that glucocorticoid-induced stimulation of protein oxidation does not persist but that a metabolic adaptation occurs to limit protein loss. **(*J Clin Endocrinol Metab* 92: 3923–3929, 2007)**

MAINTENANCE OF OPTIMAL body protein status is an essential regulatory process for good health. Protein loss is a cause of substantial morbidity. Protein malnutrition increases mortality in chronic renal failure (1), AIDS (2), and chronic obstructive pulmonary disease (3). Endogenous glucocorticoid overproduction in Cushing's syndrome causes a marked reduction in lean body mass (LBM) (4–6) and leads to skin thinning and muscle wasting and weakness (7). Therapeutic use of glucocorticoids results in a similar but usually milder physical phenotype.

Insights into the regulation of whole-body protein metabolism have been made possible by the application of steady-state tracer methodology, such as the leucine turnover technique. This has allowed accurate and noninvasive estimation of whole-body rates of protein breakdown, oxidation, and synthesis, the key components of protein metabolism. High doses of glucocorticoids (*e.g.* prednisolone 40–60 mg/d) acutely induce protein catabolism by increasing protein breakdown and oxidation in healthy young adults (8–10). However, most patients receiving glucocorticoids for control of inflammatory or autoimmune disease are elderly and are treated with prednisolone doses of less than 10 mg/d (11, 12). Little is known about the effect of lower therapeutic doses of glucocorticoids on protein metabolism in the elderly.

The acute and chronic effects of a hormone on protein metabolism may differ. GH acutely reduces protein oxidation and increases protein mass in subjects with GH deficiency (13–15). However, during chronic GH treatment, protein oxidation returns toward baseline (16), a change that may reflect a metabolic adaptation that maintains protein mass at a new steady state (17). It is not known to what extent therapeutic doses of glucocorticoids perturb protein metabolism and whether this effect is sustained.

The aims were to assess whether 1) therapeutic glucocorticoid doses acutely induce protein catabolism and 2) acute changes in protein metabolism are maintained during chronic glucocorticoid use in elderly subjects. Two clinical studies were undertaken to address these aims. First, healthy elderly subjects were studied before and after a short course of glucocorticoids. Second, protein metabolism in subjects on

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Abbreviations: BCM, Body cell mass; BMC, bone mineral content; BMI, body mass index; CV, coefficient of variation; DHEAS, dehydroepiandrosterone sulfate; ECW, extracellular water; FM, fat mass; HOMA-β, homeostasis model of insulin secretion; HOMA-R, homeostasis model of insulin resistance; 11β-HSD, 11β-hydroxysteroid dehydrogenase; IGFBP-3, IGF-binding protein-3; KIC, α-ketoisocaproic acid; LBM, lean body mass; leucine Ra, leucine appearance; LIP, leucine incorporation into protein; Lox, leucine oxidation; REE, resting energy expenditure.

long-term glucocorticoids was compared with healthy subjects matched for age and gender. The primary endpoint of both studies was whole-body protein oxidation, which reflects the balance between whole-body protein breakdown and synthesis.

Subjects and Methods

Subjects and study design

Acute effects of glucocorticoids (study 1). Ten healthy subjects (seven women), aged at least 55 yr (range 56–78 yr) were recruited from the general public. Subjects were excluded if they were receiving glucocorticoids and if they had diabetes mellitus, an active infection, congestive heart failure, hepatic or renal disease, or a malignancy. The study design consisted of an open-label two-dose sequential study of prednisolone 5 mg/d followed by 10 mg/d for 7 d each.

Chronic effects of glucocorticoids (study 2). This was a cross-sectional study comparing 12 subjects (11 women) with documented but inactive polymyalgia rheumatica treated with glucocorticoids for at least 12 months to 12 normal subjects (11 women) of similar age. Five normal subjects (four women) had also participated in study 1. Subjects with polymyalgia rheumatica had received a stable prednisone dose (2–10 mg/d) for at least 3 months and were in remission as defined by resolution of clinical symptoms and a normal erythrocyte sedimentation rate. Subjects were excluded if they had congestive heart failure, diabetes mellitus, hepatic or renal disease, or malignancy or if they were receiving immunosuppressive therapy other than prednisone. No subject was receiving medication known to influence 11β-hydroxysteroid dehydrogenase (11β-HSD) activity (18). The Research Ethics Committee of St. Vincent's Hospital (Sydney, Australia) approved both studies, and all subjects provided written informed consent.

