



ACTH and cortisol response to combined corticotropin releasing hormone-arginine vasopressin stimulation in obese males and its relationship to body weight, fat distribution and parameters of the metabolic syndrome

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OBJECTIVES: To investigate the activity of the hypothalamic–pituitary–adrenal (HPA) axis in male obesity and its relationship with several prominent parameters of the metabolic syndrome.

DESIGN: A cross-sectional clinical study of the activity of the HPA axis in groups of obese males and normal-weight controls.

SUBJECTS: Seventeen obese non-diabetic males with a body mass index (BMI) > 28 and eight normal-weight controls were examined.

MEASUREMENTS: Fat free mass (FFM) and fat mass (FM) were measured by bioelectrical impedance, and the waist-to-hip circumference ratio (WHR) was calculated in all subjects. Baseline samples were taken for sex hormone and lipid determination, and an oral glucose tolerance test (OGTT) was performed for glucose and insulin determination. The activity of the HPA axis was determined by the combined administration of human corticotropin releasing hormone (CRH) (100 µg) and arginine vasopressin (AVP) (0.3 IU).

RESULTS: As expected, FFM and FM and the WHR were higher in obese men than in controls, as were fasting insulin and stimulated (as area under the curve (AUC)) glucose and insulin concentrations. Baseline adrenocorticotropin (ACTH) and cortisol concentrations were similar in both groups, but stimulated (as AUC), ACTH was higher ($P < 0.05$) in obese subjects than in controls, whereas no significant difference in cortisol_{AUC} was present. Since the main differences between obese subjects and controls were present during the early 30 min of the test, the correlation coefficients between total and incremental ACTH_{AUC 0–30 min} and cortisol_{AUC 0–30 min} and all other variables were analyzed. A significant correlation coefficient was present between them and all anthropometric parameters, fasting insulin and insulin_{AUC}, but not with androgens and gonadotrophins. In addition, a significant correlation was present between total and incremental ACTH_{AUC 0–30 min} and triglyceride concentrations. However, after adjusting for BMI or FM values, all correlation coefficients became non-significant, except the one between incremental ACTH_{AUC 0–30 min} and insulin_{AUC} ($P < 0.05$).

CONCLUSION: These findings indicate that obese men may also have an altered pituitary response to combined CRH/AVP stimulation, which appears to be predominantly related to body size and total body fat. ACTH hyper-responsiveness after CRH/AVP stimulation also appears to be related to hyperinsulinaemia, but underlying mechanisms of this relationship remain to be elucidated.

Keywords: Corticotropin releasing hormone; arginine vasopressin; adrenocorticotropin; obesity; men; metabolic syndrome

Introduction

There is increasing evidence that central obesity may be associated with several abnormalities of the hypothalamic–pituitary–adrenal (HPA) axis, and it is suggested that these may play a role in the development of visceral fat and related metabolic alterations, including insulin resistance (IR) and hyperinsulinaemia, glucose intolerance and lipid disorders.^{1–3}

However, the majority of available data come from studies performed in women with abdominal or visceral body fat distribution, in which exaggerated adrenocorticotropin (ACTH) and cortisol response to i.v. administration of corticotropin releasing hormone (CRH) alone⁴ or combined with arginine vasopressin (AVP),⁵ and higher than normal cortisol response to standard³ or low-dose⁴ i.v. ACTH stimulation or to acute mental stress challenge⁶ have been found. In addition, other studies have demonstrated that these women may also show hyperactivity of the HPA axis following naloxone-induced opioid blockade, which, however, can be completely reversed by increasing serotonergic receptor activation.⁷ Taken together, these results strongly support the concept that in obese women with abdominal body fat distribution,

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the HPA axis may be hyperresponsive to appropriate neuroendocrine stimulations.

