Clinical Endocrinology (2006) **64**, 436–443 doi: 10.1111/j.1365-2265.2006.02488.x

ORIGINAL ARTICLE

Characterization of the metabolic phenotypes of Cushing's syndrome and growth hormone deficiency: a study of body composition and energy metabolism

Morton G. Burt*†‡, James Gibney* and Ken K. Y. Ho*†‡

**Pituitary Research Unit, Garvan Institute of Medical Research, Sydney, NSW 2010, †Department of Endocrinology, St Vincent's Hospital, Sydney, NSW 2010 and ‡University of New South Wales, Sydney, NSW 2031, Australia*

Summary

Objective A comparison of the severity and distribution of perturbations in body composition and their relationship to energy metabolism in glucocorticoid excess and GH deficiency (GHD) has not been undertaken before. The aim of this study was to investigate the impact of Cushing's syndrome (CS) and GHD on whole and regional body composition and energy metabolism.

Design Cross-sectional study design.

Patients Eighteen subjects with CS (12 women, aged = 41·5 ± 3·0 years, 24-h urinary free cortisol = 1601 ± 361 nmol/day, normal < 300 nmol/day), 22 subjects with GHD (14 women, age = 42·9 ± 2·9 years) and 18 normal subjects (11 women, age = 46·8 ± 2·8 years).

Measurements Lean body mass (LBM), fat mass (FM) and regional body composition were assessed by dual-energy X-ray absorptiometry (DEXA). Resting energy expenditure (REE) and fat oxidation (Fox) were assessed by indirect calorimetry.

Results Mean percentage FM was significantly greater by 30% in CS ($P = 0{\cdot}002$) and 22% in GH-deficient subjects ($P = 0{\cdot}014$) than in normal subjects. LBM was significantly lower by 15% in CS ($P = 0{\cdot}002$) and 11% in GHD ($P = 0{\cdot}013$). In CS, the proportion of lean tissue in the limbs was 12% less than in normal ($P = 0{\cdot}001$) and GH-deficient subjects ($P = 0{\cdot}0005$). Truncal fat represented a greater proportion of total FM in CS (52·5 ± 1·8% *vs.* 46·9 ± 1·3%, $P = 0{\cdot}014$) than in normal subjects, but not in GHD. REE and Fox, corrected for LBM, were significantly lower in GHD ($P < 0{\cdot}02$ for both *vs.* normal) but not in CS.

Conclusion FM was higher and LBM lower in both CS and GHD. However, there is a greater abnormality of regional body composition in patients with CS who exhibit a lower limb lean mass and a greater truncal fat. Reduced REE and Fox contribute to increased adiposity in GHD. As REE and Fox are not perturbed in CS, other mechanisms must explain the marked gain in truncal and total fat.

(Received 30 October 2005; returned for revision 15 November 2005; finally revised 19 December 2005; accepted 19 December 2005)

Introduction

Assessment of body composition provides important information on health status. Perturbations of fat mass (FM) and protein mass are significant determinants of mortality and morbidity. Abdominal adiposity is strongly associated with insulin resistance and increased cardiovascular mortality.[1,2] Loss of protein mass not only results in substantial morbidity but also is an independent predictor of mortality in chronic renal failure[3] and chronic obstructive pulmonary disease.[4,5]

Cushing's syndrome (CS) and GH deficiency (GHD) are two chronic conditions that, on first inspection, share similar changes in body composition, with increased FM and reduced lean body mass (LBM) and bone mineral content (BMC). The clinical impression is that LBM may be reduced and total and truncal FM increased to a greater extent in CS; however, this has not been systematically evaluated. The acute effects of glucocorticoids and GH on energy and fat metabolism are very different, suggesting that differing metabolic mechanisms are likely to underlie changes in body fat. Whereas acute GH stimulates energy expenditure and fat oxidation,[6] acute glucocorticoids exert little effect on energy metabolism.[7–9] The chronic effects of glucocorticoids on energy balance and fat metabolism, and whether perturbations account for the increased FM in CS, have not been evaluated.

The aim of this study was to compare body composition and energy metabolism in CS, GH-deficient and normal subjects. We hypothesized that distinct differences in body composition and energy metabolism are present in CS and GHD, despite superficial similarities in body composition phenotype. These may result in important differences in the risk of cardiovascular and metabolic disease and impact on physical function.

Correspondence: Ken K. Y. Ho, Pituitary Research Unit, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, NSW 2010, Australia. Fax: + 61 29295 8481; E-mail: k.ho@garvan.org.au

Table 1. Treatment characteristics of subjects with Cushing's syndrome (CS)

Subject	Age (years)	Gender (M,F)	Cause of CS	Treatment	Hormone deficiencies	Hormone replacement
1	52	M	Mi	Nil	G	Nil
2	54	M	Mi	Nil	G	Nil
3	32	F	Mi	S	Nil	Nil
4	34	F	Mi	Nil	Nil	Nil
5	36	F	EA	Nil	Nil	Nil
6	22	F	AH	Nil	Nil	Nil
7	53	M	Mi	S	T,G	T,G
8	26	F	Mi	Nil	Nil	Nil
9	64	F	Ma	S,X	T	T
10	59	M	Ma	Nil	T	Nil
11	31	F	Mi	Nil	Nil	Nil
12	42	M	Mi	Nil	Nil	Nil
13	39	F	AA	Nil	Nil	Nil
14	43	F	Mi	Nil	Nil	Nil
15	54	F	Mi	Nil	Nil	Nil
16	30	F	Mi	S	T	T
17	49	F	Mi	S	Nil	Nil
18	27	M	Mi	Nil	Nil	Nil

Mi, microadenoma; Ma, macroadenoma; EA, ectopic ACTH production; AH, adrenal hyperplasia; AA, adrenal adenoma; S, surgery; X, radiotherapy; T, thyroid deficiency/replacement; G, gonadal deficiency/replacement.

