Enhanced circadian ACTH release in obese premenopausal women: reversal by short-term acipimox treatment

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Obese animal models are marked by an exceedingly active HPA ensemble. Genetically obese rodents have high levels of glucocorticoids (5, 6), adrenalectomy reduces body weight in these animals (12, 21), and subsequent corticosterone replacement restores the obese state (12, 22, 32, 61, 76). Adrenalectomy also attenuates diet-induced obesity. Removal of the adrenals reduces energy intake and adipose tissue weights in diet-induced obese rodents, which is reversed by glucocorticoid replacement (18, 36, 48, 62).

Various clinical studies suggest that the HPA axis is also hyperactive in human obesity. Both plasma ACTH and cortisol concentrations rise to higher levels in response to corticotropin-releasing hormone (CRH) administration alone or in combination with arginine vasopressin (AVP) in obese humans compared with normal-weight controls (51, 54, 69). Moreover, the cortisol response to ACTH is exaggerated in obese volunteers (29, 49, 53), and it has been reported that stress-induced cortisol secretion is increased in abdominally obese women (20). Furthermore, urinary free cortisol excretion appears to be elevated in abdominally obese humans (49, 53), whereas suppression of plasma cortisol levels by dexamethasone (43, 59) or hydrocortisone (35) is blunted. The cause of these endocrine perturbations remains elusive.

Considerable evidence obtained in experimental studies in rats shows that circulating free fatty acids (FFAs) are involved in the control of the HPA axis. Elevation of systemic or portal plasma FFA levels by intravenous lipid infusions enhances ACTH and cortisol secretion in rats (4, 74). Moreover, prolonged high-fat feeding raises circulating FFA levels and basal ACTH and cortisol concentrations in rodents (63). Circulating FFA concentrations are high in obese humans (15, 34).

Acipimox is a powerful inhibitor of lipolysis. Its antilipolytic action is probably mediated through suppression of intracellular cAMP levels, which inhibits cAMP-dependent protein kinase activity. This precludes proper association of hormone-sensitive lipase with triacylglycerol substrate in the lipid droplet of adipocytes, thereby hampering lipolysis and lowering circulating FFAs (13).

We hypothesized that the spontaneous secretory activity of the HPA axis is elevated and that lowering of circulating FFAs by acipimox would reduce spontaneous secretion of the HPA hormonal ensembles in obese humans. The hypothalamo-pituitary-adrenal (HPA) axis is exceedingly active in obese individuals. Experimental studies show that circulating free fatty acids (FFAs) promote the secretory activity of the HPA axis and that human obesity is associated with high circulating FFAs. We hypothesized that HPA axis activity is enhanced and that lowering of circulating FFAs by acipimox would reduce spontaneous secretion of the HPA hormonal ensemble in obese humans. To evaluate these hypotheses, diurnal ACTH and cortisol secretion was studied in 11 obese and 9 lean premenopausal women (body mass index: obese 33.5 ± 0.9 vs. lean 21.2 ± 0.6 kg/m², P < 0.001) in the early follicular stage of their menstrual cycle. Obese women were randomly assigned to treatment with either acipimox (inhibitor of lipolysis, 250 mg orally four times daily) or placebo in a double-blind crossover design, starting one day before admission until the end of the blood-sampling period. Blood samples were taken during 24 h with a sampling interval of 10 min for assessment of plasma ACTH and cortisol concentrations. ACTH and cortisol secretion rates were estimated by multiparameter deconvolution analysis. Daily ACTH secretion was substantially higher in obese than in lean women (7,950 ± 1,212 vs. 2,808 ± 329 ng/24 h, P = 0.002), whereas cortisol was not altered (obese 36,362 ± 7,969 vs. lean 37,187 ± 4,239 nmol/24 h, P = 0.912). Acipimox significantly reduced ACTH secretion in the obese subjects (acipimox 5,850 ± 769 vs. lean 33,542 ± 3,436 nmol/24 h, P = 0.039 vs. placebo), whereas cortisol release did not change (acipimox 33,542 ± 3,436 nmol/24 h, P = 0.484 vs. placebo). In conclusion, spontaneous ACTH secretion is enhanced in obese premenopausal women, whereas cortisol production is normal. Reduction of circulating FFA concentrations by acipimox blunts ACTH release in obese women, which suggests that FFAs are involved in the control of the HPA axis.

