Hypothalamic-Pituitary-Adrenal Axis Activity and Its Relationship to the Autonomic Nervous System in Women With Visceral and Subcutaneous Obesity: Effects of the Corticotropin-Releasing Factor/Arginine-Vasopressin Test and of Stress

Renato Pasquali, Bruno Anconetani, Rabih Chattat, Mimmo Biscotti, Giulio Spinucci, Francesco Casimirri, Valentina Vicennati, Anastasia Carcello, and Antonio Maria Morselli Labate

In a previous study, we demonstrated that premenopausal women with visceral obesity have hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, characterized by an exaggerated hormone response to corticotropin-releasing factor (CRF) and corticotropin (ACTH) stimulation. The hypothalamic peptide flow that stimulates the pituitary, particularly after a physiological stress challenge, involves not only CRF, but also arginine-vasopressin (AVP), which synergizes the CRF capacity to stimulate pituitary hormone secretion. Previous studies in humans have demonstrated that combining AVP with CRF permits maximal stimulation of the pituitary, providing a more appropriate method of assessing pituitary hormone reserve. We therefore investigated the response of the HPA axis to combined CRF and AVP stimuli in obese women with different obesity phenotypes. Moreover, we examined hormonal and cardiovascular responses to several mental stress tasks, according to previously standardized procedures. Two groups of age-matched premenopausal eumenorrheic obese women with visceral (V-BFD) or subcutaneous (S-BFD) body fat distribution and a group of normal-weight healthy controls were investigated. All women randomly underwent the following protocol: (1) a combined CRF/AVP test (100 μg plus 0.3 IU intravenously [IV], respectively); (2) a standardized stress test, which consisted of completing two puzzles and a mental arithmetic test; and (3) a control saline test. Blood samples for ACTH and cortisol determinations were obtained before and during each test, and measurements of arterial blood pressure and pulse rate were made at regular intervals during the stress test. After combined CRF/AVP administration, ACTH and cortisol were significantly higher in V-BFD than in the other two groups. In contrast, no significant hormonal variation was found in either group during stress tasks. During the stress test, pulse rate (but not arterial blood pressure) significantly increased after 8 and 15 minutes in the V-BFD group, whereas no significant variation was found in S-BFD and control women. A significant correlation was present between the pulse rate and change in cortisol level during the stress test at minutes 8 ($r = .54$, $P < .05$) and 15 ($r = .57$, $P < .01$) in all women considered together. Subjective emotional involvement during stressful tasks was measured by a two-dimensional short verbal scale, which revealed that the stress section had a more significant impact in obese V-BFD than in S-BFD and control women. These data therefore confirm that women with visceral obesity have hyperactivity of the HPA axis, and that the combined CRF/AVP stimulation may offer a good tool for investigating pituitary reserve in this obesity phenotype. Moreover, the results indicate that these women probably have a hyperreactive sympathetic response to acute stress that seems interrelated to that of the HPA axis.

WE HAVE PREVIOUSLY demonstrated that women with visceral obesity have a significantly greater corticotropin (ACTH) and cortisol response to corticotropin-releasing factor (CRF) and a significantly higher cortisol response to ACTH administration than obese women with subcutaneous body fat distribution (S-BFD) and control normal-weight women.[1] These findings led us to speculate that women with visceral obesity may have hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, which substantiated the impression formed some time ago that so-called android obesity may be in some way related to abnormal cortisol metabolism or activity, particularly in women.[2] Since glucocorticoid receptors appear to be more dense in visceral adipose tissue (VAT) than in subcutaneous adipose tissue (SAT),[3,4] these data may also help to better understand the development of abdominovisceral obesity in humans. They can also be useful in speculating about the relations between visceral body fat distribution (V-BFD) and related hormonal and metabolic abnormalities such as glucose intolerance, hyperinsulinemia and insulin resistance, and hypertension.[5]