Table 2. Treatment characteristics of subjects with GH deficiency (GHD)

Subject	Age (years)	Gender (M,F)	Cause of GHD	Treatment	Hormone deficiencies	Hormone replacement
1	31	M	NFT	S	A,T,G,D	A,T,G,D
2	66	M	NFT	S	A,T,G	A,T,G
3	34	F	NFT	X	G	G
4	23	M	NFT	X	A,T,G,D	A,T,G,D
5	27	M	IH	Nil	T,G	T,G
6	44	F	NFT	S,X	A,T,G	A,T,G
7	50	F	LH	Nil	A,G	A,G
8	27	F	NFT	S,X	A.T,G,D	A.T,G,D
9	53	F	CD	S	A,T,G,D	A,T,G,D
10	53	F	NFT	S,X	A,T,G	A,T,G
11	59	M	NFT	S	A,T,G,D	A,T,G,D
12	68	F	NFT	S,X	A,T,G	A,T,G
13	22	F	IH	Nil	G	G
14	48	F	IH	Nil	A,T,G,D	A,T,G,D
15	48	F	P	X	T,G	T,G
16	58	F	NFT	S	G	G
17	38	F	CD	S,X	A,T,G	A,T,G
18	41	F	IH	Nil	A,T,G	A,T,G
19	43	F	P	S,X	A,T	A,T
20	48	M	NFT	S	A,T,G	A,T,G
21	24	M	IH	Nil	G,D	G,D
22	38	M	NFT	S	Nil	Nil

NFT, nonfunctioning tumour; IH, idiopathic hypopituitarism; LH, lymphocytic hypophysitis; CD, surgery and/or radiotherapy for Cushing's disease; P, prolactinoma; S, surgery; X, radiotherapy; A, adrenal hormone deficiency/replacement; T, thyroid deficiency/replacement; G, gonadal deficiency/replacement; D, diabetes insipidus.

Methods

Subjects

Eighteen subjects (12 women) with active CS, 22 subjects with GHD (14 women) and 18 normal subjects (11 women) were studied. The cause of CS was an ACTH-producing pituitary tumour in 15 subjects, bilateral micronodular adrenal hyperplasia in one subject, an adrenal adenoma in one subject and ectopic ACTH secretion from a thymic carcinoid tumour in one subject (Table 1). All subjects with CS had active disease as defined by an elevated 24-h urinary free cortisol (mean = 1601 ± 361 nmol/day, normal < 300 nmol/day). The mean duration of symptoms consistent with CS was 42 ± 9 months. In 13 subjects, CS was newly diagnosed and these subjects were assessed prior to replacement of other pituitary hormone deficits. Central hypothyroidism was present in one subject and testosterone was below the lower limit of normal in two of the six male subjects. In five subjects (four women), CS 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 the male subject receiving testosterone replacement.

All subjects with GHD had a peak GH in response to insulin-induced hypoglycaemia (glucose < 2·2 mmol/l) of < 3 ng/ml.[10] The estimated duration of GHD was > 12 months in all subjects (range 14–360 months). The aetiology of GHD was a nonfunctioning tumour ($n = 12$), lymphocytic hypophysitis ($n = 1$), prolactinoma ($n = 2$), surgery and radiotherapy for CS ($n = 2$) and idiopathic hypopituitarism ($n = 5$) (Table 2). The two subjects treated for CS had been eucortisolaemic for 4 and 16 years, respectively. Subjects were receiving physiological hormone replacement therapy as necessary for other anterior pituitary hormone deficits (Table 2). Fifteen subjects were receiving glucocorticoid replacement for ACTH deficiency (mean hydrocortisone dose = 22·3 ± 2·4 mg/day) and 16 were receiving thyroxine replacement (mean thyroxine dose = 111 ± 5 μg/day). Seven of eight men were receiving testosterone replacement (Sustanon 250 mg every 2–3 weeks, $n = 6$; testosterone implant 600 mg every 6 months, $n = 1$) and 13 of 14 women were receiving oestrogen replacement (transdermal, $n = 5$; oral, $n = 8$). No subject had previously received GH replacement therapy.

The normal subjects comprised healthy volunteers recruited from the general population. The Research Ethics Committee of St Vincent's Hospital, Sydney, Australia approved the study and all subjects provided written informed consent.

Clinical protocol

Subjects attended the Clinical Research Facility, Garvan Institute of Medical Research, at 0830 h after an overnight fast, where they underwent indirect calorimetry and body composition measurement. Extracellular water (ECW) was measured in a subset of each group, to estimate true protein mass that was not fluid.

Assessment of body composition

Body composition was assessed using a three-compartment model. FM, LBM and BMC were measured by dual-energy X-ray absorptiometry (DEXA) using a total body scanner (Lunar model DPX, software version 3·1, Lunar Corp., Madison, WI, USA), which also quantified regional body composition of upper limb, lower limb and truncal fat. Truncal fat comprises fat in the chest, abdominal and pelvic regions, as described previously.[1] The coefficients of variation (CVs) for FM and LBM at our institution were 2·9% and 1·4%, respectively.[11]

Indirect calorimetry

Subjects were asked to remain on their normal diet for 3 days before indirect calorimetry. After subjects had rested for at least 30 min, O_2 consumption (VO_2) and CO_2 production (VCO_2) 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. VO_2 and VCO_2 were measured over two 20-min periods and averaged. Resting energy expenditure (REE) and substrate oxidation rates were calculated using the equations of Ferrannini.[12] The mean day-to-day intrasubject CV for REE at our institution is approximately 4%.[13,14]

Measurement of extracellular water

ECW was estimated using the bromide dilution technique as described previously.[15,16] 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.*[15] Serum bromide concentration was measured by high-pressure liquid chromatography after removal of plasma proteins by centrifugal ultrafiltration. The intra-assay CV at our institution is < 4%. The mean day-to-day intrasubject CV for ECW at our institution, based on repeat ECW estimation from four subjects, is 5·7%.[16] ECW was subtracted from LBM to quantify body cell mass (BCM).