Studies in rats and humans have shown a greater sensitivity of the adrenals to ACTH in females than in males.^{8–11} This could imply that the activity of the HPA axis may have a strict dependency on gender, even in obesity. Previous studies performed in men found that the cortisol response to ACTH was significantly and positively correlated with the waist-to-hip ratio (WHR), after adjusting for confusing variables.¹² In addition, other authors¹³ recently reported that men with elevated WHR experienced a decrease in the inhibition of cortisol secretion after submaximal dexamethasone oral administration, suggesting that blunted inhibition of the HPA axis with increasing abdominal fattening may be a contributory factor in determining increased axis activity.

There are no studies referring to the effects of specific releasing factors on pituitary and adrenal function in male obesity. This study was therefore carried out in obese men and appropriate normal-weight controls to investigate the ACTH and cortisol response to the administration of a combined CRH/AVP stimulus, which provides a reliable physiological test for investigating pituitary–adrenal functioning.¹⁴ An additional aim of this study was to investigate the relationship between the activity of the HPA axis and several prominent parameters of the metabolic syndrome.

Material and methods

Subjects

This study was approved by the local ethics committee and all subjects gave informed written consent. Seventeen obese (body mass index (BMI) > 28 kg/m²) men and eight age-matched normal-weight controls (BMI < 26), were included in the study. Their characteristics are reported in Table 1. The obese men had been referred to the Endocrine Unit of the Department of Internal Medicine and Gastroenterology at the University of Bologna, as outpatients, for evaluation and treatment of their obesity, whereas controls were selected from doctors and volunteers on the staff. Other endocrine and metabolic diseases were excluded on the basis of physical examination and adequate laboratory tests. None of the obese subjects had diabetes. Six obese men had hypertension and five had obstructive sleep apnea syndrome (OSAS), which was diagnosed by nocturnal polysomnographic examination, as previously described.¹⁵ None took drugs for at least one month before the study, nor were any dieting. Dietary interviews, which were performed in both obese subjects and controls, demonstrated no heavy alcohol consumption (> 30 g per day) in any subject. Four obese men, and one

Table 1 General characteristics, anthropometric parameters and baseline hormone concentrations (mean ± s.e.m.) in obese men and the control group

Parameters	Obese men (n = 17)	Control men (n = 8)
Age (y)	36.9 ± 2.4	32.6 ± 2.5
Body weight (kg)	123.7 ± 6.7*	75.8 ± 2.6
Body mass index (kg/m ²)	40.4 ± 2.0*	24.2 ± 0.7
Waist (cm)	119.0 ± 4.4*	82.6 ± 2.7
Hip (cm)	124.3 ± 4.1*	98.7 ± 0.8
Waist-to-hip ratio	0.96 ± 0.01*	0.83 ± 0.02
Fat free mass (kg)	77.3 ± 3.2*	54.3 ± 3.1
Fat mass (kg)	46.4 ± 3.8*	22.3 ± 4.1
Androstenedione (nmol/L)	0.1 ± 6.3	0.1 ± 1.6
Testosterone (nmol/L)	10.5 ± 9.5*	22.6 ± 2.1
LH (mU/mL)	3.1 ± 0.4	3.4 ± 0.5
FSH (mU/mL)	4.3 ± 0.6	3.5 ± 0.6
DHEAS (nmol/mL)	2.43 ± 0.30	2.26 ± 0.20
SHBG (nmol/L)	19.0 ± 2.1*	32.6 ± 2.8

Statistics: **P* < 0.01 between obese and control men.

LH = luteinizing hormone; FSH = follicle stimulating hormone; DHEAS = dehydroepiandrosterone sulphate; SHBG = six hormone-binding globulin

normal weight control, were smokers. None of the controls had a history of obesity.

