Predicting Cardiovascular Risk Factors From Plasma Cortisol Measured During Oral Glucose Tolerance Tests

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Increasing evidence suggests that activation of the hypothalamic-pituitary-adrenal (HPA) axis may contribute to the pathogenesis of the metabolic syndrome and obesity. The mechanisms are unknown but may involve alterations in the metabolic responses to feeding that interact with the HPA axis. As it is known that plasma cortisol falls during an oral glucose tolerance test (OGTT), changes in cortisol measured during an OGTT may be altered in the metabolic syndrome. We measured changes in plasma cortisol during OGTTs in a large study of 593 men and women to determine correlates of changes in cortisol with features of the metabolic syndrome and the extent to which these relationships are confounded by obesity. In men and women, higher cortisol area under the curve (AUC) during the OGTT was associated with higher glucose AUC and higher systolic blood pressure. Higher cortisol AUC was associated with reduced insulin increment in men, but higher 2-hour insulin and insulin AUC in women. However, the decline in plasma cortisol after glucose administration was poorly predictive of features of the metabolic syndrome. Obesity was associated with lower cortisol AUC but not with percentage decline in cortisol. Plasma cortisol and obesity had independent effects on plasma glucose and were the strongest predictors of plasma glucose in multiple regression analysis. Measurements of plasma cortisol during the OGTT reinforce the previously observed relationships of activation of the HPA axis in the metabolic syndrome. However, the altered HPA response to feeding does not appear to be primarily responsible for HPA activation in subjects with the metabolic syndrome.

EXPOSURE TO EXCESS levels of cortisol in Cushing's syndrome is associated with an increased risk of developing glucose intolerance, insulin resistance, hypertension, and dyslipidemia. Increasing evidence suggests that less profound disturbances of the hypothalamic-pituitary-adrenal (HPA) axis may contribute to the pathogenesis of these risk factors for cardiovascular disease, which are together described as the "metabolic syndrome." Men and women with these cardiovascular risk factors have activation of the HPA axis as judged by elevated morning plasma cortisol concentrations,[1-6] increased plasma cortisol after stimulation by exogenous corticotropin,[7] and urinary increased excretion of cortisol metabolites.[5,7,8] Obese subjects also have activation of the HPA axis,[9-11] but in contrast to the elevated plasma cortisol in subjects with the metabolic syndrome,[1-6] plasma cortisol concentrations are often low in obesity.[6,12]

The mechanism(s) for activation of the HPA axis in the metabolic syndrome and in obesity remain uncertain. It is predicted by low birthweight[2,3,7,13] and may result from permanent alteration in central control of the HPA as a result of events in early life.[14] In obesity, increased peripheral clearance of cortisol may account for compensatory activation of the HPA axis.[11] Alternatively, a number of metabolic responses to feeding may interact with the HPA axis, and be altered in the metabolic syndrome. It is known that plasma cortisol levels fall during an oral glucose tolerance test (OGTT)[15-17] and rise following a protein meal.[18] In the extreme, adrenal hypersensitivity to gastric inhibitory peptide can result in "food-induced Cushing's syndrome."[19] To establish whether changes in plasma cortisol during glucose tolerance tests might reveal altered control of the HPA in the metabolic syndrome, we performed a preliminary study in just 39 men but observed that plasma cortisol following an oral glucose load did not differ in subjects with glucose intolerance compared with normoglycaemic controls.[17] In another study plasma cortisol measured 2 hours following a glucose load was less predictive of cardiovascular risk than the fasting measurement,[6] suggesting that the fall in cortisol might be less in subjects with the metabolic syndrome. We have now measured changes in plasma cortisol during OGTTs in a large study of 593 men and women to determine correlates of changes in cortisol with features of the metabolic syndrome. We also examined the extent to which these relationships are confounded by relative obesity.

MATERIALS AND METHODS

The subjects had participated in two epidemiological studies examining relationships between the early environment and subsequent type 2 diabetes mellitus. In 1991, 370 men born between 1920 and 1930 in East Hertfordshire, England, underwent 75-g OGTTs. Measurements of blood pressure and height, weight, and waist and hip circumferences were recorded.[20] In 1993, a similar study was performed on 266 men and women born between 1935 and 1943 in Sharoe Green Hospital, Preston, Lancashire, England.[21] Measurements of glucose, insulin, lipids, and fasting plasma cortisol from both studies have been reported previously.[2,3,20,21] However, plasma cortisol during OGTTs has not been reported before. We assayed plasma cortisol by radioimmunoassay (RIA) in the fasting, 30-minute, and 120-minute samples from the OGTT. None of the subjects had pituitary or adrenal disease and 2 subjects on oral prednisolone treatment were excluded from the Preston analysis. There was sufficient plasma for complete cortisol results from 339 men in the Hertfordshire cohort and 254 subjects (135 men, 119 women) in the Preston cohort.