Statistical analysis

Statistical analysis was undertaken using statistical software packages Statview 4·5 PPC (Abacus Concepts, Inc, Berkeley, CA, USA) and SPSS 11·0 (SPSS Inc, Chicago, IL, USA). Results are expressed as mean ± standard error unless stated otherwise. Categorical variables were assessed using χ^2-testing, while continuous variables were assessed by factorial analysis of variance (ANOVA). Simple regression analysis was performed to assess the relationship between variables. Correction for significant covariates was made using analysis of covariance (ANCOVA) with a Bonferroni correction made when multiple comparisons were undertaken.

Results

Subject characteristics

The characteristics of the groups of normal subjects, and subjects with CS and GHD are shown in Table 3. There were no significant differences in gender distribution ($P = 0·94$), age, weight and body mass index between the three groups.

Table 3. Subject characteristics of 18 normal subjects, 18 subjects with Cushing's syndrome (CS) and 22 subjects with GH deficiency (GHD) (values represent mean ± standard error)

	Normal	CS	GHD
Gender (F/M)	11/7	12/6	14/8
Age (years)	46·8 ± 2·8	41·5 ± 3·0	42·9 ± 2·9
Weight (kg)	72·4 ± 4·2	75·1 ± 3·6	76·9 ± 2·9
BMI (kg/m^2)	25·5 ± 1·1	27·4 ± 1·1	27·5 ± 0·9

All *P*-values > 0·15.

Table 4. Whole body composition analysis in 18 normal subjects, 18 subjects with Cushing's syndrome (CS) and 22 subjects with GH deficiency (GHD) (values represent mean ± standard error)

	Normal	CS	*P*-value*	GHD	*P*-value*
Fat mass (kg)	24·2 ± 2·6	31·7 ± 2·3	0·029	30·6 ± 1·9	0·050
Fat mass (%)†	33·8 ± 2·4	43·9 ± 1·6	0·002	41·1 ± 2·1	0·014
LBM (kg)	43·1 ± 2·4	37·1 ± 1·7	0·056	40·7 ± 2·1	0·42
LBM (%)†	62·1 ± 2·3	52·7 ± 1·6	0·002	55·1 ± 1·9	0·013
BMC (kg)	2·82 ± 0·15	2·41 ± 0·08	0·027	2·76 ± 0·13‡	0·75
BMC (%)†	4·06 ± 0·13	3·46 ± 0·12	0·003	3·76 ± 0·14	0·12

**vs.* normal subjects.
†Expressed as percentage total body weight.
‡$P < 0·05$ *vs.* CS.

Body composition

Body composition was first assessed using a three-compartment model with FM, LBM and BMC expressed both in kilograms and as a percentage of total body weight (Table 4). In subjects with CS, percentage FM was approximately 30% greater ($P = 0·002$) while LBM ($P = 0·002$) and BMC ($P = 0·003$) were 15% lower than in normal subjects (Fig. 1). In subjects with GHD, percentage FM was significantly greater by 22% ($P = 0·014$) and LBM significantly lower by 11% ($P = 0·013$) than in normal subjects, while BMC was not significantly different ($P = 0·12$) (Fig. 1). There were no significant differences in FM ($P = 0·32$) and LBM ($P = 0·36$) between subjects with CS and GHD. Absolute, but not percentage, BMC was significantly lower in subjects with CS than in subjects with GHD (Table 4). Body composition was reanalysed following exclusion of the two subjects with acquired GHD after undergoing treatment for CS. Omission of these two subjects did not alter the above findings.

Regional body compartments were compared next (Fig. 2). In subjects with CS compared to normal subjects, mean percentage truncal fat was significantly higher by 46% ($P < 0·0001$), whereas limb fat mass was not significantly different. Percentage arm and leg lean mass were significantly lower by 22% ($P = 0·0008$) and 21%

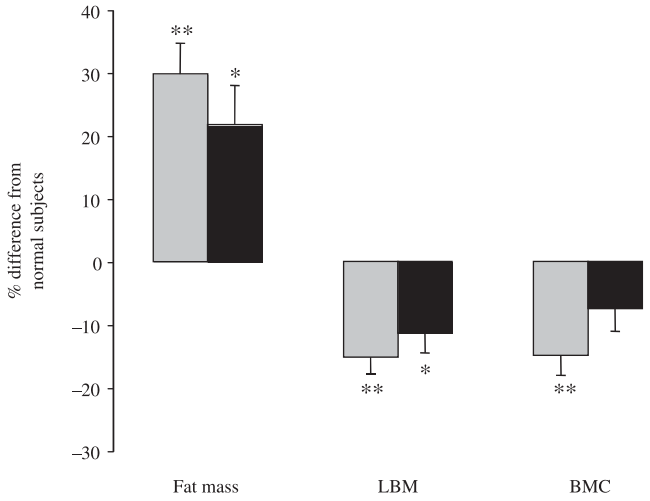

Fig. 1 Percentage total body fat mass, lean body mass (LBM) and bone mineral content (BMC) in 18 subjects with Cushing's syndrome (grey bars) and 22 subjects with GH deficiency (black bars), expressed as the mean ± standard error percentage difference from 18 normal subjects (*$P = 0{\cdot}05$ *vs.* normal subjects, **$P = 0{\cdot}01$ *vs.* normal subjects).

