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Tissue-specific dysregulation of cortisol metabolism in human obesity

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ABSTRACT Cortisol has been implicated as a pathophysiological mediator in idiopathic obesity, but circulating cortisol concentrations are not consistently elevated. The tissue-specific responses to cortisol may be influenced as much by local pre-receptor metabolism as by circulating concentrations. For example, in liver and adipose tissue cortisol is regenerated from inactive cortisone by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). In obese Zucker rats 11β-HSD1 activity is reduced in liver but enhanced in adipose tissue. This study addressed whether the same tissue-specific disruption of cortisol metabolism occurs in human obesity. 34 men were recruited from the MONICA population study in Northern Sweden to represent a wide range of body composition and insulin sensitivity. Plasma cortisol was measured at 0830h and 1230h, after overnight low-dose dexamethasone suppression, after intravenous corticotropin releasing hormone (CRH), and after oral cortisone administration. Urinary cortisol metabolites were measured in a 24 h sample. A subcutaneous fat biopsy was obtained from 16 participants to measure cortisol metabolism *in vitro*. Higher body mass index was associated with increased total cortisol metabolite excretion ($r=0.47$, $p<0.01$), but lower plasma cortisol at 1230 h and after dexamethasone, and no difference in response to CRH. Obese men excreted a greater proportion of glucocorticoid as metabolites of cortisone rather than cortisol ($r=0.43$, $p<0.02$), and converted less cortisone to cortisol after oral administration ($r=-0.49$, $p<0.01$), suggesting impaired hepatic 11β-HSD1 activity. By contrast, *in vitro* 11β-HSD1 activity in subcutaneous adipose tissue was markedly enhanced in obese men ($r=0.66$, $p<0.01$). We conclude that in obesity, reactivation of cortisone to cortisol by 11β-HSD1 in liver is impaired, so that plasma cortisol levels tend to fall, and there may be a compensatory increase in cortisol secretion mediated by a normally functioning hypothalamic-pituitary-adrenal axis. However, changes in 11β-HSD1 are tissue-specific: strikingly enhanced reactivation of cortisone to cortisol in subcutaneous adipose tissue may exacerbate obesity; and it may be beneficial to inhibit this enzyme in adipose tissue in obese patients.

In the last 15 years it has been recognised that the effects of cortisol depend as much on tissue-specific responses as they do on circulating cortisol concentrations. Of key importance is the pre-receptor metabolism of cortisol in each tissue which dictates the balance between active and inactive steroids. Thus, in the distal nephron, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts cortisol to inactive cortisone; failure of this inactivation in congenital deficiency or after liquorice administration results in cortisol-dependent mineralocorticoid excess and hypertension (1). In other tissues, a different protein, 11β-HSD type 1, reactivates cortisone to active cortisol. This reactivation appears to be important to maintain adequate cortisol levels in sites where glucocorticoid receptors regulate crucial metabolic functions, eg in liver and adipose tissue (2-4).

Subtle changes in 11β-HSD activity may be important in common clinical syndromes. Given its clinical similarities to Cushing's syndrome, many investigators have considered cortisol as a pathophysiological mediator in idiopathic obesity. Although cortisol secretion rate is enhanced in obesity (5), this may be in proportion to increased lean body mass (6), and plasma cortisol levels are not consistently elevated. Metabolic clearance rate for cortisol is also increased in obesity (7). This has been explained recently by observations that reactivation of cortisone to cortisol by 11β-HSD1 in liver is impaired, and inactivation of cortisol by 5α-reductase enzymes is enhanced (8;9). These changes in metabolism predict lower plasma cortisol levels which, by negative feedback, could explain a compensatory rise in ACTH and cortisol secretion. If any increase in cortisol secretion is only to compensate for enhanced peripheral metabolism, does this mean that the tissue actions of cortisol are unaffected, ie that cortisol does not contribute to obesity and its metabolic complications?

