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Obesity and Gender Influence Cortisol Secretion and Metabolism in Man

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ABSTRACT In obesity, urinary cortisol excretion is enhanced but plasma cortisol levels are not elevated, suggesting that metabolic clearance of cortisol is increased. Cortisol is metabolised in liver and fat by A-ring reductases but also regenerated from inactive cortisone in liver, fat, and skeletal muscle by 11β-reductase. These enzymes are regulated by estrogen. This study addressed whether there are differences in cortisol metabolism in obesity, and whether these differences are estrogen dependent.

31 men and 37 post-menopausal women (9 on estrogen replacement therapy) aged 47-53 y supplied 24 h urine for gas chromatography/mass spectrometry. Total cortisol metabolite excretion was higher in men than women, but weakly related to indices of obesity. By contrast, metabolism of cortisol favoured 5α- rather than 5β-reduction in obese men and obese women, and favoured cortisol rather than cortisone in obese men. In women compared with men ratios of 5α-/5β-reduced and cortisol/cortisone metabolites were also higher but these variables were not affected by estrogen replacement therapy.

We conclude that in obesity, inactivation of cortisol by 5α-reductase is enhanced but this is offset by impaired metabolism of cortisol by 5β-reductase in women and enhanced conversion of cortisone to cortisol by 11β-reductase in men. These observations suggest that cortisol clearance is altered in obesity, and this may account for activation of the hypothalamic-pituitary-adrenal axis. Moreover, these data predict that obese subjects will have higher concentrations of cortisol in key target tissues including liver and visceral fat. This may contribute to the adverse metabolic consequences of obesity.

Cushing's syndrome is characterised by central obesity. Conversely, primary idiopathic obesity is associated with elevated urinary free cortisol excretion (1), impaired suppression of plasma cortisol by dexamethasone (2), and enhanced sensitivity of plasma cortisol to exogenous ACTH (3). These abnormalities are most pronounced when the fat is distributed in a central or visceral pattern (4). As a result, the clinical distinction between primary obesity and Cushing's syndrome is not always easy.

However, the mechanism of activation of the hypothalamic-pituitary-adrenal axis in obesity is not established. Plasma cortisol levels are paradoxically lower in obese subjects (2) which, in the face of higher urinary cortisol excretion, suggests that the metabolic clearance rate of cortisol may be enhanced. Most metabolism of cortisol is by enzymes in the liver, including A-ring reductases (Figure 1), but additional metabolism occurs by 5α-reductase in fat and by 11β-dehydrogenase in kidney. Moreover, not all of these metabolic pathways are irreversible: the metabolic clearance rate of cortisol is influenced by the extent of regeneration of cortisol from inactive cortisone (5). This regeneration of cortisol, catalysed by 11β-reductase, occurs in many tissues including liver (6;7) and fat (8).

In the present report, we sought to relate indices of obesity with indices of cortisol secretion and metabolism by A-ring reductases, 11β-dehydrogenase, and 11β-reductase in a cross-sectional study of healthy men and post-menopausal women. Previous reports suggest that 11β-reductase (9;10) and 5α-reductase (11;12) activities are regulated by estrogen, so that our analysis took account of differences in gender and the potential influence of estrogen replacement therapy.

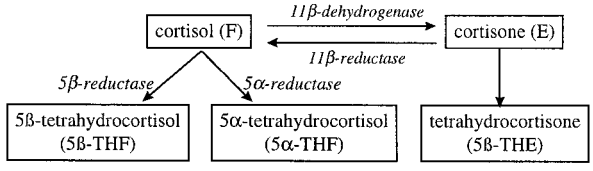

Figure 1. Principal urinary metabolites of cortisol measured by gas chromatography and mass spectrometry.

Materials and Methods

We studied 31 men and 37 post-menopausal women, aged 47-53 y, selected at random but with equal representation from each of the quintiles of insulin sensitivity defined previously in a cohort in Preston, Lancashire, UK (13). Their characteristics are shown in Table 1. Nine of the women were receiving estrogen replacement therapy. Studies were approved by the local ethical committee. Each subject collected a 24 h urine sample and had measurements of height, weight, and abdominal (at the level of the umbilicus) and hip (at the level of the greater trochanters) circumferences.

