COMMENT

Response of the Hypothalamic-Pituitary-Adrenocortical Axis to High-Protein/Fat and High-Carbohydrate Meals in Women with Different Obesity Phenotypes

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Subjects with abdominal obesity are characterized by hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis. Food intake, particularly at noon, is a well-known inducer of HPA axis activation. Whether obese subjects present an abnormal response to meals containing different macronutrient proportions is at present unknown. Therefore, this study was carried out to investigate the effect of a high-lipid/protein meal (HLP-meal) and a high-carbohydrate meal (HCHO-meal) on the HPA axis activity in women with different obesity phenotypes. Nondepressed, uncomplicated obese (body mass index greater than 28 kg/m²) women with abdominal (A-BFD) (n = 10) and peripheral body fat distribution (P-BFD) (n = 9) and a group of 11 normal-weight controls were investigated in the follicular phase of the menstrual cycle. They were randomly given an 800-kcal HCHO-meal (containing 89% carbohydrates, 11% proteins, 0% lipids) and an 800-kcal HLP-meal (containing 53% lipids, 43% proteins, 4% carbohydrates), which were eaten within 15 min at noon, with an interval of 2 d between each meal. Blood samples for ACTH, cortisol, glucose, and insulin were obtained at 15-min intervals before and after each meal. Baseline hormone and glucose concentrations in the three groups were similar. After the HLP-meal, ACTH tended to similarly but insignificantly increase in all groups, whereas cortisol increased significantly (P < 0.05) in the P-BFD group and insignificantly in the other groups. Conversely, both ACTH and cortisol significantly (P < 0.05) increased only in the A-BFD group, without any significant changes in both controls and P-BFD women. The analysis of the interaction between meals and groups clearly indicated that the cortisol response to the HLP-meal and the HCHO-meal was significantly different (P < 0.025) between the two obese groups, the A-BFD group being characterized by a significantly lower response to the HLP-meal and a significantly higher response to the HCHO-meal, compared with the P-BFD group. Considering all groups together and after adjusting for body mass index, a highly significant relationship was found between cortisol-area under the curve and ACTH-area under the curve after each meal test. However, no relationships were found between changes in ACTH and cortisol and those of glucose, insulin, and the glucose-insulin ratio after each meal. Therefore, our data demonstrate that the response of the HPA axis to meals containing different macronutrient proportions may depend on the pattern of body fat distribution. We also suggest that the activation of the HPA axis following the ingestion of large amounts of carbohydrates may have some pathophysiological relevance, specifically in women with the abdominal obesity phenotype. (J Clin Endocrinol Metab 87: 3984–3988, 2002)

There is increasing evidence that, in humans, the abdominal obesity phenotype may be characterized by a hyperactivation or hyperresponsiveness of the hypothalamic-pituitary-adrenocortical (HPA) axis (1–4). Disruption of neuroendocrine mechanisms regulating both CRH and ACTH release have been repeatedly demonstrated in abdominal obesity in both sexes (2), and results from long-term interventional studies performed in primates (5) and cross-sectional epidemiological surveys performed in humans (6, 7) indicate that they may represent maladaptive neuroendocrine mechanisms to chronic environmental stressors and adverse life events. In addition, peripheral alterations of cortisol metabolism involving both 11β-hydroxysteroid dehydrogenase and 5α-reductase activity in peripheral tissues such as the adipose tissue and liver have been described (7). However, whether other factors, such as internal signals coming from peripheral organs and tissues, may play a role in the pathophysiology of the HPA axis abnormalities of the human obesity phenotype remains to be clarified.

A strong interaction between the HPA axis and the brain-gut axis has been described in many different reports (8). Food ingestion is a well-known inducer of several peptides that may, in turn, directly influence the activity of the HPA axis (9). Strong evidence of this assumption is provided by animal models, in which both food intake and the light-dark cycle have been found to represent independent synchronizers for the circadian periodicity of cortisol secretion (10). In addition, meal timing, food composition, and the duration of premeal fasting have been shown to exert an important effect on cortisol secretion (11, 12). Specifically, there are studies demonstrating that a protein-rich intake may induce a sustained cortisol increase, whereas negligible effects have been found after a carbohydrate-rich meal (13, 14).
Studies on the response of the HPA axis in obesity, particularly the abdominal phenotype, are still controversial, because both altered (7, 15) and normal (16) cortisol responses to standard mixed meals have been reported. This study was, therefore, carried out to investigate the effects of a high-carbohydrate meal (HCHO-meal) or high-lipid/protein meal (HLP-meal) on the HPA axis activation among obese women with different patterns of body fat distribution and an appropriate group of normal-weight healthy controls.