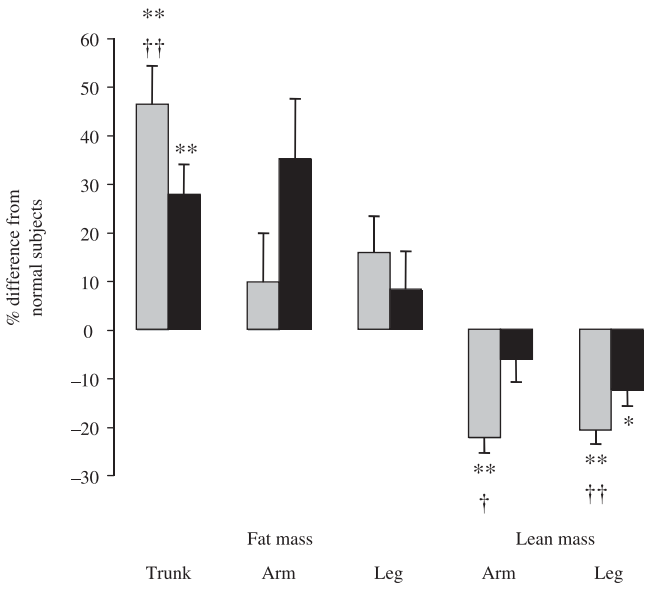

Fig. 2 Percentage regional body composition in 18 subjects with Cushing's syndrome (grey bars) and 22 subjects with GH deficiency (black bars), expressed as the mean ± standard error percentage difference from 18 normal subjects (*$P = 0{\cdot}05$ *vs.* normal subjects, **$P = 0{\cdot}01$ *vs.* normal subjects, †$P = 0{\cdot}05$ *vs.* GH-deficient subjects, ††$P = 0{\cdot}10$ *vs.* GH-deficient subjects).

($P = 0{\cdot}0001$), respectively, in CS. In subjects with GHD, truncal fat was significantly greater than normal subjects by 28% ($P = 0{\cdot}006$) and percentage arm fat was 35% higher, although this did not reach statistical significance ($P = 0{\cdot}052$). Percentage lean leg mass was significantly lower by 12% ($P = 0{\cdot}01$) while lean arm mass was not significantly different to normal subjects ($P = 0{\cdot}30$). Truncal fat tended to be greater in CS than in GHD, although this did not reach

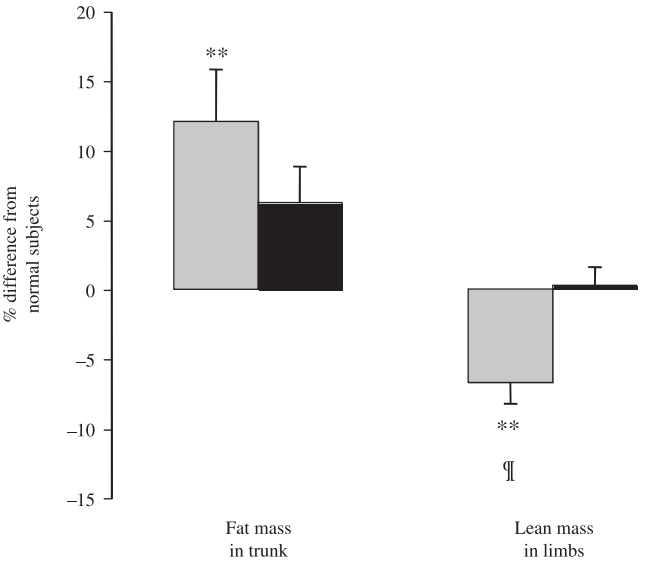

Fig. 3 Percentage of total body fat in the trunk and percentage of total lean body mass in the limbs in 18 subjects with Cushing's syndrome (grey bars) and 22 subjects with GH deficiency (black bars), expressed as the mean ± standard error percentage difference from 18 normal subjects (**$P = 0{\cdot}01$ *vs.* normal subjects, ¶$P = 0{\cdot}01$ *vs.* GH deficient subjects).

statistical significance ($P = 0{\cdot}067$). Subjects with CS had a significantly lower percentage arm lean mass than subjects with GHD ($P = 0{\cdot}0008$), while significant leg lean mass was not statistically different ($P = 0{\cdot}087$).

Finally, the proportion of total FM in the trunk (truncal fat/total FM) and the proportion of LBM in the limbs (limb lean mass/LBM) were ascertained (Fig. 3). In subjects with CS, truncal fat represented a significantly greater percentage of FM ($P = 0{\cdot}009$) and lean limb mass a smaller percentage of LBM ($P = 0{\cdot}001$) than in normal subjects. In subjects with GHD, there were no significant differences from normal subjects in the proportion of FM in the trunk ($P = 0{\cdot}14$) or in the proportion of LBM in the limbs ($P = 0{\cdot}88$). Subjects with CS had a significantly lower proportion of LBM in the limbs than subjects with GHD ($P = 0{\cdot}0005$). The proportion of fat in the trunk in subjects with CS was not significantly different to subjects with GHD ($P = 0{\cdot}17$).

Extracellular water

ECW was measured in a subset of normal subjects ($n = 6$, four women), subjects with CS ($n = 7$, four women) and subjects with GHD ($n = 7$, seven women). Although only women were in the subgroup with GHD, gender distribution in the three subgroups was not significantly different ($P = 0{\cdot}15$). There were no significant differences in age, weight or BMI between the three subgroups (data not shown).

In normal subjects there was a highly significant correlation between ECW and LBM ($P = 0{\cdot}0002$, Fig. 4a). In subjects with GHD the correlation between ECW and LBM approached statistical significance ($P = 0{\cdot}052$, Fig. 4b), whereas in subjects with CS there was

