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Pituitary–Adrenocortical Function in Abdominal Obesity of Males: Evidence for Decreased 21-Hydroxylase Activity

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Certain differences in regional fat distribution might be explicable by subtle hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis. We examined prospectively PA function relative to abdominal obesity defined by waist-to-hip circumference ratio (WHR) in 71 normotensive men aged 30–55 years. Basal PA activity was assessed by measurements of serum cortisol and plasma corticotropin (ACTH) concentrations during the oral glucose tolerance test (OGTT). Functional activity was examined by dexamethasone suppression and ACTH stimulation tests; responses of 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol (S), cortisol, dehydroepiandrosterone (DHEA), and androstenedione were determined. When the subjects were divided into tertiles for the WHR, the ratio of mean ACTH to mean cortisol during the OGTT was increased ($p < 0.05$), and the ratio of urinary cortisol to body-mass index was decreased ($p < 0.01$), whilst the net increments of cortisol ($p < 0.05$) and 17-OHP ($p < 0.05$) from 0 to 60 min, as well as the ratio of 17-OHP to S increments ($p < 0.05$) after ACTH were elevated in the highest vs lowest WHR tertile. The ratio of mean ACTH to mean cortisol ($r = 0.495$; $p < 0.001$) during the OGTT, the ratio of net 17-OHP to S increments ($r = 0.404$; $p < 0.001$), and the net DHEA ($r = 0.276$; $p = 0.020$) and 17-OHP ($r = 0.336$; $p = 0.005$) responses to ACTH at 60 min correlated with WHR. In multivariate analyses the ratio of mean ACTH to cortisol, cortisol response to ACTH, and the ratio of net 17-OHP to S increments were all significant predictors of WHR independent of smoking, physical activity, and BMI explaining 49.0% of the variance in WHR. Thus, abdominal obesity may be associated with decreased activity of adrenal 21-hydroxylase. Either obesity-related functional alteration of 21-hydroxylase activity or the high carrier prevalence of genetic defects of this enzyme may explain these findings. Copyright © 1996 Elsevier Science Ltd.

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INTRODUCTION

Of the various phenotypes of obesity, excess fat in the abdominal region carries a risk for cardiovascular and metabolic disorders in both men and women [1]. Causal factors leading to abdominal obesity remain unknown, although hereditary, behavioural and lifestyle characteristics all affect its expression [2]. Abdominal obesity is a classic sign of glucocorticoid excess, as exemplified by Cushing's disease. Some individual differences in the regional fat distribution might thus theoretically be explained by subtle hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis [3].

Cortisol metabolism in general obesity has been a subject of extensive studies; excess cortisol appears to be excluded, although the rates of production and catabolism of cortisol might be increased [4, 5]. Yet recent studies have shown that basal cortisol levels tend to be negatively related [6], and the cortisol response to exogenous CRH or ACTH stimulation [6–8] as well as the endogenous ACTH response to oral glucose were positively related to the WHR [8], a measure of abdominal obesity.

Increased androgen activity has been implicated in

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abdominal obesity in females, although the precise source of hyperandrogenism is unknown [4, 9–11]. In obese males, a decrease in testosterone levels has been well established [4, 12, 13], whereas the secretion of adrenal androgens has attracted less research interest in relation to obesity. We have examined the pituitary–adrenocortical function of healthy males by several functional tests and found alterations in cortisol and ACTH levels related to abdominal obesity [8]. We have now expanded our previous study by measuring the responses of dehydroepiandrosterone (DHEA), androstenedione, 17-hydroxyprogesterone (17-OHP), and 11-deoxycortisol (S) to dexamethasone (DXM) suppression and ACTH stimulation. We have also investigated the precursor-to-product ratios of these steroids to assess the functional activity of some adrenal steroid synthesizing enzymes in relation to abdominal obesity.

SUBJECTS AND METHODS

Subjects and protocol

This study is part of a research project, which addressed the hypothetical associations between psychological and behavioural characteristics, lifestyle factors and neuroendocrine responses and the insulin resistance syndrome. The research protocol was approved by the local ethics committee. A total of 101 middle-aged men were recruited as previously described [8, 14]. Informed consent was obtained from all subjects. Twenty-five subjects with hypertension, one with non-insulin dependent diabetes mellitus and four with coronary heart disease were excluded from this analysis, which thus includes 71 normotensive males. They received no medication and had no history or clinical evidence of liver, kidney, gastrointestinal, endocrine or coronary heart disease, as determined by clinical examination and laboratory analyses including blood cell counts, serum chemistry profiles, urinalyses and electrocardiograms.

The subjects were studied as outpatients on three consecutive days at the Helsinki University Central Hospital. On day 1, at 0730 h, after an overnight fast, blood samples were drawn to determine blood cell counts and serum chemistry profiles, followed by recording of electrocardiograms. Starting at 1800 h, 12 h urine samples were collected to determine the free cortisol excretion. Because subject number 16 was the first with a urine sample, free cortisol excretion could be determined from 60 subjects for this analysis. On day 2, at 0730 h, an oral glucose tolerance test (OGTT) was carried out. An in-dwelling cannula was inserted into an antecubital vein. Thirty minutes later, a standard dose of 75 g glucose was given. Blood samples were drawn at 0, 60 and 120 min after glucose for the determination of insulin, C-peptide, glucose, DHEA, androstenedione, 17-OHP, cortisol, and ACTH concentrations.

On day 2, each subject received 1 mg dexamethasone (DXM, Orion, Helsinki, Finland) orally at 2300 h. On day 3 at 0730 h, an antecubital vein was cannulated. Thirty minutes later, ACTH (S-Cortrophin, Organon, Oss, Holland) was injected as an intravenous bolus at a dose of 10 $\mu g/m^2$. Blood samples were drawn 30 min before, at times 0 and 30 and 60 min after the administration of ACTH for the determination of DHEA, androstenedione, 17-OHP, S and cortisol concentrations.