Materials and Methods

Subjects

In this study, 19 consecutive women, selected to cover a wide range of body fat distribution measured by the waist to hip ratio (WHR) see below for selection criteria) and with a body mass index greater than 28 kg/m², were investigated. They were referred to the Endocrine Unit of the Department of Internal Medicine, University of Bologna, as outpatients for the evaluation and treatment of their obesity. All had regular menses. On the basis of clinical history, physical examination, and laboratory data, none had diabetes; thyroid diseases; Cushing’s syndrome; hyperandrogenism; hypertension; or relevant cardiovascular, renal, hepatic, or systemic abnormalities. None took drugs for at least 1 month before the study, and all were following their usual diet, containing at least 250–300 g carbohydrates, and no more than 30 g/d alcohol intake. For comparison, 11 normal-weight healthy women, regularly menstruating and without a history of having been overweight or obese, were also investigated. None of the obese or control women were smokers. All women gave their informed and written consent to the study, which had been previously approved by the local Ethics Committee.

Psychological evaluation

Psychological evaluation was performed to exclude the presence of depressive traits, which can be associated with a dysregulation of the HPA axis (17). Two different questionnaires, the Children Depression Questionnaire (CDQ) (18) and the Center for Epidemiological Studies Depression Score (CES-D) (19), both in the Italian version, were administered. They are based on a numerical scale with a given threshold value assessing the presence or the absence of depression. In the CDQ scale, values lower than 7 exclude depression, as values lower than 21 do in the CES-D scale.

Anthropometry

Height was measured without shoes to the nearest 0.5 cm, and body weight was measured without clothes. The waist and hip circumferences were also measured, with the subjects standing, using a 1-cm-wide metal measuring tape. According to the recommendation of the World Health Organization (20), 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 between the buttocks. Women with WHR greater than or equal to 0.85 were defined as having abdominal obesity (A-BFD), and women with WHR less than 0.80 were defined as having peripheral body fat distribution (P-BFD) (20). Women having intermediate WHR values were, therefore, not included in the study.

Protocol

Meal tests were performed in the follicular phase of the menstrual cycle and never more than 10 d after the start of the previous menstrual cycle. The subjects were fasting from 2300 h of the day before the test. An iv catheter for blood collection was placed in a forearm vein of one arm and the vein was kept open with NaCl (0.9%) infusion for at least 30 min. Baseline blood samples for ACTH, cortisol, glucose, and insulin determination were taken at 2345 h and 2400 h. All subjects were then randomly given an 800-kcal HCHO-meal (containing 89% carbohydrates, 11% proteins, 0% lipids) and an 800-kcal HLP-meal (containing 53% lipids, 43% proteins, 4% carbohydrates), which were eaten within the following 15 min, with an interval of 2 d between each meal. Additional blood samples were then obtained 15, 30, 45, and 60 min thereafter.

Hormonal and biochemical assays

Blood samples for ACTH and cortisol determinations were placed in different tubes containing EDTA without or with aprotinin (500 U/ml), respectively, maintained in ice until centrifuged and plasma aliquots then stored at ~80 °C until assayed. ACTH was determined with an immunoradiometric assay method with reagents obtained from Nichols Institute (San Juan Capistrano, CA). In our laboratory, the lowest sensitivity level was approximately 0.22 pmol/liter (1 pg/ml) and inter-and intraassay coefficients of variations at concentration levels of 6.3 pmol/liter (28.6 pg/ml) and 53.8 pmol/liter (244 pg/ml) were 9.6% and 7.1%, and 7.3% and 3.7%, respectively. Cortisol was determined by RIA with reagents obtained from Diagnostic Products (Los Angeles, CA). The lowest sensitivity level was 83 nmol/liter (30 ng/ml) and inter- and intraassay coefficients of variations at concentration levels of 160.0 nmol/liter (58 ng/ml) and 990.5 nmol/liter (395 ng/ml) were 9.8%, and 8.0%, and 1.4% and 4.1%, respectively. Plasma glucose concentrations were measured immediately by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Fullerton, CA). Serum insulin was determined by RIA using commercial kits (INSIK-5, Sorin, Saluglia, Italy). The intraassay coefficient of variation was 3%. The glucose/insulin ratio at baseline and after each meal was also used as a measure of insulin sensitivity (21).

