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CLINICAL STUDY

Influence of short-term dietary weight loss on cortisol secretion and metabolism in obese men

Alexandra M Johnstone, Peter Faber, Ruth Andrew[1], Eileen R Gibney[2], Marinos Elia[3], Gerald Lobley, R James Stubbs and Brian R Walker[1]

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK, [1]Endocrinology Unit, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK, [2]Human Nutrition Research Centre, Department of Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK, [3]Institute of Human Nutrition, Level C (113), West Wing, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK

(Correspondence should be addressed to Brian R Walker; Email: B.Walker@ed.ac.uk)

Abstract

Objectives: Obesity is associated with increased inactivation of cortisol by hepatic A-ring 5α- and 5β-reductases, impaired hepatic regeneration of cortisol from cortisone by 11β-hydroxysteroid dehydrogenase type 1 (11HSD1), but increased subcutaneous adipose 11HSD1 activity enhancing local cortisol levels in fat. Cause and effect between obesity and abnormal cortisol metabolism is untested.
Design: Acute weight loss was induced by very low calorie diet (VLCD) or starvation in obese men.
Methods: Otherwise healthy males (aged 20–55 years; body mass index (BMI) 30–40 kg/m^2) were studied after 6 days on a weight maintenance diet; then after either 6 days of starvation ($n = 6$) or 3 weeks of VLCD (2.55 MJ; $n = 6$); then after 1 week of weight maintenance; and finally after 2 weeks of being allowed to feed *ad libitum*. Plasma samples were obtained from indwelling cannulae at 0930 h and 1815 h and a 24 h urine collection was completed for analysis of cortisol metabolites by gas chromatography/mass spectrometry.
Results: Data are mean±S.E.M. BMI fell (kg/m^3) from 34.8±0.8 at baseline to 31.8±1.4 on VLCD and 32.7±1.1 on starvation. Starvation caused a rise in plasma cortisol (at 0930 h from 143±17 to 216±11 nM, $P < 0.001$) but no change in total urinary cortisol metabolites. VLCD did not alter plasma cortisol and markedly reduced cortisol metabolite excretion (from 15.8±1.1 mg/day at baseline to 7.0±1.1 mg/day, $P < 0.001$). Relative excretion of 5α-reduced cortisol metabolites fell on both diets, but there were no changes in cortisol/cortisone metabolite ratios reflecting 11HSD activities.
Conclusions: Weight loss with VLCD in obesity reverses up-regulation of hepatic A-ring reductases and normalises cortisol production rate; in contrast, starvation produces acute stress and further activation of cortisol secretion. We suggest that activation of cortisol secretion is not an irreversible intrinsic abnormality in obese patients, and speculate that dietary content has an important influence on the neuroendocrine response to weight loss.

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Introduction

Idiopathic obesity has been associated with alterations in several neuroendocrine axes (1). Of particular interest is evidence for increased production of cortisol in men and women who are obese, which appears to be especially striking in those with central or abdominal obesity (2, 3). By analogy with the clinical consequences of increased cortisol secretion in Cushing's syndrome, these observations raise the possibility that increased cortisol production is of pathophysiological significance in idiopathic central obesity. However, to date no consistent abnormality of central control of the hypothalamic–pituitary–adrenal (HPA) axis has been observed which would explain increased cortisol secretion in obesity. Moreover, unlike patients with Cushing's syndrome, subjects with idiopathic obesity do not have markedly elevated plasma cortisol concentrations. Indeed, although there is some flattening of the diurnal rhythm (4), peak plasma cortisol in the morning is generally lower in obese subjects (5, 6). Lower plasma cortisol in the face of elevated cortisol production suggests that the metabolic clearance rate for cortisol is increased, and that activation of the HPA axis is compensatory. Enhanced clearance of cortisol in obesity has been demonstrated experimentally using labelled cortisol tracer (7, 8). More recent studies have attributed this, at least in part, to increased activity of enzymes which metabolise cortisol in the liver, particularly 5α-reductase (9, 10). Nevertheless, although activation of the HPA axis secondary to increased cortisol metabolism does not increase circulating cortisol concentrations, it may have adverse effects, including increased adrenal androgen production (11).

An additional level of complexity involves the influence of peripheral cortisol metabolism on intracellular cortisol concentrations within target tissues. Intracellular cortisol levels are modulated independently of circulating cortisol levels according to the activity of locally expressed enzymes. The clearest example is the influence of the two isozymes of 11β-hydroxysteroid dehydrogenase (11HSD) (12). 11HSD type 2 (11HSD2) inactivates cortisol to cortisone and is responsible for preventing cortisol gaining access to mineralocorticoid receptors, e.g. in distal nephron. 11HSD type 1 (11HSD1) catalyses reactivation of cortisone to cortisol in most, if not all, tissues where it is expressed (13). These include liver and adipose tissue, where reactivation of cortisol has a potent influence on local glucocorticoid receptor activation. 11HSD1 knockout mice show evidence of lower glucocorticoid activity in liver (reduced gluconeogenesis, increased lipid catabolism) and adipose tissue (decreased free fatty acid, increased leptin, and resistance to obesity on high-fat feeding) (14, 15). In contrast, mice with transgenic overexpression of 11HSD1 under the adipose-specific AP2 promoter have central obesity and its metabolic complications (hyperglycaemia, increased free fatty acids, insulin resistance) (16). In idiopathic obesity in man, tissue-specific disruption of 11HSD1 activity results in increased local generation of cortisol in subcutaneous adipose tissue (17–20) but decreased cortisol in liver (17, 19, 21). 11HSD1 has therefore been proposed as a key determinant of the metabolic consequences of obesity and a potentially exciting therapeutic target (22–25).