Body height was measured without shoes to the nearest 0.5 cm, and body weight without clothes. The waist (W) and hip (H) circumferences were also measured, with the subjects standing, using a 1-cm-wide metal measuring-tape, and their ratio (WHR) was calculated. Waist circumference was obtained as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the buttocks. Bioelectrical impedance analysis (BIA) measurement was performed in all subjects using a tetrapolar-phase-sensitive impedance plethysmograph (50 kHz) BIA 101S, Akern RJL System (Florence, Italy) and fat free mass (FFM) was calculated.¹⁶ Fat mass (FM) was then calculated by subtracting FFM from body weight.

Protocol

All subjects were tested while following their usual diet, providing at least 250 g carbohydrates were ingested. Smoking was not allowed the evening before and the day on which tests were performed. Blood tests were performed in the morning (08.00–08.30 h), after overnight fasting, while subjects had been quietly lying down for 15–20 min. One i.v. cannula was placed in one forearm vein for blood sampling and another was placed in the contralateral arm for infusion (at the constant rate of 30 mL/h) of saline (0.9% NaCl) or peptide. A single blood sample was obtained for baseline hormone concentrations in all subjects. The activity of the HPA axis was then examined by administering combined stimuli with CRH and AVP, as previously described.⁴ This procedure has been shown to maximally stimulate the pituitary reserve in physiological conditions.¹⁴ Human CRH (hCRH, Novabiochem, Laufelfingen,

Switzerland) and synthetic 8-e AVP (Pitressin, Parke Davis and Co, Berlin, Germany) were injected as i.v. bolus, at doses of 100 µg and 0.3 I.E., respectively. Samples for ACTH and cortisol determination were drawn in the contralateral arm in basal conditions (–15 min and 0 min) and 15 min, 30 min, 60 min, 90 min and 120 min thereafter. On a separate day, a 75 g oral glucose tolerance test (OGTT) (Curvosio Sclavo, Siena, Italy) was performed, and blood samples were obtained in basal conditions and after 30 min, 60 min, 90 min, 120 min and 180 min for plasma glucose, and after 60 min, 120 min and 180 min for serum insulin determinations.

Laboratory assays

Blood glucose concentrations were determined immediately after the OGTT, by the glucose oxidase method. Lipid determination was performed on the same day as the blood samples were taken. Total cholesterol and triglyceride were determined in plasma samples by enzymatic methods, using reagents purchased from Biochemia Boehringer Robin (Mannheim, Germany). The high-density lipoprotein (HDL) cholesterol concentration was measured after precipitation with MgCl₂ 6H₂O (0.05 mmol/L) and phosphotungstic acid (14 mmol/L) with reagents purchased from Behring (Marburg, Germany).

Gonadotropin, androgen and insulin measurements were performed on plasma or serum samples stored at –20°C until assayed and determination in each man was performed in duplicate in the same assay. Insulin was measured by reagents purchased from Eiken Chemical Corporation (Tokyo, Japan). Steroids (testosterone, androstendione and dehydroepiandrosterone-sulphate-DHEA-S) were measured by RIA after serum purification by ether (Carlo Erba, Milan, Italy) extraction followed by solid phase extraction on C18 disposable columns (Varian, Harbour City, CA). Extracts were reconstituted in suitable buffer, incubated overnight with antiserum and 3H-tracer (NEN Dupont, Brussel) and the separation between bound and free ligands was performed by dextran-coated charcoal. Gonadotropin LH and FSH (standard: 2nd WHO-IRP 80/552 and 94/632, respectively) levels were measured with reagents obtained from Ciba-Corning (Medfield, MA) by chemiluminescent methods, and SHBG with reagents obtained from DPC (Los Angeles, CA) by a solid-phase chemiluminescent enzyme immunometric assay.