The ENDOCRINE ENVIRONMENT is a powerful regulator of body fat storage. For example, the hypothalamo-pituitary-adrenal (HPA) ensemble profoundly affects body composition in animals and humans. Glucocorticoid administration promotes body weight gain in rodents (19, 26, 77), and hypercortisolism in patients with Cushing’s syndrome leads in visceral depots to excess fat, which is readily reversed by lowering plasma cortisol levels (45, 70).
Subjects

Eleven healthy, obese premenopausal women [body mass index (BMI) > 30 kg/m²] and 9 lean (BMI < 25 kg/m²) controls of similar age were recruited. All subjects enrolled in our study underwent medical screening, including medical history taking, physical examination, and standard laboratory hematology, blood chemistry, and urine tests. Acute or chronic disease, smoking, alcohol abuse, and use of medication were exclusion criteria. All participants were required to have regular menstrual cycles and did not use oral contraceptives. All subjects gave written acknowledgement of informed consent for participation.

Body fat distribution. The obese subjects were recruited so as to vary widely with respect to girth, whereas their BMI was required to fall within a relatively narrow range to enable the effect of regional body fat distribution on hormone release to be specifically judged. The total amount and location of excess body fat were determined in the obese women only. The percentage of total body fat mass (fraction of total body weight) was quantified using dual-energy X-ray absorptiometry (Hologic QDR4500) (7). Visceral and subcutaneous adipose tissue areas were assessed by MRI as described previously (41), using a multislice fast-spin echo sequence (Gyroscan-T5 whole body scanner, 0.5 Tesla; Philips Medical Systems, Best, The Netherlands). MRI images were analyzed by two observers independently.

Diet. The obese subjects were randomly assigned to 250 mg of acipimox or placebo in a double-blind crossover design by an independent investigator. Drug and placebo were taken four times daily (breakfast at 0730, lunch at 1300, dinner at 1900) and were consumed previously, using a multiparametric deconvolution method was performed as described

Clinical Protocol

The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center. All subjects were admitted at 1600 to the Clinical Research Unit of the Department of General Internal Medicine in the early follicular stage of their menstrual cycle. Obese women just before or between both study occasions. Meals were served according to a fixed time schedule. Lights were switched off at 2300, and subjects were not disturbed by drawing of blood samples during their sleep (sleep monitoring by EEG was not performed). Subjects were awakened at 0100 for drug intake. Vital signs were recorded at regular time intervals.

Assays

Blood sample handling. Each tube, except the serum tubes (because of blood clotting), was immediately chilled on ice. All samples were centrifuged at 4,000 rpm at 4°C for 20 min within 60 min of sampling. Subsequently, plasma/serum was divided into separate aliquots and frozen at −80°C until assays were performed. Plasma ACTH concentrations were measured by immunoradiometric assay (IRMA) with a detection limit of 3 ng/l (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intra-assay coefficient of variation ranged from 2.8 to 7.5%. The ACTH IRMA was calibrated against the standard obtained from the World Health Organization (WHO) (13). Plasma cortisol concentrations were measured by radioimmunoassay (RIA) with a detection limit of 25 nmol/l (DiaSorin, Stillwater, MN). The intra-assay coefficient of variation ranged from 2.0 to 4.0%. The cortisol RIA was calibrated against the Public Health Service Cortisol Reference standard.

FFA levels were determined using a NEFA-C Free Fatty Acid Kit (Wako Chemicals, Neuss, Germany). The detection limit was 30 μmol/l, and the inter- and intra-assay coefficients of variation were 1.1 and 2.6%, respectively. Basal estradiol concentrations were determined by RIA (Diagnostic Systems Laboratory, Webster, TX). The detection limit was 10 pmol/l, and the intra- and intra-assay coefficients of variation were 6.8 and 15.8%, respectively.