Clinical protocol

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

Assessment of body composition. Fat mass (FM), LBM, and bone mineral content (BMC) were measured by dual-energy x-ray absorptiometry using a total body scanner (Lunar model DPX, software version 3.1; Lunar Corp., Madison, WI), which also quantified regional body composition of the upper and lower limb along with truncal and central abdominal fat. Truncal fat comprises fat in the chest, abdominal, and pelvic regions, whereas central abdominal fat is contained within a manually traced region bordered by the upper margin of the second and the lower margin of the fourth lumbar vertebral bodies and the outer margins of the ribs (19). At our institution, the coefficients of variation (CV) for FM and LBM are 2.9 and 1.4%, respectively (20).

Estimation of extracellular water (ECW). ECW was estimated using the bromide dilution technique. ECW was calculated from the change in serum bromide concentration 140 min after injection of a known amount of bromide using the formula of Miller *et al.* (21). The intraassay CV for measurement of serum bromide concentration at our institution is less than 4% (22). The mean day-to-day intrasubject CV for ECW, based on four subjects studied on two occasions, is 5.7% (22). ECW was subtracted from LBM to calculate body cell mass (BCM), the metabolically active component of LBM (20).

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 (23, 24). $NaH^{13}CO_3$ 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 (24). Blood and breath samples were collected before (−10 and 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 of recordings were averaged.

Calculation of whole-body protein turnover. Rates of whole-body protein turnover were estimated using the reciprocal pool method (25). Because leucine has two pathways of disposal, oxidation and reincorporation into protein, the principles of steady-state kinetics allow 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 leucine when calculating its rate of appearance, because plasma KIC more accurately reflects the intracellular environment (25, 26). 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% in our laboratory (24). LIP is derived as the difference between leucine Ra and Lox. The CV 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.

Indirect calorimetry. Resting energy expenditure (REE) and substrate oxidation rates were calculated using the equations of Ferrannini (27). The mean day-to-day intrasubject CV for REE at our Institute is about 4% (20, 28).

Estimation of insulin sensitivity. Homeostasis models of insulin resistance (HOMA-R) and secretion (HOMA-β) were calculated from measures of fasting glucose and insulin as described by Matthews *et al.* (29).

Estimation of physical activity. Physical activity was assessed using a modified Baecke questionnaire. The questionnaire is designed specifically for mature subjects, assessing participation in household chores and leisure activities (30). A higher score denotes greater physical activity.

Analytical methods

KIC was extracted from plasma as described by Nissen *et al.* (31). 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), with selective monitoring of ions 301 and 302 (32). CO_2 enrichment in breath was measured at St. Thomas' Hospital, London, UK, on a SIRA series II isotope ratio mass spectrometer (VG Isotech, Cheshire, UK).

Glucose measurements were performed immediately after the samples were drawn and were analyzed by an immobilized glucose oxidase method on a glucose analyzer (model 23AM; Yellow Springs Instrument Co., Yellow Springs, OH). Serum bromide concentration was measured by HPLC after removal of plasma proteins by centrifugal ultrafiltration (22). Dehydroepiandrosterone sulfate (DHEAS) was measured using a commercial assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). 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 were assigned a value of 0.4 μmol/liter. 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 (33). Plasma insulin (Linco Research, Inc., St. Charles, MO) and IGF-binding protein-3 (IGFBP-3) (Bioclone Australia Pty. Ltd., Marrickville, New South Wales, Australia) concentrations were measured by RIA using commercial kits. The interassay CV for insulin was 4.2% at 42 μU/ml. The interassay CV for IGFBP-3 was 7.3% at 0.65 μg/ml and 10.6% at 4.2 μg/ml (34).