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Statistical Analysis

The area under the cortisol curve (cortisol AUC) was used as a summary measurement of plasma cortisol during the OGTT by calculating the area under the trapezium described by the cortisol measurements at baseline, 30 minutes, and 120 minutes (units = h · nmol/L). Likewise, the areas under the glucose (glucose AUC) and insulin (insulin AUC) curves during the OGTT were calculated. In addition, the percentage decline in cortisol from baseline to 120 minutes during the OGTT was calculated as $100 \cdot ([\log_e\text{cortisol 0 min} - \log_e\text{cortisol 120 min}]/\log_e\text{cortisol 0 min})$. The insulin increment, a previously validated index of insulin secretion,[22] was calculated from the plasma measurements during the OGTT as $\log_e([\text{insulin 30 min} - \text{insulin 0 min}]/[\text{glucose 30 min} - \text{glucose 0 min}])$. The distributions of measurements of blood pressure, triglycerides, cholesterols, cortisol, glucose, insulin, and AUCs were $\log_e$-transformed and geometric means and standard deviations are presented. As the distribution of body mass index (BMI) was positively skewed in the Preston data set but was not satisfactorily transformed by the $\log_e$ transformation, Fisher-Yates normal-scores for BMI were used as a measure of obesity. Pearson correlation coefficients were computed to examine the pairwise associations between variables. Associations between cortisol, BMI, and glucose and insulin measurements during the OGTT were investigated by multiple linear regression.

RESULTS

Subject Characteristics

Table 1 shows the characteristics of the participants and the measurements of plasma glucose, insulin, and cortisol during the OGTT. The Hertfordshire men were older but of similar BMI to the Preston men. The Preston men and women were of similar ages but the men were of higher BMI ($P = .01$) and waist-hip ratio (WHR) ($P < .0001$). The Preston men had higher glucose AUC ($P < .001$ unadjusted, $P = .002$ adjusted for BMI) and tended to have higher insulin AUC during the OGTT (P = not significant [NS]) than the women. Blood pressure was higher in men than in women.

Table 1. Characteristics of Participants

	Hertfordshire Men (n = 339)	Preston Men (n = 135)	Preston Women (n = 119)
Age (yr)	64.6 (3.2)	51.8 (2.2)	51.3 (2.4)
BMI (kg/m^2)	26.9 (3.6)	25.7 (3.4)	24.7 (4.4)
WHR	0.9 (0.1)	0.9 (0.1)	0.8 (0.1)
No. with IGT/type 2 DM*	84	14	20
Plasma glucose (mmol/L)			
Fasting	6.1 (1.2)	5.8 (1.1)	5.4 (1.1)
30 min	9.4 (1.2)	9.0 (1.3)	7.5 (1.3)
120 min	6.6 (1.4)	5.5 (1.3)	5.8 (1.3)
Glucose AUC (h · mmol/l)	16.0 (1.3)	14.7 (1.2)	13.4 (1.3)
Plasma insulin (pmol/L)			
Fasting	43 (1.9)	45 (1.7)	42 (1.6)
30 min	275 (1.9)	288 (1.7)	249 (1.7)
120 min	150 (2.4)	147 (2.2)	178 (2.0)
Insulin AUC (h · pmol/L)	425 (1.8)	439 (1.6)	420 (1.6)
Insulin increment (pmol/mmol)	70.5 (2.3)	81.4 (2.2)	94.2 (2.1)
Plasma cortisol (nmol/L)			
Fasting	320 (1.4)	427 (1.3)	403 (1.4)
30 min	349 (1.5)	425 (1.5)	362 (1.4)
120 min	259 (1.4)	276 (1.4)	245 (1.4)
Cortisol AUC (h · nmol/l)	634 (1.4)	749 (1.4)	656 (1.3)
Systolic blood pressure (mm Hg)	164 (1.2)	138 (1.2)	130 (1.2)
Diastolic blood pressure (mm Hg)	90 (1.1)	78 (1.1)	73 (1.2)
Triglycerides (mmol/L)	1.4 (1.7)	1.4 (1.7)	1.1 (1.6)
HDL cholesterol (mmol/L)	1.2 (1.3)	1.2 (1.3)	1.5 (1.3)
LDL cholesterol (mmol/L)	4.7 (1.3)	4.4 (1.3)	4.3 (1.3)

NOTE. Values are geometric mean (SD) for all variables except age and waist-hip ratio (WHR), and body mass index (BMI) for which the arithmetic means (SD) are given.

