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Cortisol and ACTH Response to Oral Dexamethasone in Obesity and Effects of Sex, Body Fat Distribution, and Dexamethasone Concentrations: A Dose-Response Study

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There is increasing evidence that the abdominal obesity phenotype may be associated with multiple alterations of the hypothalamic-pituitary-adrenocortical (HPA) axis activity in both sexes. Our hypothesis is that the lack of adequate cortisol suppression after the dexamethasone test may constitute an indirect marker of HPA axis hyperactivity in the presence of the abdominal obesity phenotype. A total of 34 normal-weight (13 men and 21 women) and 87 obese (36 men and 51 women), healthy, nondepressed subjects therefore underwent four different dexamethasone suppression tests randomly performed at varying intervals of at least 1 wk between each test. After a standard overnight 1-mg dexamethasone test, which served as a reference, three other tests were randomly performed at 1-wk intervals by administering 0.0035, 0.0070, and 0.015 mg oral dexamethasone per kilogram of body weight overnight. Blood samples were obtained for cortisol, ACTH, and dexamethasone. Results were analyzed separately in men and women as well as in normal-weight [body mass index (BMI) ≤25 kg/m^2] and overweight or obese (BMI >25 kg/m^2) subjects. The waist circumference and the waist to hip ratio (WHR) were used as markers of body fat distribution. After the standard 1-mg test, cortisol suppression was greater than 90% in all subjects. However, after each test, obese women had significantly higher values of percent cortisol and percent ACTH suppression than normal-weight women without any difference between obese and normal-weight men. Considering the response to the three variable-dose tests, a clear dose-response pattern ($P < 0.001$ for trend analysis) in percent cortisol and percent ACTH suppression was found in all subjects. After each test men had significantly higher dexamethasone levels than women, regardless of BMI. However, obese women, but not men, had significantly higher dexamethasone levels after each test than their normal-weight counterpart. Plasma dexamethasone concentrations were dose related ($P < 0.001$ for trend analysis) in all subjects, but the dose-related increase was significantly higher in normal-weight men than normal-weight women, whereas it was similar in obese subjects of both sexes. Stepwise multiple regression analysis revealed that both percent cortisol and percent ACTH variations were significantly and negatively influenced by dexamethasone levels, as well as by waist circumference values in men, and independently by BMI and waist circumference in women. However, in contrast to what has been found in men, a divergent contribution of BMI and waist circumference was found in women indicating that, with increasing waist values, a smaller suppression of the HPA axis was found with respect to that expected on the basis of BMI values. In conclusion, this study provides data of both physiological and physiopathological relevance. Overall, our data indicated that adjustment of the dexamethasone dose to body weight does not seem to substantially improve the sensitivity of the test, even in obese individuals, particularly when near-maximal doses are administered. However, this study demonstrated a highly significant effect of dexamethasone blood level concentrations on cortisol and ACTH suppression to low-dose dexamethasone tests. In addition, a significant effect of gender on postdexamethasone cortisol concentrations, suppression of the HPA axis, and dexamethasone levels were found, which may be dependent on related differences in both cortisol and dexamethasone metabolism. We showed that pituitary sensitivity to feedback inhibition by dexamethasone is preserved in obesity in both sexes even at low dosages. On the other hand, our data suggest that, at least in women, abdominal fat distribution may partially counteract the progressively greater suppressibility of the HPA axis that would be expected according to increasing BMI. (*J Clin Endocrinol Metab* 87: 166–175, 2002)

ABDOMINAL OBESITY PHENOTYPE may be associated with multiple alterations of the hypothalamic-pituitary-adrenocortical (HPA) axis activity in both sexes (1, 2). Indicators of such a dysregulation are altered ACTH pulsatile secretory dynamics (3); high secretion of cortisol after laboratory stress tests (4–6); and HPA hyperresponsiveness to different exogenous neuropeptides, including CRH alone (7), or combined with AVP (5, 8) and maximal (9) or low-dose ACTH (5). Recent epidemiological studies have also shown a strong interaction between perceived stress and abdominal fat distribution and related metabolic abnormalities, partic-

Abbreviations: BMI, Body mass index; CES-D, Center for Epidemiological Studies Depression Rating Scale; DST, dexamethasone suppression test; DST_1, 1-mg dexamethasone suppression test; HPA, hypothalamic-pituitary-adrenocortical; WHR, waist to hip ratio.

ularly in subjects with low diurnal cortisol variability, suggesting a reset of the HPA toward chronic hyperactivation by factors such as environmental stress (10). Furthermore, several alterations in the cortisol turnover and metabolism have been detected in the adipose tissue, particularly the visceral depots, indicating that peripheral factors may be involved in determining HPA axis abnormalities in the abdominal obesity phenotype (2).

The lack of adequate cortisol suppression in the abdominal obesity phenotype has been proposed by some studies to represent an indirect marker of HPA hyperactivity (10, 11). In fact, previous studies reported an inadequate suppression of morning cortisol levels following overnight low-dose (0.5 mg) dexamethasone administration in adult obese men with abdominal fat distribution, and this finding has been advocated to support the concept that the HPA axis may be hyperactivated in abdominal obesity (11).

To further investigate whether central sensitivity to low-dose exogenous glucocorticoids may be impaired in obesity, we therefore carried out a dexamethasone dose-response study in a large cohort of subjects of both sexes, ranging from normality to obesity and covering a wide spectrum of age. In fact, there is evidence that the HPA axis as well as peripheral cortisol metabolism may be differently regulated according to sex and age factors (12, 13). Moreover, because dexamethasone concentrations after oral administration are partially a function of body weight (14, 15), in contrast to other studies on this issue (10, 11), we designed a protocol in which the dose of dexamethasone administrated was not fixed in all subjects but was individually adjusted for kilogram of body weight. Finally, in this study particular care was taken in the recruitment of the patients to exclude potential confounding factors such as depression. Therefore, only subjects without depressive traits were investigated because obese individuals often have depression, which is *per se* associated with important abnormalities of the HPA axis leading to cortisol oversecretion (16–18).