Calculations and Statistics

Deconvolution analysis. Multiparametric deconvolution analysis was used to estimate various kinetic and secretory parameters of spontaneous 24-h ACTH and cortisol plasma concentration time series data. Initial waveform-independent assessments of ACTH and cortisol secretion were performed with Pulse (Unival Inc., Maryland School of Medicine) and the National Institute of Arthritis, Diabetes, and Kidney Diseases. Plasma cortisol concentrations were measured by radioimmunoassay (RIA) with a detection limit of 25 nmol/l (DiaSorin, Stillwater, MN). The intra-assay coefficient of variation ranged from 2.0 to 4.0%. The cortisol RIA was calibrated against the Public Health Service Cortisol Reference standard.

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Approximate entropy. Approximate entropy (ApEn) is a scale- and model-independent statistic that assigns a nonnegative number to time series data, reflecting regularity of these data (56). We used normalized ApEn parameters of m = 1, r = 20%, and 1,000 for the amount of runs to test for regularity in 24-h plasma ACTH and cortisol concentration time series. Hence, this member of the ApEn family is designated ApEn (1, 20%). The ApEn metric evaluates the consistency of recurrent subordinates (nonpulsatile) patterns in a time series and thus yields information distinct from and complementary to deconvolution (pulse) analyses (67). Higher absolute ApEn values

SUBJECTS AND METHODS

Visits.

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denote greater relative randomness of hormone patterns. Data are presented as normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1,000 randomly shuffled versions of the same series. Cross-ApEn was used to investigate joint regularity of the hormone pair ACTH-cortisol (55).

Statistical analysis. Means of cortisol and ACTH secretion parameters of lean and obese volunteers were compared using independent Student’s t-test. The means of ACTH and cortisol secretion parameters in obese subjects during acipimox vs. placebo treatment were compared using Student’s t-test for paired samples. Regression analysis was used to determine the correlation between BMI and ACTH and cortisol secretion in obese and normal-weight women. Stepwise multiple regression analysis, including percent body fat, subcutaneous fat area, and visceral fat area as independent variables, was used to determine the relationship between the sizes of various fat depots and diurnal ACTH and cortisol production. The same technique was employed to determine the effect of acipimox on ACTH and cortisol secretion in the obese subjects in relation to body fat distribution. Significance level was set at 0.05. Data are presented as means ± SE unless otherwise specified.

RESULTS

Subjects

Eleven obese and nine lean subjects were enrolled in this study. Mean age of both groups was similar (obese 36.6 ± 1.9 yr, P = 0.971), whereas BMI was significantly different (obese 33.5 ± 0.9 vs. lean 21.2 ± 0.6 kg/m², P < 0.001). All subjects were studied in the follicular phase of their menstrual cycle, and basal estradiol (E₂) levels in plasma were similar in both groups (obese 190 ± 31 vs. lean 208 ± 66 pmol/l, P = 0.795). Body weight of the obese subjects remained stable from 3 mo before until the end of the study period.

Features of Spontaneous 24-h ACTH and Cortisol Secretion in Lean and Obese Women

Total ACTH production was clearly higher in the obese subjects. In particular, pulsatile production, burst frequency, and burst mass were enhanced, whereas basal secretion, half-life, and secretory half-duration were not significantly different. Cortisol kinetic parameters were similar in the obese subjects compared with age-matched lean controls, except half-life, which was slightly prolonged in the obese women. A graphic illustration of representative ACTH and cortisol concentration profiles and corresponding secretion profiles of one obese and one lean woman of similar age are presented in Fig. 1. Data of ACTH kinetic parameters of the obese and lean subjects are presented in Table 1 and Fig. 2. An overview of cortisol kinetics, as estimated by deconvolution analysis, is given in Table 2.

Effect of Acipimox on Spontaneous 24-h ACTH and Cortisol Secretion Parameters in Obese Women

Mean 24-h plasma FFA levels were reduced during acipimox treatment in all subjects (placebo 0.52 ± 0.04 vs. acipimox 0.40 ± 0.03 mmol/l, P = 0.005). Total ACTH production was significantly lower in the obese subjects during acipimox treatment (Fig. 2). In particular, peak frequency and pulsatile production were reduced, whereas basal secretion, half-life, and secretory half-duration were not affected. Data of ACTH secretory and kinetic parameters during acipimox and placebo treatment are presented in Table 1.