The WHR, a measure of abdominal obesity, was determined as described [8]. The smallest girth between the rib cage and the iliac crest and the largest girth between the waist and the thigh were defined as the circumference of waist and hip, respectively. Body-mass index (BMI) was calculated as the weight in kilograms divided by the square of height in metres.

Systolic and diastolic blood pressures were measured by a standard sphygmomanometer on days 2 and 3 before the glucose tolerance and ACTH tests. The subjects had been in the supine position for at least 15 min. Three readings to the nearest even digit were recorded, and the mean of the second and third readings was defined as the blood pressure. If the blood pressures exceeded the limits defined previously [8, 14, 15] on both days, the subject was considered to have hypertension and excluded from this analysis.

Alcohol consumption, physical activity, and smoking were estimated by a standard questionnaire completed by structured interview [14]. Four categories of leisure-time exercise patterns were used: (1) no regular physical activity; (2) light physical activity; (3) moderate physical activity; and (4) strenuous physical activity.

Steroid assays

Commercial RIA kits were used for the determination of serum and urinary cortisol (Farmos Diagnostica, Oulu, Finland), and serum S (ICN Biomedicals, Carson, CA, U.S.A.). Serum DHEA, androstenedione, and 17-OHP concentrations were determined by RIA as described [14]. Plasma concentrations of intact ACTH were determined by procedure A of the double-antibody RIA (Incstar Corp., Mn., U.S.A). Means of duplicate determinations were used in all calculations. High and low value quality control samples were included in each assay. Samples were re-run, if duplicate values differed more than 10% from their calculated mean. The intra-assay and inter-assay imprecision (CV) of the RIA methods were as follows: cortisol 6.4 and 7.0%, androstenedione 4.8 and 6.1%, DHEA 3.0 and 5.8%, 17-OHP 6.1 and 9.2%, S 5.4 and 6.4%, respectively. For ACTH CVs varied from 3.4 and 23.1% (low control values) to 4.2 and 7.1% (high control values).

Statistical analysis

The mean of three measured values of DHEA, androstenedione, 17- OHP, cortisol and ACTH taken during the OGTT was used to assess the basal secretion of hormones. Adrenal steroid responses to exogenous ACTH stimulation are given by either the absolute poststimulation levels at 60 min or the net increment from 0 (DXM-suppressed level) to 60 min of various steroids. The ratio of 17-OHP to S was calculated for both absolute poststimulation levels and net increments of steroids. The ratio of DHEA to androstenedione responses and the ratio of 17-OHP to androstenedione responses were calculated from the sum of steroid values at 30 and 60 min after ACTH stimulation minus the DXM-suppressed value. One-way analysis of variance (ANOVA) was used to test differences between tertiles; the Bartlett test for homogeneity of variance was included, and variables were logarithmically transformed when necessary. The Tukey's HSD procedure was used as a *post-hoc* multiple comparison test. Relationships between variables were analysed by computing Pearson's correlation coefficients and by multiple linear regression with the Systat^{R} statistical program package.

RESULTS

Basic characteristics subjects

The mean (SD) age of the 71 study subjects was 44.6 (5.2) years (range, 30–55). The body-mass index of the subjects averaged 25.3 (3.3) kg/m^2 (range, 20.0–35.1) and the WHR 0.93 (0.06) (range, 0.78–1.12). They had a mean systolic blood pressure 123 (10) mmHg (range, 94–138), and a mean diastolic blood pressure 78 (7) mmHg (range, 62–88). Their average alcohol consumption was 174 (124) g/week (range, 0–540). Twenty-six subjects (36.6%) were smokers. Regular physical exercise three times or more per week was reported by 24 subjects (33.8%), whereas 14 (19.7%) were physically inactive. All had normal glucose tolerance according to the established criteria [16].

Steroid determinations by tertiles of WHR

To examine the relationship between abdominal obesity and steroid responses, the study group was divided into tertiles for the WHR. The tertile cutpoints of WHR were 0.900 and 0.949. The mean (SD) age, height, weight, WHR, BMI, alcohol consumption, physical activity, smoking status (yes/no), the number of cigarettes smoked per day, s-creatinine and *S*-glutamyltransferase values for the tertiles are shown in Table 1. The means of weight, WHR and BMI were significantly different between the lowest, middle and upper tertiles. Age, height, alcohol consumption and s-creatinine levels were similar in all tertiles, whereas physical activity was lower and *S*-glutamyltransferase levels higher in the upper tertile than in the lowest tertile. Although the number of smokers and cigarettes smoked tended to be higher in the upper tertile, no statistically significant differences were found between the three tertiles.

Urinary cortisol was collected before, and serum DHEA, androstenedione, 17-OHP, cortisol and ACTH were taken during the OGTT to assess the basal activity of the pituitary–adrenal axis. Table 2 shows the decreasing tendency of fasting, 1 and 2 h, of mean basal (0 + 1 + 2 h), and of mean 1 + 2 h cortisol concentrations during the OGTT. The increasing tendency of respective ACTH concentrations is also shown. The ratio of mean ACTH to mean cortisol (0 + 1 + 2 h), and the ratio of urinary cortisol to BMI differed significantly between the lowest and highest tertiles. On the contrary, the mean DHEA, androstenedione, and 17-OHP concentrations during the OGTT were not

Table 1. Anthropometric, lifestyle and basic laboratory variables by tertiles of WHR ratio