Materials and Methods

Subjects

The subjects included in the study were enrolled by investigators working in different Italian university centers and teaching hospitals. The task of each center was to enroll normal subjects and obese patients of both sexes, aged between 18 and 65 yr, including an adequate number of subjects for each age group, which included ages from 18–31, 31–50, and 51–65 yr, respectively. We investigated 34 normal-weight (13 men and 21 women) and 87 obese (36 men and 51 women) subjects. All normal-weight subjects had a body mass index (BMI) lower than 25 (body weight, in kg, divided by height, in m^2), whereas overweight and obese subjects had BMI values greater than 25. Exclusion criteria were based on both clinical examination and laboratory tests. None of the subjects had hypertension; diabetes; thyroid dysfunction; hyperandrogenism of various origin; or relevant cardiovascular, hepatic, renal, or systemic diseases. Cushing's syndrome was excluded on the basis of cortisol response to standard 1-mg dexamethasone test (blood cortisol levels lower than 138 nmol/liter) and routine 24-h free cortisol urine excretion rates (normal values in our laboratory are lower than 180 nmol/24 h). Moreover, none of the subjects had been taking oral contraceptives for 2 months or any medication for 1 month before the study or were dieting. Finally, none of the subjects were used to drinking more than 30 g alcohol per day. Thirty-seven subjects (30.6%) were habitual smokers (12 men and 25 women). None of the normal-weight individuals had a history of overweight or obesity. Fertile women were examined regardless of the phase of the cycle.

All subjects gave their informed and written consent to the study, which was approved by the Ethics Committee of the S. Orsola-Malpighi Hospital, University of Bologna, and by the institutions of the other participating research centers.

Psychological evaluation

The presence of depressive traits was investigated by means of two different questionnaires, the Children Depression Questionnaire (19) and the Center for Epidemiological Studies Depression Rating Scale (CES-D) (20), both in the Italian version. They are based on a numeric scale with a given threshold value assessing the presence or absence of depression. In the CDQ scale, values lower than 7 exclude depression, as do values lower than 21 in the CES-D scale.

Anthropometry

Body height was measured without shoes to the nearest 0.5 cm and body weight without clothes. The waist and hip circumferences were also measured, with the subjects standing, using a 1-cm-wide metal measuring tape, and their waist to hip ratio (WHR) was calculated accordingly. In agreement with the World Health Organization's recommendation (21), waist circumference was measured 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. Both waist circumference and the WHR were used to define different patterns of body fat distribution (21).

Protocol study

After a first standard 1-mg dexamethasone suppression test (DST_1), with an interval of at least 1 wk, each subject randomly underwent three additional dexamethasone suppression tests (DSTs), using 0.0035 mg, 0.007, and 0.015 mg of dexamethasone per kilogram of body weight. To obtain the individual doses for each DST, the hospital pharmacy of each center prepared three doses of dexamethasone powder in capsule form after an adequate explanation of the preparation method. Each dose was obtained by trituration of a commercial preparation of dexamethasone tablets (Decadron 0.5 mg, Merck Sharp & Dhome, Pavia, Italy), using the following formula: individual dose (mg/kg body weight) = [weight of two tablets of Decadron (equal to 1 mg) × requested dose (mg/kg body weight)/1 mg]. Each capsule therefore contained the individual dose for each test for each subject. All the dexamethasone formulations were prepared after the subject had been included in the study. During each test, dexamethasone preparations were administered at 2300 h. Blood tests were performed in the morning after overnight fasting while the subjects had been quietly sitting or lying down for at least 15 min, with a needle for blood withdrawal placed in a forearm vein with NaCl (0.9%) saline infusion. Blood samples for cortisol and ACTH determinations were obtained at 0800 h on two consecutive days, before and after taking the drug; an additional sample for dexamethasone determination was obtained after each DST on the second day.

Hormone assays

Blood samples were placed in different tubes containing EDTA without (for cortisol and dexamethasone determination) or with aprotinin (500 U/ml) (for ACTH determination) and maintained in ice until centrifugation. Plasma aliquots for hormone determinations were then stored at −80 C until assayed. All measurements were performed in duplicate. ACTH was determined with an immunoradiometric assay method with reagents obtained from the Nichols Institute Diagnostics (San Juan Capistrano, CA). The sensitivity of this assay is approximately 0.22 pmol/liter (1 pg/ml). Interassay coefficient of variation (CV) at concentrations of 1 pmol/liter (4.45 pg/ml), 7.3 pmol/liter (32.7 pg/ml), and 24.2 pmol/liter (112.3 pg/ml) are 2.4%, 8.5%, and 4.3%, respectively, whereas interassay CV at 1 pmol/liter and 24 pmol/liter were 9.9% and 3.9%, respectively. Cortisol was determined by RIA with reagents (CORT-CTK 125) (DiaSorin, Inc., Saluggia, Italy). The lowest sensitivity level is 2.7 nmol/liter (1 ng/ml). Interassay CV at concentration levels of 16.6 nmol/liter (45.9 ng/ml), 40.8 nmol/liter (112.6 ng/ml), and 198.8 nmol/liter (298.2 ng/ml) are 6.8%, 14.6, and 4.3%, respectively, whereas intraassay CV at concentrations of 9.1 nmol/liter (25.1 ng/ml), 45.9