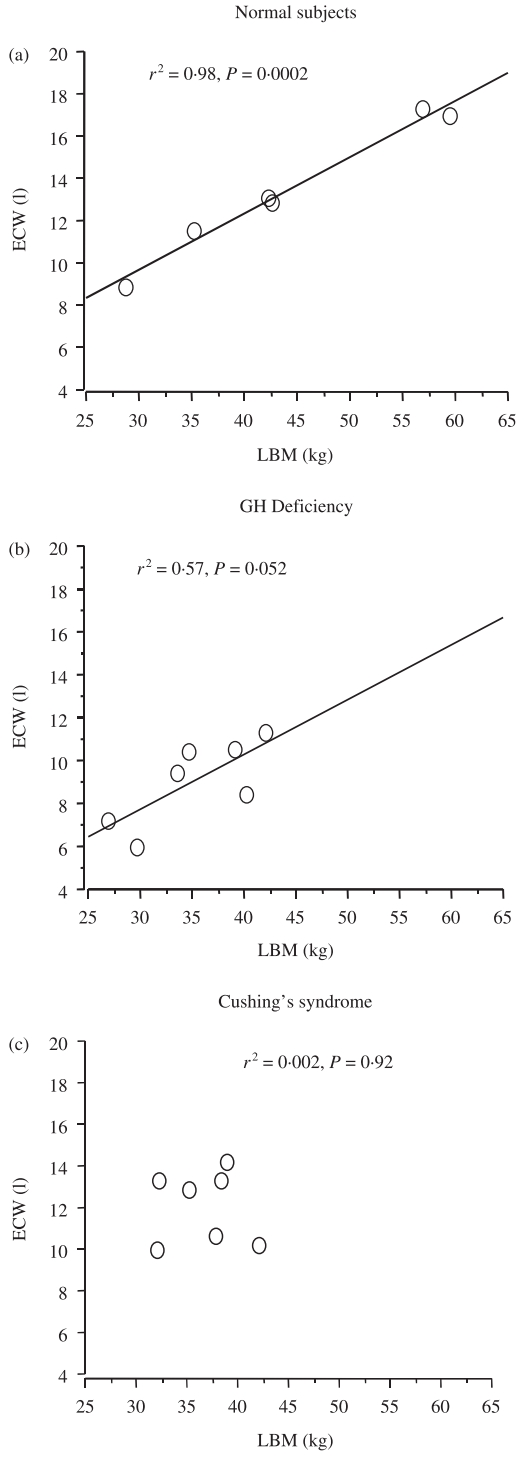

Fig. 4 Relationship between lean body mass (LBM) and extracellular water (ECW) in (a) six normal subjects, (b) seven subjects with GH deficiency and (c) seven subjects with Cushing's syndrome.

no significant correlation (Fig. 4c). ECW occupied a significantly smaller proportion of the LBM in subjects with GHD than both normal subjects ($25{\cdot}7 \pm 1{\cdot}4\%$ *vs.* $30{\cdot}7 \pm 0{\cdot}6\%$, $P = 0{\cdot}045$) and subjects with CS ($33{\cdot}3 \pm 2{\cdot}2\%$, $P = 0{\cdot}004$). The proportion of the LBM that was ECW in CS was not significantly different to normal subjects ($P = 0{\cdot}29$). BCM tended to be lower in subjects with CS than in normal subjects; however, this did not reach statistical significance ($24{\cdot}6 \pm 1{\cdot}6$ kg *vs.* $30{\cdot}7 \pm 3{\cdot}6$ kg, $P = 0{\cdot}08$). In subjects with GHD, BCM was not significantly different to normal subjects ($26{\cdot}1 \pm 1{\cdot}7$ kg *vs.* $30{\cdot}7 \pm 3{\cdot}6$ kg, $P = 0{\cdot}18$) or subjects with CS ($P = 0{\cdot}64$).

Because of apparent unequal (albeit not statistically different) gender distribution in the three subgroups, analysis of ECW and BCM was repeated following correction for gender distribution. Correction for gender distribution did not significantly alter ECW results (data not shown). When gender was included as a covariate, BCM was significantly lower in subjects with CS than in normal subjects ($23{\cdot}3 \pm 2{\cdot}1$ kg *vs.* $30{\cdot}1 \pm 2{\cdot}2$ kg, $P = 0{\cdot}34$), whereas in GHD BCM was not significantly different to normal ($27{\cdot}9 \pm 2{\cdot}1$ kg, $P = 0{\cdot}15$).

Energy metabolism

REE was significantly correlated with LBM in normal subjects ($r^2 = 0{\cdot}83$, $P < 0{\cdot}0001$), subjects with CS ($r^2 = 0{\cdot}39$, $P = 0{\cdot}008$) and GHD ($r^2 = 0{\cdot}51$, $P = 0{\cdot}0002$). After correction for LBM, REE was significantly lower in subjects with GHD than in subjects with CS ($P = 0{\cdot}011$) and normal subjects ($P = 0{\cdot}044$) (Table 5). The mean REE in CS was not significantly different from that of normal subjects. Exclusion of the three subjects with CS who did not receive replacement therapy for thyroid ($n = 1$) and gonadal ($n = 2$) hormones did not significantly alter the results.

LBM was also significantly correlated with Fox ($r^2 = 0{\cdot}15$, $P = 0{\cdot}003$). After correction for LBM, the rate of Fox was significantly lower ($P = 0{\cdot}003$) in GH-deficient than in normal subjects whereas in subjects with CS, Fox was not significantly different from normal ($P = 0{\cdot}50$) or GH-deficient subjects ($P = 0{\cdot}17$) (Table 5). Fox in GH-deficient subjects remained significantly less than normal subjects after adjustment for oral oestrogen use (data not shown). As there was not a significant correlation between CHOox and LBM, the results are reported unadjusted. CHOox was significantly greater in subjects with GHD than in normal subjects ($P = 0{\cdot}013$), whereas in CS CHOox was not significantly different to normal ($P = 0{\cdot}11$) (Table 5).

Discussion

This study, the first systematic comparison of body composition and energy metabolism in CS and GHD, reveals similarities in whole body composition between the two conditions but quite striking differences in regional body composition and energy metabolism. While perturbations of greater FM and lower LBM were quantitatively similar in CS and GHD, truncal fat (higher) and limb lean mass (lower) were more markedly affected in CS. LBM was significantly correlated with REE and Fox. After correcting for the influence of LBM, REE and Fox were lower in GHD, but not in CS.