In an animal model, the leptin-resistant obese Zucker rat, we have recently described tissue-specific changes in glucocorticoid metabolism (10). Clearance of corticosterone (the rat equivalent of cortisol) is enhanced in livers of obese animals as it is in man, due to increased 5α-reductase and impaired 11β-HSD1 activities. However, reactivation of corticosterone by 11β-HSD1 is enhanced in adipose tissue from obese rats. Moreover, obesity in these animals is glucocorticoid-dependent (11). This raises the possibility that, in the face of greater inactivation of glucocorticoid by metabolic pathways in liver, there is also enhanced reactivation of glucocorticoid in adipose tissue which might contribute to the obese phenotype. In this report we describe the same pattern of tissue-specific dysregulation of 11β-HSD1 in human obesity. These data support a new model to explain the contribution of cortisol to obesity, which provides opportunities for novel therapeutic intervention.

Materials and Methods

Participants were recruited from members of the MONICA population sample who live in the health care district of Umeå or

Luleå hospitals in Northern Sweden (12). To ensure representation from a wide range of body composition, men were selected at random from the highest and lowest quartiles of fasting plasma insulin concentrations. Thirty-five of 40 men approached agreed to participate, but one was excluded because of intermittent use of oral glucocorticoids. Characteristics of the remaining 34 are shown in Table 1. Two participants were receiving low-dose inhaled glucocorticoids (budesonide ≤400 μg/24h); two were receiving aspirin after a stroke (with no sequelae) and after mitral-valve surgery, respectively; three were receiving antihypertensive treatment (with β-blocker, ACE inhibitor, and/or calcium channel blocker). None had other clinical features of Cushing's syndrome. Diabetes mellitus and thyroid dysfunction were excluded by laboratory tests. Approval of Umeå University Hospital ethics committee and written informed consent were obtained.

Clinical measurements were made on different days separated by >24 h as follows. Days 3-5 were in random order. *Day 1)* Baseline anthropometry, blood pressure, and body composition (Akern-RJL System bioelectrical impedance instrument, EL.Dot, Fredriksvaerk, Denmark) were measured. *Day 2)* Insulin sensitivity was measured using the euglycaemic hyperinsulinaemic clamp technique, with insulin infused at 56 mU/m²/min for 110 minutes and plasma glucose maintained at 4.6±0.1 mM. *Day 3)* Plasma was obtained at 0830 h and a human CRH test (1μg/kg body weight iv) was performed at 1230 h with blood samples drawn every 15 min for 2 h; subjects did not eat until 1430 h. *Day 4)* Cortisol and its metabolites were measured in a 24 h urine sample by gas chromatography and electron impact mass spectrometry (8). *Day 5)* Conversion of cortisone to cortisol by 11β-HSD1 on first pass through the liver was measured *in vivo* after subjects took oral dexamethasone (3.5 μg/kg body weight as suspension) at 2300 h, fasted overnight, and attended at 0830 h for intravenous cannulation and oral cortisone acetate (25 mg with water)(13). Cortisol (Orion Diagnostica, PO Box 83, FIN-02101 Espoo, Finland) and corticosterone (14) were measured by radioimmunoassay on blood samples withdrawn until 1230 h. *Day 6)* Adipose 11β-HSD1 activity was measured *in vitro*. Sixteen subjects consented to return for a ~500 mg subcutaneous fat biopsy to be taken from the abdominal region under local anaesthesia. Subcutaneous fat was frozen immediately at -70C. After thawing, it was homogenised in Krebs buffer at pH 7.4 and 750 μg/ml protein was incubated at 37C with NADP 2mM and 1,2,6,7-$^3H_4$-cortisol 100 nM for 30 hours, with samples withdrawn at 3, 6, 20 and 30 hours for separation of cortisol and cortisone by HPLC with on-line liquid scintillation detection (10). 11β-HSD1 activity was measured in the dehydrogenase direction (ie cortisol to cortisone, rather than reductase cortisone to cortisol) because this is the preferred reaction when the enzyme is liberated from its intracellular environment (15); when driven with excess cofactor, this activity is proportional to total protein in whichever direction the reaction is measured (10). In these conditions, there was no evidence of conversion of cortisol to other metabolites, such as 5α-reduced cortisol.