nmol/liter (126.8 ng/ml), and 135.3 nmol/liter (373 ng/ml) were 9.9%, 5.7%, and 6.1%, respectively. Plasma dexamethasone was measured by RIA by using a specific lamb antibody, provided by Dr. Sabina Lewicka (University of Heidelberg, Heidelberg, Germany) (22). The cross-reaction of the antibody with cortisol and the other endogenous steroids was less than 0.1%. The standard curve was performed with increasing amounts of cold dexamethasone in steroid-free plasma, tritiated dexamethasone (NEN Life Science Products, Bruxelles, Belgium; specific activity 49 Ci/mmol), and diluted antibody. In parallel the plasma of the subjects was incubated with the tracer and the antibody. After overnight incubation at 4 C, the ^{3}H-dexamethasone-bound antibody was separated from the free antibody by dextrane-coated charcoal, and the radioactivity of the supernatant was counted with β-counter. Inter- and intraassay variations were 16.07% and 12%, respectively. Sensitivity was 0.012–0.025 nmol/liter (0.5–1 ng/dl).

Statistics

Data were reported as mean values ± SD. The distributions of cortisol and ACTH concentrations, as well as their percent variations observed after dexamethasone administration, were normalized by means of logarithmic transformations. To assess the normality of the transformed variables, the Kolmogorov-Smirnov test was applied. To avoid multiple comparisons, men and women, as well as normal-weight and obese subjects, were compared by means of the two-way ANOVA using a within-group nested design. The repeated-measure ANOVA trend was applied, using the same design, to test the dose effect of dexamethasone administration on percent cortisol, ACTH variations, and dexamethasone concentrations. The data obtained during the DST_1 were excluded from the trend analyses. The relationships among different variables were evaluated by univariate and stepwise multivariate regression analyses. The equality of lines was tested by means of the 1R procedure of the BMDP statistical package (23). Statistical evaluations were performed by running the SPSS, Inc./PC+ statistical package (24). Two-tailed *P* values less than 0.05 were considered statistically significant.

Results

Psychological evaluation

None of the subjects included in the study had depression, having values lower than 7 in the CDQ scale and lower than 21 in the CES-D scale.

General characteristics and tolerability

The general characteristics of the men and women included in the study are reported in Table 1. Age distribution in men and women, both obese and nonobese, was not significantly different. All tests were well tolerated and no side effects were reported by any subject.

Baseline cortisol and ACTH concentrations

Similar cortisol (Table 2) and ACTH (Table 3) baseline levels were found between normal-weight and obese men and between normal-weight and obese women. However, at each DST, lower baseline cortisol levels were found in normal-weight women than normal-weight men at each test, without any significant difference between obese men and women (Table 3).

Cortisol response to DSTs

Postdexamethasone cortisol values in obese, compared with normal-weight men, were found only after the DST using 0.0035 mg ($P = 0.010$) (Table 2). After the DST_1, mean percent cortisol suppression was more than 90% in all subjects. However, after each DST, percent cortisol suppression was significantly higher in obese than in normal-weight women, whereas no difference was found between obese and normal-weight men (Fig. 1). Considering the response to the three variable-dose DSTs, a clear dose-response pattern in the cortisol percent suppression was found in all subjects ($P < 0.001$ for trend analysis) without any significant effect of either gender or obesity (Fig. 1).

ACTH response to DSTs

ACTH levels after each DST were significantly lower in obese than normal-weight women, whereas no difference was found between obese and normal-weight men. Similarly, percent ACTH variation after each DST, including the DST_1, was significantly higher in obese than normal-weight women, without any difference between obese and nonobese men (Fig. 1). On the other hand, no significant differences were found in percent ACTH variation at each DST between normal-weight and obese men and women (Fig. 1). Considering the response to the three variable-dose DSTs, a clear dose-response suppression of ACTH concentrations was found in all groups ($P < 0.001$ for trend analysis), without any significant effect of either gender or obesity (Fig. 1).

Dexamethasone concentrations after each DST

All subjects had measurable dexamethasone levels after each DST. Both normal-weight and obese men had significantly higher plasma dexamethasone than normal-weight and obese women. No significant difference was found between obese and normal-weight men, whereas obese women had significantly higher dexamethasone levels than their normal-weight counterparts (Table 4). Plasma dexamethasone concentrations were dose related in both groups ($P <$ 0.001 for trend analysis). The dose-related increase of dexa-

TABLE 1. General characteristics and anthropometric parameters (mean ± SD) of normal-weight and obese subjects

Parameter	Men		*P*	Women		*P*
	Normal weight (13)	Obese (36)		Normal weight (21)	Obese (51)	
Age (yr)	35.6 ± 13.3	39.7 ± 13.6	0.284	31.6 ± 8.1	34.7 ± 11.0	0.301
Body weight (kg)	70.5 ± 6.5	112.5 ± 25.8	<0.001	54.4 ± 6.4[a]	92.9 ± 14.3[b]	<0.001
BMI (kg/m^2)	22.4 ± 1.8	37.3 ± 9.0	<0.001	20.6 ± 1.7	36.2 ± 5.7	<0.001
Waist circumference (cm)	82.5 ± 5.6	115.3 ± 18.3	<0.001	69.5 ± 5.1[a]	102.7 ± 12.6[b]	<0.001
Hip circumference (cm)	94.7 ± 6.9	119.1 ± 17.0	<0.001	92.8 ± 4.8	120.9 ± 11.8	<0.001
WHR	0.87 ± 0.05	0.97 ± 0.05	<0.001	0.75 ± 0.05[c]	0.85 ± 0.08[b]	<0.001

[a] $P < 0.01$ *vs.* normal-weight men.
[b] $P < 0.001$ *vs.* normal-weight men.
[c] $P < 0.001$ *vs.* obese men.