Regional body composition analysis in CS suggests that lean tissue is reduced to a greater extent in the limbs and fat is increased to a greater extent in the trunk. While total LBM was 15% lower in subjects with CS than in normal subjects (Fig. 1), the percentage of lean tissue in the limbs was also significantly lower (Fig. 3), suggesting greater loss of lean tissue from the arms and legs. Similarly, despite

Table 5. Resting energy expenditure and substrate oxidation in 18 normal subjects, 18 subjects with Cushing's syndrome (CS) and 22 subjects with GH deficiency (GHD) (values represent mean ± standard error)

	Normal	CS	*P*-value*	GHD	*P*-value*
REE† (kcal/day)	1458 ± 39	1489 ± 41	1·00	1325 ± 35‡	0·044
Fox† (mg/min)	76·0 ± 5·5	64·7 ± 5·7	0·50	49·9 ± 4·9	0·003
CHOox (mg/min)	52·4 ± 10·0	78·8 ± 16·2	0·11	90·9 ± 6·6	0·013

REE, resting energy expenditure; Fox, fat oxidation; CHOox, carbohydrate oxidation.
**vs.* normal subjects.
†Corrected for lean body mass using analysis of covariance with Bonferroni adjustment made for multiple comparisons.
‡$P < 0{\cdot}05$ *vs.* CS.

total FM being 30% higher, the percentage of fat in the trunk was significantly greater than normal subjects, suggesting predominant truncal fat accumulation (Fig. 3). Previous studies, undertaken in a smaller cohort[17] and in women only,[18] which have characterized body composition abnormalities in CS, have provided some inconsistencies. Compared to weight-matched controls, percentage LBM was reported as unchanged[17] or reduced,[18] and total and truncal FM unchanged[17] or increased.[18] The lean mass of the arm, but not the leg, was reduced in CS.[17,18] Visceral fat, measured by computer tomography, was consistently increased.[17,19] Our study in this large group of both women and men provides strong data supporting the clinical phenotype of greater loss of lean tissue from both limbs and gain of truncal but not limb fat.

Our study is consistent with previous reports of a greater whole body FM accompanied by a lower LBM in GHD of a similar magnitude.[20] Few studies, however, have characterized regional body composition in GHD. In an earlier study of elderly GH-deficient subjects, no difference was observed in the proportion of fat deposited centrally or the distribution of lean tissue assessed by DEXA.[21] However, it has been reported that visceral abdominal adipose tissue is increased to a greater extent than subcutaneous tissue.[22] GH replacement has resulted in a greater gain in lean tissue in the limbs and a greater reduction in abdominal than peripheral FM,[23,24] with the reduction predominantly visceral rather than subcutaneous adipose tissue.[25] We observed the impact of GHD on body composition to be more generalized than in CS. The proportion of lean tissue in the limbs in GH-deficient subjects was almost identical to normal subjects (Fig. 3), suggesting that loss of lean tissue tends to be generalized. The percentage of FM in the truncal region in GH-deficient subjects was also not significantly different to normal subjects. As the proportion of fat in the trunk was midway between normal subjects and subjects with CS, it is possible that the lack of a statistically significant increase in truncal fat represents a type II error, such that a larger cohort may reveal a redistribution of fat to the trunk. However, preferential development of truncal adiposity is less marked than in CS.

The status of ECW in CS has not been reported previously. ECW was assessed in a subgroup as perturbations in ECW might confound estimation of protein cell mass by DEXA. If ECW were increased in CS, this would result in an overestimation of protein mass. Conversely, failure to account for ECW in GHD will lead to underestimation of protein mass, because of the known antinatiuretic action of GH.[26] It was surprising that we did not observe increased ECW in CS in view of the known sodium-retaining effect of glucocorticoids. However, the tight relationship between ECW and LBM in normal subjects was absent in the subgroup with CS (Fig. 4), suggesting that glucocorticoid excess may influence the hydration of the LBM. These data should be interpreted with caution, given the small number of subjects in whom ECW was quantified and the relatively narrow range of LBMs in the subgroup with CS.

Adiposity is a hallmark of both CS and GHD and may arise from a disturbance in energy balance or a reduction in the oxidative metabolism of fat. In GH-deficient subjects, we found a reduction in REE, both unadjusted (data not shown) and after correcting for the reduced LBM. Three previous studies found no significant difference in REE in subjects with GHD.[6,26,27] One study reported REE in subjects with GHD to be 90% of predicted; however, the reduction was not statistically significant.[27] Another study found REE to be in the lower part of the normal range in GH-deficient adults.[6] Both these studies involved smaller numbers of subjects (nine and seven, respectively),[6,27] suggesting that the failure to find a significant reduction in REE may be due to a type II error. Our results in a larger well-matched cohort are consistent with the known positive impact of GH replacement on REE[6] and the finding of an increased rate of REE in acromegaly.[11]

Reduced Fox may also contribute to the development of adiposity in GHD because of the known stimulation of lipolysis and Fox by GH. Lean tissue is the major site of postprandial Fox[28] and explains the correlation between LBM and Fox. In this study we observed Fox in GHD to be significantly reduced even after correcting for the influence of a contracted LBM. This observation underscores the importance of GH as a potent stimulator of Fox and its physiological role as a regulator of Fox. The rate of Fox is unlikely to be confounded by altered protein oxidation, as this is not significantly different from normal in subjects with GHD.[29] Similarly, glucocorticoid, thyroid hormone and testosterone replacement were physiological and Fox remained reduced after correction for oral oestrogen use. Reduced REE and Fox are likely to relate directly to GHD and contribute to a generalized gain in adipose tissue.

