Am J Physiol Endocrinol Metab 292: E1426–E1432, 2007.
First published January 23, 2007; doi:10.1152/ajpendo.00524.2006.

Protein metabolism in glucocorticoid excess: study in Cushing's syndrome and the effect of treatment

Morton G. Burt,[1,2,3] James Gibney,[1] and Ken K. Y. Ho[1,2,3]

[1]*Pituitary Research Unit, Garvan Institute of Medical Research;* [2]*Department of Endocrinology, St. Vincent's Hospital; and* [3]*University of New South Wales, Sydney, New South Wales, Australia*

Submitted 26 September 2006; accepted in final form 19 January 2007

Burt MG, Gibney J, Ho KK. Protein metabolism in glucocorticoid excess: study in Cushing's syndrome and the effect of treatment. *Am J Physiol Endocrinol Metab* 292: E1426–E1432, 2007. First published January 23, 2007; doi:10.1152/ajpendo.00524.2006.—How protein metabolism is perturbed during chronic glucocorticoid excess is poorly understood. The aims were to investigate the impact of chronic glucocorticoid excess and restoration of eucortisolemia in Cushing's syndrome (CS) on whole body protein metabolism. Eighteen subjects with CS and 18 normal subjects (NS) underwent assessment of body composition using DEXA and whole body protein turnover with a 3-h constant infusion of L-[^{13}C]leucine, allowing calculation of rates of leucine appearance (leucine R_a), leucine oxidation (L_{ox}), and leucine incorporation into protein (LIP). Ten subjects with CS were restudied after restoration of eucortisolemia. Percentage FM was greater (43.9 ± 1.6 vs. 33.8 ± 2.4%, $P = 0.002$) and LBM lower (52.7 ± 1.6 vs. 62.1 ± 2.3%, $P = 0.002$) in CS. LBM was significantly correlated ($r^2 > 0.44$, $P < 0.005$) to leucine R_a, L_{ox}, and LIP in both groups. After correcting for LBM, leucine R_a (133 ± 5 vs. 116 ± 5 μmol/min, $P = 0.02$) and L_{ox} (29 ± 1 vs. 24 ± 1 μmol/min, $P = 0.01$) were greater in CS. FM significantly correlated ($r^2 = 0.23$, $P < 0.05$) with leucine R_a and LIP, but not L_{ox} in CS. In multiple regression, LBM was an independent determinant of all three indexes of leucine turnover, FM of leucine R_a, and LIP and CS of L_{ox}. Following restoration of eucortisolemia, L_{ox} was reduced (Δ−7.5 ± 2.6 μmol/min, $P = 0.02$) and LIP increased (Δ+15.2 ± 6.2 μmol/min, $P = 0.04$). In summary, whole body protein metabolism in CS is influenced by changes in body composition and glucocorticoid excess per se, which increases protein oxidation. Enhanced protein oxidation is a likely explanation for the reduced protein mass in CS. Successful treatment of CS reduces protein oxidation and increases protein synthesis to prevent ongoing protein loss.

whole body leucine turnover; body composition; resting energy expenditure

MAINTENANCE OF OPTIMAL BODY PROTEIN STATUS is an essential regulatory process for health. Protein loss causes substantial morbidity and increases mortality in chronic renal failure (31), acquired immunodeficiency syndrome (21), and chronic obstructive pulmonary disease (26). Protein mass is reduced in Cushing's syndrome (3, 9, 49), resulting in skin thinning, muscle wasting, and weakness (36). Protein wasting may contribute directly or indirectly to the markedly increased mortality in untreated Cushing's syndrome (34).

Body protein is constantly turned over, a process regulated by genetic, nutritional, behavioral, environmental, and hormonal factors. Steady-state tracer methodology, such as the leucine turnover technique, has allowed accurate and noninvasive estimation of whole body rates of the three main components of protein metabolism: breakdown, oxidation, and synthesis. Protein mass is lost when the rate of protein breakdown is increased relative to synthesis or conversely if synthesis is reduced relative to breakdown. Both scenarios result in an increase in irreversible loss of amino acids by oxidation.

The acute impact of pharmacological doses of glucocorticoids on whole body protein metabolism have been well characterized; they increase protein breakdown relative to synthesis, thereby increasing protein oxidation (1, 10, 17). The impact of chronic glucocorticoid exposure on protein metabolism is controversial. This has been explored in Cushing's syndrome, where protein breakdown and synthesis have been reported to be proportionately reduced (2) or not different from those in normal subjects (44). In both studies the rate of protein oxidation was similar to normal subjects (2, 44), an observation that is not consistent with the progressive protein loss that occurs in this condition. Cushing's syndrome is associated with severe perturbations of body composition (3, 9, 49). Both lean body mass (LBM) (16, 51) and fat mass (FM) (46) influence rates of whole body protein metabolism. The above studies did not take into account the confounding effects of abnormal body composition (2, 44), and thus the status of protein metabolism in Cushing's syndrome remains to be clarified.

The aim of this study was to assess the impact of chronic glucocorticoid excess and restoration of eucortisolemia on whole body protein metabolism after accounting for body composition differences in Cushing's syndrome. With this in mind, we have undertaken two studies. First, a cross-sectional study compared whole body protein metabolism in subjects with Cushing's syndrome with normal subjects matched for sex, age, and weight, and second, a longitudinal study assessed the impact of restoring eucortisolemia in subjects with Cushing's syndrome.