Statistical analysis

Statistical analysis was undertaken using statistical software packages Statview 4.5 PPC (Abacus Concepts, Inc., Berkeley, CA) and SPSS

TABLE 1. Weight, ECW, glucose, serum hormone concentrations, and indirect calorimetry in 10 healthy elderly subjects at baseline, after prednisolone 5 mg/d for 7 d and 10 mg/d for an additional 7 d

	Baseline	Prednisolone 5 mg	Prednisolone 10 mg	*P* value
Weight (kg)	64.9 ± 4.2	64.8 ± 4.1	64.6 ± 4.1	0.28
ECW (liters)	13.2 ± 0.6	13.6 ± 0.8	14.1 ± 0.9	0.55
DHEAS (μmol/liter)	1.29 ± 0.22	0.88 ± 0.16[b]	0.77 ± 0.13[b]	<0.0001
IGF-I (nmol/liter)	19.0 ± 2.0	20.6 ± 2.3	22.3 ± 2.4[a]	0.005
IGFBP-3 (μg/ml)	2.2 ± 0.2	2.2 ± 0.1	2.1 ± 0.1	0.80
Glucose (mmol/liter)	4.5 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	0.62
Insulin (μU/ml)	10.9 ± 1.8	11.1 ± 1.5	11.2 ± 1.6	0.90
HOMA-R	2.24 ± 0.47	2.27 ± 0.33	2.29 ± 0.32	0.97
HOMA-β	224 ± 28	225 ± 28	226 ± 35	0.70
REE (kcal/d)	1245 ± 60	1283 ± 61	1275 ± 61	0.21
Fox (mg/min)	50.8 ± 5.9	41.9 ± 4.6	46.6 ± 2.9	0.17
CHOox (mg/min)	66.7 ± 10.6	89.6 ± 8.8	78.0 ± 10.4	0.10

Values represent mean ± SE, and *P* values represent a treatment effect of prednisolone calculated using ANOVA with repeated measures. CHOox, Carbohydrate oxidation; Fox, fat oxidation.
[a] $P < 0.05$ *vs.* baseline.
[b] $P < 0.01$ *vs.* baseline.

11.0 (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 to determine a glucocorticoid treatment effect. *Post hoc* paired *t* tests with a Bonferroni correction were then performed to determine whether there was a difference between the 5- and 10-mg/d prednisolone doses. In study 2, unpaired *t* tests were used to compare differences between glucocorticoid users and normal subjects. Data that were not normally distributed were log-transformed for analysis or analyzed nonparametrically. Simple and multiple regression analyses were performed to examine the relationship between variables. Correction of whole-body leucine turnover for differences in body composition was made using analysis of covariance, which is the recommended technique for correction of a biological variable with a nonzero intercept (35, 36).

Results

Acute effect of glucocorticoids (study 1)

Subjects. The 10 healthy subjects' mean age was 68.2 ± 2.8 yr, mean weight was 64.9 ± 4.2 kg, and body mass index (BMI) was 24.2 ± 1.2 kg/m^2. All subjects completed the study protocol and complied with prednisolone treatment (on the basis of returned pill bottles). No subject reported any adverse events related to prednisolone use.

Endocrine and metabolic parameters. Serum DHEAS fell ($P < 0.0001$) and IGF-I increased ($P = 0.005$) during prednisolone treatment (Table 1). Prednisolone treatment did not significantly change body weight, ECW, fasting glucose or insulin, HOMA estimates of insulin sensitivity and secretion, IGFBP-3, REE, or substrate oxidation (Table 1).

Whole-body leucine turnover. Prednisolone induced a significant increase in Lox ($P = 0.008$) and a reduction in LIP that approached statistical significance ($P = 0.08$) but did not affect leucine Ra (Table 2). Lox increased after prednisolone 5 mg/d ($P = 0.03$) and 10 mg/d ($P = 0.07$), although the change with the latter dose did not quite reach statistical significance. There was no significant difference between the changes in Lox induced by the two prednisolone doses. LIP fell slightly, but not significantly, with both doses (Table 2).

The data were also analyzed with Lox expressed as a percentage of leucine Ra (Lox/leucine Ra, percent Lox), reflecting the percentage of amino acids generated from protein breakdown undergoing oxidation. As shown in Fig. 1, percent Lox rose significantly with both the 5- and 10-mg/d prednisolone doses ($P < 0.005$). No difference between the effect of the 5- and 10-mg/d prednisolone doses on percent Lox was observed (Fig. 1). Because the effects of prednisolone on body fat and lean mass over the short duration of treatment are minimal, it is unlikely that the leucine metabolic changes were influenced secondarily by a change in body composition.

Chronic effects of glucocorticoids (study 2)

Subjects. There were no significant differences in age, weight, and BMI between subjects receiving long-term glucocorticoids and normal subjects (Table 3). Glucocorticoid users had a significantly lower physical activity score than normal subjects.