Areas under curves of plasma cortisol after CRH or cortisone and of *in vitro* 11β-HSD1 activity were calculated by the trapezoidal rule. Logarithmic transformation was applied to obtain normal distribution of data for body mass index, waist/hip ratio, 0830 h plasma cortisol and corticosterone concentrations, urinary cortisol metabolites and their ratios, M/I insulin sensitivity index, area under curve of plasma cortisol after cortisone, and area under curve of *in vitro* 11β-HSD1 activity. Pearson correlation analyses and adjustments for potential confounding variables by partial correlation analyses were performed as indicated in the text.

Table 1. Subject characteristics and biochemical data

Tertile of BMI:	Lowest	Middle	Highest
n	11	11	12
Age (y)	46.8±8.7	49.6±8.5	51.9±12.1
Tobacco users	3	4	2
Body Mass Index (kg/m²)	22.9±1.4	26.4±0.7	31.7±4.0
% Body fat[c]	17.6±3.5	22.5±5.6	33.0±11.8
Waist/Hip ratio[c]	0.87±0.03	0.96±0.04	1.00±0.08
Insulin sensitivity (M/I)[c]	10.2±4.4	7.5±3.6	3.5±1.8
Systolic/diastolic[a] blood pressure (mmHg)	127/77 ±22/11	132/83 ±21/7	143/85 ±12/11
Urine cortisol metabolites (mg/day):			
Total[b]	11.3±5.1	12.8±5.3	15.3±3.4
5α-Tetrahydrocortisol (5α-THF)	2.1±1.0	2.1±0.8	3.1±1.1
5β-THF	2.8±0.9	3.3±1.4	2.9±1.0
Tetrahydrocortisone (THE)[b]	4.4±2.0	4.9±2.6	7.7±2.9
(5αTHF+5βTHF)/THE[a]	1.18±0.28	1.29±0.58	0.87±0.37
5αTHF/5βTHF	0.78±0.31	0.71±0.29	1.13±0.35
plasma Cortisol (nM):			
at 0830 h	285±79	358±108	282±96
at 1230 h[a]	292±49	301±100	215±78
after CRH (area under curve nM.h)	854±378	859±231	817±210
at 0830 h after dexamethasone[b]	338±117	253±145	168±106
plasma Corticosterone (nM):			
at 0830 h after dexamethasone[a]	30±11	25±12	20±10
120 min after cortisone	24±9	25±14	19±9
240 min after cortisone	18±5	17±6	13±5

Data are mean ± SD. From Pearson correlations with body mass index (BMI): [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$. M/I=glucose infusion rate/plasma insulin concentration during euglycaemic clamp (mg glucose.kg body weight^{-1}.min^{-1}.mU/L x 100).

Results

Characteristics of participants are shown in the Table. Obese and lean subjects were well-matched for age and smoking. Generalised obesity, indicated by higher body mass index (Table 1), was associated with insulin resistance (r=-0.69), higher diastolic blood pressure (r=0.40), and higher total urinary cortisol metabolite excretion (r=0.48), but no difference in plasma cortisol at 0830 h, no difference in plasma cortisol or ACTH response to CRH, lower plasma cortisol at 1230 h (r=-0.42), and lower plasma cortisol (r=-0.52) and corticosterone (r=-0.42) at 0830 h after overnight dexamethasone suppression (Table 1 and Figure 1). Similar relationships were observed with total fat mass measured by bioimpedance, and with central obesity indicated by higher waist/hip ratio (data not shown). These associations persisted for urinary but not plasma measurements after adjustment for potential confounding factors, including age, smoking, and blood pressure. Adjustment for lean body mass did not affect

these relationships, except that the correlations between body mass index and total cortisol metabolite excretion (adjusted r=0.31, p=0.09) and 1230 h plasma cortisol (r=-0.33, p=0.07) were no longer statistically significant.

