Central Hypothalamic-Pituitary-Adrenal Activity and the Metabolic Syndrome: Studies Using the Corticotrophin-Releasing Hormone Test

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A number of studies have suggested that the metabolic syndrome (principally, the combination of hypertension, glucose intolerance, and dyslipidemia) is associated with subtle dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis leading to raised circulating cortisol concentrations. The mechanisms underlying these observations are not known. We assessed the salivary cortisol response to awakening and pituitary-adrenal responses during a 100-μg human corticotrophin-releasing hormone (CRH) test and a dexamethasone-suppressed CRH test in a well-characterized group of 65-year-old men (n = 122). In the cohort from which this subgroup was drawn, there were associations between the components of the metabolic syndrome and 9 AM cortisol concentration in line with previous studies. However, there were no significant associations between blood pressure, glucose tolerance, and lipid concentrations and the dynamic tests of HPA activity. We therefore found no evidence to suggest that exaggerated pituitary responsiveness or increased central drive to the pituitary, as determined by CRH testing, plays a part in the development of the metabolic syndrome.

THERE IS INCREASING evidence that subtle defects of the hypothalamic-pituitary-adrenal (HPA) axis leading to mild hypercortisolism may play a part in the etiology of the metabolic syndrome. This evidence derives from case-control and cross-sectional studies which show that raised fasting plasma cortisol concentrations and exaggerated adrenal responses to corticotrophin (ACTH) are associated with components of the syndrome, including raised blood pressure, glucose intolerance, and dyslipidemia.[1-6] Several studies also demonstrate that abdominal obesity, which is strongly linked with the metabolic syndrome, is associated with exaggerated ACTH and cortisol responses to corticotrophin-releasing hormone (CRH), higher cortisol responses to ACTH stimulation, and increased HPA responses to a mental stress challenge.[7-11] However, although cortisol secretion rate is increased in obesity, its metabolic clearance rate is also increased, in part due to changes in 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD 1) and 5α-reductase activity.[12-14] Consequently, plasma levels of cortisol in obesity are generally reduced.[4] These findings suggest that the HPA abnormalities found in association with obesity differ from those observed in association with the metabolic syndrome, a conclusion supported by large population studies which suggest that the effects of raised plasma cortisol concentrations are additive to those of obesity in determining the prevalence of the metabolic syndrome or its components.[4,6,15] The nature of the HPA abnormality associated with the metabolic syndrome is currently unclear and it is uncertain whether it has a central component.

The purpose of this study was to investigate the relationship between central regulation of the HPA axis and features of the metabolic syndrome in a well-characterized population of middle-aged men. We assessed ACTH and cortisol responses to both CRH and a combined dexamethasone/CRH challenge (the latter has been proposed as a sensitive test of central HPA function as it is abnormal in patients with depression who are thought to have exaggerated drive to the pituitary gland[16]) and the wakening cortisol response.

MATERIALS AND METHODS

Study Participants

In 1998, the MRC Environmental Epidemiology Unit in Southampton began recruiting individuals born between 1931 and 1939 in Hertfordshire, UK to participate in studies examining the interactions between early life, adult diet, and lifestyle and genetics as determinants of adult disease. Men in the East Hertfordshire region were the first subjects to be recruited into this cohort. At their enrollment visit, detailed information on the participant's medical and social history, mood (Hospital Anxiety and Depression [HAD] scale[17]), socioeconomic status, exercise patterns, smoking, and alcohol consumption was obtained from a nurse-administered questionnaire.

Height was measured to the nearest 0.1 cm using a Harpenden pocket stadiometer (Chasmors Ltd, London, UK) and weight to the nearest 0.1 kg on a SECA floor scale (Chasmors Ltd). Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Waist (mid way between the costal margin and the iliac crest in the mid axillary line) and hip (greatest diameter around the gluteal region) circumferences were measured with steel tape and skinfold thickness was determined at 4 sites (biceps, triceps, subscapular, and suprailiac) using a Harpenden skinfold calliper (Chasmors Ltd. Waist-to-hip ratio (WHR) was calculated and total body fat (%) was derived from the skinfolds according to the equations of Durnin and Womersley.[18] Subscapular-to-triceps skinfold ratio (SSTR) was calculated as a measure of truncal fat. Blood pressure was recorded as the mean of 3 measurements taken with a Dinamap Model 8101 (GE Medical Systems, Slough, UK) after the subject had been seated for 5 minutes. A fasting blood sample was taken for cortisol, lipid profile, and insulin precursors, and the subjects underwent a standard 75-g oral glucose tolerance test, sampling at 0, 30, and 120 minutes for glucose and insulin.