	Tertile of WHR			
	< 0.900 (n = 24)	0.900–0.949 (n = 24)	> 0.949 (n = 23)	ANOVA (p)*
Age	44.6(7.4)	44.2(4.3)	45.0(2.8)	0.812
Height (cm)	180.8(5.5)	178.1(4.4)	178.9(6.6)	0.246
Weight (kg)	75.5(9.0)	78.0(8.2)	91.0(13.1)§¶	< 0.001
WHR	0.86(0.04)	0.93(0.02)§	0.99(0.04)§¶	< 0.001
BMI	23.1(1.9)	24.6(2.1)	28.3(3.2)§¶	< 0.001
Alcohol (g/week)	159(99)	163(127)	200(144)	0.466
Physical activity index	2.5(0.8)	2.3(0.9)	1.8(0.7)‡	0.006
Smokers (yes/no)	6/18	8/16	12/11	0.146
Cigarettes (no/day)	5.1(9.3)	6.7(11.0)	10.0(12.0)	0.288
S-creatinine (μmol/1)	94.0(9.1)	94.3(9.0)	95.4(8.9)	0.991
S-glutamyltransferase (U/1)	31.4(29.7)	45.2(41.3)	61.4(63.8)†	0.013

* One-way analysis of variance (ANOVA).
Age, weight, BMI, WHR and *S*-glutamyltransferase were log transformed for comparisons.
Post-hoc test on pairs of means using Tukey's HSD procedure.
Comparisons with the lowest tertile: † $p < 0.05$, ‡ $p < 0.01$, § $p < 0.001$.
Comparisons with the middle tertile: ¶ $p < 0.001$.

Table 2. Adrenal steroid and ACTH levels during oral glucose tolerance test and the urinary cortisol excretion in the study group by tertiles of WHR

Variables	Tertile of WHR			
	< 0.900 ($n = 24$)	0.900–0.949 ($n = 24$)	> 0.949 ($n = 23$)	ANOVA (p)*
Fasting-cortisol	406(102)	404(112)	369(76)	0.400
1 h cortisol	357(124)	328(106)	290(82)	0.091
2 h cortisol	339(127)	317(111)	277(77)	0.348
Mean cortisol (0 + 1 + 2h)	375(105)	340(74)	323(60)	0.082
Mean cortisol (1 + 2h)	348(116)	323(85)	284(61)	0.088
Fasting ACTH	15.1(7.2)	14.4(5.3)	16.7(7.0)	0.377
1 h ACTH	13.4(5.7)	13.0(3.7)	14.1(6.5)	0.849
2 h ACTH	11.8(2.3)	12.5(2.8)	14.1(4.2)	0.075
Mean ACTH (0 + 1 + 2h)	13.4(3.9)	13.3(2.8)	15.0(4.7)	0.292
Mean ACTH (1 + 2h)	12.7(3.6)	12.8(2.7)	14.1(4.7)	0.332
Ratio of mean ACTH to mean cortisol	3.82(1.19)	3.93(0.95)	4.97(1.91)†	0.015
Mean basal DHEA (nmol/1)	11.8(3.9)	12.7(4.4)	11.4(3.6)	0.629
Mean basal androstene-dione (nmol/1)	4.4(0.6)	4.5(0.8)	4.1(0.8)	0.183
Mean basal 17-OHP (nmol/1)	7.6(2.7)	6.7(2.6)	6.4(2.7)	0.281
Urinary cortisol (nmol/12 h)	264(58) ($n = 21$)	266(51) ($n = 20$)	242(91) ($n = 19$)	0.182
Ratio of urinary cortisol to BMI	11.4(2.4)	11.0(2.2)	8.7(3.4)‡	0.006

See footnote in Table 1.
* ACTH and cortisol measurements, except the ratio of urinary cortisol to BMI were log transformed for comparisons.
† $p < 0.05$, $p < 0.01$ compared with lowest tertile.
‡ $p < 0.05$ compared with middle tertile.

Table 3. Steroid responses to ACTH stimulation in the study group by tertiles of WHR

Steroid	Tertile of WHR			ANOVA (p)
	< 0.900 ($n = 24$)	0.900–0.949 ($n = 24$)	> 0.949 ($n = 23$)	
Androstenedione (nmol/1)				
Net (0–60 min)	1.8(1.3)	2.0(1.3)	2.1(2.0)	0.801
Absolute (60 min)	6.7(1.3)	6.8(1.3)	6.8(1.9)	0.957
DHEA (nmol/1)				
Net (0–60 min)	23.3(14.0)	25.5(14.8)	30.5(17.1)	0.263
Absolute (60 min)	28.2(14.5)	31.6(15.5)	36.2(17.8)	0.233
17-OHP (nmol/1)				
Net (0–60 min)	10.3(4.7)	12.3(3.9)	13.4(3.5)†	0.034
Absolute (60 min)	15.7(3.6)	17.1(3.4)	17.6(3.8)	0.183
S (nmol/1)				
Net (0–60 min)	5.5(1.4)	6.2(1.9)	5.6(1.2)	0.271
Absolute (60 min)	8.1(1.5)	8.4(1.9)	8.0(1.7)	0.731
Cortisol (nmol/1)				
Net (0–60 min)	550(113)	639(97)*	624(101)†	0.009
Absolute (60 min)	583(113)	681(93)†	667(115)†	0.005
17-OHP/S ratio				
Net (0–60 min)	1.85(0.81)	2.08(0.65)	2.41(0.49)†	0.019
Absolute (60 min)	1.96(0.40)	2.11(0.45)	2.24(0.37)	0.075
S/cortisol ratio x100				
Net (0–60 min)	1.02(0.28)	0.97(0.26)	0.91(0.18)	0.300
Absolute (60 min)	1.43(0.32)	1.24(0.26)	1.21(0.25)†	0.016
DHEA/androstenedione ratio (30 + 60–0 min)	6.0(3.7)	6.1(3.0)	7.6(5.6)	0.532
17-OHP/androstenedione Ratio (30 + 60–0 min)	3.3(1.1)	3.6(1.4)	4.5(2.7)	0.199

See footnote in Table 1.
*$p < 0.05$, † $p < 0.01$ compared with the lowest tertile.