MATERIALS AND METHODS

Subjects

Eighteen subjects (12 women) with active Cushing's syndrome were recruited through St. Vincent's Hospital, Department of Endocrinology, and 18 normal subjects (11 women) were recruited from the general population. The clinical characteristics of the group of subjects with Cushing's syndrome participating in the cross-sectional study have been reported previously (3). The cause of Cushing's syndrome was an ACTH-producing pituitary tumor in 15 subjects, bilateral micronodular adrenal hyperplasia in one subject, an adrenal

Address for reprint requests and other correspondence: K. K. Y. Ho, Pituitary Research Unit, Garvan Institute of Medical Research, 384 Victoria St., Darlinghurst NSW 2010, Australia (e-mail: k.ho@garvan.unsw.edu.au).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

adenoma in one subject, and ectopic ACTH secretion from a thymic carcinoid tumor in one subject. All subjects with Cushing's syndrome had active disease as defined by an elevated 24-h urinary free cortisol (mean = 1,408 ± 333 nmol/day, normal <300 nmol/day). The mean duration of symptoms consistent with Cushing's syndrome was 42 ± 9 mo. In 12 subjects Cushing's syndrome was newly diagnosed, and these subjects were assessed prior to replacement of other pituitary hormone deficits (which were present in only 2 subjects). Central hypothyroidism was present in one subject, and testosterone was below the lower limit of normal in one male subject. In six subjects (4 women), Cushing's disease was persistent following transsphenoidal surgery, with one subject also previously receiving radiotherapy. These subjects were receiving replacement for other pituitary hormone deficits as appropriate, including thyroxine replacement ($n = 3$), with one male subject receiving testosterone replacement.

Ten subjects with Cushing's syndrome were restudied on a second occasion, 13.6 ± 2.4 mo after their initial study, following successful treatment of Cushing's syndrome. Treatment consisted of a transsphenoidal hypophysectomy ($n = 6$), bilateral adrenalectomy ($n = 1$), stereotactic pituitary radiotherapy ($n = 2$), and a transsphenoidal hypophysectomy followed by bilateral adrenalectomy ($n = 1$). Five subjects were on no glucocorticoid replacement with a normal 24-h urinary free cortisol, and five subjects were receiving physiological glucocorticoid replacement. Postoperatively, two subjects developed central hypothyroidism. Both received physiological replacement therapy (thyroxine 100 μg/day) for ≥6 mo before the second study. The Research Ethics Committee of St. Vincent's Hospital (Sydney, Australia) approved both studies, and all subjects provided written informed consent.

Experimental Protocol

Subjects attended the Clinical Research Facility, Garvan Institute of Medical Research, at 0830 after an overnight fast, where they underwent assessment of whole body protein metabolism and body composition.

Body composition. Body composition was assessed by dual-energy X-ray absorptiometry (DEXA) using a three-compartment model. FM, LBM, and bone mineral content (BMC) were measured using a total body scanner (Lunar model DPX, software version 3.1; Lunar, Madison, WI) that also quantified regional body composition of upper and lower limb along with truncal fat. Truncal fat comprises fat in the chest, abdominal, and pelvic regions, as previously described (4). At our institution the coefficients of variation (CVs) for FM and LBM are 2.9 and 1.4%, respectively (30).

Whole body protein turnover. Whole body protein turnover was assessed using a primed constant infusion of L-[^{13}C]leucine, as previously described (11, 16, 53). $NaH^{13}CO_3$ and 99% L-[^{13}C]leucine were obtained from Cambridge Isotope Laboratories (Woburn, MA). Solutions were prepared under sterile conditions using 0.9% saline.

Following an overnight fast, a 0.1 mg/kg priming dose of $NaH^{13}CO_3$ was immediately followed by a 3-h primed constant infusion of L-[^{13}C]leucine (prime, 0.5 mg/kg; infusion, 0.5 $mg \cdot kg^{-1} \cdot h^{-1}$), based on previous studies (16, 53) demonstrating that steady state was achieved during this time period. 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. Total CO_2 production rates were measured with an open-circuit ventilated hood system (Deltatrac metabolic monitor; Datex Instrumentation, Helsinki, Finland) that was calibrated against standard gases before each study. Measurements of CO_2 production were collected during two 20-min periods and averaged.

Calculation of whole body protein turnover. Rates of whole body protein turnover were calculated using the reciprocal pool method, as previously described (37), allowing calculation of rates of leucine appearance (leucine R_a; an index of protein breakdown), leucine oxidation (L_{ox}; an index of oxidative loss of protein), and leucine incorporation into protein (LIP; an index of protein synthesis). The method is based on the principle of steady-state kinetics in which the rate of appearance of substrate equals its rate of disposal. For leucine there are two pathways of disposal: oxidation and reincorporation into protein. In the reciprocal pool method, α-ketoisocaproic acid (KIC), formed when leucine undergoes transamination, is used as a surrogate marker of true intracellular leucine enrichment as intracellular and serum levels rapidly equilibrate (37). Because leucine represents 8% of total body protein, or 590 μmol leucine represents 1 g of protein, rates of protein turnover may be estimated using these constants (27). The CVs for leucine R_a, L_{ox}, and LIP at our institution, based on seven subjects studied on two occasions, are 3.5, 6.1, and 3.5%, respectively.

Indirect calorimetry. O_2 consumption and CO_2 production were measured with the Deltatrac metabolic monitor, as described above. Resting energy expenditure (REE) and substrate oxidation rates were calculated using the equations of Ferrannini (8). At our institution, the mean day-to-day intrasubject CV for REE is ~4% (14, 29) and fat oxidation ~15% (unpublished data).