Endocrine and metabolic parameters (Table 3). DHEAS concentration was significantly lower in glucocorticoid users than in normal subjects. DHEAS was below the limit of detection

TABLE 2. Rates of leucine Ra, Lox, and LIP in 10 subjects at baseline and then after prednisolone 5 mg/d for 7 d and 10 mg/d for an additional 7 d

	Baseline	Prednisolone 5 mg	Prednisolone 10 mg	*P* value
Leucine Ra (μmol/min)	111.9 ± 8.3	109.3 ± 6.5	106.2 ± 7.5	0.44
Lox (μmol/min)	18.8 ± 1.2	22.5 ± 1.5[a]	21.5 ± 1.6[b]	0.008
LIP (μmol/min)	93.1 ± 7.3	86.8 ± 5.5	84.7 ± 6.2[b]	0.08

Values represent mean ± SE, and *P* values represent a treatment effect of prednisolone calculated using ANOVA with repeated measures.
[a] $P < 0.05$ *vs.* baseline.
[b] $P < 0.10$ *vs.* baseline.

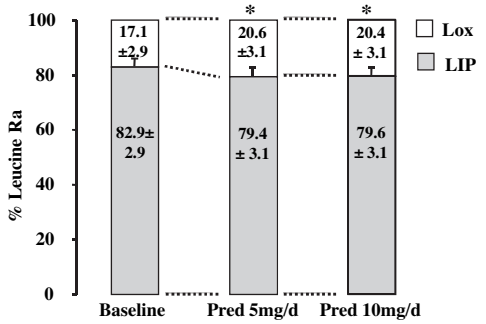

FIG. 1. Lox (*white bars*) and LIP (*gray bars*) expressed as a percentage of leucine Ra in 10 subjects at baseline and after prednisolone (Pred) 5 mg/d for 7 d and 10 mg/d for an additional 7 d. *Bars* represent mean ± SE. *, $P < 0.005$ *vs.* baseline.

(<0.4 μmol/liter) in nine of 12 glucocorticoid users compared with one of 12 normal subjects ($P = 0.0009$; $\chi^2 = 11.0$). There were no significant differences in IGF-I, IGFBP-3, fasting glucose, or glycosylated hemoglobin between the two groups. Fasting insulin tended to be higher in glucocorticoid users, although this did not reach statistical significance ($P = 0.08$). HOMA-R was significantly greater in glucocorticoid users than in normal subjects ($P = 0.04$). REE and substrate oxidation were not significantly different between glucocorticoid users and normal subjects.

Body composition (Table 4). In glucocorticoid users, percent FM was greater by 12%, LBM was lower by 8%, and BMC was lower by 11% than in normal subjects; however, these differences were not statistically significant. The mean ECW was not significantly different in the two groups, nor was there a difference in the derived BCM between the two groups. Percent truncal ($P = 0.009$) and central abdominal ($P = 0.018$) fat was about 25% greater in glucocorticoid users than in normal subjects, whereas limb fat was not significantly different (data not shown).

Whole-body leucine turnover. Because LBM (23, 24, 37) and FM (23, 38) both influence rates of whole-body protein metabolism, their relationship with leucine turnover was first assessed in simple regression analyses. Whole-body leucine turnover was subsequently adjusted for significant covariates to ensure differences in body composition between the groups did not influence results. In an analysis of the two groups combined, LBM was significantly and positively correlated with leucine Ra ($r^2 = 0.25$; $P = 0.014$), Lox ($r^2 = 0.33$; $P = 0.004$), and LIP ($r^2 = 0.17$; $P = 0.047$). The correlations between leucine turnover and BCM were similar to LBM (data not shown). FM was positively correlated with leucine Ra ($r^2 = 0.17$; $P = 0.04$) and LIP ($r^2 = 0.23$; $P = 0.02$) but not Lox ($r^2 = 0.001$; $P = 0.90$). Physical activity score was not significantly correlated with indices of leucine turnover (data not shown); therefore, data were not corrected for its influence.