Immediately after taking the blood samples, aliquots were placed in different tubes containing EDTA with or without aprotinin (500 U/mL), for cortisol and ACTH determinations, respectively, maintained in ice and then stored at –80°C until assayed. All assays of each man were performed in duplicate. ACTH was determined with an IRMA method with reagents obtained from Nichols Institute (San Juan Capistrano, CA). Sensitivity of this assay in our laboratory is approximately 1 pg/mL (0.22 pmol/L). Inter- and

intraassay coefficients of variations at concentrations of 28.6 pg/mL (6.3 pmol/L) and 244.3 pg/mL (53.8 pmol/L) are 9.6% and 7.1%, and 7.3% and 3.7%, respectively. Cortisol was determined by RIA with reagents obtained from Diagnostic Product Corporation (Los Angeles, CA). In our laboratory, the lowest sensitivity level is 30 ng/mL (8.3 nmol/L). Inter- and intraassay coefficients of variations at concentrations of 58 ng/mL (160 nmol/L), 217 ng/mL (599 nmol/L), and 359 ng/mL (990 nmol/L) are 9.8%, 6.9%, 8.0% and 1.4%, 3.1%, 4.1%, respectively.

Statistics

All results are reported as mean ± standard error of the mean (S.E.M.). Inter-group comparisons were performed by the non-parametric Mann-Whitney U test, and intra-group comparisons (that is, comparison within the test for each group) were performed by the Wilcoxon rank sum test. Total and incremental ACTH and cortisol AUC after combined CRH/AVP administration were calculated by the trapezoidal method. Incremental AUC were calculated from total AUC, after subtracting for baseline area. Simple and multiple regression analysis were used as appropriate. $P < 0.05$ was used to define statistical significance.

Results

Anthropometry and baseline hormones (Table 1)

Other than BMI, obese men had significantly higher FM, FFM, waist, hip and WHR values than controls. There were no significant differences between obese subjects and controls on basal ACTH, cortisol, androstenedione, DHEA-S and gonadotropins. On the contrary, serum testosterone ($P < 0.01$) and SHBG ($P < 0.01$) concentrations were significantly lower in obese subjects than in controls.

ACTH and cortisol response to combined CRH/AVP administration

After stimulation, ACTH and cortisol rapidly increased in control subjects, reaching peak values at 15 min ($P < 0.001$) and 30 min ($P < 0.001$), respectively, then progressively declined to values significantly below basal levels at 120 min ($P < 0.05$) for cortisol and at 90 min ($P < 0.05$) and 120 min ($P < 0.05$) for ACTH. In the obese group, there was a much more sustained and significant increase of ACTH and cortisol at times 15 min ($P < 0.001$) and 30 min ($P < 0.01$), after which they progressively declined to values below baseline (at time 90 min, $P < 0.01$, and 120 min, $P < 0.01$, for ACTH, and at time 120 min, $P < 0.05$, for cortisol). However, in obese subjects, all ACTH values from time 15 min

onwards were significantly higher than in controls, whereas no significant difference was present between cortisol values at any time (Figure 1). Total ACTH_{AUC} was significantly ($P < 0.005$) higher in obese subjects (4200 ± 382 pmol/L.min) than in controls (2313 ± 283 pmol/L.min), whereas no significant difference was observed in total cortisol_{AUC} (obese subjects: 14270 ± 695 nmol/L.min vs controls: 14035 ± 752 nmol/L.min). In view of the reduction of ACTH and cortisol below baseline values during the test (see above), we calculated total and incremental AUC, using hormone values at 15 min and 30 min after stimulation. Both total and incremental ACTH_{AUC0-30min} were significantly ($P < 0.01$) higher in obese subjects (total: 1819 ± 217 pmol/L.min, and incremental: 809 ± 171 pmol/L.min, respectively) than in the control group (total: 964 ± 146 pmol/L.min, and incremental: 304 ± 110 pmol/L.min, respectively). On the contrary, total cortisol_{AUC0-30min} was similar in both groups (5902 ± 263 nmol/L.min vs 5633 ± 412 nmol/L.min; not statistically significant (NS); incremental cortisol_{AUC0-30min} was higher in obese subjects (1643 ± 284 nmol/L.min) than in controls (822 ± 239 nmol/L.min), but this difference was not significant ($0.05 > P > 0.1$) (Figure 1).