Analytical methods. KIC was extracted from plasma as described by Nissen et al. (28). KIC enrichment was measured as the butyldimethylsilyl derivative by gas chromatography (model 5890; Hewlett-Packard, Palo Alto, CA)-mass spectrometry (MSD 5972A; Hewlett-Packard), with selective monitoring of ions 301 and 302 (38). 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).

Statistical analysis. Statistical analysis was undertaken using statistical software packages Statview 4.5 PPC (Abacus Concepts, Berkeley, CA) and SPSS 11.0 (SPSS, Chicago, IL). Results are expressed as means ± SE unless otherwise stated. Categorical variables were assessed using a chi-square test. Continuous variables were assessed using unpaired or paired *t*-tests as appropriate. When data were not normally distributed (changes in body composition with restoration of eucortisolemia), they were log transformed prior to statistical analysis; however, means are presented in the text nontransformed. Simple and multiple regression analyses were performed to examine the relationship between variables. Correction of whole body leucine turnover for the impact of differences in body composition was undertaken by analysis of covariance, rather than simple division by LBM, since this avoids any statistical bias arising from differences in body composition between the groups (35, 40, 42).

RESULTS

Cross-Sectional Study

Subject characteristics. There were no significant differences in sex distribution, age, weight, and body mass index between the groups of normal subjects and subjects with Cushing's syndrome (Table 1).

Body composition. A detailed description of whole and regional body composition in the two groups has previously been reported (3). Percentage FM was significantly greater ($P = 0.002$) and LBM ($P = 0.002$) and BMC ($P = 0.002$) lower in subjects with Cushing's syndrome (Table 1). Truncal fat ($P = 0.0002$) was significantly greater and lean arm and leg mass significantly lower ($P < 0.0005$ for both) in subjects with Cushing's syndrome (Table 1).

Whole body leucine turnover. LBM was positively correlated with all three indexes of whole body leucine turnover in normal subjects and subjects with Cushing's syndrome (Table 2). The correlation between leucine turnover and LBM was stronger in normal subjects than in subjects with Cushing's syndrome (Table 2). After correction for LBM, leucine R_a and L_{ox} were

Table 1. *Subject characteristics and whole and regional body composition of 18 normal subjects and 18 subjects with Cushing's syndrome*

	Normal Subjects	Cushing's Syndrome	*P* Value
Sex (F/M)	11/7	12/6	0.73
Age, yr	46.8±2.8	41.6±3.0	0.21
Weight, kg	72.4±4.2	74.5±3.6	0.70
BMI, kg/m^2	25.5±1.1	27.1±1.1	0.31
Fat mass, %*	33.8±2.4	43.9±1.6	0.002
Lean body mass, %*	62.1±2.3	52.7±1.6	0.002
BMC, %*	4.06±0.13	3.46±0.12	0.002
Truncal fat, %*	15.8±1.2	23.2±1.3	0.0002
Lean arm mass, %*	7.6±0.3	5.9±0.2	<0.0005
Lean leg mass, %*	22.1±0.9	17.5±0.6	<0.0005

Values represent means ± SE. F/M, females/males; BMI, body mass index; BMC, bone mineral content; *Expressed as %total body weight.

significantly greater in subjects with Cushing's syndrome than in normal subjects; LIP was also higher, but the difference did not reach statistical significance ($P = 0.06$; Table 3 and Fig. 1). If indexes of whole body leucine turnover were unadjusted (Table 3 and Fig. 1) or expressed per kilogram of body weight (data not shown), no significant differences in any of the indexes of leucine metabolism between the groups were apparent.

In subjects with Cushing's syndrome, FM was significantly positively correlated with leucine R_a and LIP, but not L_{ox} (Table 2). The correlations between FM and leucine turnover in normal subjects were not statistically significant (Table 2). When FM was included as a covariate with LBM, L_{ox} was significantly elevated in subjects with Cushing's syndrome, whereas leucine R_a and LIP were not significantly different (Table 3 and Fig. 1). Age and 24-h urinary free cortisol were not significantly correlated with indexes of whole body leucine turnover (Table 2).

A multiple regression analysis was performed to determine the independent effects of LBM, FM, and glucocorticoid excess (Cushing's syndrome defined as either present or absent) on whole body leucine turnover (Table 4). LBM was an independent determinant of all three indexes of whole body leucine turnover. FM was a significant independent determinant of leucine R_a and LIP, but not L_{ox}. Cushing's syndrome was an independent determinant of L_{ox}, but not leucine R_a or LIP.

Longitudinal Study

Subject characteristics. Ten subjects were restudied after successful treatment of Cushing's syndrome. Subjects had been eucortisolemic for an estimated 7.6 ± 1.3 mo. At the time of retesting, five subjects were on no glucocorticoid replacement with a mean 24-h urinary free cortisol of 139 ± 41 nmol/day. Five subjects were receiving physiological glucocorticoid replacement (prednisone 2.5–5 mg/day).

Body composition. Following successful treatment of Cushing's syndrome, mean weight fell by 4.2 ± 3.2 kg; however, the change was not statistically significant ($P = 0.23$). There were no significant changes in absolute FM or LBM (Table 5), although the fall in FM approached statistical significance ($P = 0.098$). Percent FM fell significantly, by 4.6 ± 2.0% ($P = 0.046$), and percent LBM increased by 4.4 ± 1.9% ($P = 0.045$) with successful treatment of Cushing's syndrome.