Statistics

All results are reported as mean ± se (SEM). General characteristics of patients were analyzed by means of one-way ANOVA, and repeated measure ANOVA was applied to hormonal and biochemical data. To avoid multiple comparisons, full-factorial ANOVA was applied by using linear contrasts and nested designs (22). Statistical analyses were performed by running the SPSS/PC+ package (SPSS, Inc., Chicago, IL; Ref. 23) on a personal computer. The two-tailed P value of 0.05 was chosen to detect significant results.

Results

Characteristics of the subjects

Obese women were divided into two groups, according to their WHR value: Nine women were included in the P-BFD group and 10 women in the A-BFD group. The clinical characteristics of obese women and normal-weight controls are summarized in Table 1.

All subjects had values lower than 7 in the CDQ scale and lower than 21 in the CES-D scale, consistent with the absence of significant depression traits.

Baseline hormone and glucose levels

There were no significant differences in fasting plasma ACTH, cortisol, insulin, and glucose concentrations measured before the HLP-meal and the HCHO-meal among the obese women with different patterns of body fat distribution.

TABLE 1. General characteristics (mean ± SEM) of obese women with A-BFD and P-BFD and of normal weight controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 11)</th>
<th>P-BFD (n = 9)</th>
<th>A-BFD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>30.4 ± 2.1</td>
<td>33.2 ± 2.4</td>
<td>35.0 ± 2.8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>60.4 ± 2.0</td>
<td>80.5 ± 2.5</td>
<td>88.1 ± 4.0b</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.3 ± 0.7</td>
<td>31.8 ± 1.1</td>
<td>33.6 ± 1.4b</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>71.4 ± 1.6</td>
<td>89.1 ± 1.9c</td>
<td>101.1 ± 3.2c</td>
</tr>
<tr>
<td>WHR</td>
<td>0.75 ± 0.01</td>
<td>0.79 ± 0.02</td>
<td>0.92 ± 0.01bc</td>
</tr>
</tbody>
</table>

a P < 0.05 P-BFD vs. controls.
b P < 0.05 A-BFD vs. controls.
c P < 0.05 A-BFD vs. P-BFD.
three groups and within each group (Table 2), with the exception of significantly higher \( P < 0.05 \) plasma insulin before the HLP-meal in the A-BFD group, compared with controls.

**ACTH and cortisol response to HLP- and HCHO-meals**

After the HLP-meal, ACTH tended to similarly but insignificantly increase in all groups, whereas cortisol concentrations significantly increased in the P-BFD group \( P < 0.05 \) and insignificantly in controls and the A-BFD women (Fig. 1).

Conversely, after the HCHO-meal, ACTH \( P < 0.05 \) and cortisol \( P < 0.05 \) significantly increased only in the A-BFD group, without any significant changes in the controls and P-BFD women (Fig. 1). Differences in the ACTH response between the two meals in each group as well as those among the three groups for each meal were not, however, significant. On the other hand, the analysis of the interaction between meals and groups clearly indicated that the cortisol response to the HLP-meal and the HCHO-meal was significantly different \( P < 0.025 \) between the two obese groups, the A-BFD group being characterized by a significantly lower response to the HLP-meal and a significantly higher response to the HCHO-meal, compared with the P-BFD group.

Considering all groups together, a highly significant relationship between cortisol-area under the curve and ACTH-area under the curve after each meal (HLP-meal: \( r = 0.679, P < 0.001 \); HCHO-meal: \( r = 0.449; P < 0.01 \)) was found. Body mass index had no effect \( P \) (NS) on the response of ACTH and cortisol to either the HLP-meal or the HCHO-meal test.