Full baseline data, including fasting 9 AM cortisol concentration, were available for 678 men (88% of the East Hertfordshire cohort) at the time of the present study. From this group, 122 men were recruited into the detailed study of HPA function. They were a random sample of subjects from the top and bottom quartile of birthweight, selection criteria required for a parallel study. The group from which the sample

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was drawn did not differ significantly from subjects in the middle birthweight quartiles when comparing baseline characteristics and hemodynamic and metabolic data. Subjects were excluded if they had a history of pituitary or adrenal disease, diabetes, or either major depression or glucocorticoid treatment in the previous 3 months. Ethical approval was obtained from the North and East Hertfordshire Local Research Ethics Committee and all subjects gave written informed consent.

Salivary Cortisol Response to Awakening

Subjects were sent 5 Salivettes (Sarstedt, Leicester, UK) with which to collect saliva. They were asked to fast from midnight and then to collect a saliva sample at 0, 15, 30, 45, and 60 minutes after waking the following morning, recording the times at which they were collected. They remained fasted during this period and were asked to refrain from brushing their teeth to prevent contamination of the saliva samples with blood. They brought the samples with them to the clinic.

Corticotrophin-Releasing Hormone Test

Subjects attended the clinic at 8 AM the same morning. A 21-gauge cannula was inserted into an antecubital vein and the subject then rested for 30 minutes in a semi-recumbent position. Baseline blood samples were drawn at −15 and −5 minutes before CRH administration. At 9 AM, 100 μg human CRH (Ferring Pharmaceuticals, Slough, UK) was injected as a bolus and flushed through with 10 mL 0.9% saline. Lyophilized CRH was reconstituted in the supplied diluent immediately prior to administration. Blood samples (10 mL) were then drawn from the cannula at 5, 15, 30, 45, 60, 90, and 120 minutes. The patency of the cannula was maintained with regular saline flushes. Blood for ACTH analysis was collected into chilled EDTA tubes, stored on ice, spun at 4°C within 20 minutes, and the plasma was immediately frozen to −80°C until assayed. Serum for cortisol analysis was prepared from clotted samples. Following removal of the cannula, subjects were given breakfast.

CRH administration resulted in facial flushing in 77% of the subjects. Five men experienced a brief vasovagal episode within 10 minutes of CRH injection and 2 men became unwell during the course of the study. Examination of the ACTH and cortisol response profiles in these subjects showed extremely high values compared with other participants and they were therefore excluded from further analysis. So too were the data from a needle phobic subject who had very high baseline values and failed to respond to CRH. Baseline blood samples were accidentally frozen in 1 subject, rendering them unsuitable for ACTH analysis.

Dexamethasone-Suppressed CRH Test

Subjects who had completed the CRH test without side effects (n = 115) were invited to attend on a second occasion for a DEX/CRH test, at least 1 month after the first study day; 103 subjects agreed to take part. They were given 1.5 mg dexamethasone to take at 11 PM on the night before the study and were asked to have breakfast as usual the following morning but to avoid caffeine-containing drinks. They attended the clinic at 12:30 PM and were given a standard sandwich lunch to ensure that all subjects had equivalent calorie and electrolyte intake before the DEX/CRH test. At 1:30 PM, a cannula was inserted into an antecubital vein and the subject then rested in a semi-recumbent position for 30 minutes. Baseline blood samples were withdrawn at −15 and −5 minutes prior to injection of 100 μg human CRH. Following the injection, blood was sampled at 15, 30, 45, 60, 75, 90, 105, and 120 minutes for ACTH and cortisol. Samples were prepared as described above.