Whole body leucine turnover. Changes in leucine metabolism were analyzed after correction for changes in LBM. Following successful treatment of Cushing's syndrome, L_{ox} fell ($P = 0.02$) and LIP increased ($P = 0.04$) significantly, whereas leucine R_a did not change (Fig. 2). There were no significant differences in LBM-adjusted leucine R_a ($\Delta+9.3 \pm 6.8$ μmol/min, $P = 0.18$), L_{ox} ($\Delta-3.2 \pm 2.1$ μmol/min, $P = 0.13$), and LIP ($\Delta+12.5 \pm 6.6$ μmol/min, $P = 0.07$) between treated subjects with Cushing's syndrome and normal subjects.

Indirect calorimetry. There were no significant changes in REE (1,431 ± 79 vs. 1,385 ± 62 kcal/day, $P = 0.49$), fat oxidation (71.5 ± 10.5 vs. 79.7 ± 10.3 mg/min, $P = 0.57$), or carbohydrate oxidation (55.2 ± 22.0 vs. 31.2 ± 20.2 mg/min, $P = 0.41$) following successful treatment of Cushing's syndrome. Results were not significantly different after correction for the change in LBM (data not shown).

DISCUSSION

This is the first study assessing postabsorptive whole body protein metabolism in Cushing's syndrome that accounts for the confounding effects of differences in body composition and the first to assess LBM-adjusted changes in protein metabolism after successful treatment. Percentage FM was significantly greater and LBM and BMC lower in Cushing's syndrome. LBM significantly and positively influenced all indexes of leucine turnover in both groups. In the cross-sectional study, comparison of raw unadjusted data revealed no difference in any of the indexes of leucine turnover between subjects with Cushing's syndrome and normal subjects. After correction for the reduced LBM, L_{ox} was significantly higher in Cushing's, brought about by a greater increase in leucine R_a than LIP. FM significantly and positively influenced leucine R_a and LIP in subjects with Cushing's syndrome. After accounting for the difference of FM and LBM, patients with Cushing's syndrome displayed a significant increase in L_{ox} only. In the longitudinal study, restoration of eucortisolism resulted in a significant reduction in percentage FM and a reciprocal increase in percentage LBM. This was associated with a significant reduction in L_{ox} and an increase in LIP to a level indistinguishable from normal subjects.

This study shows that elucidation of the effect of glucocorticoid excess on postabsorptive whole body protein metab-

Table 2. *Simple regression analyses of correlation between leucine R_a, L_{ox}, and LIP and LBM, FM, age, and 24-h UFC in 18 normal subjects and 18 subjects with Cushing's syndrome*

	Normal Subjects			Cushing's Syndrome		
	Leucine R_a	L_{ox}	LIP	Leucine R_a	L_{ox}	LIP
LBM, kg	0.84†	0.69†	0.80†	0.53†	0.54†	0.44†
FM, kg	0.15	0.02	0.15	0.23*	0.11	0.23*
Age, yr	0.14	0.14	0.12	0.02	0.004	0.03
UFC, nmol/day	NA	NA	NA	0.03	0.03	0.03

Leucine R_a, rate of leucine appearance; L_{ox}, leucine oxidation; LIP, leucine incorporation into protein; LBM, lean body mass; FM, fat mass; UFC, urinary free cortisol; NA, not assessed. Figures denote r^2 values. *$P < 0.05$; †$P < 0.005$.

Table 3. *Rates of leucine R_a, L_{ox}, and LIP in 18 normal subjects and 18 subjects with Cushing's syndrome unadjusted and corrected for LBM alone and LBM and fat FM by analysis of covariance*

	Leucine R_a		L_{ox}		LIP	
	Normal	Cushing's	Normal	Cushing's	Normal	Cushing's
Unadjusted	125.6±7.8	123.8±8.0	25.6±1.8	26.8±2.1	99.6±6.4	97.0±6.4
LBM	116.2±4.7	133.2±4.7*	23.8±1.3	28.9±1.3*	92.4±4.1	104.2±4.1
LBM and FM	119.4±4.7	130.0±4.7	23.9±1.4	28.9±1.4*	95.5±4.1	101.1±4.1

Values represent means ± SE and are in μmol/min. *$P < 0.05$ vs. normal subjects.

olism in Cushing's syndrome requires distinction of the effects of body composition differences from the effects of glucocorticoid excess per se. Differences in LBM account for 60–80% of the explained variability of rates of all three indexes of leucine turnover (16, 51), and correction of whole body leucine turnover for the size of the LBM is now standard practice. Because LBM is markedly reduced in Cushing's syndrome, failure to correct for it in previous studies (2, 44) would have resulted in underestimation of all indexes of leucine metabolism. Had we failed to correct for LBM, we would have come to a similar erroneous conclusion, that L_{ox} is normal in Cushing's syndrome (Table 3).

Glucocorticoid excess increased postabsorptive L_{ox}, independent of differences in both LBM and FM, in Cushing's syndrome (Table 3 and 4). Because oxidation results in irreversible loss of amino acids, this finding is consistent with ongoing protein loss. Based on the constants described by Matthews et al. (27), a difference in L_{ox} of 5 μmol/min equates to a rate of protein loss of ~8.5 μg/min or ~4.5 kg/yr. However, this calculation was based solely on measures of protein metabolism in the postabsorptive state. Although acute glucocorticoids increase fasting and postprandial L_{ox} to a similar extent (1, 17), the effect of chronic glucocorticoid excess on postprandial protein metabolism is unclear.