A multiple regression analysis was performed to ascertain the independent effects of LBM, FM, and glucocorticoid use (defined as present or absent) on whole-body leucine turnover (Table 5). LBM was an independent determinant of all three indices of leucine turnover. FM was an independent determinant of leucine Ra and LIP but not Lox. Glucocorticoid use was not an independent determinant of any index of whole-body leucine turnover. Rates of whole-body leucine turnover in glucocorticoid users and normal subjects were then calculated unadjusted and after correction for LBM alone and LBM and FM (Table 6). In all analyses, there were no significant differences in leucine Ra, Lox, or LIP between glucocorticoid users and normal subjects, supporting the findings from multiple regression analysis.

Discussion

These longitudinal and cross-sectional studies provide strong evidence that the acute and chronic effects of similar doses of glucocorticoids on protein metabolism differ. In therapeutic doses, acute glucocorticoids significantly increased Lox and reduced LIP to a level that approached statistical significance. After chronic administration of similar doses, none of the indices of leucine metabolism were significantly different from age- and gender-matched controls in a careful analysis that accounted for the confounding effects of body composition differences. Therefore, the data

TABLE 3. Subject characteristics, hormone levels, and insulin sensitivity of 12 normal subjects and 12 subjects receiving long-term glucocorticoids for polymyalgia rheumatica (glucocorticoid users)

	Normal	Glucocorticoid users	*P* value
Gender distribution (female/male)	11/1	11/1	
Prednisone (mg)	0	5.0 ± 0.8	
Age (yr)	69.0 ± 1.8	73.3 ± 2.1	0.13
Weight (kg)	63.8 ± 1.8	69.2 ± 3.6	0.19
BMI (kg/m^2)	25.0 ± 0.8	27.5 ± 1.3	0.11
Physical activity score	19.3 ± 2.4	10.3 ± 1.9	0.008
DHEAS (μmol/liter)	1.36 ± 0.24	0.49 ± 0.07	0.0009
IGF-I (nmol/liter)	11.7 ± 1.1	13.3 ± 1.4	0.39
IGFBP-3 (μg/ml)	2.40 ± 0.19	2.44 ± 0.19	0.86
Glucose (mmol/liter)	4.6 ± 0.2	5.0 ± 0.2	0.12
HbA1c (%)	5.8 ± 0.1	5.9 ± 0.1	0.92
Insulin (μU/ml)	13.4 ± 1.1	18.0 ± 2.5	0.08
HOMA-R	2.8 ± 0.3	4.1 ± 0.7	0.04
REE (kcal/d)	1375 ± 102	1376 ± 160	0.98
Fox (mg/min)	75.6 ± 4.1	75.3 ± 5.7	0.97
CHOox (mg/min)	29.1 ± 11.4	30.0 ± 12.8	0.95

Values represent mean ± SE. CHOox, Carbohydrate oxidation; Fox, fat oxidation; HbA1c, glycosylated hemoglobin.

TABLE 4. Whole- and regional-body composition in 12 normal subjects and 12 subjects receiving long-term glucocorticoids for polymyalgia rheumatica (glucocorticoid users)

	Normal	Glucocorticoid users	*P* value
FM (%)	40.2 ± 2.5	45.0 ± 2.5	0.19
BMC (%)	3.5 ± 0.2	3.1 ± 0.1	0.11
LBM (%)	56.1 ± 2.2	51.7 ± 2.4	0.20
ECW (%)	16.4 ± 0.8	15.6 ± 0.9	0.48
BCM (%)	39.7 ± 1.8	36.1 ± 2.5	0.26
Truncal fat (%)	19.0 ± 4.5	24.2 ± 4.5	0.009
Central fat (%)	2.6 ± 0.6	3.2 ± 0.6	0.018

Results are expressed as a percentage of total body weight and represent mean ± SE.

suggest that protein oxidation is acutely increased but returns to normal during long-term treatment.