Laboratory Methods

All hormone assays were performed under the supervision of Dr Peter Wood in the Regional Endocrine Laboratory at Southampton General Hospital. Salivary cortisol was measured using a time-resolved fluorescent immunoassay ("DELFIA" system). The assay has a lower limit of detection of 0.4 nmol/L and an interassay coefficient of variation (CV) of 5% to 10% between 2 and 15 nmol/L cortisol. Serum cortisol was assayed using an in house radioimmunoassay (CV, 7.4% to 10.3%) and plasma ACTH was measured with a commercial assay (Nichols Institute Diagnostics, San Juan Capistrano, CA) with an interassay CV of 6.8% to 7.8% between 36 and 358 pg/mL ACTH. Intact insulin, proinsulin, and 32,33 split proinsulin were measured by in-house immunofluorimetric 2-site assays (DELFIA system) based on published methods.[19] Interassay imprecision ranged from 6% to 10 % (CV%) for low-, medium-, and high-quality control samples for intact insulin and from 7% to 15% for insulin precursors. Glucose and lipids were assayed on an Advia 1650 autoanalyser (Bayer Diagnostics, Newbury, UK).

Statistical Analysis

Insulin resistance and insulin secretion were estimated using the homeostasis model assessment (HOMA).[20] The total integrated salivary cortisol concentration in the first hour after waking was assessed by the area under the curve (AUC), calculated by the trapezoidal rule. The cortisol response to awakening was assessed by the incremental area under the curve (iAUC = AUC − area under the baseline). Unstimulated, fasting 9 AM cortisol and ACTH concentrations in the CRH test and dexamethasone-suppressed values in the DEX/CRH test were derived from the mean of the 2 baseline samples. The response to CRH stimulation in both tests was assessed using the iAUC. Log_e-transformation of skewed variables was performed where necessary and corresponding geometric means and standard deviations (gsd) are presented. ACTH iAUC in the CRH test and cortisol and ACTH iAUC in the DEX/CRH test were transformed to normality using Fisher-Yates normal scores as negative values prevented logarithmic transformation. Descriptive statistics are presented as untransformed median values for simplicity.

Associations between baseline and stimulated pituitary-adrenal activity and the components of the metabolic syndrome were assessed using Pearson correlation coefficients, correcting for the possible confounding effects of age, obesity, and adult lifestyle variables (smoking, alcohol, physical activity, social class) in multiple linear regression models. All analyses were performed using Stata Statistical Software Release 7.0 (Stata Corp, College Station, TX).

RESULTS

Baseline Assessment

Initial assessment of the influence of HPA activity on cardiovascular risk factors was based on the cohort of 678 men who had a fasting 9 AM cortisol concentration measured at their enrollment visit. The baseline characteristics of the cohort are detailed in Table 1. BMI, WHR, and body fat percentage were closely correlated ($r = 0.56$ to 0.75, $P < .001$), whereas SSTR was only weakly correlated with the other obesity measures ($r = 0.14$ to 0.16, $P < .001$). Fasting 9 AM cortisol concentration was inversely related to measures of obesity (BMI: $r = -0.11$, $P = .003$; WHR: $r = -0.09$, $P = .02$; total body fat: $r = -0.09$, $P = .03$; SSTR: $r = -0.05$, $P = .2$) and positively correlated with age ($r = 0.10$, $P = .01$). The 9 AM cortisol

Table 1. Characteristics of the Study Participants at Enrollment

	Whole Cohort	HPA Study Group
N	678	122
Age (yr)	64.3 (2.6)	64.1 (2.7)
Height (m)	1.74 (0.07)	1.74 (0.07)
Weight (kg)*	80.8 (1.2)	79.7 (1.1)
BMI (kg/m^2)*	26.7 (1.1)	26.4 (1.1)
WHR	0.96 (0.06)	0.95 (0.06)
Total body fat (%)	28.7 (5.3)	27.9 (5.1)
SSTR*	171 (1.4)	165 (1.4)
Current smoker (%)	17.1	18.0
Alcohol (U/wk)†	10 (3-23)	10 (4-25)
Social class I-IIINM (%)	36.7	38.5
Systolic BP (mm Hg)	137 (19)	135 (17)
Diastolic BP (mm Hg)	76 (11)	75 (10)
Fasting glucose (mmol/L)*	5.9 (1.2)	5.5 (1.1)‡
2-h glucose (mmol/L)*	6.5 (1.5)	5.7 (1.4)‡
Triglyceride (mmol/L)*	1.4 (1.6)	1.3 (1.6)
9 AM cortisol (nmol/L)*	339 (1.4)	342 (1.3)

NOTE. Data are mean (SD).

Abbreviations: BMI, body mass index; WHR, waist-to-hip ratio; SSTR, subscapular:triceps skinfold ratio; BP, blood pressure.

*Geometric mean (SD).

†Median (interquartile range).