In the obese group, there were no significant effects of smoking, hypertension, OSAS, or impaired glucose

tolerance on fasting and stimulated (as AUC) ACTH or cortisol concentrations.

Glucose, insulin and lipids

Fasting values of triglycerides (1.40 ± 0.10 mmol/L vs 0.80 ± 0.10 mmol/L, $P < 0.01$), glucose (5.1 ± 0.2 mmol/L vs 4.2 ± 0.2 mmol/L, $P < 0.01$), and insulin (198.0 ± 31.3 pmol/L vs 36.8 ± 5.7 pmol/L, $P < 0.01$) were significantly higher in obese subjects than in controls, whereas no significant difference was present in total (5.4 ± 0.2 mmol/L vs 4.4 ± 0.3 mmol/L) and (HDL) cholesterol (1.10 ± 0.10 mmol/L vs 1.20 ± 0.10 mmol/L) concentrations. Six obese subjects had impaired glucose tolerance, according to the World Health Organization (WHO) Expert Committee criteria.¹⁷ Therefore, glucose_{AUC} ($P < 0.05$) was significantly higher in obese subjects (1269 ± 64 mmol/L.min) than in controls (880 ± 58 mmol/L.min) and, as expected, insulin_{AUC} was significantly ($P < 0.01$) higher in the former (193.0 ± 40.7 pmol/L.min. 10^{-3}) than in the latter (38.6 ± 6.2 pmol/L.min. 10^{-3}).

Relationship between ACTH_{AUC} and cortisol_{AUC} and anthropometry, sex hormones, insulin, and lipids.

Since the main differences between obese subjects and controls were present during the early 30 min of