Table 4. Pearson's correlations between hormone levels and body-mass index, WHR and smoking in the whole study group (n = 71)

Steroid determination	BMI	WHR	Smoking
Mean ACTH (0 + 1 + 2h)*	0.118	0.238†	0.054
2 h ACTH	0.173	0.352‡	0.054
Mean basal cortisol	-0.404§	-0.425§	-0.102
Ratio of mean ACTH to mean cortisol	0.384‡	0.495§	0.117
Net DHEA increment	0.325‡	0.276†	0.290†
Net Adione increment	-0.021	0.136	0.288†
Net 17-OHP increment	0.318‡	0.3311‡	0.356‡
Net S increment	0.048	0.009	0.080
Net cortisol increment	0.191	0.215	0.269†
Ratio of net 17-OHP/S increments	0.314‡	0.404§	0.320‡
Urinary cortisol excretion (12 h)	0.058	0.139	0.104

* Mean ACTH and cortisol determinations during the oral glucose tolerance test.
† $p < 0.05$, ‡ $p < 0.01$, § $p < 0.001$.

significantly different between the tertiles. No significant differences were found in responses of cortisol, DHEA, androstenedione, 17-OHP, and S to DXM suppression between the tertiles (data not shown).

Table 3 shows the adrenocortical steroid responses to exogenous ACTH stimulation after DXM suppression across the WHR tertiles. Steroid concentrations are given by either the net increment in steroid levels from 0–60 min or the absolute poststimulation levels at 60 min. The ratios of 17-OHP to S, DHEA to androstenedione, 17-OHP to androstenedione, and S to cortisol after ACTH stimulation were used as indices of 21-hydroxylase, 3ß-hydroxysteroid dehydrogenase. 17,20-lyase and 11ß-hydroxylase activities, respectively.

The net increments of 17-OHP and the calculated ratios of 17-OHP to S net increments were significantly higher in the upper than the lower tertile of WHR. The net increments and absolute responses of cortisol were similar in the middle and upper tertiles, but significantly higher than in the lowest tertile. In contrast, the ratio of absolute S to cortisol responses was significantly lower in the highest tertile than in the lowest tertile. Androstenedione, DHEA, and S responses as well as the DHEA to androstenedione and 17-OHP to androstenedione ratios were not significantly different across the three WHR tertiles.

Correlations between anthropometric and lifestyle variables and steroid determinations

Table 4 shows the Pearson correlations between steroid determinations and BMI, WHR and smoking. Age, physical activity and alcohol consumption were not significantly associated with any of the steroid measurements (data not shown). WHR was positively correlated with the mean and 2 h ACTH during the OGTT. Both BMI and WHR were negatively related to the mean basal cortisol and positively related to the ratio of mean ACTH to cortisol during the OGTT. In addition, BMI and WHR were positively related to the net increments of DHEA, 17-OHP, and the ratio of net 17-OHP to S increments after ACTH stimulation.

Smoking was positively correlated with the net increments of DHEA, androstenedione, 17-OHP and cortisol, as well as with the ratio of the net 17-OHP to S increments. Alcohol consumption was positively related to the mean ACTH during the OGTT (data not shown). In addition, the calculated ratio of urinary cortisol to BMI was negatively correlated with WHR ($r = -0.532$; $p < 0.001$).

The relationships of WHR with the ratio of mean ACTH to mean cortisol during the OGTT (Fig. 1), the ratio of urinary cortisol to BMI (Fig. 2) and the ratio of net increments of 17-OHP to S (Fig. 3) are also illustrated.

Table 5 shows the partial correlation coefficients for BMI and WHR and steroid determinations adjusted for age, physical activity, smoking and alcohol consumption. The correlations were slightly attenuated, but most of the

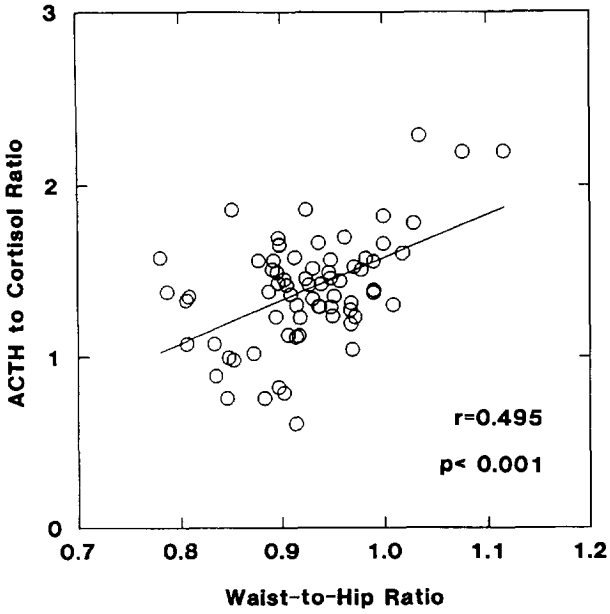

Fig. 1. The ratio of mean ACTH to mean cortisol during oral glucose tolerance test vs WHR in the whole study group ($n = 71$).