TABLE 2. Cortisol concentrations (nmol/liter) (mean ± SD) before and after four overnight DSTs performed with oral dexamethasone at different doses

Dexamethasone test	Men		*P*	Women		*P*
	Normal weight (13)	Obese (36)		Normal weight (21)	Obese (51)	
DST_1						
Before	468.7 ± 168.0 (333–984)	357.0 ± 98.2 (187–565)	0.125	346.5 ± 146.0[a] (132–582)	348.2 ± 142.4 (148–728)	0.610
After	24.3 ± 14.9 (5–55)	25.4 ± 23.7 (2–118)	0.533	22.3 ± 13.2 (8–66)	17.7 ± 14.1 (2–80)	0.058
$DST_{0.0035}$						
Before	451.0 ± 78.6 (292–571)	343.8 ± 88.0 (176–538)	0.112	357.3 ± 149.8[b] (190–755)	364.5 ± 148.4 (154–783)	0.898
After	291.1 ± 119.5 (16–457)	143.7 ± 126.9 (11–325)	0.010	210.5 ± 152.6 (27–540)	129.1 ± 125.5 (10–560)	0.018
$DST_{0.007}$						
Before	420.7 ± 108.2 (193–540)	356.5 ± 91.3 (137–526)	0.193	318.4 ± 117.8[b] (132–562)	363.6 ± 156.4 (140–736)	0.277
After	77.5 ± 62.6 (16–212)	58.8 ± 73.7 (2–309)	0.135	91.0 ± 111.2 (2–391)	45.5 ± 106.5[c] (2–604)	0.008
$DST_{0.015}$						
Before	412.7 ± 75.6 (311–526)	348.7 ± 88.0 (212–584)	0.156	350.9 ± 136.0[b] (162–629)	350.9 ± 176.3 (137–960)	0.682
After	24.6 ± 7.4 (11–38)	26.4 ± 25.3 (2–104)	0.301	25.1 ± 23.2 (5–110)	14.6 ± 9.4[c] (2–41)	0.021

Values in *parentheses* are range values. DST_1, $DST_{0.0035}$, $DST_{0.007}$, and $DST_{0.015}$ identify DSTs performed by administering 1 mg, 0.035 mg/kg, 0.007 mg/kg, and 0.015 mg/kg body weight.

[a] $P < 0.01$ *vs.* normal-weight men.

[b] $P < 0.05$ *vs.* normal-weight men.

[c] $P < 0.05$ *vs.* obese men.

TABLE 3. ACTH concentrations (pmol/liter) (mean ± SD) before and after four overnight DSTs performed with oral dexamethasone at different doses

Dexamethasone test	Men		*P*	Women		*P*
	Normal weight (13)	Obese (36)		Normal weight (21)	Obese (51)	
DST_1						
Before	7.3 ± 3.8 (2–16)	7.4 ± 5.7 (1–31)	0.657	5.3 ± 2.9 (1.8–14)	6.4 ± 5.8 (0.8–28)	0.318
After	1.0 ± 0.3 (0.2–1)	0.8 ± 0.8 (0.2–2)	0.352	1.3 ± 1.2 (0.3–5)	0.7 ± 0.4 (0.2–1)	0.004
$DST_{0.0035}$						
Before	5.6 ± 2.24 (1–8)	6.8 ± 4.1 (1–18)	0.615	5.1 ± 2.7 (1–13)	6.3 ± 5.0 (0.7–29)	0.560
After	3.4 ± 1.6 (0.8–6)	3.4 ± 2.8 (0.4–14.9)	0.486	3.9 ± 2.3 (0.6–10)	2.8 ± 2.6 (0.6–14)	0.023
$DST_{0.007}$						
Before	6.4 ± 2.2 (3–9)	7.0 ± 4.1 (0.2–17)	0.662	5.9 ± 4.0 (1–20)	6.4 ± 5.3 (1–29)	0.992
After	2.4 ± 1.5 (0.2–5)	1.3 ± 1.0 (0.2–4)	0.073	3.2 ± 3.1 (0.4–11)	1.7 ± 3.0 (0.2–19)	0.001
$DST_{0.015}$						
Before	8.4 ± 4.2 (3–17)	6.7 ± 4.2 (1–18)	0.138	5.4 ± 3.1 (1–15)	6.0 ± 4.8 (1–27)	0.958
After	0.8 ± 0.3 (0.2–1)	0.8 ± 0.7 (0.2–4)	0.706	1.3 ± 1.0 (0.2–4)	0.7 ± 0.5 (0.2–3.3)	0.004

Values in *parentheses* are range values. DST_1, $DST_{0.0035}$, $DST_{0.007}$, and $DST_{0.015}$ identify DSTs performed by administering 1 mg, 0.0035 mg/kg, 0.007 mg/kg, and 0.015 mg/kg body weight.

methasone levels was significantly higher in normal-weight men than in normal-weight women ($P = 0.016$), without differences between obese subjects of both sexes.

In men significant simple correlations between plasma dexamethasone levels after the three variable-dose DSTs were found with age (r = 0.214; $P = 0.015$) but not with BMI, waist circumference, or WHR. On the contrary, plasma dexamethasone levels in women significantly correlated with BMI (r = 0.286; $P < 0.001$), waist circumference (r = 0.322; $P < 0.001$), and WHR (r = 0.179; $P = 0.014$) but not with age.

