CLINICAL STUDY

Diminished adrenal sensitivity and ACTH efficacy in obese premenopausal women

Ferdinand Roelfsema, Hanno Pijl, Daniel M Keenan 1 and Johannes D Veldhuis 2

Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Albinusdreef 2, 2333ZA Leiden, The Netherlands, 1Department of Statistics, University of Virginia, Charlottesville, Virginia 22904, USA and 2Endocrine Research Unit, Mayo Medical and Graduate Schools, Clinical Translational Research Center, Mayo Clinic, Rochester, Minnesota 55901, USA

(Correspondence should be addressed to F Roelfsema; Email: f.roelfsema@lumc.nl)

Abstract

Background: The ACTH–cortisol axis in women is activated and associated with decreased ACTH potency, estimated by relating ACTH and cortisol pulse masses. Recently, a new accurate method for constructing the endogenous dose–response relationship was introduced, which is based on the relation between ACTH concentrations and associated cortisol secretion rates within cortisol bursts.

Hypothesis: The endogenous dose–response relation between ACTH and cortisol in obesity is changed, leading to diminished responsiveness.

Subjects: Twenty-five obese premenopausal women and 16 normal weight premenopausal women were studied by 10-min blood sampling for 24 h.

Outcomes: ACTH and cortisol secretion rates, analytical dose–response estimates of endogenous ACTH efficacy (maximal cortisol secretion), dynamic ACTH potency, and adrenal sensitivity (slope term) from 24-h ACTH–cortisol profiles were quantified.

Results: The initial potency (negative logarithm) was K 7.83 ± 0.75 (mean ± S.E.M.) in obese women and K 10.14 ± 1.08 in lean women (P = 0.10), and the corresponding values for the recovery phase were K 26.62 ± 2.21 and K 36.67 ± 1.66 (P = 0.004). The sensitivity (curve slope) amounted to 0.468 ± 0.05 in obese women and 0.784 ± 0.09 in normal weight women (P = 0.004). The efficacy (maximal value) was 17.6 ± 4.9 nmol/l per min in obese women and 26.3 ± 3.8 nmol/l per min in normal weight women (P = 0.009). Basal secretion rate, inflection point, and EC 50 values were not different. Bromocriptine or acipimox did not change the dose–response curve.

Conclusion: The ACTH–cortisol relation in obesity in women is characterized by decreased sensitivity and efficacy, thus explaining non-elevated serum cortisol concentrations despite increased plasma ACTH levels.

European Journal of Endocrinology 167 633–642

Introduction

Obesity impacts many organ systems, including the endocrine system. Well known is the emergence of insulin resistance with major implications to health. However, other endocrine ensembles are also affected in obesity. For instance, GH secretion is severely blunted, unlike that of true GH deficiency, but IGF1 concentrations remain within normal limits. Furthermore, the GH increase after stimulation tests, including hypoglycemia, GHRH, hexarelin and arginine, is diminished (1, 2, 3). In addition, TSH and prolactin secretion is amplified, while gonadotropin secretion can be reduced (4, 5, 6).

Studies on the effect of obesity on the hypothalamic–pituitary–adrenal (HPA)-axis have demonstrated increased glucocorticoid secretion and/or urinary excretion with different techniques (7, 8, 9). Furthermore, plasma ACTH concentration and secretion estimated by deconvolution techniques are increased in obesity. Comparison of ACTH secretion with that of cortisol suggested diminished responsiveness of the adrenal gland in obesity (10). A recent analytical method allows noninvasive estimation of physiological (nonlinear) dose–response functions from serially sampled hormone-effector response pairs, such as LH and testosterone, or ACTH and cortisol, without injection of hormone agonists, antagonists, or labeled compounds (11). Relevant hormone pairing (i.e. fitted ACTH concentrations and cortisol secretion rates calculated across the 24-h cycle) permits dose–response estimation under conditions of allowable (but not required) downregulation of target gland responses. For allowable downregulation, the two-potency model was used, which can be described as a shift of the curve in the rightward horizontal direction during the
recovery phase (i.e. during the decline of ACTH concentration pulses), compared with the rising phase. The selection of this model is based on statistical considerations. The first point of interest is whether the ACTH concentration–effect relation is changed in obesity and which parameters are involved, and second whether medically induced normalization of ACTH secretion or improving metabolic abnormalities without body weight reduction can restore the relationship between ACTH and adrenal output, when analyzed with specific and sensitive new tools.