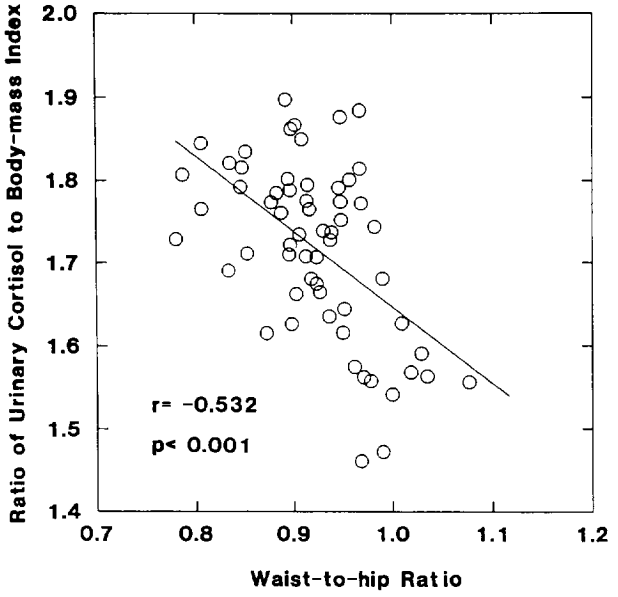

Fig. 2. The ratio of urinary cortisol excretion to body-mass index vs WHR ($n = 60$).

above associations between BMI, WHR and steroid determinations remained significant.

Steroid determinations and WHR in non-smokers

Because smoking inhibits adrenal 21-hydroxylase [14] and the number of smokers tended to be higher in the upper WHR category, Pearson's correlations were also computed between WHR and BMI and the steroid determinations of the non-smoking study participants ($n = 45$).

WHR was positively correlated with the ratio of mean ACTH to cortisol ($r = 0.448$; $p = 0.002$) during the OGTT and with the net increment of 17-OHP ($r = 0.306$; $p = 0.041$) and with the ratio of the net 17-OHP to S increments ($r = 0.325$; $p = 0.029$) after ACTH stimulation. On the contrary, WHR was negatively correlated with physical activity ($r = -0.380$; $p = 0.001$), the mean cortisol ($r = 0.331$; $p = 0.027$) during the OGTT, as well as with the ratio of urinary cortisol excretion to BMI ($r = -0.564$; $p = 0.001$). BMI was associated with WHR ($r = 0.733$; $p < 0.001$), and with the ratio of net increments of 17-OHP to S ($r = 0.340$; $p = 0.022$), but not with the other variables. The relationships of WHR with the ratio of mean ACTH to mean cortisol during the OGTT, the ratio of urinary cortisol to BMI, and with the ratio of net increments of 17-OHP to S and WHR and BMI are also shown in Figs 4, 5 and 6, respectively.

Table 5. Partial correlations between body-mass indices and WHRs with steroid determinations (adjusted for age, smoking, physical activity and alcohol consumption) in the whole study group (n = 71)

Steroid determination	BMI	WHR
Mean ACTH	0.144	0.237
2 h ACTH	0.250*	0.432‡
Mean basal cortisol	-0.310*	-0.396‡
Ratio of mean ACTH to mean cortisol	-0.304*	-0.401‡
Net DHEA increment	0.226	0.255*
Net Adione increment	-0.143	0.053
Net 17-OHP increment	0.318†	0.261*
Net S increment	0.085	-0.007
Net cortisol increment	0.303*	0.203
Ratio of net 17-OHP/S increments	0.297*	0.363†
Ratio of urinary cortisol/BMI	-0.547‡	-0.441‡

See footnote in Table 4.
*$p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$.

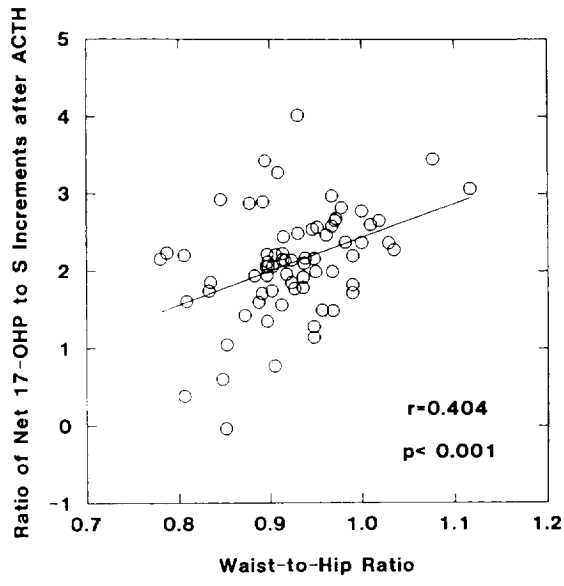

Fig. 3. The ratio of the net 17-hydroxyprogesterone to 11-deoxycortisol increments after ACTH stimulation vs WHR the whole study group ($n = 71$).

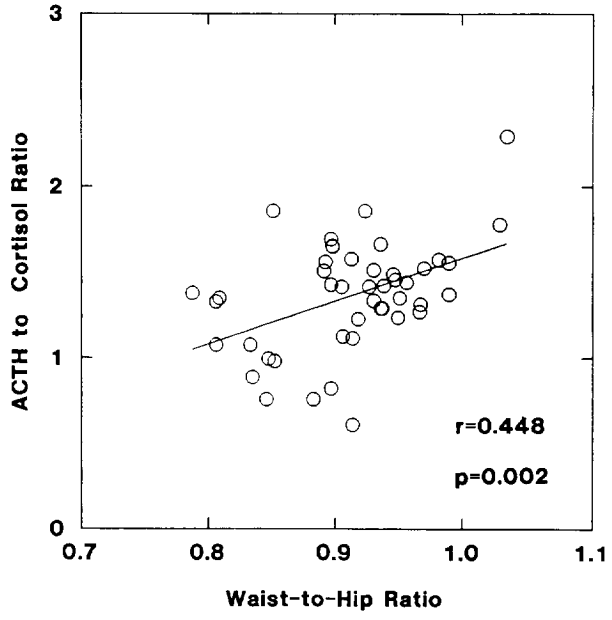

Fig. 4. The ratio of mean ACTH to mean cortisol during the OGTT vs WHR in non-smokers ($n = 45$).