Factors influencing percent cortisol and percent ACTH variation after the DSTs

Percent cortisol variation after the three variable-dose DSTs was negatively correlated with waist values ($P = 0.038$) and WHR ($P = 0.030$) in men and with BMI ($P < 0.001$) and

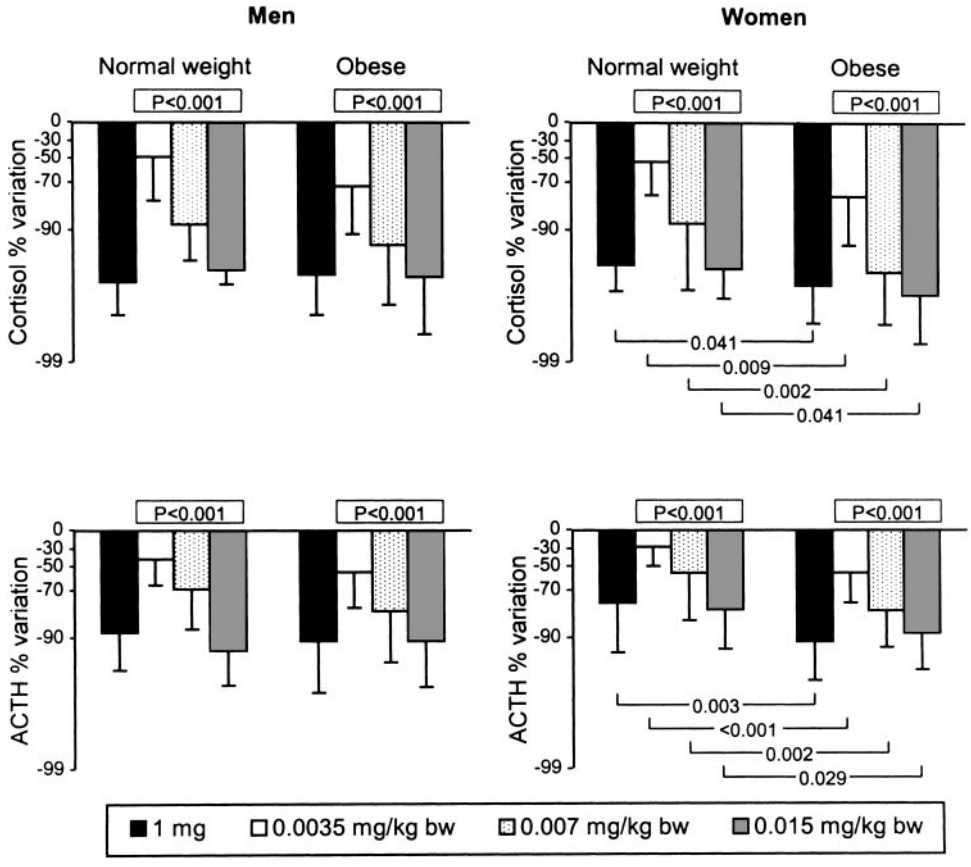

FIG. 1. Percent cortisol and ACTH variations (log scale; mean ± SD) after each dexamethasone test in normal-weight and obese men (*left*) and women (*right*). *P* values included in the *box* refer to the ANOVA trend evaluating the dose effect, taking into account the data of three dexamethasone suppression tests at variable doses.

TABLE 4. Dexamethasone plasma concentrations (nmol/liter) (mean ± SD) after four overnight DSTs performed at different doses

Dexamethasone test	Men		*P*	Women		*P*
	Normal weight (13)	Obese (36)		Normal weight (21)	Obese (51)	
DST_1	7.0 ± 3.6 (0.3–11)	9.3 ± 3.6 (2–15)	0.096	4.3 ± 2.0[a] (0.2–7)	8.5 ± 3.3 (1–15)	<0.001
$DST_{0.0035}$	1.6 ± 1.0	2.5 ± 1.9	0.081	0.9 ± 0.8[b]	1.8 ± 1.4[c]	0.026
$DST_{0.007}$	3.9 ± 2.4	5.0 ± 2.7	0.152	1.8 ± 1.5[b]	4.1 ± 2.1[d]	<0.001
$DST_{0.015}$	7.7 ± 3.5[e] (4–15)	9.2 ± 3.5[e] (2–15)	0.229	4.1 ± 2.6[b,e] (0.2–9)	7.1 ± 3.8[c,e] (1–15)	0.004

Values in *parentheses* are range values. DST_1, $DST_{0.0035}$, $DST_{0.007}$, and $DST_{0.015}$ identify DSTs performed by administering 1 mg, 0.0035 mg/kg, 0.007 mg/kg, and 0.015 mg/kg body weight.

waist circumference values in women ($P < 0.001$) (Fig. 2). Accordingly, significant simple correlations were found between percent ACTH variations and BMI (women: r = −0.265, $P < 0.001$) and waist circumference (men: r = −0.196; $P = 0.021$; women: r = −0.224, $P = 0.002$).

Dexamethasone levels were also negatively correlated with percent cortisol variation in both men and women (Fig. 3). Similar correlations were also found for percent ACTH variations (men: r = −0.269, $P = 0{,}002$; women: r = −0.347, $P < 0.001$).