‡Significant difference between those in the HPA study and those not ($P < .001$)

concentration was higher in those men who drank 10 units of alcohol or more per week than those with minimal alcohol intake (350 *v* 330 nmol/L, $P = .01$), but it was not related to smoking, social class, physical activity, or depression score.

Subjects with higher 9 AM cortisol concentrations had higher systolic ($r = 0.11$, $P = .003$; r = partial correlation coefficient, corrected for age and BMI) and diastolic blood pressure ($r = 0.08$, $P = .047$), higher fasting ($r = 0.15$, $P < .001$) and post load glucose ($r = 0.10$, $P = .01$), but did not differ in insulin resistance (HOMA, $r = 0.00$) or fasting triglyceride concentration ($r = 0.02$) (Table 2). There was an inverse relationship between cortisol concentration and insulin secretion ($r = -0.15$, $P < .001$). To ensure that these relationships were not driven by the presence of diabetes, we repeated the analysis without those individuals with diabetes (n = 77) and found similar associations for all variables except post load glucose, which was no longer significantly related to fasting cortisol (data not shown). We also examined the impact of adiposity on the relationship between cortisol and cardiovascular risk factors. There was a significant interaction between cortisol and adiposity in determining fasting plasma glucose (FPG) concentrations ($P = .009$). The correlation between cortisol and FPG increased across BMI tertiles (BMI < 25.2: $r = 0.09$, $P = .2$; BMI 25.2 to 28: $r = 0.11$, $P = .1$; BMI ≥ 28: $r = 0.20$, $P = .002$). Cortisol and adiposity were independent predictors of the other cardiovascular risk factors, but the interaction terms were not statistically significant.

Detailed Study of HPA Function

The 122 subjects who took part in this study did not differ from the cohort as a whole in age, anthropometry, lifestyle variables, or the majority of the cardiovascular risk factors measured at baseline (Table 1). Glucose tolerance was better in this subgroup as expected given that diabetes was an exclusion criterion for the HPA study. The participants were evenly split between 2 birthweight groups (see Methods) and correcting for birthweight group did not influence any of the results described below.

BMI and WHR were closely correlated ($r = 0.74$, $P < .001$) and it was not possible to define a group of men who differed in WHR for a given BMI to assess the effect of central versus peripheral obesity. BMI was a slightly stronger predictor of the cardiovascular risk factors than WHR (systolic blood pressure: $r = 0.36$ *v* $r = 0.34$; diastolic blood pressure: $r = 0.32$ *v* $r = 0.27$; fasting glucose: $r = 0.26$ *v* $r = 0.22$; 2-hour glucose: $r = 0.34$ *v* $r = 0.32$; and triglycerides: $r = 0.37$ *v* $r = 0.34$) and was used to correct for adiposity in multiple linear regression analysis.

Salivary Cortisol Response to Awakening

Salivary cortisol concentrations rose in 82% subjects over the first hour after waking, peaking on average at 30 minutes (Fig 1). Mean total integrated salivary cortisol secretion (AUC)

Table 2. Relationship Between Fasting 9 AM Cortisol and Cardiovascular Risk Factors in 678 Men Aged 64 Years

Cortisol Quintile (nmol/L)	N	Age (yr)	BMI (kg/m^2)†	WHR	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Fasting Glucose (mmol/L)†	2-h Glucose (mmol/L)†	Insulin Resistance (HOMA)†	Insulin Secretion (HOMA)†	Triglyceride (mmol/L)†
<259	136	63.9	27.4	0.97	134	76	5.8	6.4	4.0	140	1.5
−314	136	64.1	27.1	0.96	137	76	5.8	6.3	3.6	125	1.5
−371	136	64.4	26.3	0.95	137	76	5.9	6.2	3.5	113	1.3
−453	136	64.7	26.1	0.95	137	76	5.9	6.6	3.4	110	1.4
>453	134	64.5	26.5	0.96	140	78	6.1	6.9	3.6	106	1.4
All	678	64.3	26.7	0.96	137	76	5.9	6.5	3.6	118	1.4
SD		2.6	1.1	0.06	19	11	1.2	1.5	2.0	1.8	1.6
P		.01	.003	.02	.005	.1	.001	.04	.2	<.001	.6
*P**					.003	.047	<.001	.01	.9	<.001	.5

NOTE. Data presented are mean values in each cortisol quintile.