The underlying mechanisms for these complex changes in cortisol metabolism in obesity are not known. In the Zucker obese rat, a model which recapitulates changes in A-ring reductases (5α- and 5β-reductases) and 11HSD1 observed in human idiopathic obesity (26), altered glucocorticoid-metabolising enzymes are normalised by preventing weight gain by adrenalectomy (27). Whether differences in cortisol production or metabolism between lean and obese men and women reflect constitutive differences, or are secondary to weight gain, has not been tested. In the current study, to assess plasticity of cortisol metabolism and associated increased cortisol production in obesity, we examined effects of acute weight loss, induced by fasting or by very low calorie diet (VLCD), on cortisol production and metabolism in obese men.

Subjects and methods

Participants

Otherwise healthy, obese (body mass index (BMI) 30–40 kg/m^2) males aged 20–55 years were recruited by newspaper advertisement. Subjects were only included if they were not on any special religious or prescribed diet; were non-smokers; had stable weight (weight change of no more than 2 kg in the previous 3 months); had normal medical examination, screening blood tests (full blood count, renal, liver and thyroid function) and electrocardiogram; and took no regular prescribed medication, vitamin or mineral supplements. These inclusion criteria were also checked with the participant's primary care physician. The study was approved by the Joint Ethical Committee of Grampian Health Board and the University of Aberdeen. Written informed consent was obtained.

Study protocols

Participants were admitted to the Human Nutrition Unit at the Rowett Research Institute, Aberdeen. They were resident in the Unit at night for the duration of the study (5–7 weeks), and during the daytime when calorimetry and other intensive measurements were being performed, but were allowed to leave the Unit during the day at other times to attend their workplace or home. All food and drink consumed during weight loss and weight maintenance was supplied by dietetic staff in the Unit, with food weighed before and after consumption to determine intake.

On days 1–6 (maintained baseline period), subjects consumed a mandatory maintenance diet (13% protein, 30% fat and 57% CHO), calculated to meet energy requirements (estimated at 1.6 × resting metabolic rate, RMR). Following this, subjects were allocated randomly to one of two weight loss diets, either starvation for 6 days or VLCD for 3 weeks (weight loss period). Starvation was intended to lose ~5% of original body weight; VLCD was intended to lose ~10% of original body weight. During starvation, subjects were only allowed to consume water. The VLCD comprised: daily weight 642 g, energy 2.55 MJ, protein 49.4 g (0.84 MJ), carbohydrate 0.85 MJ, and fat 0.85 MJ. After the period of weight loss, subjects were maintained at their new lower body weight for 1 week (reduced weight maintenance period). During this time, subjects were fed a diet calculated at 1.4 × RMR. Finally, subjects were studied while feeding *ad libitum* for 2 weeks, determining their own energy and nutrient intake from a selection of 45 supermarket type foods, as previously described (28).

In order to maintain a habitual level of energy expenditure, the subjects had compulsory exercise targets to achieve every day during the weight loss periods. The starvation group volunteers had two cycling periods of 40 min at 65–75 W and two step box sessions to maintain energy expenditure at approximately 1.5–1.6 × RMR, similar to an average everyday level of activity (29). This was necessary because subjects within the starvation group were resident in a whole-body calorimeter (dimensions, 3.5 × 1.9 × 2.2 m) for the duration of the fast and were otherwise inactive. The VLCD group were not resident in the calorimeter throughout their weight loss period, and they exercised

for 40 min per day on a bicycle ergometer (Tunturi, Helsinki, Finland) at 50–90 W. The volunteers were requested not to undertake any other strenuous physical activity during the study. Total 24 h energy expenditure was estimated from heart rate measurements (Polar Sports tester, Polar Electro Oy, Kempele, Finland) (30). To equate heart rate to energy expenditure, a regression line between these variables was established individually for each subject, weekly, by simultaneously measuring heart rate, breath-by-breath VO_2 and VCO_2 (Vmax29 metabolic cart, Sensor Medics, Yarba Linda, CA, USA) at incremental workloads, on a bicycle ergometer (Tunturi E850, Tunturi, Finland), using the modified FLEX method (31).

Clinical measurements

Subject height was measured to the nearest 0.1 cm using a stadiometer (Holtain Ltd Crymych, Dyfed, Wales, UK). Body weight was measured daily, to the nearest 50 g, by a member of staff, before eating and after emptying the bladder, with subjects wearing the same dressing gown each morning on a portable digital scale (DIGI DS-410, CMS Weighing Equipment, London, UK). Body composition was assessed by a four-compartment model (32), including dual energy X-ray absorptiometry scanning (DEXA; Norland XR-26, Mark II-high speed pencil beam scanner, Norland Corporation, White Plains, NY, USA, equipped with dynamic filtration, with version 2.5.2 of the Norland software), air displacement whole body plethysmography (BodPod Body Composition System, Life Measurement Instruments, Concord, CT, USA), and total body water by deuterium dilution (33).

Compliance with the dietary regime was monitored by daily body weight, respiratory quotient (RQ) and plasma concentrations of β-hydroxybutyrate and glucose (data not shown).

For assessment of cortisol metabolites, a 24 h urine collection was obtained in both groups at the end of the baseline period, after 5% weight loss, and at the end of 2 weeks of feeding *ad libitum*. In addition, in the VLCD group, urine was collected after 10% weight loss, and at the end of the reduced maintenance period after weight loss. Blood samples were obtained from indwelling cannulae for plasma cortisol and corticosteroid binding globulin (CBG) measurement at 0900 h and 1815 h in both groups at the end of the baseline period, after 5% weight loss, and at the end of the weight maintenance period.