Acipimox did not affect cortisol kinetic and secretory parameters in the obese women. An overview of cortisol kinetics,

Fig. 1. Representative 24-h ACTH (A and B) and cortisol (C and D) concentration profiles (A and C) and corresponding diurnal secretion plots (B and D) of one lean (●) and one obese woman (○). Lean woman age 33 yr, body mass index (BMI) 18.3 kg/m²; obese woman age 31 yr, BMI 39.4 kg/m²; ACTH concentration (24-h, ng/l) and corresponding secretion (ng l⁻¹ min⁻¹) profiles. Cortisol concentration (24-h, nmol/l) and corresponding secretion (nmol l⁻¹ min⁻¹) profiles.

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DIURNAL ACTH AND CORTISOL SECRETION IN OBESITY  

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Table 1. Twenty-four-hour ACTH secretory parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 9)</th>
<th>Obese Subjects (n = 11)</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak frequency, no./24 h</td>
<td>23 ± 2</td>
<td>32 ± 1*</td>
<td><strong>0.001</strong></td>
<td>0.054</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>16 ± 1</td>
<td>14 ± 1</td>
<td>0.116</td>
<td>0.299</td>
</tr>
<tr>
<td>Secretory half-duration, min</td>
<td>20 ± 2</td>
<td>23 ± 2</td>
<td>0.267</td>
<td>0.258</td>
</tr>
<tr>
<td>Peak amplitude, ng/l V&lt;sub&gt;d&lt;/sub&gt;</td>
<td>1.3 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>0.103</td>
<td>0.456</td>
</tr>
<tr>
<td>Burst mass, ng/l V&lt;sub&gt;d&lt;/sub&gt;-peak&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>27.9 ± 4.2</td>
<td>40.8 ± 4.7</td>
<td>0.059</td>
<td>0.191</td>
</tr>
<tr>
<td>Basal production, ng/l V&lt;sub&gt;d&lt;/sub&gt;-1/24 h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>533 ± 62</td>
<td>603 ± 108</td>
<td>0.467</td>
<td>0.307</td>
</tr>
<tr>
<td>Pulse production, ng/l V&lt;sub&gt;d&lt;/sub&gt;-1/24 h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>606 ± 89</td>
<td>1,320 ± 181*</td>
<td><strong>0.004</strong></td>
<td>0.051</td>
</tr>
<tr>
<td>Total production, ng/l V&lt;sub&gt;d&lt;/sub&gt;-1/24 h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1,139 ± 105</td>
<td>2,000 ± 289*</td>
<td><strong>0.019</strong></td>
<td><strong>0.043</strong></td>
</tr>
<tr>
<td>Total production, ng/24 h&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2,808 ± 529</td>
<td>7,950 ± 1,212*</td>
<td><strong>0.002</strong></td>
<td>0.039</td>
</tr>
<tr>
<td>ApEn</td>
<td>0.45 ± 0.04</td>
<td>0.56 ± 0.03</td>
<td><strong>0.033</strong></td>
<td>0.092</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. ApEn, approximate entropy; Vd, distribution volume. Multiparameter deconvolution analysis was used to estimate various kinetic and secretory parameters of spontaneous 24-h ACTH concentration time series data. *P value obese vs. lean; statistical analysis was performed by independent Student’s t-test. **P value placebo vs. acipimox; statistical analysis was performed by paired-samples t-test. *Vd = 40 ml/kg (65). *P < 0.05 obese vs. lean subjects; †P < 0.05 placebo vs. acipimox obese subjects. Boldface indicates P < 0.05.

as estimated by deconvolution analysis, during acipimox and placebo treatment is shown in Table 2.