However, the origin of this abnormality is not clearly defined, and several mechanisms may in fact be involved. First of all, this phenomenon may be primarily due to a neuroendocrine alteration leading to an increased sensitivity to CRF of ACTH-producing cells at the pituitary level or to increased CRF flow toward the pituitary gland. Alternatively, functional cortisol resistance may be present, since glucocorticoid receptors can undergo several structurally acquired alterations, as recent studies in AIDS patients have demonstrated.[6] Finally, it could be suggested that this abnormality represents part of an altered response to acute or chronic stress, which can be independent of the mechanisms responsible for feedback regulation.[5] Several studies have in fact demonstrated that a similar neuroendocrine adaptation takes place during the reaction behavior in laboratory animals exposed to various socioenvironmental stressors.[7-9] Theoretically, women with visceral obesity may

From the Institute of Clinical Medicine 1, Endocrine Unit, and Department of Psychology, University Alma Mater, Bologna; and the Radiology Service, S. Orsola-Malpighi Hospital, Bologna, Italy.
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Address reprint requests to Renato Pasquali, MD, Istituto di Clinica Medica 1, Endocrinologia, Policlinico S. Orsola-Malpighi, Via Massarenti 9, 40138 Bologna, Italy.

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have hyperactivity of the HPA axis as a consequence of maladaptation to chronic stress exposure. Some time ago, Bjorntorp[5] proposed this mechanistic model to explain the development of visceral obesity and its typically associated metabolic alterations and diseases. In this model, a key role should be played by the complex of events involving a maladaptation to an altered coping reaction to chronic stress. These abnormalities include increased or unbalanced ACTH and cortisol response.[10]

At the neuroendocrine level, the hypothalamic peptide flow that stimulates the pituitary following stressful challenge involves not only CRF but also arginine-vasopressin (AVP) release,[10,11] which synergizes the CRF capacity to stimulate pituitary hormone secretion.[11,12] Previous studies performed in humans have shown that combined use of CRF and AVP can increase by severalfold the ACTH secretion elicited by CRF alone.[12,13] Since maximal stimulation of the pituitary can therefore be achieved with this procedure, it has been suggested that combined stimulation may be a more appropriate method of assessing pituitary ACTH reserve.[13] We therefore investigated the response of the HPA axis to combined CRF and AVP stimuli in obese women with different patterns of BFD and an age-matched group of healthy normal-weight controls. Moreover, we examined hormonal and cardiovascular responses to several mental stress tasks, according to standardized procedures previously used in our laboratory.[14,15]

SUBJECTS AND METHODS

Subjects

Sixteen obese women participated in the study. All subjects were fully informed in advance as to the nature and aim of the study, and they gave informed, written consent to participate. They had been referred to the Endocrine Section of the Institute of Clinical Medicine & Gastroenterology, University of Bologna, as outpatients for evaluation and treatment of obesity. All had normal menses, and 10 had previously been pregnant. Other endocrine and metabolic diseases were excluded on the basis of physical examination and laboratory tests. In particular, none of the subjects had Cushing's syndrome or congenital adrenal hyperplasia, hirsutism, acanthosis nigricans, or polycystic ovaries. Seven women had mild hypertension but had never been treated for it. None took drugs for at least 1 month before the study, nor were any dieting. All obese women but one were nonsmokers. Six age-matched normal-weight healthy women with regular menstrual cycles served as a control group; three were smokers.

Anthropometry and Definition of BFD

Body height was measured without shoes to the nearest 0.5 cm, and body weight was measured without clothes. All obese women had a body mass index (weight in kilograms divided by height in meters squared) greater than 30. Waist and hip circumferences were also measured, with subjects standing, using a 1-cm–wide metal measuring tape as described elsewhere,[16] and the waist to hip ratio 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. BFD was defined by computed tomography.[17] Total VAT area, VAT and SAT areas in the abdomen, and the VAT to SAT area ratio were evaluated by a single scan at the L4-S1 level.[17] The criteria used to determine V-BFD versus S-BFD were in agreement with those reported by others.[17,18] The general characteristics of obese women with V-BFD or S-BFD and of control normal-weight women are reported in Table 1. Four of seven women in the V-BFD group and three of nine women in the S-BFD group had mild hypertension. The mean age in the two obese groups were not significantly different. The table also reports smoking habits for each group.

Protocol

Women were examined in the follicular phase of the menstrual cycle and never more than 10 days after the start of the previous menstrual cycle. Before testing, they were asked to follow a diet containing at least 300 g carbohydrate. Tests were randomly performed in the morning (8 to 9 AM) after overnight fasting, while subjects had been quietly lying down for at least 15 to 20 minutes after an indwelling catheter had been placed in an antecubital vein kept patent with slowly infused normal saline.