The findings of study 1 suggest that glucocorticoids in therapeutic doses acutely induce protein catabolism by increasing whole-body protein oxidation. Protein oxidation represents the difference between protein breakdown and synthesis that is irreversibly lost from the body. A prednisolone dose of 5 mg/d, about 1.5 times physiological glucocorticoid production, significantly increased protein oxidation by 20%. The failure to find a greater change in protein oxidation with the prednisolone 10-mg/d dose could reflect the sequential study design, with an acute increase in protein oxidation attenuated during the second week of treatment. Previous studies using prednisolone doses of about 40–60 mg/d have reported glucocorticoids acutely increase protein oxidation by 55–110% (8–10, 39), suggesting there is a dose response to glucocorticoid. One recent study reported that a prednisone dose of 0.5 mg/kg/d (35 mg/d for a 70-kg man) for 6 d did not significantly alter whole-body protein turnover or skeletal muscle fractional synthetic rate and amino acid kinetics in the lower limb (40). The authors concluded that moderate glucocorticoid doses were not catabolic (40). These differing findings may reflect the different investigative techniques used, because Short *et al.* (40) did not quantify whole-body protein oxidation, which was increased in our study. The contrasting interpretations of the two studies may reflect that protein oxidation is a more sensitive measure of a small imbalance between protein breakdown and synthesis or that glucocorticoids exert a greater effect on nonskeletal muscle sources of protein (*e.g.* skin or the splanchnic bed).

A major finding of this study is that the acute and chronic effects of similar doses of glucocorticoids are different. Although the paired longitudinal data have greater statistical power than the unpaired cross-sectional data, the finding that Lox is not significantly increased in chronic glucocorticoid users is unlikely to represent a type II error, because the rate of Lox was, if anything, lower than in normal subjects (Table 6). Furthermore, the groups were well matched for age and gender distribution, and differences in conversion of prednisone to prednisolone by 11β-HSD are unlikely to be responsible for this finding, because subjects were not receiving medication known to influence 11β-HSD activity. Based on the observations in study 1, a sample size of 12 subjects per group in study 2 would have a power of 90% to detect a 20% greater Lox in glucocorticoid users at the 0.05 significance level. Therefore, the findings suggest that stimulation of protein oxidation by therapeutic glucocorticoids wanes over time, so that the rate of protein oxidation returns to baseline. Normalization of protein oxidation may represent an adaptive or corrective metabolic mechanism to limit protein loss and achieve a new steady state of protein mass. A similar phenomenon has been observed for GH treatment, in that the acute change in protein oxidation is no longer present after chronic treatment (16).

TABLE 5. Multiple regression analysis assessing the independent effects of LBM, FM, and glucocorticoid use on rates of leucine Ra, Lox, and LIP in 12 normal subjects and 12 subjects on long-term glucocorticoids

	P values		
	Leucine Ra	Lox	LIP
LBM (kg)	0.001	0.004	0.005
FM (kg)	0.008	0.36	0.006
Glucocorticoid use	0.47	0.48	0.29

The finding of a normal rate of protein oxidation in chronic glucocorticoid users stands in contrast to our report in Cushing's syndrome, a pathological state of chronic glucocorticoid excess where protein oxidation was persistently increased (23). The likely explanation for this difference is the degree of glucocorticoid excess. In subjects with Cushing's syndrome, the mean 24-h urinary free cortisol excretion was about 10 times physiological glucocorticoid production (23), whereas a prednisone dose of 5 mg/d is about 1.5 times physiological levels. The magnitude of increase in Lox of 55–110% induced acutely by high-dose glucocorticoids (8–10, 39) is greater than in Cushing's syndrome where mean Lox was increased by only 20% (18). Some adaptation probably occurs during chronic high-dose glucocorticoid excess in Cushing's syndrome but is not sufficient to return Lox to normal. Therefore, the difference in the rates of Lox between Cushing's syndrome and therapeutic glucocorticoid use is likely to reflect incomplete metabolic compensation to the greater glucocorticoid effect in the former.

Cushing's syndrome results in unequivocal changes in body composition with greater total and truncal adiposity and reduced LBM (5, 6, 41). In glucocorticoid users, a similar pattern of body composition change was found; however, only truncal and central abdominal fat were significantly different from normal subjects (Table 4). Measurement of visceral fat was not undertaken in this study but would provide further insight into the effect of low-dose glucocorticoids on regional fat accumulation. Changes in abdominal adiposity may have contributed to increased insulin resistance as measured by HOMA in subjects on chronic glucocorticoid therapy. Previous studies reported subjects receiving long-term therapeutic doses of glucocorticoids for giant cell arteritis (42) and congenital adrenal hyperplasia (43, 44) had greater truncal and total adiposity but no significant difference in LBM. Body composition was not the primary endpoint in the present study and was quantified to ensure that differences did not confound estimates of leucine turnover. Because the sample size of the two groups was relatively small, the negative results could represent a type II error. However, the finding of a normal rate of protein oxidation in chronic glucocorticoid users provides a meta-