Abbreviations: AUC, area under the curve; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*IGT, impaired glucose tolerance (2-hour glucose 7.8 to 11.0 mmol/L); type 2 DM, type 2 diabetes mellitus (2-h glucose ≥11.1 mmol/L).

Plasma Cortisol Concentrations During the OGTT

In all subjects plasma cortisol concentrations fell during the OGTT ($P < .0001$). Table 2 shows the associations between cortisol AUC and clinical features of the metabolic syndrome. In men and women, higher cortisol AUC was associated with higher glucose AUC and higher systolic blood pressure. Higher cortisol AUC was associated with reduced insulin increment in men, but higher 2-hour insulin and insulin AUC in women. In contrast, the change in plasma cortisol following the oral glucose load was not a strong predictor of the clinical features of the metabolic syndrome (data not shown). The only statistically significant associations were in the Preston men where a smaller decline in plasma cortisol was associated with higher 2-hour glucose ($P = .02$) and glucose AUC ($P = .06$), lower systolic blood pressure ($P = .04$), higher high-density lipoprotein (HDL) cholesterol ($P = .001$), and reduced insulin increment ($P = .04$).

Obesity

Obesity, as measured by BMI, was associated with both lower fasting cortisol (Hertfordshire $P = .02$; Preston $P = .04$ unadjusted, $P = .02$ adjusted for gender) and a lower cortisol AUC (Hertfordshire $P = .0001$; Preston $P = .05$ unadjusted, $P = .01$ adjusted for gender), but not with the percentage decline in cortisol. Table 2 shows that after adjustment for the confounding effect of obesity, the associations between cortisol AUC and clinical features of the metabolic syndrome were strengthened.

Independent Effects of Plasma Cortisol and Obesity on Plasma Glucose

The independent effects of plasma cortisol and obesity on plasma glucose are illustrated in Table 3 and Table 4. In both cohorts at any BMI, higher cortisol AUC was associated with higher glucose AUC, while at any cortisol AUC, higher BMI was associated with higher glucose AUC. Thus, the highest glucose AUC was observed in subjects with highest cortisol AUC and highest BMI, and the lowest in those with lowest cortisol AUC and least BMI. In a multiple regression analysis with glucose AUC as the dependent variable and cortisol AUC, BMI, age, and gender as independent variables, cortisol AUC ($P = .001$), BMI ($P < .001$), and age ($P = .01$) were all significant predictors of glucose AUC in Hertfordshire, and cortisol AUC ($P < .001$), BMI ($P < .001$), and gender ($P = .03$, women with lower glucose AUC than men) were strongest

Table 2. Associations Between Cortisol AUC During OGTT and Features of the Metabolic Syndrome

	Hertfordshire Men (n = 339)			Preston Men (n = 135†)			Preston Women (n = 119†)		
	r	*P* Value	*P* Value*	*r*	*P* Value	*P* Value*	*r*	*P* Value	*P* Value*
2-h glucose (mmol/L)	0.07	.14	.03	0.19	.03	.002	0.05	.61	.36
Glucose AUC (h · mmoL)	0.12	.02	.001	0.23	.006	<.001	0.21	.02	.01
2-h insulin (pmol/L)	−0.04	.41	.90	−0.00	.96	.73	0.21	.02	.04
Insulin AUC (h · pmol)	−0.08	.13	.69	0.05	.58	.41	0.31	.001	.001
Insulin increment (pmol/mmol)	−0.19	.001	.001	−0.2	.02	.01	−0.01	.89	.95
Systolic BP (mm Hg)	0.14	.006	<.001	0.10	.25	.05	0.20	.03	.02
Diastolic BP (mm Hg)	0.14	.01	<.001	0.14	.09	.02	0.01	.93	.76
Triglycerides (mmol/L)	−0.00	.98	.22	0.07	.45	.19	0.10	.29	.14
HDL cholesterol (mmol/L)	0.10	.06	.13	0.21	.01	.04	0.05	.63	.97

**P* value for % change in cortisol AUC adjusted for BMI.

†Five Preston men and 3 Preston women had missing values for BMI.

predictors of glucose AUC in Preston. There was no significant interaction between cortisol AUC and obesity in either cohort (Hertfordshire $P = .73$, Preston $P = .99$). The association between glucose AUC and both cortisol AUC and obesity was also observed after exclusion of the 84 Hertfordshire and 34 Preston subjects with IGT or type 2 diabetes mellitus, although the strengths of the associations were reduced.