Combined CRF and AVP test. Human CRF (hCRF; Novabiochem, Laufelfingen, Switzerland) and synthetic 8-AVP (Pitressin; Parke-Davis Co, Germany) were injected as an intravenous (IV) bolus at doses of 100 μg and 0.3 IE, respectively. Blood samples for ACTH and cortisol determination were drawn during basal conditions (−15 and 0 minutes) and at 15, 30, 60, 90, and 120 minutes thereafter. Previous studies in normal subjects have in fact demonstrated that simultaneous administration of IV AVP at doses of 0.3 IU with CRF significantly amplified the ACTH response following IV CRF alone.[13]

Experimental stress tasks. Subjects were asked to complete two different puzzles selected from the Psychovisceral Reactivity Test, according to previously reported procedures.[14,15] A puzzle made up of four different parts had to be put together to form a square; this puzzle is especially difficult to solve because of the shape of the pieces. The second puzzle was made up of seven parts that had to be put together to form a circle. Seven minutes were given to complete each of these tasks. More detailed information and figures related to these tests are reported elsewhere.[15] In addition, each subject was asked to complete a mental calculation consisting of a progressive subtraction. Subjects were aware that they had a short but adequate time to complete the task. If the tasks were not completed in the allotted time, subjects were not allowed to complete them. The total time given for stress testing was 20 minutes. At the beginning and end of the stress session, each subject was asked to report subjective emotions on a short two-dimensional verbal scale related to feelings of tension/relaxation and agreeableness/disagreeableness. Blood samples for cortisol and ACTH determination were obtained in basal conditions (−15 and 0 minutes) and 8, 15, 30, 60, 90, and 120 minutes

Table 1. Characteristics of Obese Women With S-BFD and V-BFD and Control Women Participating in the Study

Characteristic	S-BFD	V-BFD	Controls
No. of subjects	9	7	6
Age (yr)	28.3 ± 7.1†	33.9 ± 7.4*	26.6 ± 7.2
Body weight (kg)	100.0 ± 28.4†	104.0 ± 9.9†	51.1 ± 9.9
Body mass index (kg/m²)	37.6 ± 9.1†	40.9 ± 4.7†	21.0 ± 1.1
Waist to hip ratio	0.80 ± 0.09†	0.88 ± 0.11†§	0.72 ± 0.04
VAT (cm²)	72.5 ± 31.3†	186.5 ± 69.0†§	16.9 ± 6.6
SAT (cm²)	554.8 ± 144.6†	593.7 ± 86.9†	94.5 ± 74.4
VAT/SAT ratio	0.13 ± 0.03*	0.31 ± 0.11*‡	0.22 ± 0.09
Nonsmokers/smokers	9/0	6/1	3/3

*$P < .05$, †$P < .01$: *v* control women.
‡$P < .05$, §$P < .01$: S-BFD *v* V-BFD.

after the stress challenge. At the same times, pulse rate and arterial blood pressure were monitored.

Control test. A control saline study was performed for each woman, repeating exactly the same procedure used for the previous tests, ie, blood samples for hormone determination were obtained and pulse rate and arterial blood pressure were measured at the same times.

Hormone Assays

Immediately after withdrawal, blood samples were placed in different tubes containing EDTA without or with aprotinin (500 U/mL) for cortisol and ACTH determinations, respectively, and maintained on ice until stored. All plasma samples for hormone determinations were stored at −80°C until assayed. All assays for each woman were performed in duplicate. ACTH was determined with an immunoradiometric assay with reagents obtained from Nichols Institute (San Juan Capistrano, CA). Sensitivity of this assay in our laboratory was approximately 1 pg/mL (0.22 pmol/L). Interassay and intraassay coefficients of variation at concentrations of 28.6 pg/mL (6.3 pmol/L) and 244.3 pg/mL (53.8 pmol/L) were 9.6% and 7.1% and 7.3% and 3.7%, respectively. Cortisol was determined by radioimmunoassay with reagents obtained from Diagnostic Products (Los Angeles, CA). In our laboratory, the lowest sensitivity level was 30 ng/mL (8.3 nmol/L). Interassay and intraassay coefficients of variation at concentrations of 58 ng/mL (160 nmol/L), 217 ng/mL (599 nmol/L), and 359 ng/mL (990 nmol/L) were 9.8%, 6.9%, 8.0% and 1.4%, 3.1% and 4.1%, respectively.