Regularity of Plasma ACTH and Cortisol Concentration Time Series

ApEn ratios of plasma ACTH concentration time series were significantly higher in obese women compared with controls (0.56 ± 0.03 vs. 0.45 ± 0.04, respectively, P = 0.033), whereas the regularity of the 24-h cortisol concentration time series was similar in both groups (0.51 ± 0.03 vs. 0.52 ± 0.02, respectively, P = 0.738; Fig. 3). Cross-ApEn statistics showed that joint regularity of ACTH-cortisol hormone pairs was not significantly different between both groups (cross-ApEn ratio = 0.55 ± 0.03 vs. 0.49 ± 0.02, P = 0.143).

Acipimox did not impact the orderliness of plasma ACTH concentration time series of the obese women (ApEn ratios: placebo 0.56 ± 0.03 vs. acipimox 0.66 ± 0.05, P = 0.092), whereas the regularity of 24-h cortisol concentration time series was significantly less regular during acipimox treatment (placebo 0.51 ± 0.03 vs. acipimox 0.56 ± 0.03, P = 0.009; Fig. 3). Cross-ApEn statistics showed that the joint regularity of ACTH-cortisol hormone pairs was lower during acipimox treatment (placebo vs. acipimox: cross-ApEn ratio 0.55 ± 0.03 vs. 0.63 ± 0.04, P = 0.029).

Correlation Between ACTH and Cortisol Concentration Time Series

Cross-correlation analysis revealed a high correlation between ACTH and cortisol concentration values, which was significantly higher in the obese women (obese: R = 0.85 ± 0.02 vs. lean: R = 0.77 ± 0.02, P = 0.016). ACTH was leading cortisol with a time lag of 10 min in both groups. Cross-correlation between ACTH and cortisol hormone pairs in the obese subjects was not significantly altered after acipimox treatment (placebo 0.85 ± 0.02 vs. acipimox 0.74 ± 0.06, P = 0.065). ACTH was leading cortisol with a similar time lag during both treatments (placebo 10 ± 2 vs. acipimox 30 ± 24 min, P = 0.426).

BMI vs. Daily ACTH and Cortisol Secretion in Lean and Obese Women

Both obese and lean subjects (n = 21) were included in the correlation analysis of BMI (range 18.3–39.4 kg/m²) vs. daily ACTH and cortisol production. A highly significant positive correlation was found for BMI vs. total ACTH production (R² = 0.39, P = 0.003; Fig. 4). Also, BMI was positively related to peak frequency (R² = 0.39, P = 0.003) and peak burst mass (R² = 0.26, P = 0.022). Total cortisol secretion parameters were not related to BMI (total cortisol vs. BMI: R² = 0.04, P = 0.413).

Body Fat Distribution vs. Daily ACTH and Cortisol Secretion and Effect of Acipimox in Obese Women

The obese subjects had a mean BMI of 33.5 (30.3–39.4) kg/m². The mean of their percent total body fat mass (%total body wt) was 40.7 (36.9–46.3)%. Mean sizes of their visceral and subcutaneous fat area were 392 (274–539) cm² and 1,326 (1,106–1,709) cm², respectively. Multiple regression analysis, with percent total body fat mass and sizes of visceral and subcutaneous fat areas as independent variables, revealed that there was no significant correlation between any of these specific body composition parameters and total daily ACTH production, 24-h cortisol production, or decrease of total daily

Fig. 2. Features of diurnal ACTH secretion in obese women during placebo and acipimox treatment and in lean controls. Vd, distribution volume. Error bars of the box plot represent SE. *P < 0.05, obese vs. lean women; statistical analysis was performed using independent Student’s t-test. **P < 0.05, placebo vs. acipimox obese women; statistical analysis was performed using paired-samples t-test.
ACRTH production during acipimox treatment. Additionally, regression analysis revealed that the reduction of FFA levels was not related to the reduction of ACTH secretion after acipimox treatment in the obese women [ΔFFA (mmol/l) vs. ΔACTH (ng/24 h); \( R^2 = 0.05, P = 0.550 \)].

**DISCUSSION**

This study delineates differences of spontaneous diurnal ACTH and cortisol secretion in obese and lean premenopausal women and evaluates the effects of acipimox, a powerful inhibitor of lipolysis, on the HPA hormonal ensemble in obese individuals.