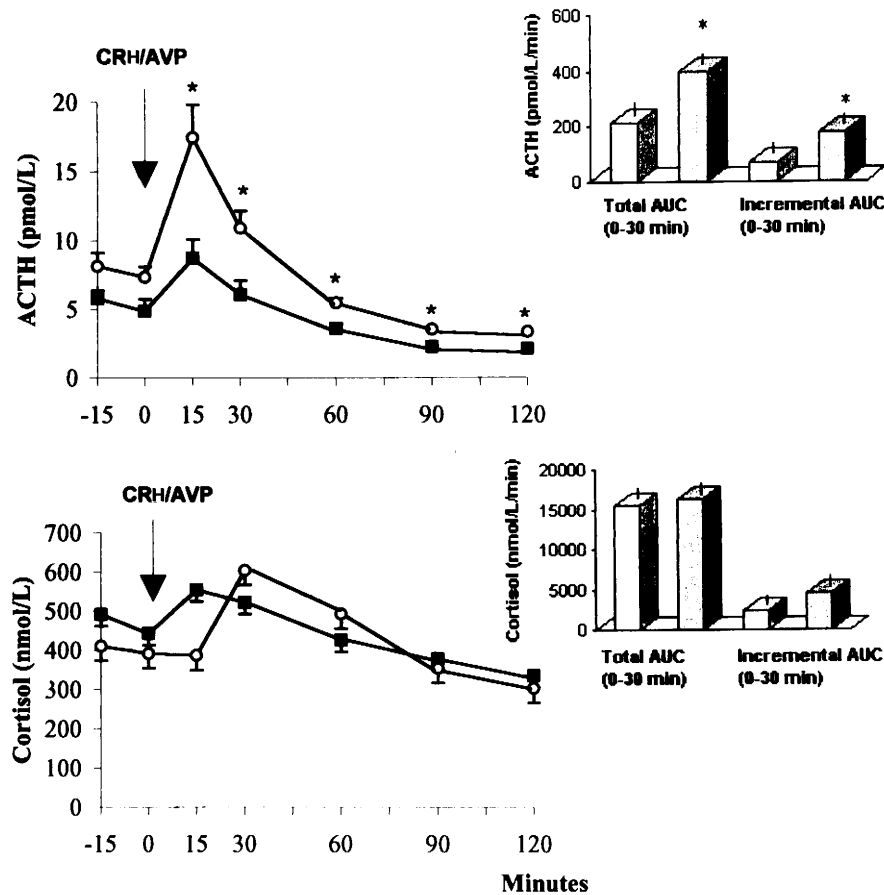


Figure 1 Plasma ACTH and cortisol concentrations after corticotropin releasing hormone (CRH)/arginine vasopressin (AVP) test and total and incremental ACTH_{AUC0-30min} and Cortisol_{AUC0-30min} (mean \pm s.e.m) in obese and normal weight control men (* $P < 0.05$). AUC_{0-30min} = the area under the curve (AUC) calculated based on hormone values measured 15 min and 30 min after CRH/AVP administration.

the test, we analyzed the correlation coefficients between total and incremental $ACTH_{AUC0-30\text{ min}}$ and $cortisol_{AUC0-30\text{ min}}$ and all anthropometric and metabolic variables. A significant correlation coefficient was present between them and all anthropometric parameters (BMI, waist circumference, WHR, FFM and FM), fasting insulin and $insulin_{AUC}$, but not with androgens and gonadotropins. In addition, a significant correlation was present between total and incremental $ACTH_{AUC0-30\text{ min}}$ and triglyceride concentrations. However, after adjusting for BMI or FM values, all correlation coefficients became non-significant, except the one between incremental $ACTH_{AUC0-30\text{ min}}$ and $insulin_{AUC}$ ($P < 0.05$) (Figure 2).

Discussion

The results of this study indicate that obese males may have ACTH hyper-responsiveness to combined CRH/AVP stimulation, similar to what was previously reported in women with the abdominal obesity phenotype.⁴ However, this abnormality was apparently less marked than that described in such obese women. This difference between the sexes is not an unexpected finding, since there is evidence that females may have a greater sensitivity in the adrenals to different stimulatory neuropeptides, including ACTH.^{10,11,18,19} The lack of correlation between the activity of the HPA axis and anthropometric indices of body fat distribution, contrary to what was previously reported in women,^{3,5} may be explained by the fact that obesity in males is almost always associated with a parallel increase of abdominal and visceral fat, which means that the central distribution of body fat

depends on the actual presence of obesity in males. This is exemplified by the very high correlation coefficients between BMI and FM and waist circumference (in this study: $r = 0.97$, $P < 0.0001$, and $r = 0.89$, $P < 0.0001$, respectively) but not with the hip circumference (NS), which is commonly found in a male population.²⁰ Therefore, net ACTH response to CRH/AVP in obese males appears to be proportional to the increase of body size and total body fat, rather than to the amount of abdominal or visceral fat.

A further finding of this study was that the ACTH response was significantly correlated with $insulin_{AUC}$ during the OGTT, regardless of BMI and FM values. The theoretical basis for this association does not necessarily imply a cause-effect relationship. However, hyperinsulinaemia represents a distinct characteristic of obesity, particularly of the visceral phenotype. However, since CRH/AVP-induced ACTH hypersecretion and increased insulin concentrations are distinct alterations simultaneously present in male obesity, it cannot be excluded that they are in some way partially related to common regulatory mechanisms. There are, in fact, conditions such as, for example, food ingestion, in which insulin and the HPA axis activity may change in the same direction, probably due to the intervention of neural factors, particularly the sympathetic nervous system, which is involved in the regulation of both insulin secretion and the HPA axis surge.²¹ Recently, it has been found that ACTH and cortisol response to mixed meals may be supranormal in obese women, at least in those with the abdominal phenotype.²² Whether altered neural-mediated mechanisms are involved in the regulation of altered HPA axis activity and in its relation with insulin secretion in obesity, either after meal ingestion or after hypothalamic or hypophyseal stimulation, remains an obscure question that needs more detailed investigation.