Statistical Analysis

Results are expressed as the mean ± SEM unless otherwise stated. Areas under the curve (AUCs) of each parameter measured during the tests were calculated by the trapezoidal method. The Kolmogorov-Smirnov test was applied to verify normal distribution of the data.[19] Because of the multiway experimental design, intragroup and intergroup comparisons were performed by ANOVA, taking into account all competent factors. Linear regression analysis was used to evaluate correlations between variables. All statistical evaluations were performed using SPSS/PC$^+$ software (SPSS Inc, Chicago, IL) on an Epson AX3.25 personal computer (Nagano, Japan). P less than .05 was used to define statistical significance.

RESULTS

CRF/AVP Test

There were no differences in baseline hormone levels between the three groups or between within-group values observed before the CRF/AVP test and the control study. During the saline study, ACTH levels did not significantly differ from baseline in any group. In contrast, cortisol concentrations decreased significantly ($P < .05$) from 15 minutes onward in all groups; however, there were no significant differences in percent variations of cortisol versus baseline values between the three groups.

After CRF/AVP administration, ACTH and cortisol blood levels increased significantly ($P < .01$) at times 15, 30, and 60 minutes in all groups. However, absolute (Fig 1) increases of ACTH and cortisol after stimulation were significantly higher in V-BFD versus S-BFD and control women. In contrast, no significant difference was found in hormone response during the test between the other two groups. Both $cortisol_{AUC}$ and $ACTH_{AUC}$ were significantly ($P < .05$) higher in V-BFD women (68,798 ± 7,532 nmol/L · min and 1,962 ± 1,056 pmol/L · min, respectively) than in the S-BFD group (57,246 ± 7,079 nmol/L · min and 1,133 ± 556 pmol/L · min, respectively) and the control group (60,174 ± 6,006 nmol/L · min and 1,249 ± 380 pmol/L · min, respectively). There were no significant differences in these parameters between S-BFD and control women. Hormone response in smokers and nonsmokers was not apparently different in the obese groups or in controls.

Experimental Stress Test

Subjective Ratings. After performing the stress task, subjects reported a significant increase of tension on the tension/relaxation dimension, particularly in the obese groups. However, only V-BFD women showed significant changes in the dimension of agreeableness/disagreeableness, such as an unpleasant feeling toward the task (Fig 2). These findings obviously validated the emotional impact of the stressful task on the subjects, although different patterns were observed between the three groups.

Cardiovascular response and hormones. There was no statistically significant variation of ACTH and cortisol concentrations in each of the three groups during the test, and values for both hormones did not significantly differ with respect to those observed during the control saline study (Fig 3). Systolic and diastolic arterial blood pressure in the V-BFD group were significantly higher than in the other groups both in basal conditions and during the test. No significant variation in blood pressure occurred in the S-BFD group or in the controls, whereas a significant increase in pulse rate occurred at 8 and 15 minutes with respect to baseline values in the V-BFD group. Despite the fact that the variations of hormone concentrations in the early phase following stressful challenge were not significantly different from baseline, in the whole sample of women considered together, a positive and significant correlation between the change in cortisol level and pulse rate at minutes 8 ($r = .54$, $P < .05$) and 15 ($r = .57$, $P < .01$) was found.

Finally, there were no smoking-related differences in hormone and cardiovascular responses in both obese groups or in controls.