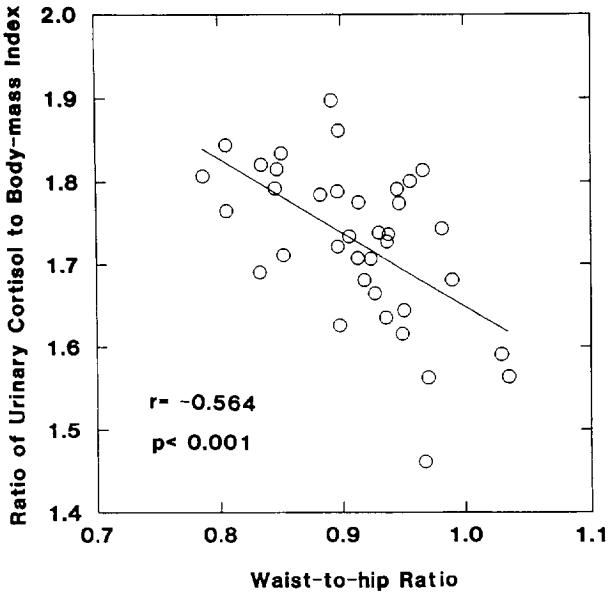

Fig. 5. The ratio of urinary cortisol excretion to body-mass index vs WHR in non-smokers ($n = 38$).

Prediction of the WHR by multivariate analyses

Of the steroid determinations, the ratio of mean ACTH to cortisol during the OGTT ($r = 0.495$; $p < 0.001$), and absolute cortisol ($r = 0.240$; $p = 0.044$) and DHEA ($r = 0.295$; $p = 0.013$) responses at 60 min, as well as the ratio of net 17-OHP to S increments ($r = 0.404$; $p < 0.001$) correlated closely with WHR. Because smoking ($r = 0.268$; $p = 0.024$) and physical activity ($r = -0.329$; $p = 0.005$) were also associated with WHR and, in addition, smoking was related to DHEA ($r = 0.293$; $p = 0.013$), androstenedione ($r = 0.268$; $p = 0.024$) and 17-OHP ($r = 0.357$; $p = 0.002$) responses to ACTH at + 60 min, multiple linear regression analyses were applied to unravel the relations between these lifestyle variables, steroid responses and WHR. Age and alcohol consumption were not related to WHR and, therefore, were omitted from regression analyses.

Table 6 shows, that the ratio of mean ACTH to mean cortisol during the OGTT, the absolute cortisol response to ACTH at 60 min, and the ratio of net 17-OHP to S increments after ACTH all were significant and independent predictors of WHR, when entered simultaneously into the regression model (Model 1). These three steroid measurements together explained as much as 45.7% of the variation in WHR. The ratio of mean ACTH to cortisol was marginally related to cortisol response to ACTH ($r = -0.234$; $p = 0.050$), but otherwise the intercorrelations of these variables were insignificant.

The DHEA response to ACTH at 60 min correlated with the ratio of net 17-OHP to S increments ($r = 0.352$; $p = 0.003$), but not with the ratio of mean ACTH to cortisol ($r = 0.144$; $p = 0.23$) nor the cortisol response to ACTH ($r = 0.059$; $p = 0.63$). When the ratio of net 17-OHP to S increments was substituted by the DHEA response to ACTH at 60 min, all three steroid measurements were still closely related to the WHR, although the explanatory power of the model slightly decreased (Model 2). Smoking alone explained 7.2% and physical activity alone 10.8% of the variance in WHR. However, after entering either physical activity or both these variables simultaneously with steroid measurements into the regression model, only physical activity was related to the WHR (Models 3 and 4). As expected, BMI was highly significantly related to the WHR, but adjustment for BMI did not alter the relation between WHR and the ratio of mean ACTH to cortisol or cortisol response to ACTH. However, the ratio of net 17-OHP to S increments to ACTH remained only a marginally significant predictor of the WHR (Model 5). Thus, pituitary–adrenocortical function appears to be more closely related to the WHR than are smoking and physical activity.

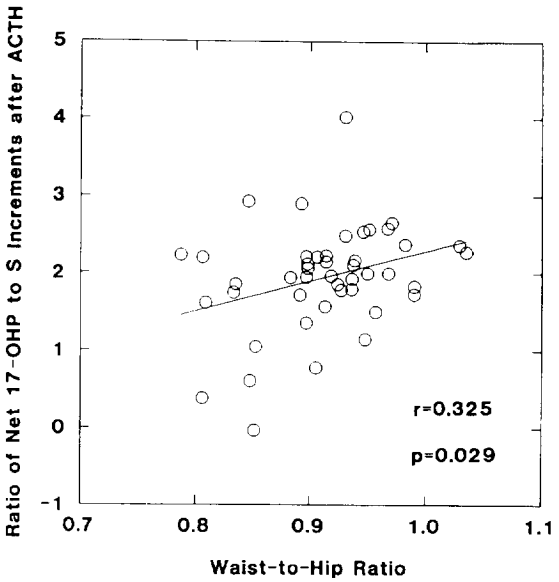

Fig. 6. The ratio of the net 17-hydroxyprogesterone to 11-deoxycortisol increments after ACTH stimulation vs WHR in non-smokers ($n = 45$).