P values based on continuous analysis, *adjusted for age and BMI.

†Geometric mean.

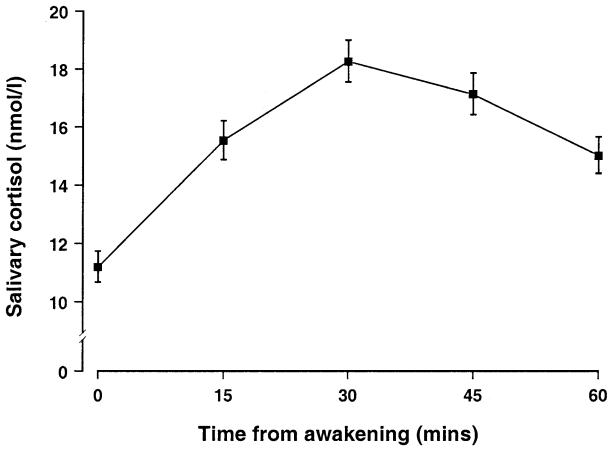

Fig 1. Salivary cortisol response to awakening in 122 men aged 65 years. Data are geometric mean ± SEM.

was 1,060 (SD 375) nmol/L · min and the mean salivary cortisol response to awakening (iAUC) was 282 (SD 420) nmol/L · min. The AUC was not related to any of the obesity measures. There was a trend towards a lower iAUC with increasing obesity (Table 3), but it was not related to age, adult lifestyle variables (social class, smoking, alcohol consumption, physical activity) or depression score. There were no significant associations between any of the parameters derived from the salivary cortisol curve over the first hour after waking and blood pressure or any of the metabolic variables (Table 4).

CRH Test

Mean fasting 9 AM serum cortisol concentration was 304 (gsd 1.3) nmol/L prior to the CRH test and was closely correlated with enrolment clinic cortisol ($r = 0.56$, $P < .001$), although absolute values were 12% lower on average ($P < .001$). Relationships with obesity measures and features of the metabolic syndrome were similar to those detailed above for the whole cohort except for post load glucose, as subjects with diabetes were excluded from the HPA study, weakening the association (Tables 3 and 4). ACTH and cortisol concentrations rose in all subjects following CRH administration. ACTH peaked between 15 and 30 minutes and the cortisol peak followed at 45 and 60 minutes (Fig 2). Median ACTH and mean cortisol responses (iAUC) to CRH were 652 (interquartile range [IQR] 344 to 1,002) pmol/L · min and 13,823 (SD 9,881) nmol/L · min, respectively. ACTH and cortisol responses to CRH were positively associated with adiposity (Table 3), but age, adult lifestyle variables, and depression score were not determinants of the CRH response. In detailed analysis, neither the ACTH nor cortisol responses to CRH were related to blood pressure, glucose tolerance, insulin sensitivity, or plasma lipid concentrations in this cohort of 65-year-old men (Table 4).

DEX/CRH Test

The mean cortisol concentration following dexamethasone result was 23.2 (gsd 1.7) nmol/L. All subjects responded to CRH, but the range of responses was considerable: ACTH increment = 0.07 to 42.8 pmol/L and cortisol increment = 1 to 520 nmol/L. The peak occurred on average at 60 minutes for ACTH and 75 minutes for cortisol (Fig 2). The median ACTH and cortisol responses (iAUC) during the DEX/CRH test were 283 pmol/L · min (IQR 131 to 537) and 4,264 nmol/L · min (IQR 986 to 13,534), respectively. There were no relationships between age or current size and the results of the DEX/CRH test (Table 3). There was a tendency for the responses during the DEX/CRH test to be lower in men who consumed more than 10 units of alcohol per week ($P = .07$), but they were not predicted by the other lifestyle variables or depression score. The dexamethasone-suppressed cortisol concentration (DST result) and the subsequent ACTH and cortisol responses to CRH did not predict any of the hemodynamic or metabolic variables (Table 4).

DISCUSSION

In this study, we have confirmed that fasting 9 AM cortisol concentration is inversely related to adiposity and positively correlated with blood pressure and glucose tolerance and that cortisol and adiposity interact in predicting fasting plasma glucose. However, detailed testing of the pituitary-adrenal axis in a large number of subjects did not suggest that increased central drive or pituitary responsiveness was responsible for the associations between unstimulated cortisol concentrations and the components of the metabolic syndrome, despite some associations with adiposity.