**Glucose and insulin response and relationship with changes of the HPA axis**

After the HLP-meal, glucose levels rose in all groups, although significantly only in the P-BFD \( P < 0.05 \) and the A-BFD women \( P < 0.05 \). The glucose increase after the HCHO-meal was, conversely, highly significant in all three groups \( P < 0.001 \). Differences in the glucose response after each meal among the groups were not significant. Insulin concentrations significantly increased after both meals \( P < 0.001 \) but significantly more \( P < 0.001 \) after the HCHO-meal than the HLP-meal in all groups, without, however, any significant difference of the insulin response to each meal among the groups. Because of certain variability in both glucose and insulin, particularly after the HCHO-meal, we investigated the response of the glucose to insulin ratio to each meal and the relationship with ACTH and cortisol changes. Baseline glucose to insulin ratios before each meal were similar, and a significant \( P < 0.001 \) decrease occurred after both meals in all groups. The reduction, however, was significantly \( P < 0.05 \) greater after the HCHO-meal than the HLP-meal. There were no significant differences in the response to the HLP-meal among the groups and the HCHO-meal between controls and P-BFD women. At variance, after the HCHO-meal, the glucose to insulin ratio decreased significantly \( P < 0.05 \) less from time 30 min onward in the A-BFD group, suggesting impaired late insulin response (data not shown).

No relationships were found between changes in ACTH and cortisol and those of glucose, insulin, and the glucose to insulin ratio after each meal.

**Discussion**

In this study, we have demonstrated that both obesity and the pattern of body fat distribution may be associated with some differences in the HPA axis response to an HLP-meal or HCHO-meal. In fact, similar to control subjects, even

**TABLE 2.** Basal ACTH, cortisol, insulin, and glucose concentrations (mean ± SEM) before the HLP-meal and the HCHO-meal in women with A-BFD and P-BFD obesity and in normal weight controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls ((n = 11))</th>
<th>P-BFD ((n = 9))</th>
<th>A-BFD ((n = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting ACTH HLP-meal ((pmol/liter))</td>
<td>3.59 ± 0.41</td>
<td>3.16 ± 0.5</td>
<td>4.00 ± 1.27</td>
</tr>
<tr>
<td>Fasting ACTH HCHO-meal ((pmol/liter))</td>
<td>4.30 ± 0.79</td>
<td>3.40 ± 0.56</td>
<td>3.30 ± 0.48</td>
</tr>
<tr>
<td>Fasting cortisol HLP-meal ((nmol/liter))</td>
<td>234 ± 18.4</td>
<td>243 ± 36.7</td>
<td>229 ± 21.0</td>
</tr>
<tr>
<td>Fasting cortisol HCHO-meal ((nmol/liter))</td>
<td>272 ± 30.5</td>
<td>296 ± 35.3</td>
<td>203 ± 21.6</td>
</tr>
<tr>
<td>Fasting glucose HLP-meal ((nmol/liter))</td>
<td>4.33 ± 0.14</td>
<td>4.30 ± 0.34</td>
<td>4.63 ± 0.31</td>
</tr>
<tr>
<td>Fasting glucose HCHO-meal ((nmol/liter))</td>
<td>4.72 ± 0.18</td>
<td>5.17 ± 0.51</td>
<td>5.06 ± 0.22</td>
</tr>
<tr>
<td>Fasting insulin HLP-meal ((pmol/liter))</td>
<td>25.40 ± 2.02</td>
<td>34.10 ± 7.74</td>
<td>54.20 ± 7.72*</td>
</tr>
<tr>
<td>Fasting insulin HCHO-meal ((pmol/liter))</td>
<td>32.60 ± 4.68</td>
<td>42.11 ± 11.13</td>
<td>74.92 ± 28.64</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \) vs. controls.
though significantly more pronounced, the P-BFD group had a significant cortisol increase after the HLP-meal with insignificant changes after the HCHO-meal. On the contrary, the activation of the HPA axis in the A-BFD group occurred only after the HCHO-meal, whereas insignificant changes were found after the HLP-meal. The relevance of these findings, however, appears to be partially limited by the small, although significant, changes of both ACTH and cortisol after each meal, particularly in the obese groups. This is unlikely related to the small sample size but rather to the wide range of intersubject response in both obese and normal-weight controls. This, however, was partially predictable, based on the literature data reporting cortisol response to meals, particularly given at noon, in small groups of healthy subjects (10, 11). On the other hand, we found the same individual variability in another study, in which we examined the response of the HPA axis to a standard mixed meal given at noon in both normal-weight and obese subjects (16). This probably indicates the need to extend such studies to larger groups of individuals, including healthy overweight and obese subjects, to investigate all characteristics of low-responsive individuals, including healthy overweight and obese sub-