Prediction of the smoking status by multivariate analyses

We were still concerned about the possibility that the above findings were an artefact attributable to the smoking-induced inhibition of adrenal 21-hydroxylase, because of the higher number of smokers in the upper WHR tertile than in the two lower tertiles. Therefore, we also used stepwise linear regression analysis to predict the smoking status (yes/no) of the study subjects. Table 7 shows the results of these analyses. Alone, the ratio of net 17-OHP to S increments ($R^2 = 0.102$; $p = 0.007$) and WHR ($R^2 = 0.072$; $p = 0.024$) and the cortisol response to ACTH at 60 min ($R^2 = 0.081$; $p = 0.016$) were significant predictors of the smoking status. In contrast, physical activity ($R^2 = 0.037$; $p = 0.109$) and the ratio of mean basal ACTH to cortisol ($R^2 = 0.014$; $p = 0.333$) were not

Table 6. Multiple regression models for the prediction of WHR in the study participants (n = 71) *

Model	Independent variable(s)	Standardized coefficient of regression	*p*-value (2-tail)	Model R^2†
1.	Ratio of mean ACTH to cortisol‡	0.533	< 0.001	0.457
	Cortisol response to ACTH + 60	0.322	0.001	
	Ratio of net 17-OHP to S§	0.287	0.003	
2.	Ratio of mean ACTH to cortisol	0.551	< 0.001	0.416
	Cortisol response to ACTH + 60	0.357	< 0.001	
	DHEA response to ACTH + 60	0.194	0.045	
3.	Ratio of mean ACTH to cortisol	0.511	< 0.001	0.490
	Cortisol response to ACTH + 60	0.313	0.001	
	Ratio of net 17-OHP to S	0.248	0.009	
	Physical activity	-0.186	0.045	
4.	Ratio of mean ACTH to cortisol	0.511	< 0.001	0.490
	Cortisol response to ACTH + 60	0.312	0.002	
	Ratio of net 17-OHP to S	0.246	0.012	
	Physical activity	-0.186	0.049	
	Smoking status (yes/no)	0.005	0.958	
5.	Body-mass index	0.526	< 0.001	0.681
	Ratio of mean ACTH to cortisol	0.300	< 0.001	
	Ratio of net 17-OHP to S	0.149	0.052	
	Cortisol response to ACTH + 60	0.172	0.028	
	Physical activity	-0.109	0.145	

*Independent variables in the regression models have been used in groups as shown to predict the WHR.

†R^2 (the square of the multiple regression coefficient) gives the proportion of the total variation in WHR that is explained by the variables in the model.

‡The ratio of the mean of three ACTH determinations to the mean of three cortisol determinations during the oral glucose tolerance test.

§The ratio of the net increments of 17-hydroxyprogesterone to 11-deoxycortisol from 0 (dexamethasone suppressed level) to 60 min after exogenous ACTH stimulation

significantly related to smoking. When the cortisol measurements, the ratio of 17-OHP to S increments, physical activity, and WHR were entered simultaneously into a stepwise regression model, only cortisol response to ACTH at 60 min and the ratio of 17-OHP to S increments remained significantly related to smoking status (Model 1).

Smoking was significantly related to the net increments of cortisol, DHEA, and androstenedione after ACTH, as well as to the ratio of net 17-OHP to S increments as shown in Table 4. Only androstenedione response remained an significant and independent predictor of the smoking status when these steroid measurements were entered simultaneously with WHR to the stepwise regression model. This combination accounted for 25.4% of the variance in the smoking status (Model 2).

Prediction of the WHR in non-smokers by multivariate analyses

Table 8 shows that the relations between WHR and steroid measurements were slightly attenuated as compared with the results above (Table 6), when non-smokers were analysed by multivariate models.

Table 7. Stepwise multiple regression models for the prediction of the smoking status (yes/no) in the study participants (n = 71) *

Model	Independent variables	Standardized coefficient of regression	*p*-value (2-tail)	Model R^2
1.	Ratio of net 17-OHP to S	0.284	0.014	0.160
	Cortisol response to ACTH + 60	0.243	0.034	
2.	Net increment of androstenedione to ACTH	0.255	0.020	0.254
	Net increment of cortisol to ACTH	0.203	0.064	
	Net increment of DHEA to ACTH	0.185	0.108	
	Ratio of net 17-OHP to S	0.226	0.052	

*See footnote in Table 6.

Model 1. Independent variables entered were the ratio of mean ACTH to cortisol, the cortisol response to ACTH at 60 min, the ratio of net increments of 17-OHP to S to ACTH, physical activity and WHR.

Model 2. Independent variables entered were the net increments of cortisol, DHEA and androstenedione to ACTH, the ratio of net increments of 17-OHP to S and WHR.

Forward and backward stepping gave the same results in both models

Table 8. Multiple regression models for the prediction of WHR in the non-smoking study participants (n = 45) *

Model	Independent variables	Standardized coefficient of regression	*p*-value (2-tail)	Model R^2
1.	Ratio of basal ACTH to cortisol	0.448	0.002	0.200
2.	Ratio of net 17-OHP to S	0.325	0.029	0.106
3.	Physical activity	-0.380	0.010	0.144
4.	Cortisol response to ACTH +60	0.121	0.428	0.015
5.	Ratio of basal ACTH to cortisol	0.515	< 0.001	0.452
	Physical activity	-0.304	0.017	
	Cortisol response to ACTH +60	0.239	0.063	
	Ratio of net 17-OHP to S	0.237	0.064	

*See footnote in Table 6

The ratio of mean ACTH to cortisol during the OGTT was most closely related to the WHR, followed by physical activity, the cortisol response to ACTH at 60 min, and by the ratio of net 17-OHP to S increments in descending order of significance. The model containing all four variables explained 45.2% of the variance in the WHR. The intercorrelations of these variables were non-significant varying from $r = -0.236$ to $r = 0.237$.

DISCUSSION

Evidence for the decreased activity of 21-hydroxylase in abdominal obesity was the WHR-related: (1) decrease in basal cortisol and compensatory increase in ACTH secretion during the oral glucose tolerance test; (2) decrease in urinary cortisol excretion in relation to BMI; (3) increase in the 17-OHP response to ACTH stimulation (measured by the net increment of the steroid); (4) increase in the stimulated ratios of 17-OHP to S; and (5) increase in the cortisol response to ACTH stimulation presumably as a result of slight adrenal hyperplasia and of the ACTH dose (about 1000-fold more than the threshold dose of 20 ng necessary for cortisol secretion [19]. Our previous interpretation of the hypothalamic aetiology of the altered ACTH-cortisol secretion in abdominal obesity has thus to be at least partly revised [8]. The primary reason for these alterations appears to be a subtle defect in cortisol biosynthesis as a result of decreased 21-hydroxylase activity, which may cause all the other observed changes.