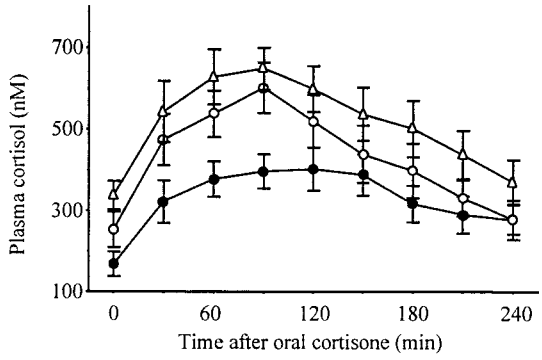

Figure 1 Plasma cortisol after overnight dexamethasone suppression and oral cortisone. Data are mean ± SE for subjects from lowest (open triangle), middle (open circle), and highest (filled circle) tertiles of body mass index. Plasma cortisol at 0830 h was lower in men with higher body mass index (r=-0.52, p<0.01). Area under curve of plasma cortisol was lower in men with higher body mass index (r=-0.49, p<0.01), independently of age, tobacco use, and blood pressure.

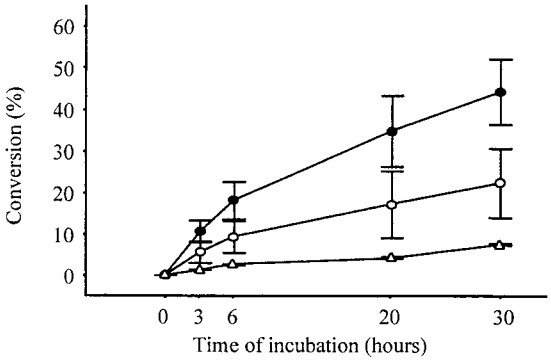

Figure 2 *In vitro* 11β-HSD1 activity in subcutaneous fat biopsy. Data are mean ± SE for % conversion of cortisol to cortisone at fixed protein concentration for subjects from lowest (open triangle, n=4), middle (open circle, n=7), and highest (filled circle, n=5) tertiles of body mass index. Areas under the curve were higher in men with higher body mass index (r=0.66, p<0.01), independently of age, tobacco use, and blood pressure.

Men with higher body mass index excreted relatively more cortisol in urine as derivatives of cortisone (tetrahydrocortisone; THE) than cortisol (Table 1), suggesting impaired reactivation of cortisone to cortisol by 11β-HSD1. Conversion of cortisone administered by mouth to cortisol in peripheral plasma was measured after overnight dexamethasone suppression, and was impaired in obese men (Figure 1). The basal 0830 h plasma cortisol before cortisone administration was lower in obese men. To assess the influence of this difference in endogenous cortisol on levels of plasma cortisol after cortisone administration we measured corticosterone, another ACTH-dependent adrenal steroid. Corticosterone was also lower after dexamethasone, but suppressed to a similar degree in obese and non-obese men after cortisone administration (Table 1). Thus, in this test, most cortisol in plasma is derived from exogenous cortisone rather than endogenous adrenal secretion. Obese men also showed trends towards higher excretion of 5α-tetrahydrocortisol (5α-THF) than 5β-THF (r=0.29).

There were no differences between participants who consented or did not consent to subcutaneous adipose biopsy (data not shown). By contrast with lower cortisol/cortisone urinary metabolite ratio and hepatic impaired conversion of cortisone to cortisol, obese men had substantially higher 11β-HSD1 activity measured *in vitro* in subcutaneous adipose tissue (Figure 2).