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 (14;15). Epi-cortisol and epi-tetrahydrocortisol were used as internal standards. Total cortisol metabolite excretion was calculated as tetrahydrocortisols (THFs) + tetrahydrocortisone (THE) + cortols + cortolones (16). Relative reduction by 5α- and 5β-reductases was inferred from the 5β-THF/5α-THF ratio. Total A-ring reduction of cortisol was inferred from the ratio of cortisol/THFs (17) and 5β-reductase activity from the ratio of cortisone/THE. 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 (15;18).

Results are mean±SD. Ratios of urinary cortisol metabolites were normalised by logarithmic conversion for statistical comparison but are presented in the tables as absolute values. Stepwise multiple regression analyses were performed to identify influences of gender (assigned values of 0 or 1), estrogen replacement therapy (0 or 1), and indices of obesity (height, weight, body mass index, waist/hip circumference ratio, abdominal circumference, and hip circumference) in predicting cortisol metabolites. These analyses identified gender, and hip and abdominal circumferences as the only independent correlates of cortisol metabolite excretion and ratios. However, there was covariance of gender and indices of obesity (e.g. there was no overlap in waist/hip ratios for men and women) which made it impossible to infer whether gender or body composition were independent determinants of urinary cortisol metabolites. For this reason, the relationships between indices of obesity and cortisol metabolite excretion were examined in men and women independently, including only those indices of obesity which were significant in the multiple regression analyses.

Results

Indices of obesity did not correlate with urinary free cortisol and correlated only weakly with total cortisol metabolite excretion in men (Table 2). In both men and women, increased abdominal and hip circumferences were associated with lower 5β-/5α-THF ratios. In men, but not women, indices of obesity were associated with increased ratios of THFs/THE (Figure 2).

Comparing men and women (Table 1), women had: (i) lower total cortisol metabolite excretion; (ii) lower ratio of 5β-/5α-reduced cortisol metabolites and a lesser proportion of cortisol excreted as A-ring metabolites (THFs or THE) rather than free cortisol or cortisone; and (iii) a greater proportion of cortisol excreted as THFs (from cortisol) rather than THE (from cortisone), but no difference in ratio of free cortisol/cortisone. None of these differences were affected by estrogen replacement therapy.

Discussion

Previous studies have inferred from measurements of urinary and plasma cortisol levels that the hypothalamic-pituitary-adrenal axis is activated in obese women (1;4) and men (2;3). Our data show a difference in urinary free cortisol excretion between men and women, but no relationship to obesity. Since only a small proportion of cortisol is excreted as urinary free cortisol, we also measured total cortisol metabolite excretion by gas chromatography and mass spectrometry and this gave similar results. However, the present study was cross-sectional, and the difference between the most and least obese was smaller than in previous reports.

Our second aim was to examine relationships between indices of obesity and cortisol metabolism. To our knowledge this is the first study of its kind in a healthy cohort: a previous study in hypopituitary patients suggested that cortisol/cortisone metabolite ratios were reduced in obesity (19) but those patients were receiving fixed doses of hydrocortisone replacement therapy. Our measurements in healthy subjects demonstrate much more striking differences in cortisol metabolism than we observed for indices of cortisol secretion.

Table 1. Characteristics of participants and principal results. Steroids are shown as mg/day in a 24 h urine sample.

	Males	Females	p from t test
n	31	37	
Age (y)	51.9±2.6	52.0±2.5	0.85
Height (m)	1.71±0.07	1.57±0.06	<0.0001
Weight (kg)	77.4±9.8	62.4±9.5	<0.0001
Abdominal girth (cm)	92.6±7.8	79.0±10.9	<0.0001
Hip girth (cm)	100.1±6.0	98.4±7.1	0.29
Body mass index (kg/m^2)	26.6±3.4	25.2±4.1	0.12
Cortisol	0.095 ±0.049	0.218 ±0.144	<0.0001
Cortisone	0.078 ±0.031	0.213 ±0.208	<0.001
5β-tetrahydrocortisol (5β-THF)	2.8±2.2	2.0±0.8	0.04
5α-tetrahydrocortisol (5α-THF)	1.4±1.5	1.6±0.8	0.44
Tetrahydrocortisone (THE)	2.9±1.6	2.1±1.4	0.04
Total cortisol metabolites	9.2±5.5	6.9±3.4	0.04
5β-THF/5α-THF	4.7±4.6	1.4±0.5	<0.0001
Cortisol/ (5β-THF+5α-THF)	0.03±0.03	0.06±0.03	<0.0001
Cortisone/THE	0.04±0.03	0.11±0.06	<0.0001
(5β-THF+5α-THF)/ THE	1.5±0.7	2.0±0.9	<0.0001
Cortisol/Cortisone	1.3±0.4	1.4±1.6	0.47

Table 2. Correlations between variables in men (M) and women (F). Data are r values. *p<0.04; $^{\$}$p<0.001.