DISCUSSION

These findings confirm that women with visceral obesity, in which different patterns of BFD were directly defined by computed tomographic scans, have hyperactivity of the HPA axis as previously reported by our group.[1] The fact that in this study, contrary to the previous one in which we used only CRF, we obtained the same result by administering AVP associated with CRF may have a certain importance. It has been shown that ACTH response to AVP depends on ambient endogenous levels of CRF and that AVP stimulation of ACTH secretion can be used as an in vivo bioassay of endogenous CRF.[20] Moreover, it has been suggested that combined CRF/AVP administration may elicit maximal pituitary ACTH secretion.[12,13] Therefore, combining AVP with CRF may provide a better means of investigating HPA axis reactivity in pathophysiological

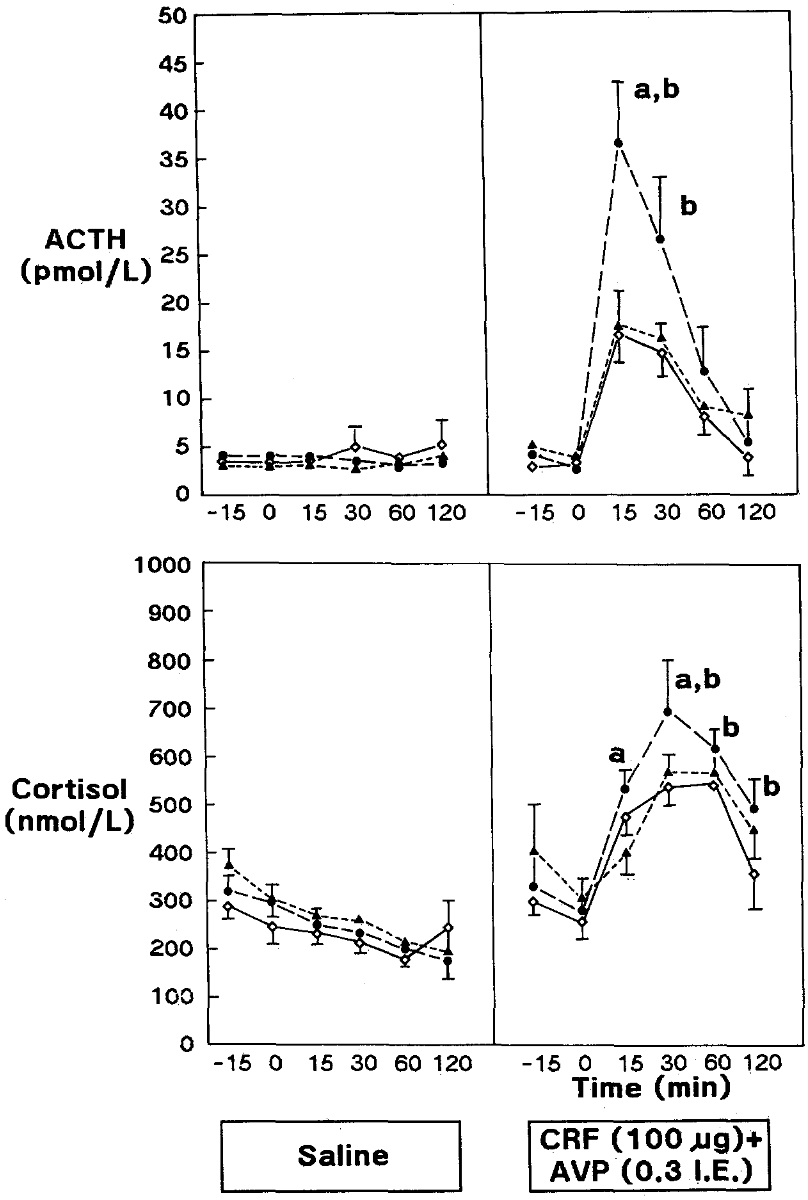

Fig 1. Plasma ACTH and cortisol concentrations before and during the CRF/AVP test and the saline control study in obese women with V-BFD (●) and S-BFD (◇) and in normal-weight healthy controls (▲). [a]$P < .05$, V-BFD *v* controls; [b]$P < .05$, V-BFD *v* S-BFD.

states. As expected, we found that the magnitude of hormone increase after combined stimulation was more than double what we previously observed after administration of CRF alone in both obese groups and controls, although both ACTH and cortisol responses to combined infusion were greater in V-BFD versus S-BFD and control women. Although the combined test did not have better discriminating power than CRF alone, these findings indicate that the presence of a hyperactive HPA axis in premenopausal V-BFD women can be confirmed even when maximal stimulation of the pituitary is achieved.