DISCUSSION

In both of these populations in whom raised fasting plasma cortisol was associated with clinical features of the metabolic syndrome,[2,3] high cortisol AUC during the OGTT was also associated with cardiovascular risk factors. The associations were sex-specific: higher cortisol AUC was associated with higher systolic blood pressure in both sexes. In women, high cortisol AUC was also associated with high glucose and insulin AUCs. However, in men high cortisol AUC was associated with high glucose AUC but not with hyperinsulinaemia, suggesting insulin deficiency rather than insulin resistance. Consistent with this, high cortisol AUC was associated with reduced insulin increment, a good correlate of first phase insulin secretion,[22] which is interesting as glucocorticoids can directly inhibit insulin release from pancreatic β cells.[23]

However, the decline in plasma cortisol after glucose administration was poorly predictive of features of the metabolic syndrome, being weakly statistically significant only in the Preston men. In other studies plasma cortisol measured 2 hours following a glucose load has been less predictive of cardiovascular risk than the fasting measurement.[6] This may not be surprising as the decline in plasma cortisol following an oral glucose load is influenced by the diurnal fall in cortisol secretion, which is altered in glucose intolerant subjects,[17,24] as well as by the effect of oral glucose to raise plasma cortisol.[17]

This study also confirms the previously reported contrasting effects of relative obesity and cortisol on glucose intolerance.[6] Several studies have shown that regulation of the HPA axis is altered in obesity,[9-11] notably that obesity is associated with lower plasma cortisol concentrations[12] and increased peripheral clearance of cortisol.[25] Consistent with the hypothesis that elevated plasma cortisol and obesity represent different mechanistic pathways leading to cardiovascular risk, the effects of BMI and plasma cortisol in this study, as in a previous study,[6] were independent and additive. Thus, increasing BMI strengthened the associations between cortisol and glucose intolerance such that the highest glucose AUC was observed in subjects with the combination of obesity and high plasma cortisol concentrations. Such findings, however, may not be applicable in morbid obesity.

Table 3. Effects of Cortisol AUC and BMI on Glucose AUC in Hertfordshire Men

Tertiles of Cortisol AUC (h · nmol/L)	Tertiles of BMI (kg/m^2) Lowest, <25.5	Middle, 28.0	Highest, ≥28.0	All
Lowest, ≤552	13.8 (n = 27)	15.6 (n = 42)	16.3 (n = 44)	15.4 (n = 113)
Middle, 720	15.2 (n = 42)	15.5 (n = 36)	17.0 (n = 36)	15.9 (n = 114)
Highest, >720	16.2 (n = 44)	16.2 (n = 36)	18.7 (n = 32)	16.9 (n = 112)
All	15.3 (n = 113)	15.8 (n = 114)	17.2 (n = 112)	16.0 (n = 339)

NOTE. Values are geometric mean of glucose AUC. The geometric SD ranged between 1.2 and 1.4.

Table 4. Effects of Cortisol AUC and BMI on Glucose AUC in Preston Men and Women

Tertiles of Cortisol AUC (h · nmol/L)	Tertiles of BMI (kg/m^2) Lowest, ≤27* and ≤24†	Middle, 27* and 26†	Highest, >27* and >26†	All
Lowest, ≤635* and ≤573†	12.8 (n = 27)	13.5 (n = 29)	14.1 (n = 25)	13.4 (n = 81)
Middle, 841* and 740†	13.5 (n = 33)	13.7 (n = 22)	14.2 (n = 27)	13.8 (n = 82)
Highest, >841* and >740†	14.2 (n = 40)	14.5 (n = 25)	16.9 (n = 18)	14.8 (n = 83)
All	13.6 (n = 100)	13.9 (n = 76)	14.8 (n = 70)	14.0 (n = 246)

NOTE. Values are geometric mean of glucose AUC with results combined for men and women for presentation. The geometric SD ranged between 1.1 and 1.3.

*Tertiles of cortisol AUC and BMI for men.

†Tertiles of cortisol AUC and BMI for women.

The glucose tolerance test is commonly used in epidemiological studies of cardiovascular risk and also provides a dynamic test of the HPA axis since oral glucose raises plasma cortisol levels. However, although measurement of plasma cortisol during the glucose tolerance test reinforces our interpretation of the correlations between fasting plasma cortisol and cardiovascular risk factors, we did not find that it offered additional value over measurement of fasting cortisol, and further interventional studies are needed. While subtle differences in diurnal variation of plasma cortisol and in response to oral glucose might have obscured each other, it seems unlikely that altered HPA response to feeding is primarily responsible for HPA activation in subjects with the metabolic syndrome.

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