The data show that daily ACTH secretion rates are substantially higher, whereas the ACTH release process is less regular (as evidenced by ApEn statistics) in obese than in lean women. Moreover, ACTH release rates correlate strongly with BMI, whereas the sizes of various fat areas (including visceral and subcutaneous fat depots) do not appear to be independently associated with ACTH production. The high ACTH secretion rate in obese subjects results from augmented peak frequency and secretory burst mass rather than enhanced basal secretion. Short-term treatment with acipimox apparently restores these kinetic anomalies (except release process randomness) to a large extent, which suggests that circulating FFA concentrations may be involved in the pathophysiology. In contrast, cortisol production is not different in obese and lean premenopausal women, and acipimox does not significantly affect the secretory dynamics of this hormone (except for a slight increase in secretory process randomness).

To our knowledge, this is the first study to estimate the secretion rates of pituitary-adrenal hormones in obese vs. lean humans by deconvolution analysis. A few previous papers reported that diurnal plasma ACTH concentrations are higher in obese individuals, whereas circulating cortisol levels are similar to those in lean controls (44, 52), which is in line with the results of the present study. Moreover, various other clinical studies showed that the incremental ACTH peak response to different exogenous stimuli is elevated in obese humans, which also corroborates our data (51, 54, 69).

### Table 2. Twenty-four-hour cortisol secretory parameters

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 9)</th>
<th>Obese Subjects (n = 11)</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P Value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak frequency, no./24 h</td>
<td>24 ± 1</td>
<td>21 ± 1</td>
<td>0.126</td>
<td>0.148</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>61 ± 2</td>
<td>73 ± 5</td>
<td>0.045</td>
<td>0.995</td>
</tr>
<tr>
<td>Secretory half-duration, min</td>
<td>12 ± 1</td>
<td>14 ± 3</td>
<td>0.668</td>
<td>0.337</td>
</tr>
<tr>
<td>Peak amplitude, nmol/1Vd</td>
<td>13.5 ± 0.9</td>
<td>13.0 ± 1.6</td>
<td>0.811</td>
<td>0.191</td>
</tr>
<tr>
<td>Burst mass, nmol⁻¹·peak⁻¹</td>
<td>172 ± 16</td>
<td>154 ± 22</td>
<td>0.550</td>
<td>0.899</td>
</tr>
<tr>
<td>Total production, nmol/24 h⁻¹·24 h⁻¹</td>
<td>4,134 ± 369</td>
<td>3,305 ± 579</td>
<td>0.267</td>
<td>0.453</td>
</tr>
<tr>
<td>Total production, nmol/24 h⁻¹</td>
<td>37,186 ± 4,239</td>
<td>36,362 ± 5,639</td>
<td>0.912</td>
<td>0.484</td>
</tr>
<tr>
<td>ApEn</td>
<td>0.52 ± 0.02</td>
<td>0.51 ± 0.03</td>
<td>0.56 ± 0.03†</td>
<td>0.738</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Multiparameter deconvolution analysis was used to estimate various kinetic and secretory parameters of spontaneous 24-h cortisol plasma concentration time series data. *P value obese vs. lean; statistical analysis was performed by independent Student’s t-test. †P value placebo vs. acipimox; statistical analysis was performed by paired-samples t-test. Vd = 5.3 liters/body surface area (m²); body surface area was calculated by the Dubois formula (37, 71).

**Fig. 3.** Regularity of plasma ACTH and cortisol concentration time series in obese women during placebo and acipimox treatment and in lean controls. Higher absolute approximate entropy (ApEn) values denote greater relative randomness of hormone patterns. Data are presented as normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1,000 randomly shuffled versions of the same series. Vertical marks indicate median ApEn ratio in each group. Mean ApEn ratios of plasma ACTH concentration time series were significantly higher in obese women compared with controls, and the regularity of 24-h cortisol concentration time series was significantly less regular during acipimox treatment. \( P < 0.05 \) obese vs. lean women; statistical analysis was performed using independent Student’s t-test. \( \times P < 0.05 \) placebo vs. acipimox obese women; statistical analysis was performed using paired-samples t-test.