To investigate the relative contribution of age, anthropometric parameters and dexamethasone concentrations on both percent cortisol and percent ACTH variations, a stepwise multiple regression model was applied separately in each sex. In both men and women, the greater predictive capacity of percent cortisol and percent ACTH variation was related to dexamethasone concentrations (Table 5). In men, after the effect of dexamethasone was taken into account, waist circumference proved to be negatively related both to percent cortisol and percent ACTH suppression. In the subsequent step, no other variables entered the analysis, except age, which was positively related to percent cortisol variation, indicating a counteractive effect of age on percent cortisol suppression at each DST. On the contrary, in women, after the effect of dexamethasone was taken into account, BMI was the sole variable significantly related to both percent cortisol and percent ACTH variation and entered the procedure. After BMI was also included in the procedure, waist circumference became significant with a change in sign showing a positive relationship with percent cortisol and percent ACTH suppression. The entering of waist circumference with a positive coefficient produced the effect of decreasing the negative relationship of BMI. This indicates that the effect of waist circumference was not an independent effect but represented an adjustment of the relationship with the BMI. Therefore, with increasing waist circumference val-

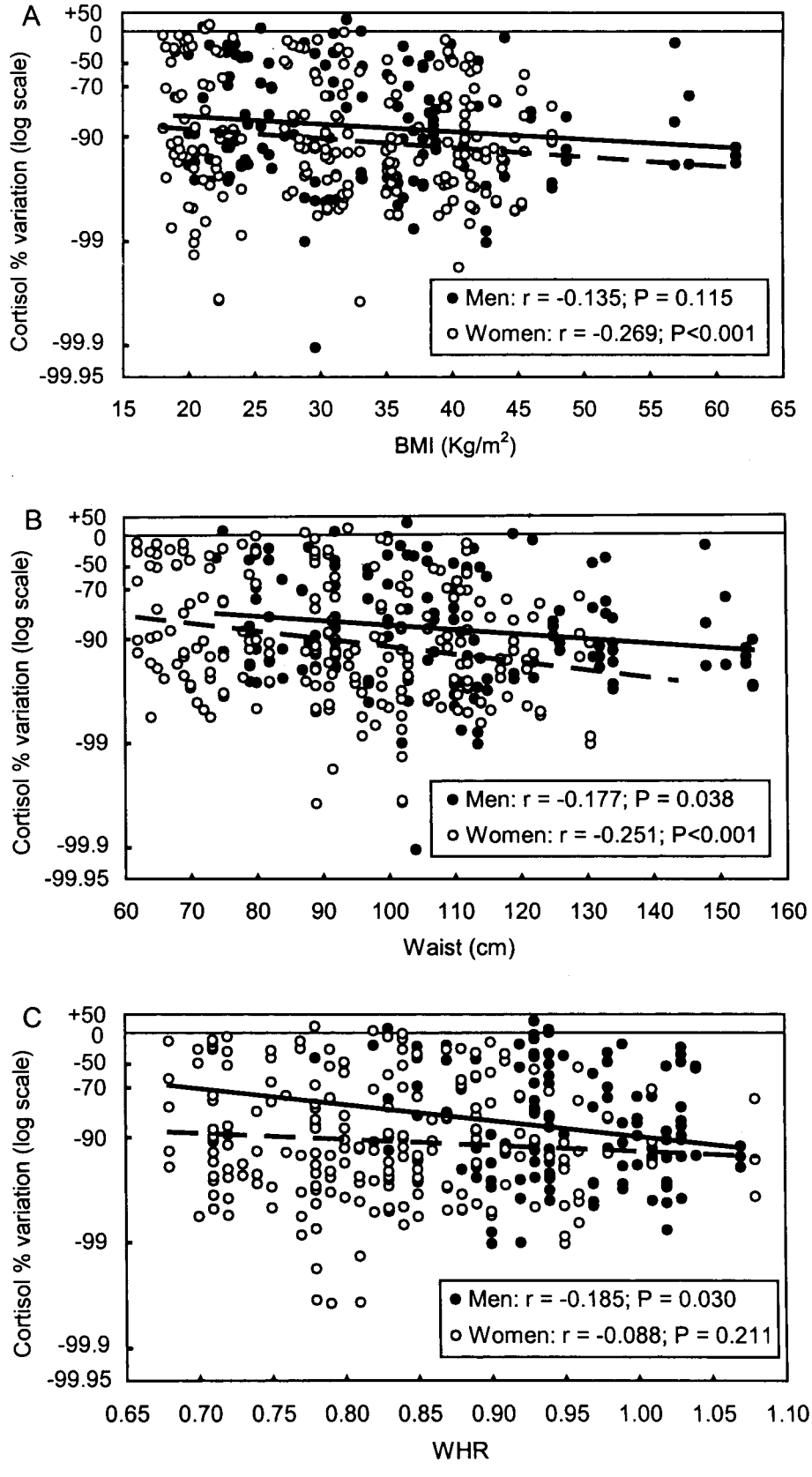

FIG. 2. Relationships among percent cortisol variation (log scale) and BMI (A), waist circumference (B), and WHR (C) in men (*filled circles* and *continuous lines*) and women (*open circles* and *dashed lines*). Only the three dexamethasone tests at variable doses were taken into account.