In contrast with the significant increase in L_{ox} in Cushing's syndrome, REE, fat, and carbohydrate oxidation were not different from normal subjects (3), nor did these measures change following successful treatment. These results are consistent with most (13, 18, 39), but not all (6), studies showing that neither REE, fat, nor carbohydrate oxidation change following acute pharmacological glucocorticoid administration. However, if REE is not affected by glucocorticoid excess, an increase in protein oxidation must be associated with a reduction in the oxidation of another substrate. This is most likely to be oxidation of fat, which was lower in Cushing's syndrome by ~11 mg/min (3) and increased by ~8 mg/min following successful treatment, although the changes were not statistically significant. Carbohydrate oxidation is unlikely to be reduced, since this tended to be higher in Cushing's syndrome and fell with successful treatment. The day-to-day reproducibility in the assessment of fat oxidation by indirect calorimetry is relatively poor (47, 52) and is likely to have contributed to the failure to find a statistically significant change.

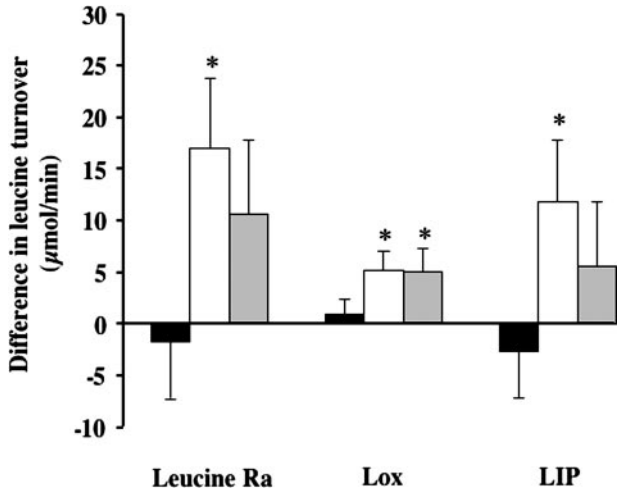

Fig. 1. Difference in rates of leucine appearance (leucine R_a), leucine oxidation (L_{ox}), and leucine incorporation into protein (LIP) between 18 subjects with Cushing's syndrome and 18 normal subjects presented unadjusted (black bars) and adjusted for lean body mass only (white bars) or lean body mass and fat mass (gray bars) using analysis of covariance. Bars represent means ± SE differences between the groups. *$P < 0.05$.

Contrary to a proposed direct effect of glucocorticoid excess on protein oxidation, postabsorptive leucine R_a and LIP were independently related to FM in subjects with Cushing's syndrome and not glucocorticoid excess. A similar relationship between adiposity and leucine R_a and LIP has previously been reported in healthy women (46), with most (5, 19, 20, 50), but not all (41), studies reporting that LBM-adjusted leucine R_a and LIP are increased in obesity. However, the mechanisms by which FM influences leucine turnover are unclear. Whereas adipose tissue accounts for 6–12% of whole-body proteolysis (7, 32), the contribution of adipose tissue to leucine R_a was similar in lean and markedly obese individuals (32). This suggests that the increased leucine R_a in obesity is not because of increased protein breakdown within adipose tissue but that adipose tissue may influence protein breakdown in lean tissue via an indirect mechanism. Speculation on the relationship between FM and leucine R_a has centered on the possibility that the increased leucine R_a in obesity may arise from reduced sensitivity to insulin. Insulin-induced inhibition of proteolysis is attenuated in obese (25) and insulin-resistant subjects (15, 45). However, no correlation was found between leucine R_a and insulin sensitivity measured by euglycemic hyperinsuline-

Table 4. *Multiple regression analysis assessing independent effects of LBM, FM, and GC excess on rates of leucine R_a, L_{ox}, and LIP in 18 subjects with Cushing's syndrome and 18 normal subjects*

	Leucine R_a	L_{ox}	LIP
LBM, kg	<0.0001	<0.0001	<0.0001
FM, kg	0.04	0.93	0.02
GC excess	0.15	0.027	0.38

GC, glucocorticoid. Figures denote *P* values.

mic clamp (46). Thus, the basis of the relationship between FM and protein breakdown is yet to be clearly defined.

Following treatment of Cushing's syndrome there was a significant fall in percentage FM and an increase in percentage, but not absolute, LBM. Previous studies (23, 33, 48) have reported that, although successful control of glucocorticoid excess in Cushing's syndrome reduces FM, LBM remains subnormal and does not significantly increase. We found that postabsorptive L_{ox} fell and LIP increased after successful treatment, whereas leucine R_a was not significantly affected. Therefore, a greater proportion of amino acids generated from protein breakdown are reincorporated back into protein rather than oxidized. However, L_{ox} was reduced to the level of normal subjects, not significantly below it, which is necessary for protein anabolism. It is possible that we missed a transient reduction in L_{ox} below levels found in normal subjects, because subjects were studied more than 6 mo on average after successful treatment. However, a normal rate of protein oxidation would predict a stable, but not increasing, protein mass.

A limitation of the whole body leucine turnover technique is that the results represent the net effect in all tissues and do not provide information on regional contribution to protein turnover. There is major interest in the impact of glucocorticoid excess on skeletal muscle protein metabolism, since limb lean tissue is preferentially lost in Cushing's syndrome (3, 9, 49). Although skeletal muscle represents the largest mass of body protein, it accounts for only 30–50% of whole body protein breakdown, oxidation, and synthesis (43). Therefore, changes in whole body protein metabolism may not reflect that in skeletal muscle. In contrast to the acute glucocorticoid-induced increase in whole body protein breakdown and oxidation, forearm and lower limb studies (22, 24, 39) have not observed any significant change in skeletal muscle protein metabolism following acute glucocorticoid administration in the postabsorptive state. However, one study (12) reported a reduction in skeletal muscle fractional synthesis rate in subjects on long-term glucocorticoids. The discrepancy between whole body and regional studies may reflect a greater effect of glucocorticoids on nonskeletal protein sources, such as the splanchnic bed, or the sensitivity or end points of the method under conditions of the study. For example, regional studies based on arteriovenous differences in amino acid enrichment do not directly quantify amino acid oxidation, which was increased in the whole body study.