The ratio of the net 17-OHP to S increments was also correlated with the smoking status and the number of cigarettes smoked. These results expand our previous finding that smokers may have an acquired adrenal hyperplasia [14, 20], resulting from the functional inhibition of adrenal 21-hydroxylase by nicotine and cotinine [21]. Therefore, we were concerned about the possible bias that might arise from the fact that the upper WHR tertile contained a larger number of smokers than the two lower tertiles. None the less, the results of the multiple regression analyses in the whole study group and non-smoking group were almost identical. Combination of the following four variables, the ratio of mean ACTH to cortisol during the OGTT, the cortisol response to ACTH, the ratio of net 17-OHP to S increments and physical activity accounted for 49.0% and 45.2% of the variation in the WHR in the whole and non-smoking groups, respectively. Hence, it is less likely, that the association of WHR with decreased 21-hydroxylase activity can be explained by the biased distribution of the smokers in the three WHR tertiles.

The elevated production of cortisol in general obesity [22] appears to be related to the increased cellular mass, because urinary excretion of cortisol metabolites is similar in obese and lean subjects when adjusted for urinary creatinine [23]. It has been proposed that slightly decreased blood cortisol levels in obesity result from the enhanced turnover rate of cortisol in adipose tissue [4, 24]. The observed WHR-related low basal cortisol levels, increased basal ACTH levels and elevated cortisol response to exogenous ACTH stimulation would be compatible with that idea. However, when cortisol excretion was expressed as a function of BMI, it was negatively related to WHR, suggesting that cortisol production is in fact decreased in abdominal obesity.

Could abdominal obesity cause a functional impairment of 21-hydroxylase activity? The association of abdominal obesity with insulin resistance and hyperinsulinemia is well established [25]. There is evidence that insulin may affect adrenal steroid synthesizing enzymes: high physiological concentrations of insulin induced by the clamp technique inhibit adrenal 17,20-lyase activity resulting in a decreased response of DHEA and androstenedione to ACTH, although the cortisol response remains unaltered [26]. In our study, the ratio of 17-OHP to androstenedione responses to ACTH, an index of 17,20-lyase activity, was not different across the tertiles, neither did the ratio of net 17-OHP to 11-OHC increments correlate with fasting insulin nor other indices of insulin sensitivity (Hautanen and Adlercreutz, submitted for publication). Hence, it is unlikely that the decreased 21-hydroxylase

activity in abdominal obesity is caused by hyperinsulinemia.

Food-dependent Cushing's syndrome has recently been described [27, 28]. These subjects had subnormal morning plasma cortisol and suppressed ACTH concentrations but elevated postprandial cortisol concentrations resulting from the abnormal responsiveness of their adrenal cells to physiological secretion of gastric inhibitory polypeptide (GIP). Our study subjects were outpatients, who otherwise carried out their normal daily activities. For logistic reasons, we collected 12 h urine for cortisol measurements, followed by three serum cortisol and plasma ACTH determinations during the OGTT. Urine collection started at 1800 h and a 12 h fast proceeding the OGTT at 2000 h thus allowing at least a 2 h period for eating and probably an even longer period for food-induced cortisol secretion. Nevertheless, we cannot exclude the possibility, that by a 24 h urine collection we could have detected some subjects with a daytime hyperexcretion of cortisol. Yet the detailed analyses of cortisol and ACTH responses at 0 min, 60 and 120 min after the OGTT did not reveal any subject with abnormal change in either cortisol or ACTH levels in response to glucose ingestion.

Such alterations in pituitary–adrenal function can also be found in mild or asymptomatic forms of congenital adrenal hyperplasia (CAH), which may result from a relative deficiency of several enzymes necessary for the biosynthesis of cortisol [17, 18]. Various defects of 21-hydroxylation account for over 95% of patients with CAH. In European populations, a conservative estimate of the incidence of severe, classic forms is about 1 in 15,000 births and of mild or asymptomatic non-classic forms about 1 in 1000 births. Corresponding heterozygote frequencies are 1 in 63 and 1 in 14, respectively [17, 18]. Carriers of both classic and non-classic genes have the same range of elevation of 17-OHP, which is revealed only by ACTH stimulation [29].

Thus, some study subjects, who had an exaggerated 17-OHP response to ACTH stimulation may be carriers of either the classic or non-classic genes. A weakness of this interpretation is the clinical experience that the patients with classic CAH are usually not obese. As far as we know, no published data exist on the regional fat distribution of male carriers of various CAH forms. Yet reduced insulin sensitivity, a common concomitant of abdominal obesity may occur in females with adrenal hyperplasia due to the non-classical 21-hydroxylase deficiency [30].

In clinical perspective, the combination of abdominal obesity and a relative hypocortisolemia is a confusing issue, considering that patients with Cushing's disease are characterized by obesity and patients with Addison's disease by leanness. However, the extent to which simultaneously elevated levels of adrenal androgens in the former and their lack in the latter disease contribute to the altered accumulation of abdominal fat is not known. It should also be stressed that the cross-sectional study precludes conclusions on the sequence of events — we cannot, therefore, exclude the possibility that subjects with abdominal obesity have been hypercortisolemic in the past.

In summary, abdominal obesity appears to be associated with several alterations in the pituitary–adrenocortical function compatible with a subtle decrease in the activity of the adrenal 21-hydroxylase. Either an obesity-related functional alteration of the enzyme activity, possibly exaggerated by smoking, or the high carrier prevalence of genetic defects of this enzyme in general population might explain these findings.

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