Visceral obesity has been suggested to depend on multiple factors, including maladaptation to chronic stress exposure.[5] In this model, a key role should be represented by the complex of events involving maladaptation to altered coping reaction to chronic stress. These abnormalities include increased or unbalanced HPA axis activity and therefore both ACTH and cortisol overproduction. Studies in animals have clearly demonstrated that a similar neuroendocrine alteration takes place during the reaction behavior that follows various socioenvironmental stressors.[7-9] AVP is actively involved in the reaction of the HPA axis to stress factors. Animal studies have shown that, whereas in nonstress situations both CRF and AVP are secreted in the portal system in a pulsatile fashion with good concordancy of the pulses,[21] during stress exposure the amplitude of AVP pulsation is significantly increased.[21] Moreover, in conscious sheep, both endogenous CRF and AVP have been shown to increase simultaneously in the hypophysial-portal circulation following intracerebroventricular norepinephrine injection.[22] Activation of the sympathetic system represents the main factor of endocrine regulation of

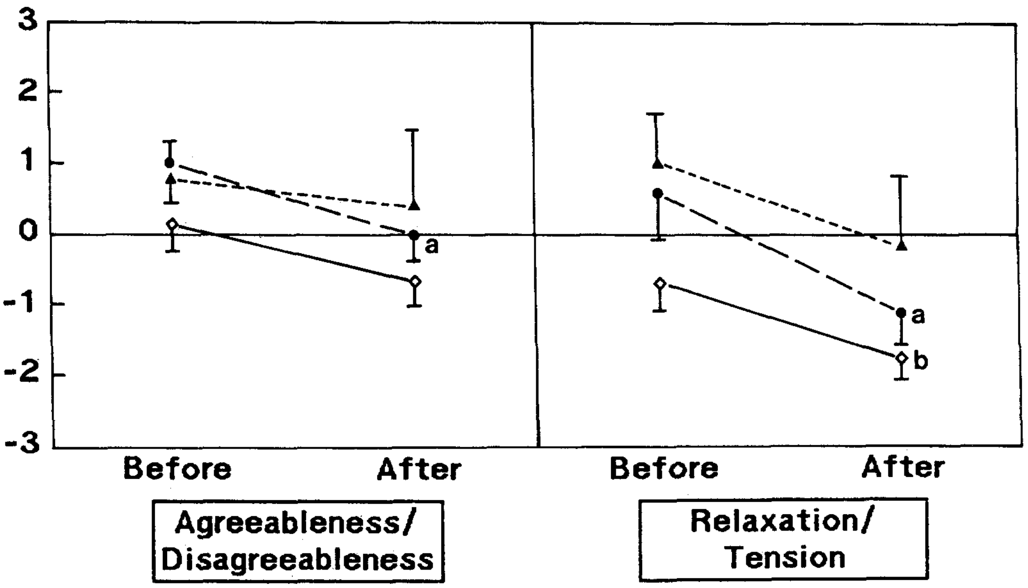

Fig 2. Subjective emotions related to feelings of tension/relaxation and agreeableness/disagreeableness measured on a short two-dimensional verbal scale before and after the stress test in obese women with V-BFD (●) and S-BFD (◇) and in normal-weight healthy controls (▲). All subjects reported a significant increase of tension and disagreeableness such as an unpleasant feeling toward the task. However, both scale indices significantly changed only for the V-BFD group ([a]$P < .05$). No significant variation was found in the controls, whereas S-BFD women showed a significant variation only in the agreeableness/disagreeableness scale ([b]$P < .01$).