Circumferences:	Abdominal		Hip	
	M	F	M	F
Urine free cortisol	+0.09	-0.05	+0.14	+0.24
Total cortisol metabolites	+0.20	+0.12	+0.37*	+0.19
5β-/5α-THF	-0.38*	-0.43$^{\$}$	-0.41*	-0.50$^{\$}$
Cortisol/THFs	-0.20	-0.20	-0.22	-0.02
Cortisone/THE	+0.07	-0.07	+0.04	+0.10
THFs/THE	+0.38*	-0.03	+0.42*	+0.24
Cortisol/Cortisone	-0.11	-0.08	-0.06	+0.05

A-Ring Reductases

In men and women, obesity was associated with a lower ratio of 5β-/5α-THF. This ratio reflects the balance between 5β-reductase and 5α-reductase activities in liver and probably in other tissues, including fat. The lack of difference in cortisone/THE ratio in obesity suggests that 5β-reductase activity is normal. Therefore, 5α-reductase activity is enhanced, as might be expected in obesity in both men and women, since fat contains 5α- but not 5β-reductase activity.

The difference in 5β-/5α-reduced metabolite ratios between men and women is also consistent with enhanced 5α-reductase in women, since previous work in rodents shows sexual dimorphism in 5α-reductase expression (11;12).

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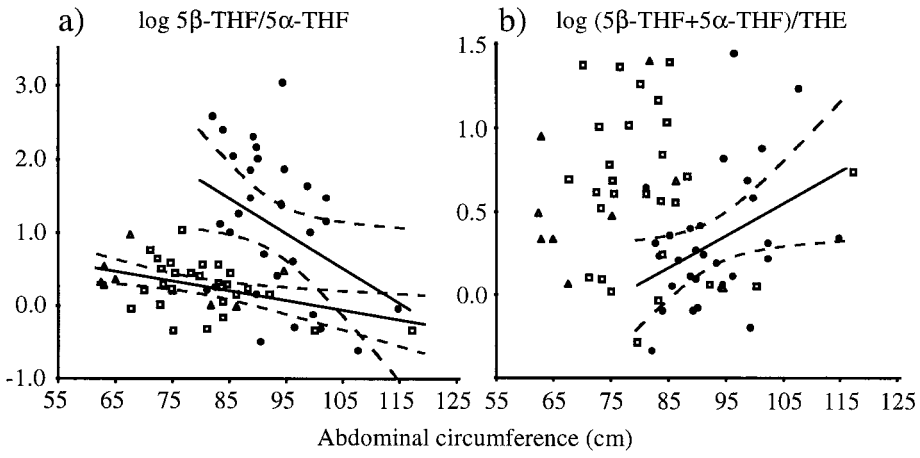

Figure 2. Correlations between abdominal girth and indices of cortisol metabolism by (a) A-ring 5α- and 5β-reductases; (b) 11β-dehydrogenase and reductase. Data points are for men (closed circles), women not taking estrogen (open squares) and women taking estrogen (open triangles) Lines show statistically significant regression slopes for men and women in Fig 2a) and men only in Fig 2b). Dotted lines show 95% confidence intervals for slopes. Correlation coefficients are in Table 2.

However, by contrast with obese subjects, in women total A-ring reduction and 5β-reduction of cortisone were impaired suggesting that 5β-reductase activity was substantially lower. The differences in 5β-reductase activity are more difficult to explain than those in 5α-reductase activity, but they could reflect variations in 11β-reductase (see below), since increased cortisol/cortisone metabolite ratios are associated with impaired 5β-reductase activity for unknown reasons.

