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

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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) and how protein metabolism is influenced by changes in body composition and glucocorticoid per se. The acute impact of pharmacological doses of glucocorticoids on whole body protein metabolism was 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, 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

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adenoma in one subject, and ectopic ACTH secretion from a thymic carcinoid tumor in one subject. All subjects with Cushings 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 Cushings syndrome was 42 ± 9 mo. In 12 subjects Cushings 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), Cushings 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 Cushings syndrome were restudied on a second occasion, 13.6 ± 2.4 mo after their initial study, following successful treatment of Cushings 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. Vincents 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 arm 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-[13C]leucine, as previously described (11, 16, 53). NaH13CO3 and 99% L-[13C]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 NaH13CO3 was immediately followed by a 3-h primed constant infusion of L-[13C]leucine (prime, 0.5 mg/kg; infusion, 0.5 mg·kg−1·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 CO2 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 CO2 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 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). 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 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**. O2 consumption and CO2 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 butyl-dimethylsilyl 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). CO2 enrichment in breath was measured at St. Thomas’ Hospital, London, UK, on a SIRA Series II isotope ratio mass spectrometer (VG Isotec, 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 Cushings 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 Cushings 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 Cushings 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 Cushings syndrome (Table 2). The correlation between leucine turnover and LBM was stronger in normal subjects than in subjects with Cushings syndrome (Table 2). After correction for LBM, leucine Ra and Lox were...
Table 1. Subject characteristics and whole and regional body composition of 18 normal subjects and 18 subjects with Cushing’s syndrome

<table>
<thead>
<tr>
<th></th>
<th>Normal Subjects</th>
<th>Cushing’s Syndrome</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>11/7</td>
<td>12/6</td>
<td>0.73</td>
</tr>
<tr>
<td>Age, yr</td>
<td>46.8 ± 2.8</td>
<td>41.6 ± 3.0</td>
<td>0.21</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72.4 ± 4.2</td>
<td>74.5 ± 3.6</td>
<td>0.70</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.5 ± 1.1</td>
<td>27.1 ± 1.1</td>
<td>0.31</td>
</tr>
<tr>
<td>Fat mass, %*</td>
<td>33.8 ± 2.4</td>
<td>43.9 ± 1.6</td>
<td>0.002</td>
</tr>
<tr>
<td>Lean body mass, %*</td>
<td>62.1 ± 2.3</td>
<td>52.7 ± 1.6</td>
<td>0.002</td>
</tr>
<tr>
<td>BMC, %*</td>
<td>4.06 ± 0.13</td>
<td>3.46 ± 0.12</td>
<td>0.002</td>
</tr>
<tr>
<td>Truncal fat, %*</td>
<td>15.8 ± 1.2</td>
<td>23.2 ± 1.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>Lean arm mass, %*</td>
<td>7.6 ± 0.3</td>
<td>5.9 ± 0.2</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Lean leg mass, %*</td>
<td>22.1 ± 0.9</td>
<td>17.5 ± 0.6</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

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

Table 2. Simple regression analyses of correlation between leucine Ra, Lox, and LIP and LBM, FM, age, and 24-h UFC in 18 normal subjects and 18 subjects with Cushing’s syndrome

<table>
<thead>
<tr>
<th></th>
<th>Normal Subjects</th>
<th>Cushing’s Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine Ra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBM, kg</td>
<td>0.84†</td>
<td>0.69†</td>
</tr>
<tr>
<td>FM, kg</td>
<td>0.05</td>
<td>0.22†</td>
</tr>
<tr>
<td>Age, yr</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>UFC, nmol/day</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Lox</td>
<td>LIP</td>
</tr>
<tr>
<td>Leucine Ra</td>
<td>0.53†</td>
<td>0.54†</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>0.23*</td>
<td>0.11</td>
</tr>
<tr>
<td>FM, kg</td>
<td>0.02</td>
<td>0.004</td>
</tr>
<tr>
<td>Age, yr</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>UFC, nmol/day</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Leucine Ra, rate of leucine appearance; Lox, 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.
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 Lox is normal in Cushing’s syndrome (Table 3).

Glucocorticoid excess increased postabsorptive Lox, 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 Lox 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 Lox 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 Lox 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.

Contrary to a proposed direct effect of glucocorticoid excess on protein oxidation, postabsorptive leucine Ra and LIP were independently related to FM in subjects with Cushing’s syndrome and not glucocorticoid excess. A similar relationship between adiposity and leucine Ra 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 Ra 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 Ra was similar in lean and markedly obese individuals (32). This suggests that the increased leucine Ra 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 Ra has centered on the possibility that the increased leucine Ra 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 Ra and insulin sensitivity measured by euglycemic hyperinsuline-
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 normal subjects, which is necessary for protein anabolism. 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 whole 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 post-absorptive 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 splanchic 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)

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

<table>
<thead>
<tr>
<th></th>
<th>Before Treatment</th>
<th>After Treatment</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat mass, kg</td>
<td>32.1±3.3</td>
<td>27.2±2.1</td>
<td>0.098</td>
</tr>
<tr>
<td>Fat mass, %*$</td>
<td>43.7±2.4</td>
<td>39.0±2.4</td>
<td>0.046</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>38.2±2.7</td>
<td>39.9±2.6</td>
<td>0.14</td>
</tr>
<tr>
<td>LBM, %*$</td>
<td>53.1±2.3</td>
<td>57.5±2.3</td>
<td>0.045</td>
</tr>
<tr>
<td>BMC, kg</td>
<td>2.3±0.1</td>
<td>2.4±0.1</td>
<td>0.34</td>
</tr>
<tr>
<td>BMC, %*$</td>
<td>3.3±0.2</td>
<td>3.4±0.1</td>
<td>0.45</td>
</tr>
</tbody>
</table>

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

We 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.

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.

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