TABLE 6. Rates of leucine Ra, Lox, and LIP in 12 normal subjects and 12 subjects on long-term glucocorticoids unadjusted and corrected for LBM alone and LBM and FM by analysis of covariance

	Leucine Ra (μmol/min)		Lox (μmol/min)		LIP (μmol/min)	
	Normal	Glucocorticoid users	Normal	Glucocorticoid users	Normal	Glucocorticoid users
Unadjusted	100.9 ± 2.5	110.4 ± 7.1	19.0 ± 1.0	18.2 ± 1.7	81.9 ± 2.0	92.2 ± 6.0
Adjusted for LBM	100.7 ± 4.7	110.6 ± 4.7	19.0 ± 1.2	18.2 ± 1.2	81.8 ± 4.1	92.4 ± 4.1
Adjusted for LBM and FM	103.5 ± 4.1	107.8 ± 4.1	19.2 ± 1.2	18.0 ± 1.2	84.3 ± 3.6	89.9 ± 3.6

Values represent mean ± SE. All P values for comparisons between the groups were > 0.05.

bolic mechanism that may explain the lack of significant reduction in LBM.

Several limitations should be considered when interpreting the results of this study. Because subjects were studied only during the fasting state, the effect of glucocorticoids in the nonfasted state has not been assessed. However, previous studies have reported that glucocorticoids exert a qualitatively and quantitatively similar acute effect on fasting and postprandial protein oxidation (8, 9). Another area unexplored by this study is the effect of glucocorticoids on protein metabolism in skeletal muscle. Rates of whole-body protein turnover represent the net effect of protein turnover in all tissues and may not be representative of skeletal muscle. The acute (40, 45, 46) and chronic (47) effects of glucocorticoids in skeletal muscle have been reported to differ from those found at the whole-body level. Another issue that is not clear is whether changes in protein metabolism are, in part, influenced by changes in other hormones, such as DHEAS and IGF-I. A recent study reported that DHEA replacement in hypoadrenal women did not alter whole-body or skeletal muscle protein metabolism (48). It is uncertain whether a reduction in DHEAS of 30–40% or increase in IGF-I of less than 20% would have a significant effect on protein metabolism. Finally, for clinical reasons, we were unable to withdraw prednisone in chronic glucocorticoid users to ascertain whether this would alter protein metabolism.

In summary, therapeutic glucocorticoids acutely induce protein catabolism by increasing irreversible oxidative loss of amino acids. However, during chronic glucocorticoid use, the rate of protein oxidation is not significantly different from normal. Normalization of the rate of oxidative loss of protein may represent a metabolic adaptation that protects against ongoing loss of body protein and provides an explanation for the lack of a significant reduction in LBM in glucocorticoid users. A proposed model demonstrating time-dependent changes in protein metabolism during glucocorticoid therapy is depicted in Fig. 2. Glucocorticoids acutely increase protein oxidation, leading to a reduction in protein mass. However, during chronic glucocorticoid use, the rate of protein oxidation returns to baseline; therefore, protein loss is not ongoing, and a new steady state of protein mass is achieved. Finally, the time-dependent changes in the dynamics of protein metabolism indicate that the timing of study after intervention is critical to the interpretation of results.

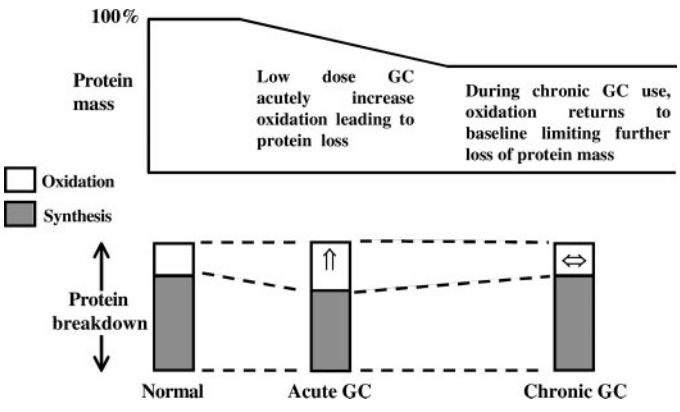

FIG. 2. Proposed model demonstrating the effect of therapeutic glucocorticoids (GCs) on protein metabolism.

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