11β-Hydroxysteroid Dehydrogenases

In men, obesity was associated with a higher ratio of THFs/THE. This ratio was also higher in women than men. This ratio reflects the whole-body equilibrium between cortisol and cortisone, determined by the balance of tissue-specific activities of 11β-dehydrogenase (the product of the 11β-hydroxysteroid dehydrogenase type 2 gene; 11β-HSD2) and 11β-reductase (the product of the 11β-hydroxysteroid dehydrogenase type 1 gene; 11β-HSD1). 11β-HSD2 inactivates cortisol to cortisone in the kidney and other mineralocorticoid target tissues, thereby protecting mineralocorticoid receptors from inappropriate activation by cortisol. Impaired 11β-HSD2 activity results in increased urinary free cortisol/cortisone ratio (15;18). In the current study increased ratios of THFs/THE were not accompanied by increased urinary free cortisol/cortisone ratios, suggesting that 11β-HSD2 is not related to obesity and not different between genders. This is consistent with experiments in animals which suggest that 11β-HSD2 is a constitutive enzyme which is not hormonally regulated. Thus, it is more likely that variations in 11β-HSD1 activity are responsible for observed differences.

11β-HSD1 converts cortisone to cortisol in liver, fat and many other glucocorticoid target tissues (6). Studies in rats show that 11β-HSD1 expression is down-regulated by insulin (7) and estrogen (9), the latter effect being dependent on a female pattern of growth hormone pulsatility. Our results suggest that enhanced conversion of cortisone to cortisol by 11β-HSD1 occurs in obese men and in post-menopausal women. In obese men up-regulation of 11β-HSD1 could be explained by resistance to the normal repression of 11β-HSD1 by insulin, altered growth hormone patterns in obesity, or increased mass of adipocytes expressing 11β-HSD1. In women, estrogen deficiency is the most likely explanation, especially since previous preliminary studies in pre-menopausal women showed that THFs/THE ratios were lower compared with men (10;19).

Against the background of estrogen regulating both 5α-reductase and 11β-reductase, we were surprised not to find that estrogen replacement therapy restored cortisol metabolism towards a pre-menopausal pattern. This may be because the number of subjects on estrogen was small (n=9), they were not matched to the subjects not receiving estrogen (allocation of therapy was not randomised), the route and preparation of estrogen therapy differed between subjects, or the age-related decline in growth-hormone secretion may remove an important mediator of the effects of estrogen (9).

Conclusions

A key inference from these results is that differences in cortisol metabolism in obesity may lead to differences in the secretion and action of glucocorticoids. The increased metabolic clearance of cortisol by 5α-reductase will impair negative feedback control of the hypothalamic-pituitary-adrenal axis and enhance cortisol secretion. However, this may be offset by decreased metabolism of cortisol by 5β-reductase in all women and increased regeneration of cortisol by 11β-reductase activity in obese men. These opposing effects might explain the weak association between obesity and total cortisol metabolite excretion rate in our study. However, in more extreme obesity, variations in cortisol metabolism could still be responsible for activation of the hypothalamic-pituitary-adrenal axis, since it may be that the balance between these enzymes in liver and fat is different as fat mass increases. This balance may also be sensitive to the distribution of body fat, since cell culture studies raise the possibility that the expression of enzymes is different in subcutaneous and visceral fat (8), so that it could explain the specific association of central obesity with enhanced cortisol secretion (1;4).

Another important effect of altered cortisol metabolism in obesity may be its influence on locally active tissue concentrations of cortisol. Reactivation of cortisol from

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cortisone by 11β-reductase influences regulation of hepatic gluconeogenic enzymes, such that transgenic mice with disrupted 11β-HSD1 are relatively hypoglycaemic (20). Inhibition of 11β-reductase in man also enhances insulin sensitivity (21). Increased 11β-reductase activity in visceral fat or liver may therefore be a determinant of insulin resistance in obese men, independently of circulating cortisol concentrations. Indeed enhanced regeneration of cortisol in visceral fat may provide a signal delivered via the portal circulation which explains the particular association of visceral obesity with insulin resistance. In principle, differences in A-ring reduction will also influence local cortisol concentrations and activity, but this has yet to be confirmed experimentally.

In conclusion, these data demonstrate differences in cortisol metabolism in relatively obese men and women which could contribute both to activation of the hypothalamic-pituitary-adrenal axis in more severe obesity and to insulin resistance and increased lipogenesis in obese subjects. The pattern of increased 5α-reduced cortisol metabolites echoes that seen in essential hypertension (22) and polycystic ovary syndrome (23). Together with the differences in cortisol metabolism between men and women, these data support the contention that a subtle increase in cortisol action could contribute to adverse cardiovascular risk in post-menopausal women and the amplification of cardiovascular risk by obesity in men.

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