**Fig. 4.** Correlation of BMI vs. diurnal ACTH secretion. Both obese and lean women (n = 21) were included in correlation analysis of BMI (range 18.3–39.4 kg/m²) vs. daily ACTH production. Total (24-h) ACTH production is calculated per liter distribution volume.
The fact that ACTH release in obese women was blunted during acipimox treatment is in keeping with data from experimental studies showing that elevation of circulating FFAs by Intralipid infusion raises plasma levels of ACTH (and corticosterone) (73, 74). It has been suggested that the acute stimulatory effect of FFA infusion on blood pressure in rodents is mediated by afferent vagal inputs modulating central adrenergic receptors (27). The hypothalamic paraventricular nucleus (PVN) contains a high density of both α₁ and α₂-adrenoceptors, and there is considerable evidence that these receptors are involved in facilitating the secretion of CRH/AVP into the hypophysial portal system, which ultimately leads to stimulation of ACTH secretion (2, 3). Therefore, FFA-induced vagal inputs into PVN neurons may partake in the control of HPA activity. Alternatively, fatty acids are taken up by the brain (58) and exert direct effects on the electrical properties of neurons (46). Application of fatty acids into the ventromedial hypothalamus (VMH) inhibits neuronal firing in that area (63) and the VMH, in its turn, downregulates pituitary adrenal activity (16). Thus FFAs may directly reduce feedback restraint of the VMH on HPA activity at the hypothalamic level, ultimately leading to enhanced ACTH secretion by the pituitary gland. Collectively, these data suggest that FFAs enhance HPA output through effects on neuronal control systems in brain centers at the suprapituitary level and that circulating FFAs are involved in the pathophysiology of pituitary-adrenal hyperactivity in obese humans.

This inference is in apparent conflict with the results of a recent study, showing that elevation of circulating FFAs through intravenous infusion of a lipid-heparin solution reduces plasma ACTH and cortisol levels in (normal-weight) women (40). However, as those authors state in their discussion, the physiological relevance of their findings might be limited, because FFA plasma concentrations induced by Intralipid infusion were 5- to 10-fold higher than those usually found in (obese) humans. Also, because various types of fatty acids (i.e., long-chain/short-chain, saturated/un saturated) may have a differential impact on neuronal membrane function (75), it seems unlikely that exogenous and endogenous lipids exert similar effects on the brain. Thus, although valuable in itself, the data reported by Lanfranco et al. (40) do not necessarily argue against the position that elevation of circulating FFAs is involved in the pathophysiology of HPA hypersecretion in obese humans.

Interestingly, there was no correlation between the reduction of FFA levels and the decrease of ACTH production after acipimox treatment in the obese subjects. Therefore, it is conceivable that acipimox impacts ACTH release directly, through mechanistic pathways independent of its effect on plasma FFA levels. Acipimox is a nicotinic acid derivative that can bind to nicotinic acid receptors. Activation of various subtypes of nicotinic acid receptors modulates neuronal activity in a variety of different regions in the central nervous system (17, 25). To our knowledge, it is unknown whether neural circuits involved in the control of pituitary-adrenal activity contain nicotinic acid receptors. Also, it is unclear whether acipimox can cross the blood-brain barrier. However, we cannot exclude that acipimox affects ACTH secretion directly at the level of the brain.

In the present study, ApEn values of ACTH secretion data were significantly higher in the obese subjects compared with normal-weight controls. Higher ApEn values denote greater irregularity (or higher process randomness). Regularity of hormonal secretion patterns mirrors the net result of feedforward signaling and feedback restraint (68). Because it seems unlikely that negative feedback restraint by cortisol per se can explain the enhanced ACTH secretion of the HPA axis in the present study, because daily cortisol secretion was not altered, the feedforward drive activating the HPA axis may be increased in obese humans. CRH is one of the most potent hypothalamic secretagogues stimulating pituitary ACTH release. Thus elevated CRH might lead to increased randomness of ACTH release. Indeed, it has been demonstrated that CRH levels are elevated in hypothalamic areas and neurons involved in the regulation of HPA axis activity in the brains of obese rodents compared with their wild-type counterparts (5, 6).