Despite our efforts to correct data for body compositional change, it is not possible to account for all variables that could influence protein metabolism in a cross-sectional study. We (3) have previously reported that the mean extracellular water volume was not significantly different in a subset of the subjects from each group, and therefore, hydration of the LBM is unlikely to affect results. Furthermore, it is unlikely that deficiencies of other hormones significantly influenced results. Although subjects were not formally evaluated for growth hormone (GH) deficiency, protein oxidation in GH-deficient adults is not significantly different to normal subjects (16). The one subject with untreated androgen deficiency is unlikely to have markedly confounded results, and omission of this subject's data did not influence the findings. The paired longitudinal data, where restoration of eucortisolemia resulted in a reduction in L_{ox} with no change in leucine R_a, corroborate the cross-sectional data showing that the independent effect of glucocorticoid excess is to increase L_{ox}. Increased protein oxidation in Cushing's syndrome is likely to be directly related to glucocorticoid excess.

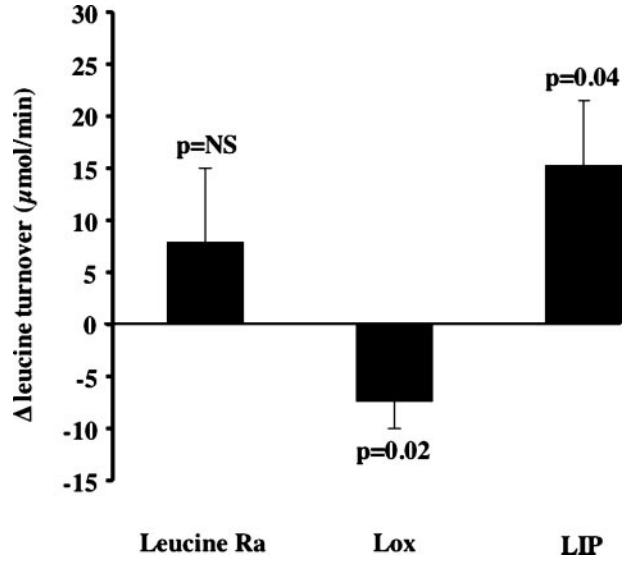

Fig. 2. Change in leucine R_a, L_{ox}, and LIP in 10 subjects with Cushing's syndrome after successful therapy. Leucine turnover is adjusted for the change in lean body mass using analysis of covariance. *P* values relate to the change in leucine turnover following therapy, and differences are expressed as means ± SE. NS, not significant.

Table 5. *Whole body composition in 10 subjects with Cushing's syndrome before and after restoration of eucortisolemia*

	Before Treatment	After Treatment	*P* Value
Fat mass, kg	32.1±3.3	27.2±2.1	0.098
Fat mass, %*	43.7±2.4	39.0±2.4	0.046
LBM, kg	38.2±2.7	39.9±2.6	0.14
LBM, %*	53.1±2.3	57.5±2.3	0.045
BMC, kg	2.3±0.1	2.4±0.1	0.34
BMC, %*	3.3±0.2	3.4±0.1	0.45

Values represent means ± SE. *Expressed as a %body weight.

In conclusion, we have shown that postabsorptive whole body protein metabolism in Cushing's syndrome is influenced both by changes in body composition and by glucocorticoid excess per se. By accounting for the changes in FM and LBM in Cushing's syndrome, we have shown that glucocorticoid excess causes a sustained but reversible stimulation of protein oxidation. This observation reconciles previous reports (2, 44) finding no perturbation in protein oxidation, which is at odds with clinical observations. Restoration of eucortisolemia results in a redistribution of amino acids from oxidative to synthetic pathways to prevent further protein loss.

ACKNOWLEDGMENTS

We thank the clinical research nurses and in particular Angela Peris and Margot Hewett for assistance with whole body leucine turnover studies, Dr. David Hoffman, Dr. Troels Wolthers, and Associate Prof. Gudmundur Johannsson for assistance with subject recruitment and whole body leucine turnover studies, Dr. Tuan Nguyen for statistical advice, Ann Poljak from the Bioanalytical Mass Spectrometry Facility, University of New South Wales, for assistance with gas chromatography-mass spectrometry, and Prof. Margot

Umpleby and Dr. Nicola Jackson from the Department of Diabetes and Endocrinology, Guy's, King's and St. Thomas' School of Medicine, St. Thomas' Hospital, London, UK, for assistance with measurement of CO_2 enrichment.