Several large cross-sectional studies have found positive associations between fasting plasma cortisol concentration and cardiovascular risk factors.[1,3,4,15] In men, the strongest relationships tend to be between cortisol and blood pressure, whereas in women insulin sensitivity and lipid concentrations are closer correlates. Higher cortisol concentrations generally add to effect of obesity, but evidence of an interaction between cortisol and adiposity in determining cardiovascular risk factors has

Table 3. Associations Between HPA Study Results and Measures of Obesity

	Salivary Cortisol iAUC	9 AM Cortisol	CRH Test ACTH iAUC	CRH Test Cortisol iAUC	Cortisol Post DEX	DEX/CRH ACTH iAUC	DEX/CRH Cortisol iAUC
N	117	121	113	114	103	101	101
BMI	−0.17 (.06)	−0.29 (.001)	0.24 (.01)	0.36 (<.001)	−0.06 (.6)	0.03 (.8)	−0.05 (.6)
WHR	−0.19 (.04)	−0.19 (.03)	0.20 (.03)	0.31 (<.001)	−0.01 (.9)	−0.03 (.8)	−0.05 (.6)
Total body fat	−0.14 (.1)	−0.19 (.04)	0.09 (.3)	0.31 (<.001)	−0.03 (.7)	−0.08 (.4)	−0.08 (.4)
SSTR	−0.16 (.09)	−0.09 (.4)	0.12 (.2)	0.10 (.3)	−0.17 (.09)	−0.01 (.9)	0.05 (.6)

NOTE. Each cell contain the Pearson correlation coefficient (*P* value) relating the 2 variables.
Abbreviation: DST, dexamethsone suppression test (see text for details).

Table 4. Associations Between HPA Study Results and the Components of the Metabolic Syndrome

	Salivary Cortisol iAUC	9 AM Cortisol	CRH Test ACTH iAUC	CRH Test Cortisol iAUC	Cortisol Post DEX	DEX/CRH ACTH iAUC	DEX/CRH Cortisol iAUC
N	117	121	113	114	103	101	101
Systolic BP	0.01	−0.02	0.08	0.06	0.01	−0.10	−0.11
	0.06	0.11	−0.01	−0.07	0.02	−0.06	−0.06
Diastolic BP	−0.08	−0.02	0.12	0.11	0.05	0.02	−0.02
	−0.02	0.08	0.04	−0.01	0.07	0.02	0.00
Fasting glucose	0.00	0.02	0.23*	0.11	−0.14	0.11	0.07
	0.03	0.10	0.18	0.04	−0.13	0.11	0.10
2-h glucose	0.01	−0.12	0.16	0.08	0.04	0.01	−0.05
	0.06	−0.02	0.09	−0.05	0.06	0.03	−0.01
Insulin resistance	−0.16	−0.12	0.21*	0.17	0.02	0.09	0.03
	−0.08	0.05	0.09	−0.03	0.06	0.06	0.04
Insulin secretion	−0.17	−0.18	0.04	0.08	0.14	0.02	−0.03
	−0.09	−0.08	−0.06	−0.06	0.17	−0.02	−0.04
Triglyceride	−0.08	−0.06	0.09	0.10	−0.04	−0.07	−0.15
	−0.01	0.06	0.00	−0.05	−0.02	−0.09	−0.14

NOTE. Each cell contains the Pearson correlation coefficient relating the 2 variables and beneath it the partial correlation coefficient corrected for age and BMI.

*$P < .05$.

been found in some cohorts.[2] Our current data therefore support previous work in this field.

To explore the underlying basis of these associations further we have performed the first large-scale assessment using tests of central HPA function in men. Previous studies have focused mainly on the effects of CRH stimulation in different patterns of obesity using small case-control studies. Initial reports suggested that the ACTH response to CRH was blunted in obese individuals, but these results were not reproduced in a subsequent larger study from the same group.[21,22] Women with abdominal obesity have elevated ACTH and cortisol responses to CRH.[8] In men, however, published data vary with one group reporting exaggerated ACTH responses in generalized obesity, while others have found a reduced cortisol response in men with a high WHR.[23,24] In the current study, we have shown positive correlations between BMI and both ACTH and cortisol responses to CRH. The close correlation between BMI and WHR in this group of 65-year-old men prevented any subgroup analysis of the differences between central and peripheral obesity. A small study (n = 13) of men in this age group found that SSTR was the only obesity measure to predict CRH responses.[25] We found no association between SSTR and any parameter derived from the CRH test or any of the metabolic variables.