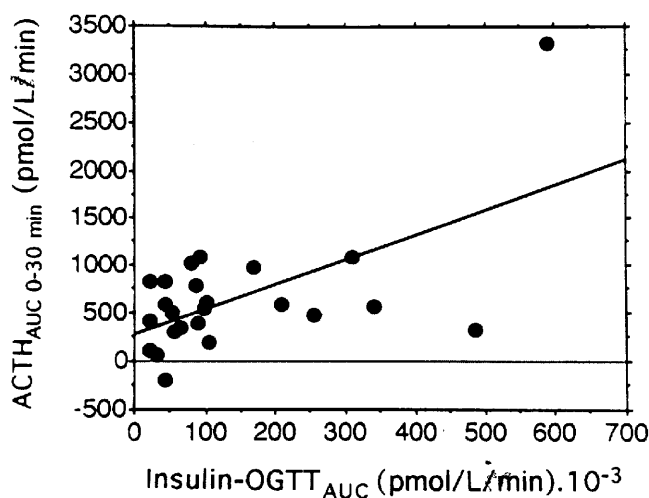


Figure 2 Correlation coefficient between incremental $ACTH_{AUC0-30\text{ min}}$ and $insulin_{AUC-OGTT}$ in obese and controls men (after adjusting for body mass index (BMI): $t = 2.1$, $P < 0.05$; after adjusting for fat mass (FM): $t = 2.4$, $P < 0.05$). AUC = area under the curve; OGTT = oral glucose tolerance test.

Conclusion

Similar to what was previously reported in women with abdominal obesity, we found that obese men may have ACTH hyperresponsiveness to combinational CRH/AVP stimulation. This alteration is closely correlated with BMI and not with parameters of body fat distribution. The independent correlation between ACTH increase after stimulation and stimulated insulin during the OGTT is an interesting finding which may stimulate further investigation.

References

- 1 Björntorp P. "Portal" adipose tissue as a generation of risk factors for cardiovascular disease and diabetes. *Atherosclerosis* 1990; **10**: 493–496.
- 2 Björntorp P. Visceral obesity: A "civilization syndrome". *Obes Res* 1993; **1**: 206–222.