Laboratory measurements

Plasma cortisol (34) and CBG (Medgenix Diagnostics, Brussels, Belgium) were measured by radioimmunoassays. Cortisol and its metabolites were measured in urine by electron impact gas chromatography/mass spectrometry following Sep-Pak C18 extraction, hydrolysis with β-glucuronidase, and formation of the methoxime-trimethylsilyl derivatives (35). Epi-cortisol and epi-tetrahydrocortisol were used as internal standards. Total cortisol metabolite excretion was calculated as tetrahydrocortisols (THFs) + tetrahydrocortisone (THE) + cortols + cortolones (36) (Fig. 1). Relative metabolism by 5α- and 5β-reductases were inferred from the 5β-THF/5α-THF ratio. A-ring reduction of cortisol was inferred from the ratios of THFs/cortisol (37) and 5β-reductase activity from the ratio of THE/cortisone. Whole-body equilibrium between cortisol and cortisone, determined by the balance of tissue-specific activities of 11β-reductase and 11β-dehydrogenase activities, was inferred from the ratio of THFs/THE. Renal 11β-dehydrogenase activity was inferred from the urinary free cortisol/cortisone ratio (35, 38).

Statistics

The starvation and VLCD studies were analysed independently. Data are expressed as mean±S.E.M., and were compared by repeated measures ANOVA with *post hoc* paired Student's *t*-tests where appropriate.

Results

Weight loss and body composition

Baseline characteristics of participants are shown in Table 1. Participants in the VLCD group tended to be older with higher % body fat than participants in the

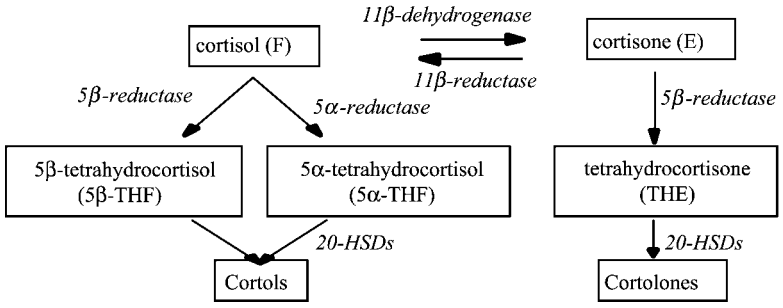

Figure 1 Principal pathways of metabolism of cortisol. 20-HSDs = 20α- and 20β-hydroxysteroid dehydrogenases.

Table 1 Baseline characteristics of participants.

	Starvation study	VLCD study
Number of men	6	6
Age (years)	39±5 (19–52)	46±4 (28–56)
Height (m)	1.76±0.02 (1.67–1.84)	1.75±0.02 (1.68–1.83)
Body weight (kg)	107.2±4.7 (93.8–123.5)	107.3±6.1 (85.2–124.1)
BMI (kg/m^2)	34.7±1.0 (30.9–38.5)	34.9±1.4 (30.3–39.5)
Body fat (% of body weight)	36.1±1.5 (32.4–41.6)	41.9±1.7 (35.5–46.6)
Systolic blood pressure (mmHg)	118±2 (117–122)	137±6 (117–160)
Diastolic blood pressure (mmHg)	77±3 (68–85)	86±6 (69–95)
Fasting plasma glucose (mM)	5.3±0.2 (4.7–5.8)	5.8±0.2 (5.5–6.5)

Data are means±S.E.M. (range).

starvation study. Changes in body weight and % body fat are shown in Table 2.

Urinary cortisol metabolites

VLCD was associated with reduced urinary excretion of cortisol and all of its principal metabolites (Fig. 2 and Table 3). This was progressive, with some reduction after 5% weight loss, a further reduction after 10% weight loss, further reduction still during the week of weight maintenance diet, and then 'recovery' towards baseline values after 2 weeks of being allowed to feed *ad libitum*. VLCD also had effects on cortisol metabolism, as judged by ratios of urinary metabolites (Table 3). There was no change in ratios reflecting 11HSD activities (either cortisol/cortisone

Table 2 Changes in body composition measured using the four-compartment model (32).

Measurement	Phase of study	Starvation	VLCD
Body weight (kg)	Maintained basal	107.2±4.7	107.3±6.1
	5% weight loss	101.1±5.0***	102.1±5.8***
	10% weight loss		98.1±5.8***
	Maintained weight loss	102.4±4.9***	97.9±5.8***
	Ad libitum	100.6±5.0***	96.3±5.3***
BMI (kg/m^2)	Maintained basal	34.7±1.0	34.9±1.4
	5% weight loss	32.7±1.1***	33.1±1.5***
	10% weight loss		31.8±1.5***
	Maintained weight loss	33.1±1.1***	31.8±1.5***
	Ad libitum	32.6±1.2***	31.2±1.4***
% Body fat	Maintained basal	36.1±1.5	41.9±1.7
	5% weight loss	35.0±1.4***	40.5±1.9***
	10% weight loss		38.2±2.0***
	Maintained weight loss	33.2±1.6***	36.9±2.2***
	Ad libitum	32.5±1.8***	36.3±2.0***
Fat mass (kg)	Maintained basal	38.8±2.7	45.3±4.1
	5% weight loss	35.5±2.6***	41.7±3.9***
	10% weight loss		37.9±3.9***
	Maintained weight loss	34.1±2.8***	36.4±3.9***
	Ad libitum	33.0±3.2***	35.1±3.3***
Fat-free mass (kg)	Maintained basal	68.4±2.9	62.0±2.8
	5% weight loss	65.6±3.1***	60.4±2.9***
	10% weight loss		60.3±2.8***
	Maintained weight loss	68.3±2.9	61.5±3.1***
	Ad libitum	67.6±2.7	61.1±3.3***
Total body water (kg)	Maintained basal	50.8±1.9	46.7±2.1
	5% weight loss	47.6±2.3***	45.4±2.3
	10% weight loss		44.8±2.3***
	Maintained weight loss	50.1±2.1	45.6±2.5
	Ad libitum	49.4±1.9	45.4±2.5
Bone mineral mass (kg)	Maintained basal	3.42±0.1	3.27±0.2
	5% weight loss	3.38±0.1	3.31±0.2
	10% weight loss		3.25±0.2
	Maintained weight loss	3.39±0.1	3.30±0.2
	Ad libitum	3.39±0.1	3.27±0.2

Data are means±S.E.M.
Versus maintained basal measurement: ***$P < 0.001$.