![graph](https://example.com/graph.png)

**Fig. 2.** Insulin and glucose changes (expressed as absolute Δ at each time vs. basal values) after the HLP-meal (●) and the HCHO-meal (□) in obese women with A-BFD or P-BFD and in the normal-weight control group. Statistics: a, $P < 0.05$ vs. baseline values; b, $P < 0.001$ vs. baseline values; c, $P < 0.001$ for response between the meal tests in each group.

...echolamines are involved in the regulation of the HPA axis (26) through the activation of both α1- and α2-adrenoreceptors (27) on the CRH-ACTH system. Studies on norepinephrine turnover performed in experimental animals have shown that food intake stimulates sympathetic nervous system activity (28). In humans, it has been found that food ingestion, particularly proteins, can stimulate α1-adrenoreceptors, possibly via the activation of neurotransmitter amines (29). In the fa/fa Zucker rats, activation of α2-adrenoreceptors at the paraventricular nuclei level after administration by norepinephrine has been found to be associated with altered pathways of food intake, body weight gain, energy expenditure, and increased plasma corticosterone levels (30). In these rats the presence of a tonically elevated drive to the HPA axis seems also to reflect a state of chronic stress response because pretreatment with α2-adrenoreceptor antagonists reinforces the stimulatory effect of exposure to chemical stressors on ACTH secretion (31). In agreement with this concept are findings recently reported by our group in obese women with the abdominal obesity phenotype (32). Whether an increased noradrenergic activity may lead to a different drive of the HPA axis response to meals in subjects with abdominal obesity, particularly when large amounts of carbohydrates are ingested, is unknown. However, because oral glucose administration has been found to activate the noradrenergic system (33), we speculate that the increase in the cortisol response to the HCHO-meal in the A-BFD group may be favored, at least in part, by an increase of the noradrenergic system activity or signaling.

...Among others, insulin may represent a potential factor participating in the regulation of the HPA axis response to meals. Two studies investigated the role of mild to moderate hyperinsulinemia in the regulation of the HPA axis in vivo using the euglycemic hyperinsulinemic clamp technique (34, 35). They reported disparate results, however, with one study demonstrating a diminished cortisol response to CRH in conditions of moderate hyperinsulinemia (34) and the other reporting an increase of ACTH and cortisol blood levels with increasing insulin levels during the clamp study, therefore suggesting a stimulatory effect of insulin on the HPA axis secretory capacity. No studies have investigated this issue in obese subjects. Therefore, whether a meal-induced increase of insulin blood levels stimulates the HPA axis in obesity, particularly the abdominal phenotype, is at present uncertain. The lack of relationship between insulin and cortisol or ACTH levels after meals, particularly the HCHO-meal, makes this fairly improbable.

The potential role of gut peptides in regulating meal-induced HPA axis stimulation seems to indicate interesting developments in this area. The recently discovered new gut peptide, ghrelin, an endogenous ligand for the growth hormone secretagogue receptor (36), seems to be involved in the control of food intake and energy balance. In fact, centrally injected ghrelin produces a sustained food intake in rodents, and ghrelin blood concentrations and mRNA expression in the stomach are increased by fasting and decreased by feeding (37). However, recent data have suggested a possible stimulatory effect of ghrelin on the HPA axis activity in experimental animals (38). Moreover, studies in humans have demonstrated a positive effect of ghrelin on glucose...
levels and negative effects on insulin concentrations (39). Ghrelin concentrations are decreased in human obesity (40). Whether ghrelin may play a role in the regulation of altered HPA response to different meals in different obesity phenotypes, therefore, represents an interesting tool to be further investigated.

In summary, we demonstrated that the response of the HPA axis to an HLP-meal or HCHO-meal in obese women depends on their pattern of body fat distribution and that the activation of the HPA following the ingestion of large amounts of complex carbohydrates may have some pathophysiological relevance, specifically in women with the abdominal obesity phenotype.

Acknowledgments

Received December 13, 2001. Accepted April 18, 2002.

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