- 3 Marin P, Darin N, Anemiya T, Anderson B, Jern S, Björntorp P. Cortisol secretion in relation to body fat distribution in obese premenopausal women. *Metabolism* 1992; **41**: 882–886.
- 4 Pasquali R, Cantobelli S, Casimirri F, Capelli M, Bortoluzzi F, Flaminia R, Morselli-Labate AM, Barbara L. The hypothalamic–pituitary–adrenal axis in obese women with different patterns of body fat distribution. *J Clin Endocrinol Metab* 1993; **77**: 341–346.
- 5 Pasquali R, Anconetani B, Chattat, Biscotti M, Spinucci G, Casimirri F, Vicennati V, Carcello A, Moselli-Labate AM. hypothalamic–pituitary–adrenal axis activity and its relationship to the autonomic nervous system in women with visceral and subcutaneous obesity: effects of corticotropin-releasing factor/arginine-vasopressin test and of stress. *Metabolism* 1996; **45**: 351–356.
- 6 Moyer AE, Rodin J, Grilo CM, Cummings N, Larson LM, Rebuffé-Scrive M. Stress-induced cortisol response and fat distribution in women. *Ob Res* 1994; **2**: 255–261.
- 7 Boushaki FZ, Rasio E, Serri O. Hypothalamic–pituitary–adrenal axis in abdominal obesity: effects of dexfenfluramine. *Clin Endocrinol (Oxf)* 1997; **46**: 461–466.
- 8 Kitay JF, Coyne MD, Newson W, Nelson K. Relation of the ovary to adrenal corticosterone production and adrenal enzyme activity in the rat. *Endocrinology* 1965; **77**: 902–907.
- 9 Roelfsema F, Vandenberg G, Frolich M, Veldhuis JD, van Eijk A, Buurman MM, Etman BHB. Sex dependent alteration in cortisol response to endogenous adrenocorticotropin. *J Clin Endocrinol Metab* 1993; **77**: 234–240.
- 10 Horrocks PM, Jones AF, Ratcliffe WA, Holdere G, White A, Holder R, Ratcliffe JB, London DR. Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. *Clin Endocrinol (Oxf)* 1990; **32**: 127–134.
- 11 Born J, Dietschuneit I, Schreiber M, Dodt C, Fehm HL. Effect of age and gender on pituitary–adrenal responsiveness in humans. *Eur J Endocrinol* 1995; **132**: 705–711.
- 12 Hautanen A, Adlercreutz H. Pituitary-adrenocortical function in abdominal obesity of males: evidence for decreased 21-hydroxylase activity. *J Steroid Biochem Mol Biol* 1996; **58**: 123–133.
- 13 Ljung T, Anderson B, Bengtsson BA, Björntorp P, Marin P. Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose-response study. *Obes Res* 1997; **4**: 277–282.
- 14 Lamberts SWJ, Verleun T, Oosterom R, De Jong F, Han-keng WHL. Corticotropin releasing factor (ovine) and vasopressin exert a synergistic effect on adrenocorticotropin release in men. *J Clin Endocrinol Metab* 1984; **58**: 298–303.
- 15 Pasquali R, Colella P, Cirignotta F, Mondini S, Gerardi R, Buratti P, Rinaldi Ceroni A, Tartari F, Schiavina M, Melchionda N, Lugaresi E, Barbara L. Treatment of obese patients with obstructive sleep apnea syndrome (OSAS): effect of weight loss and interference of otorhinolaryngoiatric pathology. *Int J Obes* 1990; **14**: 207–217.
- 16 Segal KR, Van Loan M, Fitzgerald P, Hodgdon IA, Van Itallie TB. Lean body mass estimated by bioelectrical impedance analysis: Four site cross-validation study. *Am J Clin Nutr* 1988; **47**: 7–14.
- 17 World Health Organization Expert Committee. *Second Report on Diabetes Mellitus*. Technical Report, Series 727. WHO, Geneva, 1985.
- 18 Chalew S, Nagel H, Shore S. The hypothalamic–pituitary–adrenal axis in obesity *Obes Res* 1995; **3**: 371.
- 19 Hermus ARMM, Pieters GFMM, Amals AGH, Benraad THJ, Kloppenborg PWC. Plasma adrenocorticotropin, cortisol, and aldosterone response to corticotropin-releasing factor: modular effects of basal cortisol levels. *J Clin Endocrinol Metab* 1984; **58**: 187–191.
- 20 Young WF, Zinsmeister AR, Twomey CK, Kao PC, Jiang NS, Carpenter PC. Ovine corticotropin releasing hormone stimulation test: normal value study. *Mayo Clin Proc* 1990; **65**: 943–948.
- 21 Seidell JC, Oosterlee A, Deurenberg P, Hautvast JGAJ, Ruijs JHJ. Abdominal fat depots measured with computed tomography: effects of degree of obesity, sex and age. *Eur J Clin Nutr* 1988; **42**: 805–815.
- 22 Al-Damluji S, Iveson T, Thomas JM, Pendlebury DJ, Rees LH, Bessere GM. Food-induced cortisol secretion is mediated by central alpha-1 adrenoceptor modulation of pituitary ACTH secretion. *Clin Endocrinol (Oxf)* 1987; **26**: 629–636.
- 23 Korbonits M, Trainer PJ, Nelson ML, Howse I, Kopelman PG, Besser G, Grossman AB, Svec F. Differential stimulation of cortisol and dehydroepiandrosterone levels by food in obese and normal subjects: relation to body fat distribution. *Clin Endocrinol (Oxf)* 1996; **45**: 699.