GRANTS

M. G. Burt is supported by a scholarship from the National Health and Medical Research Council of Australia.

REFERENCES

1. **Beaufrere B, Horber FF, Schwenk WF, Marsh HM, Matthews D, Gerich JE, Haymond MW.** Glucocorticosteroids increase leucine oxidation and impair leucine balance in humans. *Am J Physiol Endocrinol Metab* 257: E712–E721, 1989.
2. **Bowes SB, Benn JJ, Scobie IN, Umpleby AM, Lowy C, Sonksen PH.** Leucine metabolism in patients with Cushing's syndrome before and after successful treatment. *Clin Endocrinol (Oxf)* 39: 591–598, 1993.
3. **Burt MG, Gibney J, Ho KK.** Characterization of the metabolic phenotypes of Cushing's syndrome and growth hormone deficiency: a study of body composition and energy metabolism. *Clin Endocrinol (Oxf)* 64: 436–443, 2006.
4. **Carey DG, Jenkins AB, Campbell LV, Freund J, Chisholm DJ.** Abdominal fat and insulin resistance in normal and overweight women: direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes* 45: 633–638, 1996.
5. **Chevalier S, Marliss EB, Morais JA, Lamarche M, Gougeon R.** Whole-body protein anabolic response is resistant to the action of insulin in obese women. *Am J Clin Nutr* 82: 355–365, 2005.
6. **Chong PK, Jung RT, Scrimgeour CM, Rennie MJ.** The effect of pharmacological dosages of glucocorticoids on free living total energy expenditure in man. *Clin Endocrinol (Oxf)* 40: 577–581, 1994.
7. **Coppack SW, Persson M, Miles JM.** Phenylalanine kinetics in human adipose tissue. *J Clin Invest* 98: 692–697, 1996.
8. **Ferrannini E.** The theoretical bases of indirect calorimetry: a review. *Metabolism* 37: 287–301, 1988.
9. **Garrapa GG, Pantanetti P, Arnaldi G, Mantero F, Faloia E.** Body composition and metabolic features in women with adrenal incidentaloma or Cushing's syndrome. *J Clin Endocrinol Metab* 86: 5301–5306, 2001.
10. **Garrel DR, Moussali R, De Oliveira A, Lesiege D, Lariviere F.** RU 486 prevents the acute effects of cortisol on glucose and leucine metabolism. *J Clin Endocrinol Metab* 80: 379–385, 1995.
11. **Gibney J, Wolthers T, Johannsson G, Umpleby AM, Ho KK.** Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. *Am J Physiol Endocrinol Metab* 289: E266–E271, 2005.
12. **Gibson JN, Poyser NL, Morrison WL, Scrimgeour CM, Rennie MJ.** Muscle protein synthesis in patients with rheumatoid arthritis: effect of chronic corticosteroid therapy on prostaglandin F2 availability. *Eur J Clin Invest* 21: 406–412, 1991.
13. **Gravholt CH, Dall R, Christiansen JS, Moller N, Schmitz O.** Preferential stimulation of abdominal subcutaneous lipolysis after prednisolone exposure in humans. *Obes Res* 10: 774–781, 2002.
14. **Greenfield JR, Samaras K, Hayward CS, Chisholm DJ, Campbell LV.** Beneficial postprandial effect of a small amount of alcohol on diabetes and cardiovascular risk factors. *J Clin Endocrinol Metab* 90: 661–672, 2005.
15. **Halvatiotis P, Short KR, Bigelow M, Nair KS.** Synthesis rate of muscle proteins, muscle functions, and amino acid kinetics in type 2 diabetes. *Diabetes* 51: 2395–2404, 2002.
16. **Hoffman DM, Pallasser R, Duncan M, Nguyen TV, Ho KK.** How is whole body protein turnover perturbed in growth hormone-deficient adults? *J Clin Endocrinol Metab* 83: 4344–4349, 1998.
17. **Horber FF, Haymond MW.** Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J Clin Invest* 86: 265–272, 1990.
18. **Horber FF, Marsh HM, Haymond MW.** Differential effects of prednisone and growth hormone on fuel metabolism and insulin antagonism in humans. *Diabetes* 40: 141–149, 1991.
19. **Jensen MD, Haymond MW.** Protein metabolism in obesity: effects of body fat distribution and hyperinsulinaemia on leucine turnover. *Am J Clin Nutr* 53: 172–176, 1991.
20. **Kanaley JA, Haymond MW, Jensen MD.** Effects of exercise and weight loss on leucine turnover in different types of obesity. *Am J Physiol Endocrinol Metab* 264: E687–E692, 1993.
21. **Kotler DP, Tierney AR, Wang J, Pierson RN Jr.** Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. *Am J Clin Nutr* 50: 444–447, 1989.
22. **Liu Z, Jahn LA, Long W, Fryburg DA, Wei L, Barrett EJ.** Branched chain amino acids activate messenger ribonucleic acid translation regulatory proteins in human skeletal muscle, and glucocorticoids blunt this action. *J Clin Endocrinol Metab* 86: 2136–2143, 2001.
23. **Lonn L, Kvist H, Ernst I, Sjostrom L.** Changes in body composition and adipose tissue distribution after treatment of women with Cushing's syndrome. *Metabolism* 43: 1517–1522, 1994.
24. **Louard RJ, Bhushan R, Gelfand RA, Barrett EJ, Sherwin RS.** Glucocorticoids antagonize insulin's antiproteolytic action on skeletal muscle in humans. *J Clin Endocrinol Metab* 79: 278–284, 1994.
25. **Luzi L, Castellino P, DeFronzo RA.** Insulin and hyperaminoacidemia regulate by a different mechanism leucine turnover and oxidation in obesity. *Am J Physiol Endocrinol Metab* 270: E273–E281, 1996.
26. **Marquis K, Debigare R, Lacasse Y, LeBlanc P, Jobin J, Carrier G, Maltais F.** Midthigh muscle cross-sectional area is a better predictor of mortality than body mass index in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 166: 809–813, 2002.
27. **Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM.** Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. *Am J Physiol Endocrinol Metab* 238: E473–E479, 1980.
28. **Nissen SL, Van Huysen C, Haymond MW.** Measurement of branched chain amino acids and branched chain alpha-ketoacids in plasma by high-performance liquid chromatography. *J Chromatogr* 232: 170–175, 1982.
29. **O'Sullivan AJ, Crampton LJ, Freund J, Ho KK.** The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. *J Clin Invest* 102: 1035–1040, 1998.
30. **O'Sullivan AJ, Kelly JJ, Hoffman DM, Freund J, Ho KK.** Body composition and energy expenditure in acromegaly. *J Clin Endocrinol Metab* 78: 381–386, 1994.
31. **Owen WF Jr, Lew NL, Liu Y, Lowrie EG, Lazarus JM.** The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *N Engl J Med* 329: 1001–1006, 1993.
32. **Patterson BW, Horowitz JF, Wu G, Watford M, Coppack SW, Klein S.** Regional muscle and adipose tissue amino acid metabolism in lean and obese women. *Am J Physiol Endocrinol Metab* 282: E931–E936, 2002.
33. **Pirlich M, Biering H, Gerl H, Ventz M, Schmidt B, Ertl S, Lochs H.** Loss of body cell mass in Cushing's syndrome: effect of treatment. *J Clin Endocrinol Metab* 87: 1078–1084, 2002.
34. **Plotz D, Knowlton AL, Ragan C.** The natural history of Cushing's disease. *Am J Med* 13: 597–614, 1952.
35. **Poehlman ET, Toth MJ.** Mathematical ratios lead to spurious conclusions regarding age- and sex-related differences in resting metabolic rate. *Am J Clin Nutr* 61: 482–485, 1995.
36. **Ross EJ, Linch DC.** Cushing's syndrome—killing disease: discriminatory value of signs and symptoms aiding early diagnosis. *Lancet* 2: 646–649, 1982.
37. **Schwenk WF, Beaufrère B, Haymond MW.** Use of reciprocal pool specific activities to model leucine metabolism in humans. *Am J Physiol Endocrinol Metab* 249: E646–E650, 1985.
38. **Schwenk WF, Berg PJ, Beaufrere B, Miles JM, Haymond MW.** Use of t-butyldimethylsilylation in the gas chromatographic/mass spectrometric analysis of physiologic compounds found in plasma using electron-impact ionization. *Anal Biochem* 141: 101–109, 1984.
39. **Short KR, Nygren J, Bigelow ML, Nair KS.** Effect of short-term prednisone use on blood flow, muscle protein metabolism, and function. *J Clin Endocrinol Metab* 89: 6198–6207, 2004.
40. **Short KR, Vittone JL, Bigelow ML, Proctor DN, Nair KS.** Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am J Physiol Endocrinol Metab* 286: E92–E101, 2004.
41. **Solini A, Bonora E, Bonadonna R, Castellino P, DeFronzo RA.** Protein metabolism in human obesity: relationship with glucose and lipid metabolism and with visceral adipose tissue. *J Clin Endocrinol Metab* 82: 2552–2558, 1997.
42. **Tanner JM.** Fallacy of per-weight and per-surface area standards, and their relation to spurious correlations. *J Appl Physiol* 2: 1–15, 1949.
43. **Tessari P, Garibotto G, Inchiostro S, Robaudo C, Saffioti S, Vettore M, Zanetti M, Russo R, Deferrari G.** Kidney, splanchnic, and leg protein turnover in humans. *J Clin Invest* 98: 1481–1492, 1996.