Few data have been published relating responses to CRH stimulation to the components of the metabolic syndrome. One study of 28 men found an association between ACTH response and insulin resistance (assessed by HOMA), but another found no relationship between either ACTH or cortisol responses and the insulin sensitivity index during a hyperinsulinemic euglycemic clamp.[13,23] Bano et al compared 2 groups of Asian women matched for BMI and found no difference in CRH response in those with and without diet-controlled type 2 diabetes.[26] Our data support these findings, showing that in a large group of men cortisol responses to 100 μg CRH did not relate to blood pressure, glucose tolerance, insulin sensitivity, or lipid concentrations. There was a significant association between the ACTH response to CRH and fasting plasma glucose concentrations. However, given the large number of analyses performed, this one significant finding may represent a type 1 error and without any other supporting data it is not considered important.

These results imply that alterations in corticotroph number and CRH sensitivity are not responsible for the association between raised circulating cortisol concentrations and components of the metabolic syndrome and also, in comparison with patients suffering from major depression who have hypercortisolaemia and reduced ACTH responses to CRH,[27] that CRH drive to the pituitary is not increased. It should be noted that a fixed dose of CRH was used in this study, in line with a significant proportion of the published data. Other groups have used 1 μg/kg,[13,23] but the results do not differ in these studies. This method of examining pituitary sensitivity does not exclude the possibility that sensitivity to lower CRH concentrations delivered via the physiological route may be responsible for differences in circulating cortisol concentrations, but studies to examine this hypothesis would be difficult in humans.

We also examined responses to 2 recently developed tests of HPA function that have not previously been used in this field of research. The salivary free cortisol response to awakening has been proposed as a useful measure of HPA activity, avoiding the stress of venepuncture.[28] Individuals under chronic stress due to work overload have an exaggerated cortisol rise after waking, which is thought to reflect a stress response in anticipation of the demands of the day ahead.[29] Recently, Edwards et al have shown that the total integrated free cortisol concentration over the first hour (AUC) is a good correlate of diurnal cortisol rhythm (ie, basal HPA activity), whereas the cortisol response (iAUC) was not, further suggesting that this measure may be a dynamic test of HPA function.[30] We found that cortisol response to awakening was inversely related to adiposity, but there were no associations between the components of