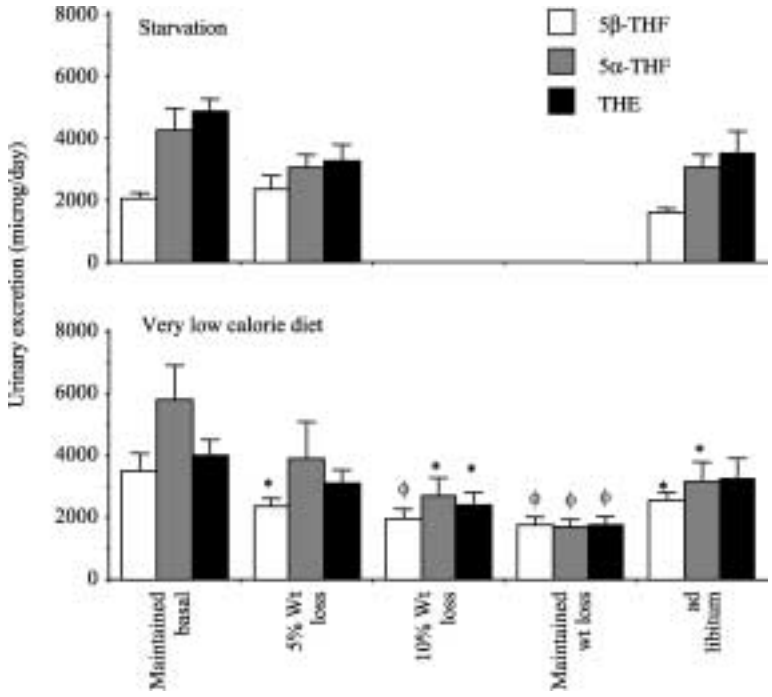

Figure 2 Twenty-four hour urinary cortisol metabolite excretion. Data are means±S.E.M. for $n = 6$ subjects in the starvation study (upper panel) or the VLCD study (lower panel). By repeated measures ANOVA, differences were significant only following VLCD ($P < 0.01$ for 5β-THF, $P < 0.02$ for 5α-THF, $P < 0.05$ for THE). In *post hoc* paired Student's *t*-tests, $^*P < 0.05$ and ϕ $P < 0.01$ compared with basal measurements.

or (5β- + 5α-THF)/THE). However, VLCD did induce a fall in 5α-reductase activity (5α-THF/cortisol) and, during weight maintenance only, reduced 5β-reductase metabolism of cortisone (THE/cortisone) but not cortisol (5β-THF/cortisol).

Fewer measurements were performed in the starvation study, but there were no statistically significant differences from baseline in absolute excretion of cortisol or cortisol metabolites after 5% weight loss or after subjects were allowed to feed *ad libitum*. However, starvation did affect cortisol metabolism, with increases in cortisol/cortisone and 5β-/5α-THF during starvation, which reverted to baseline values after subjects were allowed to feed *ad libitum*. The 5β-THE/cortisone ratio was also lower during the *ad libitum* diet than at baseline.

Plasma cortisol

CBG did not change during VLCD or starvation studies (Table 3). Plasma cortisol and its diurnal variation between 0900 h and 1815 h were not significantly affected by VLCD (Fig. 3). However, during starvation, plasma cortisol was markedly elevated both in the morning and evening compared with pre-starvation values (Fig. 3).

Discussion

These data show that acute weight loss (of ~10%) in obese men induced by VLCD over 3 weeks is associated with a substantial decline in cortisol production together with evidence of reduced metabolism of cortisol and cortisone, by 5α-reductase and 5β-reductase respectively, and no change in plasma cortisol. These findings are consistent with published evidence that activation of the HPA axis in obesity occurs as a compensatory response to increased peripheral clearance of cortisol (7, 8), particularly by A-ring reductases (9); thus, during calorie restriction, a decrease in peripheral cortisol clearance is associated with a compensatory fall in cortisol production to maintain a normal plasma cortisol. However, these effects of low calorie diet were acutely reversible when subjects returned to a diet where they were allowed to feed *ad libitum*, despite there being no regain of weight. This suggests that abnormalities in cortisol metabolism and HPA axis function in obesity are either only temporarily reversed by calorie restriction and/or weight loss in obesity, or are acutely responsive to nutritional status rather than to obesity *per se*.

More modest weight loss (of ~5%) induced more quickly by starvation appeared to show a different pattern of effects on cortisol. Fewer measurements were made during the starvation protocol, the time course of intervention was different, and the subjects were not well-matched with those in the calorie restriction study, so that direct comparison of the two groups is not appropriate. Nevertheless, in apparent contrast with men on VLCD, in starved subjects plasma cortisol rose and urinary cortisol metabolite excretion did not

Table 3 Effects of short-term weight loss on urinary cortisol metabolite excretion and plasma CBG.