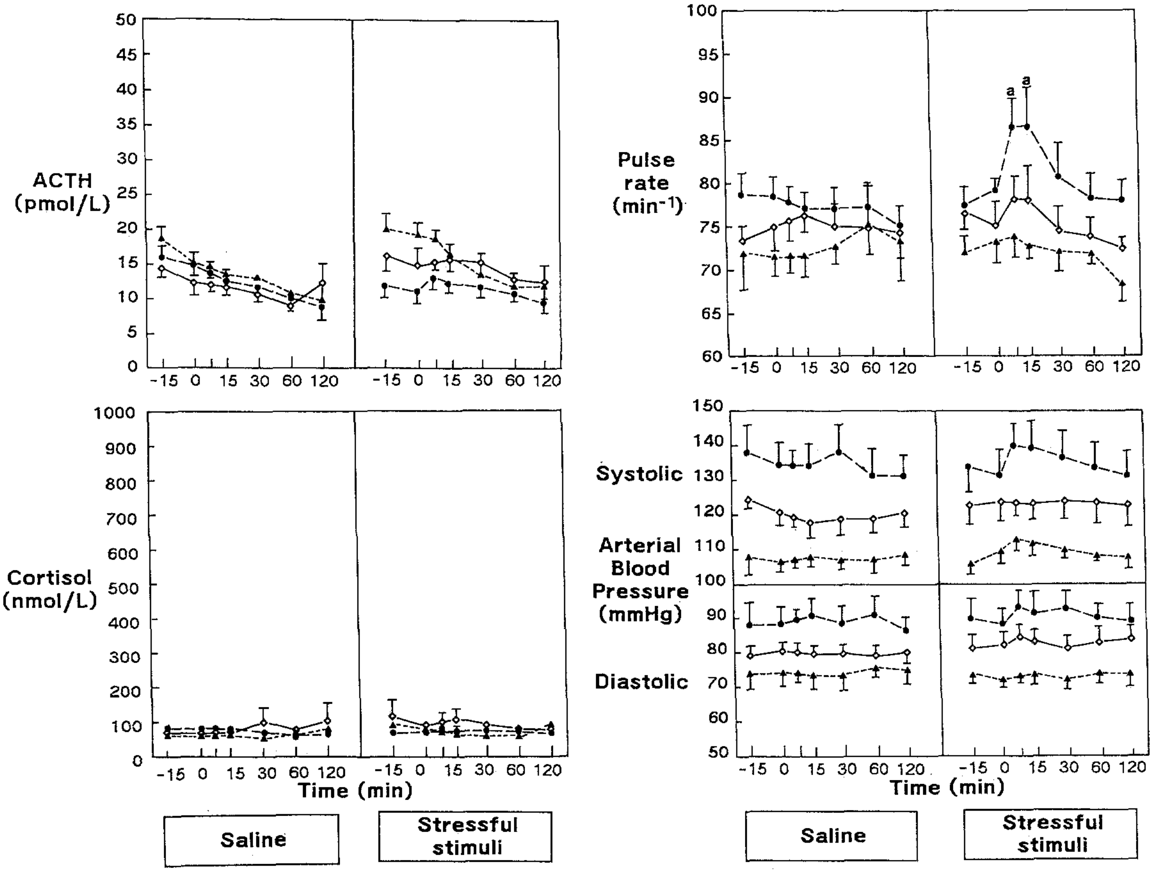

Fig 3. Plasma ACTH and cortisol concentrations, pulse rate, and arterial blood pressure before and during the stress test and the saline control study in obese women with V-BFD (●) and S-BFD (◇) and in normal-weight healthy controls (▲). ([a]$P < .05$ at minutes 8 and 15 *v* basal value in the V-BFD group).

adaptation to stress,[10] and it appears to be significantly correlated with stimulation of the HPA axis.[23] A hyperactivity of the sympathetic pathways and of the HPA axis following acute stress challenge has been reported, for example, in subjects with high cardiac reactivity to stress.[23] Stress findings reported in the present study seem to indicate that, compared with their S-BFD counterparts and normal-weight controls, women with V-BFD obesity may have a greater heart rate response and higher blood cortisol concentration during the early phase following acute mental stress. This suggests that they probably had a greater catecholaminergic activation after the stress test. This has been previously observed by Cacioppo[23] and Sgoutas-Emch et al[24] in normal-weight individuals. In fact, they found that subjects who were characterized by a higher heart rate reaction to a stress test also had higher stress-related levels of cortisol, in contrast to low–heart rate reactors. Whether women with visceral obesity represent a class of individuals in whom a stress-related increase of sympathetic activity may be associated with a hyperactivity of the HPA axis is an attractive hypothesis that needs to be further investigated. On the other hand, since norepinephrine (together with AVP) is synergistic with CRF in regulation of the stress response,[25] it can be speculated that increased sympathetic tone may be involved in the development of the HPA axis hyperactivity we have further demonstrated in women with visceral obesity. Further studies are in progress in this area.

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