By contrast, neither REE nor Fox were significantly reduced in CS after accounting for the reduced LBM. Previous studies have reported that acute administration of glucocorticoids does not alter REE[8,9,30,31] or Fox in most,[8,9,30] but not all,[31] studies. Our study extends these observations by demonstrating for the first time that energy and fat metabolism are not perturbed chronically. This observation suggests that mechanisms other than those involving major perturbation

of energy expenditure are likely to contribute to weight gain and fat deposition in CS. Glucocorticoids have been shown to markedly stimulate appetite[32] and promote differentiation of adipocytes.[33] One likely mechanism is the stimulation of lipoprotein lipase, the main enzyme responsible for hydrolysis of triglycerides into fatty acids allowing uptake and storage in fat tissue. Glucocorticoids stimulate lipoprotein lipase in visceral adipocytes to a greater extent than in subcutaneous adipocytes.[34] In subjects with CS, lipoprotein lipase activity was increased in retroperitoneal[35] but not gluteal adipose tissue.[36] Increased visceral lipoprotein lipase activity will result in the preferential gain in truncal adipose tissue found in this and other studies.[17,19]

A limitation of a cross-sectional study is that it does not control for all possible variables. However, we have attempted to account for variables that may have influenced results. Three subjects with CS had untreated deficiencies of the thyroid or gonadal axis; however, the energy metabolism findings were not significantly different if these subjects were excluded from the analysis. It is possible that concomitant GHD may have contributed to the body compositional changes in subjects with CS. However, while the subjects with CS were not tested for GHD, only six of 18 had a macroadenoma or had previously surgery/radiotherapy (Table 1). Therefore, it is unlikely that GH secretion was impaired in the majority. Moreover, as regional body compositional changes were more severe in CS, the additional perturbations are likely to be secondary to glucocorticoid excess. The subjects with GHD were a more homogeneous group, all were GH-deficient for more than 12 months and receiving stable physiological replacement of other pituitary hormone deficits. In two subjects the GHD resulted from treatment for CS; however, both had been eucortisolaemic for many years. In a previous study, LBM and FM in subjects with GHD following treatment for CS were not significantly different from subjects with GHD from other causes.[37]

In conclusion, GHD and CS display similar global but quite different regional disturbances in body fat. Preferential truncal fat deposition may increase the risk of cardiovascular disease and the marked reduction in limb lean tissue results in greater functional disability in CS. A reduction in REE and Fox is likely to contribute to adiposity in GHD. As whole body energy metabolism is not significantly perturbed in CS, other mechanisms must explain the significant differences in total and regional fat accumulation.

Acknowledgements

We thank clinical research nurses Angela Peris, Amanda Idan, Olivia Wong, Bronwyn Heinrich and Maria Males for assistance with indirect calorimetry and Dr Judith Freund and the Department of Nuclear Medicine, St Vincent's Hospital, Sydney, Australia for assistance with DEXA scanning. Dr Burt is supported by a scholarship from the NHMRC of Australia.

References

1 Carey, D.G., Jenkins, A.B., Campbell, L.V., Freund, J. & Chisholm, D.J. (1996) 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.

2 Lakka, H.-M., Laaksonen, D.E., Lakka, T.A., Niskanen, L.K., Kumpusalo, E., Tuomilehto, J. & Salonen, J.T. (2002) The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *Journal of the American Medical Association*, **288**, 2709–2716.

3 Owen, W.F., Lew, N.L., Liu, Y., Lowrie, E.G. & Lazarus, J.M. (1993) The urea reduction ratio and serum albumin concentration as predictors of mortality in patients undergoing hemodialysis. *New England Journal of Medicine*, **329**, 1001–1006.

4 Marquis, K., Debigare, R., Lacasse, Y., LeBlanc, P., Jobin, J., Carrier, G. & Maltais, F. (2002) Midthigh muscle cross-sectional area is a better predictor of mortality than body mass index in patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, **166**, 809–813.

5 Mador, M.J. (2002) Muscle mass, not body weight, predicts outcome in patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, **166**, 787–789.

6 Stenlof, K., Sjostrom, L., Lonn, L., Bosaeus, I., Kvist, H., Tolli, J., Lindstedt, G. & Bengtsson, B.-A. (1995) Effects of recombinant human growth hormone on basal metabolic rate in adults with pituitary deficiency. *Metabolism*, **44**, 67–74.

7 Horber, F.F. & Haymond, M.W. (1990) Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *Journal of Clinical Investigation*, **86**, 265–272.

8 Short, K.R., Nygren, J., Bigelow, M.L. & Nair, K.S. (2004) Effect of short-term prednisone use on blood flow, muscle protein metabolism, and function. *Journal of Clinical Endocrinology and Metabolism*, **89**, 6198–6207.

9 Gravholt, C.H., Dall, R., Christiansen, J.S., Moller, N. & Schmitz, O. (2002) Preferential stimulation of abdominal subcutaneous lipolysis after prednisolone exposure in humans. *Obesity Research*, **10**, 774–781.

10 Hoffman, D.M., Nguyen, T.V., O'Sullivan, A.J., Baxter, R.C. & Ho, K.K. (1994) Diagnosis of growth hormone deficiency in adults. *Lancet*, **344**, 482–483.

11 O'Sullivan, A.J., Kelly, J.J., Hoffman, D.M., Freund, J. & Ho, K.K.Y. (1994) Body composition and energy expenditure in acromegaly. *Journal of Clinical Endocrinology and Metabolism*, **78**, 381–386.

12 Ferrannini, E. (1988) The theoretical bases of indirect calorimetry: a review. *Metabolism*, **37**, 287–301.

13 O'Sullivan, A.J., Crampton, L.J., Freund, J. & Ho, K.K.Y. (1998) The route of estrogen replacement therapy confers divergent effects on substrate oxidation and body composition in postmenopausal women. *Journal of Clinical Investigation*, **102**, 1035–1040.

14 Greenfield, J.R., Samaras, K., Hayward, C.S., Chisholm, D.J. & Campbell, L.V. (2005) Beneficial postprandial effect of a small amount of alcohol on diabetes and cardiovascular risk factors. *Journal of Clinical Endocrinology and Metabolism*, **90**, 661–672.

15 Miller, M.E., Cosgriff, J.M. & Forbes, G.B. (1989) Bromide space determination using anion-exchange chromatography for measurement of bromide. *American Journal of Clinical Nutrition*, **50**, 168–171.