Measurement	Phase of study	Starvation	VLCD
Total cortisol metabolite excretion (mg/day)	Maintained basal	16.1±1.5	15.8±1.1
	5% weight loss	11.9±20.0	13.4±0.7
	10% weight loss		9.8±1.8**
	Maintained weight loss		7.0±1.1***
	Ad libitum	12.1±1.8	13.1±1.7
5β-THF/5α-THF	Maintained basal	0.52±0.07	0.70±0.15
	5% weight loss	0.77±0.09**	0.75±0.12
	10% weight loss		0.83±0.12
	Maintained weight loss		1.03±0.08
	Ad libitum	0.57±0.09	0.98±0.21
(5β-THF + 5α-THF)/THE	Maintained basal	1.34±0.17	2.71±0.72
	5% weight loss	1.86±0.31	2.75±1.30
	10% weight loss		1.91±0.18
	Maintained weight loss		2.08±0.20
	Ad libitum	1.53±0.25	1.90±0.21
5β-THF/cortisol	Maintained basal	15.7±2.4	41.2±8.4
	5% weight loss	15.4±2.5	31.5±4.4
	10% weight loss		36.0±3.5
	Maintained weight loss		26.9±2.9
	Ad libitum	10.6±1.0	37.1±3.1
5α-THF/cortisol	Maintained basal	32.9±6.3	70.5±17.1
	5% weight loss	22.5±5.7	51.0±13.1
	10% weight loss		47.8±7.6
	Maintained weight loss		26.6±3.3**
	Ad libitum	22.0±4.7	44.8±6.7
THE/cortisone	Maintained basal	56.7±6.1	55.9±6.5
	5% weight loss	48.3±6.0	59.6±7.3
	10% weight loss		65.9±5.7
	Maintained weight loss		34.7±6.7*
	Ad libitum	43.3±6.5*	58.7±4.2
Cortisol/cortisone	Maintained basal	1.76±0.39	1.24±0.08
	5% weight loss	2.64±0.50*	1.46±0.10
	10% weight loss		1.53±0.15
	Maintained weight loss		1.28±0.15
	Ad libitum	1.99±0.32	1.36±0.15
CBG (μg/ml)	Maintained basal	32.4±2.2	30.6±4.7
	5% weight loss	41.8±6.0	33.0±3.3
	10% weight loss		33.7±3.6
	Maintained weight loss	26.7±3.2	22.2±4.0

Data are means±S.E.M.
Versus maintained basal measurement: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

significantly fall. Starvation induced a relative increase in urinary free cortisol, and a shift in favour of 5β- rather than 5α-reduction of cortisol. These features are in keeping with stress and 'central' activation of the HPA axis.

A few previous studies have assessed the HPA axis following weight loss, but have only examined plasma cortisol concentrations (39–41). These data are broadly in keeping with our findings, since they suggest that weight loss induced by calorie restriction produces either no change or a marginal increase in plasma cortisol (41, 42) and has no effect on suppression of plasma cortisol by dexamethasone (40), while starvation induces more marked elevation in cortisol levels in saliva (39) and plasma (43, 44). The lack of change in CBG is consistent with previous studies of acute starvation (44) or high fat feeding in burned patients (45), although there may be an effect of high protein intake to increase CBG levels (46). To our knowledge, no previous studies have examined effects of weight loss on cortisol secretion rate or metabolism in humans.

The most striking finding in our data is the observation that total cortisol metabolite excretion falls substantially during weight loss with VLCD. We were unable to administer 'placebo' dietary manipulations, so that comparison is made with baseline measurements. Cortisol production might be expected to fall as subjects acclimatised to the study environment. However, it appears unlikely that such an 'order effect' explains these results. The stress response to a new environment is manifest as increased plasma cortisol especially in the evening, and usually resolves within 48 h (47); our subjects had basal measurements at the end of the first week of study and did not exhibit any fall in plasma cortisol later. Also, we included measurements during *ad libitum* feeding at the end of the dietary manipulations to show that the cortisol

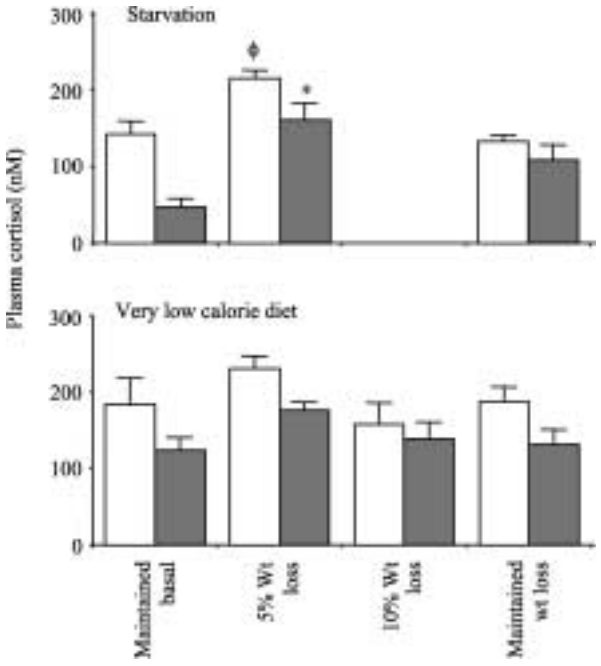

Figure 3 Plasma cortisol. Data are means±S.E.M. for $n = 6$ subjects in the starvation study (upper panel) or the VLCD study (lower panel). By repeated measures ANOVA, differences were significant only following starvation ($P < 0.001$ for 0930 h cortisol and $P < 0.05$ for 1815 h cortisol). In *post hoc* paired Student's *t*-tests, *$P < 0.05$ and ϕ $P < 0.01$ compared with basal measurements at the same time of day.

measurements returned towards baseline with a return to unrestricted food intake. However, it is notable that, during the *ad libitum* period, participants did not actually gain weight (Table 2). This is probably because of self-imposed calorie restriction by the participants at the end of the study, because they were motivated by a desire to lose weight and maintain their weight loss. It is possible that the effects of weight loss are transient and that measurements would have reverted towards baseline even if formal calorie restriction had been maintained and weight loss continued. Nonetheless, this observation suggests that the influence of VLCD on the HPA axis is mediated by nutritional signals which alter in advance of changes in weight.

The cause of the activation of the HPA axis in obesity is contentious. The failure of many studies to document any consistent abnormality of central feedback or 'feed forward' control of the axis, together with the key observation that plasma cortisol levels are not elevated in obesity, has reinforced the hypothesis that activation of the axis is secondary to enhanced metabolic clearance of cortisol. The current study does not test this hypothesis directly, but the findings are consistent with it, since the reduction of cortisol production induced by VLCD was accompanied by small but significant changes in urinary ratios reflecting the flux of metabolites through different pathways of cortisol metabolism.