44. **Tessari P, Inchiostro S, Biolo G, Marescotti MC, Fantin G, Boscarato MT, Merola G, Mantero F, Tiengo A.** Leucine kinetics and the effects of hyperinsulinaemia in patients with Cushing's syndrome. *J Clin Endocrinol Metab* 68: 256–262, 1989.
45. **Tessari P, Nosadini R, Trevisan R, De Kreutzenberg SV, Inchiostro S, Duner E, Biolo G, Marescotti MC, Tiengo A, Crepaldi G.** Defective suppression by insulin of leucine-carbon appearance and oxidation in type 1, insulin-dependent diabetes mellitus. *J Clin Invest* 77: 1797–1804, 1986.
46. **Toth MJ, Tchernof A, Rosen CJ, Matthews DE, Poehlman ET.** Regulation of protein metabolism in middle-aged, premenopausal women: roles of adiposity and estradiol. *J Clin Endocrinol Metab* 85: 1382–1387, 2000.
47. **Toubro S, Christensen NJ, Astrup A.** Reproducibility of 24-h energy expenditure, substrate utilization and spontaneous physical activity in obesity measured in a respiration chamber. *Int J Obes* 19: 544–549, 1995.
48. **Ueland T, Kristo C, Godang K, Aukrust P, Bollerslev J.** Interleukin-1 receptor antagonist is associated with fat distribution in endogenous Cushing's syndrome: a longitudinal study. *J Clin Endocrinol Metab* 88: 1492–1496, 2003.
49. **Wajchenberg BL, Bosco A, Marone MM, Levin S, Rocha M, Lerario AC, Nery M, Goldman J, Liberman B.** Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. *J Clin Endocrinol Metab* 80: 2791–2794, 1995.
50. **Welle S, Barnard RR, Statt M, Amatruda JM.** Increased protein turnover in obese women. *Metabolism* 41: 1028–1034, 1992.
51. **Welle S, Nair KS.** Relationship of resting metabolic rate to body composition and protein turnover. *Am J Physiol Endocrinol Metab* 258: E990–E998, 1990.
52. **White MD, Bouchard G, Buemann B, Almeras N, Despres JP, Bouchard C, Tremblay A.** Reproducibility of 24-h energy expenditure and macronutrient oxidation rates in an indirect calorimeter. *J Appl Physiol* 80: 133–139, 1996.
53. **Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, Ho KK.** Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. *Am J Physiol Endocrinol Metab* 281: E1191–E1196, 2001.