16 Johannsson, G., Gibney, J., Wolthers, T., Leung, K.-C. & Ho, K.K.Y. (2005) Independent and combined effects of testosterone and growth hormone on extracellular water in hypopituitary men. *Journal of Clinical Endocrinology and Metabolism*, **90**, 3989–3994.

17 Wajchenberg, B.L., Bosco, A., Marone, M.M., Levin, S., Rocha, M., Lerario, A.C., Nery, M., Goldman, J. & Liberman, B. (1995) 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. *Journal of Clinical Endocrinology and Metabolism*, **80**, 2791–2794.

18 Garrapa, G.G.M., Pantanetti, P., Arnaldi, G., Mantero, F. & Faloia, E. (2001) Body composition and metabolic features in women with adrenal incidentaloma or Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism*, **86**, 5301–5306.

19 Mayo-Smith, W., Hayes, C.W., Biller, B.M.K., Klibanski, A., Rosenthal, H. & Rosenthal, D.I. (1989) Body fat distribution measured with CT: correlations in healthy subjects, patients with anorexia nervosa, and patients with Cushing's syndrome. *Radiology*, **170**, 515–518.

20 Carroll, P.V., Christ, E.R., Bengtsson, B.-A., Carlsson, L., Christiansen, J.S., Clemmons, D., Hintz, R., Ho, K., Laron, Z., Sizonenko, P., Sonksen, P.H., Tanaka, T. & Thorner, M. (1998) Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. *Journal of Clinical Endocrinology and Metabolism*, **83**, 382–395.

21 Toogood, A.A., Adams, J.E., O'Neill, P.A. & Shalet, S.M. (1996) Body composition in growth hormone deficient adults over the age of 60 years. *Clinical Endocrinology*, **45**, 399–405.

22 Snel, Y.E., Doerga, M.E., Brummer, R.M., Zelissen, P.M. & Koppeschaar, H.P. (1995) Magnetic resonance imaging-assessed adipose tissue and serum lipid and insulin concentrations in growth hormone-deficient adults. Effect of growth hormone replacement. *Arteriosclerosis, Thrombosis and Vascular Biology*, **15**, 1543–1548.

23 Hansen, T.B., Vahl, N., Jorgensen, J.O.L., Christiansen, J.S. & Hagen, C. (1995) Whole body and regional soft tissue changes in growth hormone deficient adults after one year of growth hormone treatment: a double-blind, randomized, placebo-controlled study. *Clinical Endocrinology*, **43**, 689–696.

24 Bell, W., Davies, J.S., Evans, W.D. & Scanlon, M.F. (2000) Effect of recombinant human growth hormone on regional tissue distribution in growth hormone-deficient males. *Annals of the New York Academy of Science*, **904**, 576–583.

25 Bengtsson, B.-A., Eden, S., Lonn, L., Kvist, H., Stokland, A., Lindstedt, G., Bosaeus, I., Tolli, J., Sjostrom, L. & Isaksson, O.G.P. (1993) Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *Journal of Clinical Endocrinology and Metabolism*, **76**, 309–317.

26 Hoffman, D.M., O'Sullivan, A.J., Freund, J. & Ho, K.K.Y. (1995) Adults with growth hormone deficiency have abnormal body composition but normal energy metabolism. *Journal of Clinical Endocrinology and Metabolism*, **80**, 72–77.

27 Chong, P.K.K., Jung, R.T., Scrimgeour, C.M., Rennie, M.J. & Paterson, C.R. (1994) Energy expenditure and body composition in growth hormone deficient adults on exogenous growth hormone. *Clinical Endocrinology*, **40**, 103–110.

28 Kelley, D.E. (2005) Skeletal muscle fat oxidation: timing and flexibility are everything. *Journal of Clinical Investigation*, **115**, 1699–1702.

29 Hoffman, D.M., Pallasser, R., Duncan, M., Nguyen, T.V. & Ho, K.K.Y. (1998) How is whole body protein turnover perturbed in growth hormone-deficient adults? *Journal of Clinical Endocrinology and Metabolism*, **83**, 4344–4349.

30 Horber, F.F., Marsh, H.M. & Haymond, M.W. (1991) Differential effects of prednisone and growth hormone on fuel metabolism and insulin antagonism in humans. *Diabetes*, **40**, 141–149.

31 Chong, P.K.K., Jung, R.T., Scrimgeour, C.M. & Rennie, M.J. (1994) The effect of pharmacological dosages of glucocorticoids on free living total energy expenditure in man. *Clinical Endocrinology*, **40**, 577–581.

32 Tataranni, P.A., Larson, D.E., Snitker, S., Young, J.B., Flatt, J.P. & Ravussin, E. (1996) Effects of glucocorticoids on energy metabolism and food intake in humans. *American Journal of Physiology*, **271**, E317–E325.

33 Ahdjoudj, S., Lasmoles, F., Oyajobi, B.O., Lomri, A., Delannoy, P. & Marie, P.J. (2001) Reciprocal control of osteoblast/chondroblast and osteoblast/adipocyte differentiation of multipotential clonal human marrow stromal F/STRO-1(+) cells. *Journal of Cell Biochemistry*, **81**, 23–38.

34 Fried, S.K., Russell, C.D., Grauso, N.L. & Brolin, R.E. (1993) Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *Journal of Clinical Investigation*, **92**, 2191–2198.

35 Sakayama, K., Masuno, H., Kidani, T., Matsuda, Y., Yamamoto, H. & Okuda, H. (2001) Synthesis of active high mannose-type lipoprotein lipase in human adipose tissues. *Atherosclerosis*, **155**, 29–35.

36 Taskinen, M.R., Nikkila, E.A., Pelkonen, R. & Sane, T. (1983) Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism*, **57**, 619–626.

37 Johannsson, G., Sunnerhagen, K.S. & Svensson, J. (2004) Baseline characteristics and the effects of two years of growth hormone replacement therapy in adults with growth hormone deficiency previously treated for Cushing's disease. *Clinical Endocrinology*, **60**, 550–559.