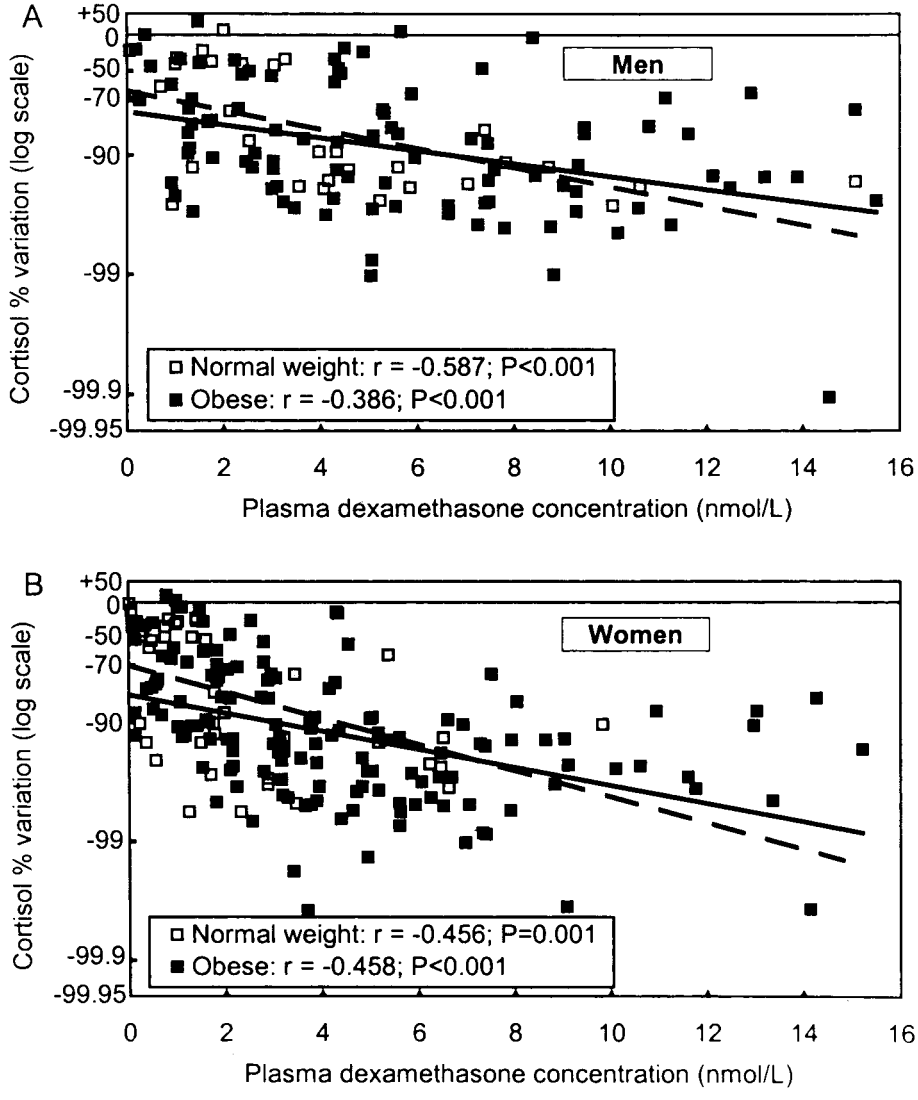

FIG. 3. Relationships between percent cortisol variation (log scale) and dexamethasone plasma concentration in men (A) and women (B) divided into normal-weight (*open squares* and *dashed lines*) and obese (*filled squares* and *continuous lines*) subjects. Correlation coefficients of normal-weight and obese subjects evaluated separately are reported. No significant differences were found in the regression line slopes and intercepts between normal-weight and obese subjects either in men ($F = 0.6$, $P = 0.532$) or women ($F = 2.2$, $P = 0.114$). Only the data of the three dexamethasone tests at variable doses were taken into account.

TABLE 5. Results of the stepwise multiple regression analysis

Variable		Men		Women	
Dependent	Independent	r	*P*	r	*P*
Percent cortisol variation					
	Age	+0.160	0.044		
	BMI			−0.151	0.019
	Waist circumference	−0.196	0.013	+0.169	0.048
	Dexamethasone concentrations	−0.443	<0.001	−0.430	<0.001
Percent ACTH variation					
	Age				
	BMI			−0.190	0.007
	Waist circumference	−0.192	0.026	+0.142	0.041
	Dexamethasone concentrations	−0.251	0.004	−0.308	<0.001

Percent cortisol and percent ACTH (log values) after the three DSTs at different doses were the dependent variables in each model (only significant r values are reported).

ues, a lesser suppression of the HPA axis was found with respect to that expected on the basis of BMI values in women.

Discussion

Physiopathological evaluation of the HPA axis in non-Cushing's syndrome is difficult because of the complexity of the functions and reciprocal interactions among the various centers making up this system. Obesity, and in particular the abdominal phenotype, represents a paradigmatic case of HPA axis alteration because of a series of modifications in the interactions between cerebral centers and peripheral organs and tissues, combined with possible variations in adrenal

steroid synthesis, steroid receptor function, and peripheral steroid metabolism (25, 26). This study aimed to gain more insight into the ability of low-dosage dexamethasone tests to reveal an alteration in the negative feedback action of glucocorticoids on the HPA axis in the abdominal obesity phenotype, also taking into account the contribution provided by the age and gender of the subjects examined.

The standard 1-mg dexamethasone suppression test has generally been proposed as a simple test to evaluate the integrity of the HPA axis. However, this test does not make it possible to discriminate between obese and nonobese subjects, the suppression of the HPA activity being provided by 1 mg-dexamethasone maximal or near-maximal (27). Therefore, this test may not reflect subtle changes in central feedback regulation. For this reason, it has recently been suggested that the utilization of lower dexamethasone doses may give better insight into the individual feedback sensitivity of the HPA axis (10, 11, 28, 29).