Insulin resistance, reflected in lower M/I values from a euglycaemic hyperinsulinaemic clamp, was associated with lower 0830 h plasma cortisol (r=0.59, p<0.001) and corticosterone (r=0.58, p<0.001) after dexamethasone and impaired conversion of cortisone to cortisol (r=0.64, p<0.001) but not with other indices of cortisol secretion or metabolism. After correction for the effect of body mass index in partial correlation analyses these relationships persisted regarding post-dexamethasone hormone levels but not for conversion of cortisone to cortisol (adjusted r=0.37, 0.43, 0.27 and p=0.03, 0.01, 0.13, respectively).

Discussion

There are several widely held explanations for increased secretion of cortisol in obesity. One is that increased cortisol secretion is appropriate to the increase in total body (lean plus fat) mass in obesity (6). A second invokes a primary neuroendocrine abnormality causing enhanced central drive to CRH, ACTH and cortisol secretion (5). A third invokes enhanced peripheral metabolism of cortisol with compensatory changes in the hypothalamic-pituitary-adrenal axis (7). In this study, we have investigated both central regulation of cortisol secretion and peripheral metabolism of cortisol. We found that increased lean body mass contributed to, but may not account entirely for, enhanced cortisol secretion. We found no evidence for enhanced central drive to cortisol secretion in these obese men, since responses to CRH were not altered and suppression with threshold doses of dexamethasone (approximately ED_{50} for suppression of plasma cortisol) was enhanced rather than impaired. More strikingly, however, our data support previous reports that peripheral metabolism of cortisol is increased due to a combination of enhanced 5α-reductase activity (8) and impaired regeneration of cortisol from cortisone by 11β-HSD1 in liver (9). One of the consequences is that levels of cortisone metabolites (THE) are elevated in obesity.

However, in animal studies dysregulation of 11β-HSD1 in obesity is tissue-specific, such that down-regulation in liver is accompanied by up-regulation in omental adipose tissue (10). Tissue-specific dysregulation of 11β-HSD1 could also explain conflicting reports of urinary cortisol/cortisone metabolite

ratios in human obesity (8;9;16), since there may be a balance between impaired regeneration of cortisol in liver and enhanced regeneration in other sites. Indeed, it has been hypothesised that increased 11β-HSD1 in omental adipose tissue is responsible for central obesity in man (4). The possibility of tissue-specific dysregulation of 11β-HSD1 is difficult to test in human studies. Measurements in omental fat have been limited to primary cell culture in non-obese subjects (4), and *in vivo* characteristics may not be retained in these cultured cells. Attempts to measure 11β-HSD1 *in vivo* in subcutaneous fat, either by arteriovenous sampling (17) or by *in vivo* microdialysis (authors' unpublished data), have produced highly variable results. In this study, we measured 11β-HSD1 activity in freshly obtained subcutaneous fat. As has been observed in extensive studies with animal tissues, 11β-HSD1 is a predominant dehydrogenase enzyme (converting cortisol to cortisone) in homogenised tissue, but the extent of this reaction reflects changes in 11β-HSD1 protein and reductase activity *in vivo* (10). The striking increase in activity in obese men predicts enhanced local reactivation of cortisone to cortisol in adipose tissue. In combination with increased supply of cortisone substrate, this predicts markedly enhanced intra-adipose cortisol levels.

We have not examined the mechanisms for tissue-specific dysregulation of peripheral cortisol metabolism, but we know that the relevant enzymes are regulated by insulin, growth hormone, and sex steroids, all of which are altered in obesity. Indeed, we found that insulin resistance was associated with impaired conversion of cortisone to cortisol in liver but not with differences in adipose 11β-HSD1 activity, but we could not confirm that this was independent of relationships with obesity. Whatever the mechanism, these observations reinforce the potential value of specific inhibitors of 11β-HSD1 to enhance insulin sensitivity (2) and limit weight gain in obesity.

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