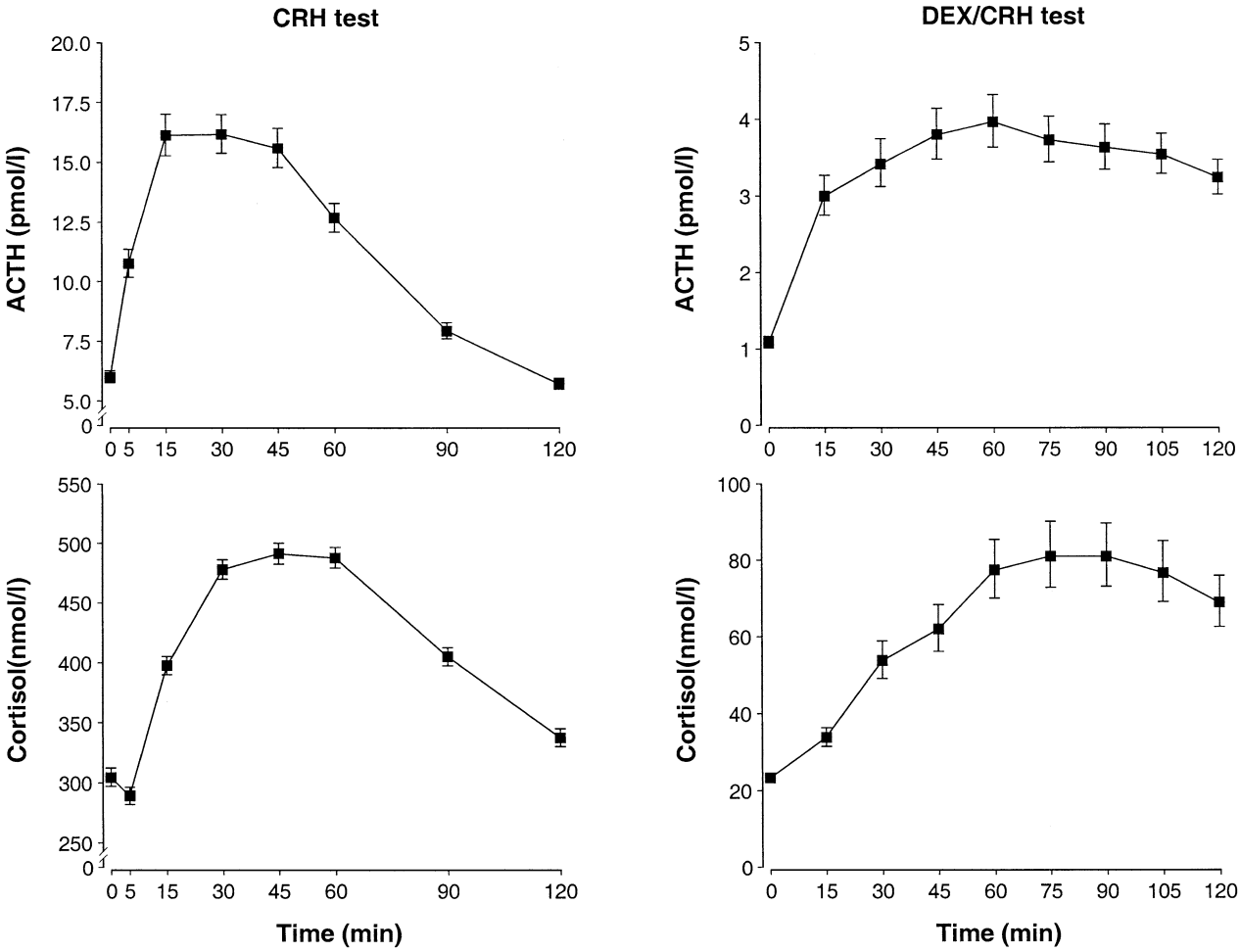

Fig 2. ACTH and cortisol responses during the CRH test (n = 114) and dexamethasone-suppressed CRH test (n = 103) in men aged 65 years. Data are geometric mean ± SEM.

the metabolic syndrome and any aspect of the salivary response in this study.

The DEX/CRH test has been developed as a sensitive tool to investigate subtle HPA dysfunction. It is suggested that this test is a marker of vasopressinergic drive to the pituitary. Dexamethasone, a synthetic steroid, does not freely pass the blood-brain barrier and the majority of its suppressive action is at the level of the pituitary.[31] This may result in a state of relative glucocorticoid depletion within the brain and a reduction of negative feedback. In depression, where an increased proportion of CRH-containing neurones in the paraventricular nucleus coexpress vasopressin (AVP), this leads to increased release of AVP into the hypophysial portal circulation, which acts synergistically with exogenous CRH to overcome dexamethasone suppression at the pituitary.[16]

Cortisol response in the DEX/CRH test increases with age, but there are no references to associations with obesity in the literature. In this study, we did not find a relationship between adiposity and the ACTH or cortisol responses during the DEX/CRH test. Likewise, there were no associations with any of the hemodynamic or metabolic variables.

It is noteworthy that we excluded participants with diabetes from the HPA study and thus the range of glucose tolerance was necessarily restricted. This may have reduced the likelihood of finding associations between pituitary-adrenal responses to the challenge tests and cardiovascular risk factors. In our older Hertfordshire cohort, where strong associations between adrenal responsiveness to low-dose ACTH and disease were found, individuals with diabetes were included.[6] However, in the current cohort, the relationships between fasting 9 AM cortisol concentration at the baseline assessment and the components of the metabolic syndrome remained even after exclusion of those individuals with diabetes, suggesting that this was a suitable subgroup in which to perform a study looking for the mechanism behind these associations.

In conclusion, it would appear from this detailed study of HPA function that neither increased pituitary responsiveness nor exaggerated CRH or AVP drive to the pituitary are likely to be responsible for the associations between higher morning cortisol concentrations and the components of the metabolic syndrome. Other groups have proposed that altered cortisol clearance is more likely to mediate the link between

glucocorticoids and the metabolic syndrome.[13,32] Alternatively, as fasting morning cortisol concentration taken on the first visit to an unfamiliar clinic is likely to represent a stress response, individuals with heightened stress reactivity may go on to develop cardiovascular risk factors. Our data suggest that future research should be concentrated in these areas.

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