Alterations in peripheral cortisol metabolism in obesity have been characterised only recently. It appears that two discrete components contribute to enhanced metabolic clearance rate of cortisol: inactivation of cortisol by 5α-reductase and 5β-reductase is enhanced (9, 10), while reactivation of cortisol from cortisone by 11HSD1 in liver is impaired (17, 19, 21). In contrast, however, intra-adipose reactivation of cortisone to cortisol by 11HSD1 is enhanced in obesity (17, 19, 20). The changes in 5α- and 5β-reductases are detected as increased relative excretion of their products in urine (see Fig. 1). However, probably because of up-regulation of 11HSD1 in liver and down-regulation in adipose tissue, urinary cortisol/cortisone metabolite ratios are unpredictable in obesity, being higher when increased fat mass predominates (e.g. in women (9, 19)) and lower perhaps when decreased hepatic 11HSD1 predominates (17, 21). Thus, changes in enzyme activity with weight loss may be easier to detect for 5α- and 5β-reductase than for 11HSD1. The same pattern of tissue-specific alterations in glucocorticoid metabolism has been described in Zucker obese rats (26). In these animals, when weight gain was prevented by adrenalectomy, alterations in glucocorticoid-metabolising enzymes largely resolved (27). Here, we found evidence for normalisation of 5α- and 5β-reductase with weight loss, but could not detect changes in 11HSD1. More detailed studies, for example measuring *in vivo* conversion of cortisone to cortisol (17, 19, 21), using deuterated cortisol tracer (48) or taking adipose biopsies (17) would be required to exclude changes in 11HSD1 during weight loss. In the current studies, repeated adipose biopsies were not taken because we judged that the protocol was already sufficiently invasive for the subjects, and dynamic tests involving cortisone administration were not performed to avoid confounding effects of exogenous glucocorticoids during nutritional manipulation.

The mechanism for the increase in A-ring reductase activities in obesity, and normalisation of this abnormality with weight loss, is obscure. There are two isozymes of 5α-reductase and both probably contribute to cortisol metabolism, since the type 1 predominates in liver while the type 2 inhibitor finasteride alters urinary cortisol metabolite excretion (49). Information on promoter regulation of these genes remains scanty and relates predominantly to sex steroid regulation (50, 51), so it is unclear whether they are transcriptionally controlled by 'nutrient' receptors such as peroxisome proliferator activating receptor (PPARs). Importantly, the present results suggest that abnormalities in cortisol metabolism in obesity are potentially reversible, but apparently only in the short term. Whether longer term weight loss achieved by less dramatic nutritional manipulation would reverse abnormalities in A-ring reductase activities and HPA axis function in obesity requires separate investigation. The current data suggest, however, that acute changes

in nutritional status can alter cortisol metabolism in humans and that the signals for this process may not be closely associated with body fat content. More detailed dissection of metabolic signals associated with altered cortisol secretion and metabolism during nutritional manipulation is now justified.

The apparently discrepant changes in cortisol measurements when 5% weight loss was induced by starvation or VLCD are intriguing. These results must be interpreted with caution as the two groups are not comparable in several respects. With complete starvation, we interpret the increased plasma cortisol levels as reflecting a stress response, which presumably obscures any tendency for cortisol production rate to fall with weight loss. With increased ACTH-dependent cortisol production, inactivation of cortisol to cortisone by 11HSD2 may be impaired (52, 53), resulting in elevated urinary free cortisol/cortisone (38). The elevation of 5β-THF/5α-THF in this circumstance is more difficult to explain, since increased cortisol production rate is usually associated with increased 5α-THF excretion (54), but it may reflect a similar effect of weight loss to normalise 5α-reductase activity as is observed in the 5α-THF/cortisol ratio with VLCD. This might explain why total cortisol metabolite excretion is not increased during what we interpret as stressful activation of the HPA axis with starvation. Further, it appears that the HPA axis activation in response to starvation is relieved when subjects are allowed to feed *ad libitum* in advance of any regain of body weight. This may reflect a temporary response of the HPA axis to starvation, removal of the psychological stress of maintaining a starvation diet, or reversal of a direct signal of nutritional deprivation.

What implications do these results have for therapy in obesity? Clearly, the results in these acute studies are not generalisable to the dietary management of obese patients. Further, the participants here represent an unusual group of highly motivated and otherwise healthy individuals. Nonetheless, one might speculate that different cortisol responses to different dietary manipulations contributes to their success in improving the metabolic profile. Thus, the failure of acute starvation, and relative success of sustained low calorie diets, in inducing sustained weight loss, might conceivably reflect contrasting effects on cortisol production. If maintained in the longer term, the results could also provide an explanation for the clinical benefits of weight loss in hyperandrogenised women. Reduced ACTH-dependent cortisol production will also be accompanied by reduced adrenal androgen synthesis, probably contributing to beneficial reproductive function with weight loss in polycystic ovarian disease (55). These results suggest that interventions which normalise cortisol metabolism in obesity, including 5α-reductase inhibitors, should be tested for their contribution to normalisation of cortisol and androgen production, and potential normalisation of metabolic profile in obesity. Finally, these data raise the intriguing possibility that peripheral cortisol metabolism is acutely regulated by nutritional intake, and that tissue cortisol exposure is modified as part of the physiological response to variations in nutrient availability. In this light, the disruption of cortisol metabolism in obese individuals may represent a maladaptive physiological response.