There is experimental evidence that leptin receptors are abundant in these CRH-containing neurons in the PVN of the rat brain (28), and intracerebroventricular leptin administration in animals enhances hypothalamic CRH content (23, 33, 60, 64). Human obesity is marked by elevated plasma leptin concentrations (14, 47), which may promote hypothalamic CRH release and thereby enhance ACTH release process irregularity. This might also explain the fact that circulating ACTH is only partially lowered by acipimox in obese women.

As alluded to earlier, our finding that diurnal plasma cortisol levels are normal in obese women in the face of increased ACTH concentrations corroborates other clinical studies (44) but remains unexplained. Although some studies report that urinary cortisol excretion is increased in (abdominal) obesity [implying that adrenal cortisol production is enhanced in the presence of normal circulating levels (50, 53)] and others suggest that 5β-reductase activity (which converts cortisol to inactive cortisone) is increased in obese humans (72), plasma half-life of cortisol was slightly (but significantly) longer in our obese subjects, which obviously does not support the notion that obesity is associated with enhanced cortisol clearance. Thus, the currently available clinical data suggest that the dynamics of the ACTH and cortisol ensemble are altered in obese humans, in the sense that cortisol release appears to be somewhat diminished in proportion to circulating ACTH. (In fact, even in absolute terms, cortisol production was slightly lower in our obese vs. normal-weight women, although the difference was not significant. Unfortunately, because of the relatively small group size, this study lacks the statistical power to significantly detect a 10% reduction of cortisol production, which could be physiologically relevant.) Various mechanistic explanations for this phenomenon were proposed, including insensitive adrenals (44) and reduced 21-hydroxylase activity [which would direct cortisol precursors toward androgen synthesis (29)]. Alternatively, enhanced sympathetic neuronal inputs into the adrenocortical cells may be involved. Evidence from experimental animal studies suggests that the sensitivity of the adrenal cortex to ACTH is centrally regulated by the suprachiasmatic nuclei via the autonomic nervous system (11). Sympathetic inputs particularly desensitize adrenocortical cells to ACTH action. Because obesity appears to be associated with increased sympathetic activity (31), this might explain the occurrence of relatively low cortisol levels in the face of elevated ACTH in the obese women enrolled in the present study. As a second alternative, it has also been described that leptin inhibits cortisol release directly at the
adrenal gland (57). Leptin receptor expression was demonstrated in rat and human adrenal tissue, and exposure of primary cultured rat and human adrenal cells to leptin led to a dose-dependent decrease of ACTH-stimulated adrenocorticosteroid secretion, whereas no effect was found in adrenal cells obtained from db/db mice, which lack a functional leptin receptor (9, 24, 57). It has been reported that there is a strict reciprocal diurnal relation between leptin and cortisol levels in both rats and humans (8, 42). Also, reduced leptin levels are associated with enhanced cortisol secretion rates in narcoleptic humans (38, 39). Thus leptin-mediated peripheral inhibition of adrenal glucocorticoid production appears to be another possible mechanistic explanation for the relatively low cortisol secretion in the obese ob/ob mice. Conclusive evidence to support either one of these postulates has not been reported to date.

The mere fact that plasma cortisol levels are normal in obese humans may limit the (patho)physiological meaning of the current findings, as cortisol is considered to be the main messenger conveying HPA signals to target tissues. In this context, it is important to keep in mind that adipocytes express ACTH receptors and that ACTH is a powerful lipolytic hormone, at least in some species (10). Therefore, a high circulating ACTH concentration in itself may promote lipolysis in obese subjects. Also, melanocortin receptors are distributed widely throughout the body, which suggests that these peptides partake in the control of a variety of (partly unknown) physiological functions (1). Thus the exact implications of high plasma ACTH concentrations in the face of normal cortisol levels remain to be established. Furthermore, given the well-known sex and age effects on HPA activity (30), one has to take into account that these results are not necessarily applicable to men or postmenopausal women.

In conclusion, this study documents enhanced circadian ACTH release in obese premenopausal women in the face of normal circulating cortisol concentrations. Reduction of plasma FFA levels by acipimox blunts ACTH secretion in obese individuals, which suggests that circulating FFAs are involved in the pathophysiology of this neuroendocrine perturbation associated with obesity.

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