Our data clearly indicate a dose-response pattern in the percent cortisol and percent ACTH suppression to the three variable-dose DSTs in all groups, regardless of body weight and gender. This seems to be consistent with the concept that, whatever the dose of dexamethasone used, the majority of obese subjects have a normal feedback drive, similarly to normal-weight individuals. Notably, at higher dexamethasone doses (DST using 0.015 mg), which in many cases exceeded 1 mg, particularly in obese subjects, no better suppression was achieved with respect to that obtained after the standard 1-mg dose, which indicates achievement of the maximum suppressibility of the HPA axis with both tests, during which, moreover, plasma dexamethasone concentrations were not significantly different. Overall, our data indicate that adjustment of the dexamethasone dose to body weight does not seem to substantially improve the sensitivity of the test, even in obese individuals, particularly when near-maximal doses are administered. Interestingly, after each DST, including the standard DST_1, percent cortisol suppression was significantly higher in obese than in normal-weight women, whereas no difference was found between obese and normal-weight men. This seems to have some physiological significance. In addition, obese women had significantly higher dexamethasone levels than female normal-weight subjects at all three tests, including the standard DST_1, whereas such a difference was not present in obese *vs.* normal-weight men. Our results confirm previous findings in which dexamethasone concentrations were positively correlated with BMI only in females but not in males (28). Therefore, this may support the concept that the greater amounts of adipose tissue present in women with respect to men, particularly in obese individuals, may play an important role in the metabolism of both endogenous and exogenous steroids and on their pharmacokinetics (see below).

The observation that the obese women had higher dexamethasone levels than normal subjects is clearly consistent with their greater percent cortisol (and percent ACTH) suppression. On the other hand, men showed systematically higher dexamethasone concentrations than their female counterpart, regardless of body weight. As mentioned above, altogether these results are consistent with some difference in the pharmacokinetics and/or metabolism of dexamethasone between the two sexes, although it cannot be excluded that subtle differences in the sensitivity of the HPA axis to circulating dexamethasone levels might also be present as well. In fact, it is well known that, although dexamethasone availability is an important determinant of cortisol suppression (30–32), rates of dexamethasone metabolism vary widely among subjects (33). Kinetic studies performed after oral dexamethasone administration in both obese and normal-weight individuals of both sexes have shown that the lag time (*i.e.* the appearance time of dexamethasone in plasma), distribution, and metabolic clearance of dexamethasone are higher in obese than in normal-weight subjects (14, 15). These patterns are also evident when low dexamethasone doses are administered (14). The activity of the 11β-hydroxysteroid dehydrogenase type 1 and 2, which catalyze not only the reversible interconversion of cortisol to cortisone (34) but also that of dexamethasone to 11-dehydrodexamethasone (35, 36) may explain the difference between the sexes. Previous studies have shown that obesity and gender may have different effects on 11β-hydroxysteroid dehydrogenase activity and, consequently, on cortisol concentrations (37, 38) and HPA axis responsiveness to CRH and AVP (8). Therefore, it clearly appears that gender differences should be taken into account when interpreting the DST in both physiological and pathological conditions.

There is controversy regarding the effect of age on the HPA axis in humans (12, 13, 39) and whether this relationship may be dependent on gender (13, 40). Our data, in agreement with those of other authors (41), showed an independent role of age on HPA axis suppressibility in men but not in women.

Another important finding of this study is the different contribution of either BMI and fat distribution on HPA axis suppressibility in males and females. In fact, simple regression analysis confirmed that only in women was there a significant negative association between percent cortisol suppression and BMI (Fig. 2), whereas the association with waist circumference (and WHR) was significant in both sexes. However, the multiple regression model (Table 4) indicates that the waist circumference completely replaced BMI in predicting HPA axis suppression in men. On the contrary, both BMI and waist circumference values entered the analysis in women. Specifically, the effect of waist circumference was not observed to play an independent role, coming into account only with BMI. This gender-related difference is not surprising because it should be considered that in both sexes the relationship between BMI and abdominal fatness may be different. In fact, although waist circumference is positively related to BMI in both sexes, the relationship is stronger in men than women (42). Moreover, it has been shown that, although men tend to progressively increase abdominal fat depots with increasing total adiposity at each age, a tendency to develop different obesity phenotypes throughout the lifespan occurs more clearly in women, particularly after the menopausal age (42). Therefore, it seems that, unlike in men, the increased abdominal fatness in women significantly counteracted the progressively greater suppressibility of the HPA axis that would be expected according to increasing BMI. As a consequence, it could be speculated that women

with the abdominal obesity phenotype seem to be partially resistant to the inhibitory effect of dexamethasone on the HPA axis, and this may be in agreement with the concept that the impact of abdominal fatness on the HPA axis function and regulation may be somewhat different according to gender (31).

In conclusion, this study demonstrated that pituitary sensitivity to feedback inhibition by dexamethasone is preserved in obesity in both sexes even at low dosages. Altogether, these results confirm a significant effect of gender on postdexamethasone cortisol concentrations, suppression of the HPA axis, and dexamethasone levels, which may be partially dependent on differences in both cortisol and dexamethasone metabolism. In addition, our results suggest that, at least in women, abdominal fat distribution may partially counteract the progressively greater suppressibility of the HPA axis that would be expected according to increasing BMI. Whether this might mean a difference in sensitivity and/or hyperresponsiveness of the hypothalamic centers, as well as adrenal regulation or peripheral cortisol metabolism, still remains unclear and requires more detailed studies.

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International Symposium
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Low thyroid reserve due to autoimmune thyroiditis is increasingly recognized as a serious health problem. In the field of type 1 diabetes mellitus it has already been a goal for several years to try and attenuate the destructive autoimmune—insulitis—process. Because of recent developments in basic and clinical immunology, the organizers of the meeting found the time appropriate to bring together experts in the fields of thyroid and islet autoimmunity, early detection of endocrine autoimmune diseases, and tolerance induction. There will be ample time for such discussions during oral and poster presentations and plenary sessions.

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