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References

1 Bjorntorp P. Endocrine abnormalities in obesity. *Diabetes Reviews* 1997 **5** 52–68.
2 Marin P, Darin M, Amemiya T, Andersson B, Jern S & Bjorntorp P. Cortisol secretion in relation to body fat distribution in obese premenopausal women. *Metabolism* 1992 **4** 1882–1886.
3 Pasquali R, Cantobelli S, Casimirri F, Capelli M, Bortoluzzi L, Flamia R *et al.* The hypothalamic–pituitary–adrenal axis in obese women with different patterns of body fat distribution. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 341–346.
4 Rosmond R, Dallman MF & Bjorntorp P. Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and haemodynamic abnormalities. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1853–1859.
5 Ljung T, Andersson B, Bengtsson B, Bjorntorp P & Marin P. Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose–response study. *Obesity Research* 1996 **4** 277–282.
6 Walker BR, Soderberg S, Lindahl B & Olsson T. Independent effects of obesity and cortisol in predicting cardiovascular risk factors in men and women. *Journal of Internal Medicine* 2000 **247** 198–204.
7 Strain GW, Zumoff B & Strain JJ. Cortisol production in obesity. *Metabolism: Clinical and Experimental* 1980 **29** 980–985.
8 Lottenberg SA, Giannella-Neto D, Derendorf H, Rocha M, Bosco A, Carvalho SV *et al.* Effect of fat distribution on the pharmacokinetics of cortisol in obesity. *International Journal of Clinical Pharmacology and Therapeutics* 1998 **36** 501–505.
9 Andrew R, Phillips DIW & Walker BR. Obesity and gender influence cortisol secretion and metabolism in man. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1806–1809.
10 Fraser R, Ingram MC, Anderson NH, Morrison C, Davies E & Connell JMC. Cortisol effects on body mass, blood pressure, and cholesterol in the general population. *Hypertension* 1999 **33** 1364–1368.
11 Stewart PM, Shackleton CHL, Beastall GH & Edwards CRW. 5alpha-reductase activity in polycystic ovarian syndrome. *Lancet* 1990 **335** 431–433.
12 Stewart PM & Krozowski ZS. 11Beta hydroxysteroid dehydrogenase. *Vitamins and Hormones* 1999 **57** 249–324.
13 Seckl JR & Walker BR. 11β-Hydroxysteroid dehydrogenase type 1 – a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 2001 **142** 1371–1376.
14 Kotelevtsev YV, Holmes MC, Burchell A, Houston PM, Scholl D, Jamieson PM *et al.* 11β-Hydroxysteroid dehydrogenase type 1

knockout mice show attenuated glucocorticoid inducible responses and resist hyperglycaemia on obesity and stress. *PNAS* 1997 **94** 14924–14929.
15 Morton NM, Holmes MC, Fievet C, Staels B, Tailleux A, Mullins JJ *et al.* Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11β-hydroxysteroid dehydrogenase type 1 null mice. *Journal of Biological Chemistry* 2001 **276** 41293–41300.
16 Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR *et al.* A transgenic model of visceral obesity and the metabolic syndrome. *Science* 2001 **294** 2166–2170.
17 Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DEW, Johnson O *et al.* Tissue-specific dysregulation of cortisol metabolism in human obesity. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1418–1421.
18 Lindsay RS, Tataranni A, Permana P, Livingstone DEW, Wake DJ & Walker BR. Subcutaneous adipose 11β-hydroxysteroid dehydrogenase type 1 activity and mRNA levels are associated with adiposity and insulinaemia in Pima Indians and Caucasians. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 2738–2744.
19 Rask E, Walker BR, Soderberg S, Livingstone DEW, Eliasson M, Johnson O *et al.* Tissue-specific changes in peripheral cortisol metabolism in obese women increased adipose 11β-hydroxysteroid dehydrogenase type 1 activity. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 3330–3336.
20 Paulmyer-Lacroix O, Boullu S, Oliver C, Alessi M-C & Grino M. Expression of the mRNA coding for 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue from obese patients: an *in situ* hybridization study. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 2701–2705.
21 Stewart PM, Boulton A, Kumar S, Clark PMS & Shackleton CHL. Cortisol metabolism in human obesity: impaired cortisone–cortisol conversion in subjects with central adiposity. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 1022–1027.
22 Walker BR, Connacher AA, Lindsay RM, Webb DJ & Edwards CRW. Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 3155–3159.
23 Bujalska IJ, Kumar S & Stewart PM. Does central obesity reflect 'Cushing's disease of the omentum'? *Lancet* 1997 **349** 1210–1213.
24 Alberts P, Engblom L, Edling N, Forsgren M, Klingstrom G, Larsson C *et al.* Selective inhibition of 11beta-hydroxysteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice. *Diabetologia* 2002 **45** 1528–1532.
25 Andrews RC, Rooyackers O & Walker BR. Effects of the 11beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone on insulin sensitivity in men with type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 285–291.
26 Livingstone DEW, Jones GC, Smith K, Andrew R, Kenyon CJ & Walker BR. Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology* 2000 **141** 560–563.
27 Livingstone DEW, Kenyon CJ & Walker BR. Mechanisms of dysregulation of 11β-hydroxysteroid dehydrogenase type 1 in Zucker obese rats. *Journal of Endocrinology* 2000 **167** 533–539.
28 Stubbs RJ, O'Reilly LM, Johnstone AM, Franklin MF & Reid C. Description and evaluation of an experimental model to examine changes in qualitative and quantitative feeding behaviour in humans. *European Journal of Clinical Nutrition* 1999 **53** 13–21.
29 Black AE, Coward WA, Cole TJ & Prentice AM. Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. *European Journal of Clinical Nutrition* 1996 **50** 72–92.
30 Spurr GB, Prentice AM, Murgatroyd PR, Goldberg GR, Reina JC & Christman NT. Energy expenditure from minute-by-minute heart-rate recording: comparison with indirect calorimetry. *American Journal of Clinical Nutrition* 1988 **48** 552–559.
31 Ceesay SM, Prentice AM, Day KC, Murgatroyd PR, Goldberg GR & Scott W. The use of heart rate monitoring in the estimation of energy expenditure: a validation study using indirect whole body calorimetry. *British Journal of Nutrition* 1989 **71** 175–186.
32 Fuller NJ, Jebb SA, Laskey MA, Coward WA & Elia M. Four-compartment model for the assessment of body composition in humans: comparison with alternative methods and evaluation of the density and hydration of fat-free mass. *Clinical Science* 1992 **82** 687–693.
33 Pullicino E, Coward WA, Stubbs RJ & Elia M. Bedside and field methods for assessing body composition: comparison with the deuterium dilution technique. *European Journal of Clinical Investigation* 1990 **40** 753–762.
34 McConway MG & Chapman RS. Development and evaluation of a simple, direct, solid-phase radioimmunoassay of serum cortisol from readily available reagents. *Clinica Chimica Acta* 1986 **158** 59–70.
35 Best R & Walker BR. Additional value of measurement of urinary cortisone and unconjugated cortisol metabolites in assessing the activity of 11β-hydroxysteroid dehydrogenase *in vivo*. *Clinical Endocrinology* 1997 **47** 231–236.
36 Zumoff B, Fukushima DK & Hellman L. Intercomparison of four methods for measuring cortisol production. *Journal of Clinical Endocrinology and Metabolism* 1974 **38** 169–175.
37 Ulick S, Tedde R & Wang JZ. Defective ring A reduction of cortisol as the major metabolic error in the syndrome of apparent mineralocorticoid excess. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 593–599.
38 Palermo M, Shackleton CHL, Mantero F & Stewart PM. Urinary free cortisone and the assessment of 11beta-hydroxysteroid dehydrogenase activity in man. *Clinical Endocrinology* 1996 **45** 605–611.
39 Fichter MM, Pirke K-M & Holsboer F. Weight loss causes neuroendocrine disturbances: experimental study in healthy starving subjects. *Psychiatry Research* 1986 **17** 61–72.
40 Yanovski SZ, Yanovski JA, Gwirtsman HE, Bernat A, Gold PW & Chrousos GP. Normal dexamethasone suppression in obese binge and nonbinge eaters with rapid weight loss. *Journal of Clinical Endocrinology and Metabolism* 1993 **76** 675–679.
41 Wabitsch M, Hauner H, Heinze E, Bockmann A, Benz R, Mayer H *et al.* Body fat distribution and steroid hormone concentrations in obese adolescent girls before and after weight reduction. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 3469–3475.
42 Hainer V, Stich V, Kunesova M, Parizkova J, Zak A & Wernischova V. Effect of 4-wk treatment of obesity by very-low-calorie diet on anthropometric, metabolic, and hormonal indexes. *American Journal of Clinical Nutrition* 1992 **56** 281S–282S.
43 Haluzik M, Matoulek M, Svacina S, Hilgertova J & Haas T. The influence of short-term fasting on serum leptin levels, and selected hormonal and metabolic parameters in morbidly obese and lean females. *Endocrine Research* 2001 **27** 251–260.
44 Fraser DA, Thoen J, Selvaag A-M, Djoseland O, Forre O & Kjeldsen-Kragh J. A preliminary study of circadian serum cortisol concentrations in response to a 72-hour fast in rheumatoid arthritis patients not previously treated with corticosteroids. *Clinical Rheumatology* 2001 **20** 85–87.
45 Bernier J, Jobin N, Emptoz-Bonneton A, Pugeat MM & Garrel DR. Decreased corticosteroid-binding globulin in burn patients: relationship with interleukin-6 and fat in nutritional support. *Critical Care Medicine* 1998 **26** 452–460.
46 Anderson KE, Rosner W & Khan MS. Diet–hormone interactions: protein/carbohydrate ratio alters reciprocally the plasma levels of testosterone and cortisol and their respective binding globulins in man. *Life Sciences* 1987 **40** 1761–1768.
47 Kaye TB & Crapo L. The Cushing syndrome: an update on diagnostic tests. *Annals of Internal Medicine* 1990 **112** 434–444.
48 Andrew R, Smith K, Jones GC & Walker BR. Use of multideuterated cortisol to distinguish activities of 11β-hydroxysteroid dehydrogenases *in vivo* in man. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 277–285.

49 Imperato-McGinley J, Shackleton C, Orlic S & Stoner E. C19 and C21 5beta/5alpha metabolite ratios in subjects treated with the 5alpha-reductase inhibitor finasteride: comparison of male pseudohermaphrodites with inherited 5alpha-reductase deficiency. *Journal of Clinical Endocrinology and Metabolism* 1990 **70** 777–782.
50 Pratis K, O'Donnell L, Ooi GT, Stanton PG, McLachlan RI & Robertson DM. Differential regulation of rat testicular 5α-reductase type 1 and 2 isoforms by testosterone and FSH. *Journal of Endocrinology* 2003 **176** 393–403.
51 Matsui D, Sakari M, Sato T, Murayama A, Takada I, Kim M *et al.* Transcriptional regulation of the mouse steroid 5α-reductase type II gene by progesterone in brain. *Nucleic Acids Research* 2002 **30** 1387–1393.
52 Ulick S, Wang JZ, Blumenfeld JD & Pickering TG. Cortisol inactivation overload: a mechanism of mineralocorticoid hypertension in the ectopic adrenocorticotropin syndrome. *Journal of Clinical Endocrinology and Metabolism* 1992 **74** 963–967.
53 Walker BR, Campbell JC, Fraser R, Stewart PM & Edwards CRW. Mineralocorticoid excess and inhibition of 11β-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. *Clinical Endocrinology* 1992 **27** 483–492.
54 Phillipou G. Investigation of urinary steroid profiles as a diagnostic method in Cushing's syndrome. *Clinical Endocrinology* 1982 **16** 433–439.
55 Pasquali R, Antenucci D, Casimirri F, Venturoli S, Paradisi R, Fabbri R *et al.* Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. *Journal of Clinical Endocrinology and Metabolism* 1989 **68** 173–179.

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