Materials and methods

Overview

Normal weight and obese volunteers were hospitalized the evening before the sampling studies. On the following morning, an indwelling i.v. cannula was inserted in a large vein of the forearm. Blood samples (2.0 ml) were withdrawn at 10-min intervals for 24 h beginning at 0900 h. A slow i.v. infusion of 0.9% NaCl and heparin (1 U/ml) was used to keep the line open. Ambulation was permitted to the lavatory only. Vigorous exercise, daytime sleep, snacks, caffeinated beverages, and cigarette smoking were disallowed. Meals were provided at 0800, 1230, and 1730 h, and room lights were turned off between 2200 and 2400 h depending upon individuals’ sleeping habits. Blood was collected in prechilled siliconized tubes containing EDTA (ACTH) or heparin (cortisol), centrifuged at 4 °C to separate plasma, and frozen at −20 °C within 30 min of collection. Total blood loss was < 360 ml. Volunteers were compensated for the time spent in the study. None of the 24-h data have been published or presented previously or analyzed in the present manner.

Subjects

Twenty-five obese premenopausal women, with a mean age of 36.9 ± 1.5 years and BMI 33.2 ± 0.5 kg/m², volunteered for this study while 16 community-dwelling normal-weight premenopausal women served as control subjects. The mean age of the normal weight group was 41.4 ± 2.4 years (not significant (NS) vs obese women), and the BMI was 23.2 ± 2.4 kg/m² (P < 0.001 vs obese women). Criteria for exclusion were: recent use of psychotropic or neuroactive drugs; drug or alcohol abuse, psychosis, depression, mania, anorexia/bulimia or severe anxiety; endocrinopathy, nightshift work or recent transmeridian travel (exceeding three time zones within 14 days of admission); acute weight change (loss or gain of > 2 kg in 6 weeks); abnormal hepatorenal function; glucocorticoid, anabolic steroid, or reproductive hormone therapy; and/or unwillingness to provide written informed consent. All subjects were studied in the follicular phase of the menstrual cycle and provided written informed consent. Eleven obese women were treated with acipimox in a double-blind crossover design in an attempt to normalize decreased GH secretion and amplified ACTH secretion. The dose used was 250 mg four times per day starting on the day before blood sampling till the end of sampling. Free fatty acids (FFAs) decreased from 0.52 ± 0.04 to 0.40 ± 0.03 mmol/l, P = 0.005. GH secretion increased by 70%, and ACTH secretion decrease by 30%, as reported previously (12, 13). Fourteen other obese women were treated with 5 mg bromocriptine in two divided doses for 10 days in an attempt to improve the metabolic state. Changes observed included a decrease of fasting insulin levels by 18%, glucose by 9.5%, and leptin by 11% (14, 15). The study was approved by the Leiden University Institutional Review Board.

Assays

Plasma ACTH was measured using a two-site sandwich assay designed to detect intact ACTH molecules. The IRMA consisted of a soluble ^125I-labeled (indicator) MAB directed to the N-terminus of ACTH as well as a second polyclonal ACTH antibody directed to the C-terminus. The second antibody was covalently conjugated to biotin to react with avidin-coated plastic beads. All incubation reagents including antibodies, human ACTH(1–39) standard and avidin-coated beads were from Nichols Institute (Allegro IRMA, San Juan Capistrano, CA, USA). Each sample was assayed in duplicate, and all samples from any one subject were assayed in the same run. Sensitivity of the IRMA was 1.0 ng/l or 0.22 pmol/ml, and intraassay precision was 3.2–5.8% (range of median intrasample coefficients of variation in all individuals). Cross-reactivity with β-endorphin, TSH, LH, FSH, GH or prolactin was < 0.1%. Plasma cortisol concentrations were measured by RIA (Sorin Biomedica, Milan, Italy). The detection limit of this assay was 25 nmol/l. The intra- and interassay precisions varied from 2 to 4%.

Analytical methods

Details of the dynamic dose–response methodology were described in several earlier papers (11, 16, 17, 18, 19). The goal here was to relate time-varying ACTH concentrations (input, effector) to time-varying cortisol secretion rates (output, response) via both a classic four-parameter logistic dose–response function and a five-parameter logistic dose–response function that contain two potencies to accommodate allowable (possible, but not obligatory) downregulation of ACTH drive. Simultaneous estimation of two potencies allows for a possible right-shifted inhibitory (negative) adaptation of the dose–response process within each coupled ACTH–cortisol pulse pair after an estimable time lag. The
details of the modeling and the estimation procedures are described in the section ‘Modeling and estimation procedures’.

ACTH (145 samples) and cortisol (145 samples) time series in each subject were first deconvolved by automated analysis. Deconvolution reconstructs hormone concentrations as a train of variable-amplitude secretory bursts superimposed upon basal (time-invariant) nonpulsatile secretion with hormone elimination proceeding via a biexponential function (20). The fast component of cortisol was 2.41 min and for ACTH 3.5 min and they were fixed, as well as the fractional component of cortisol secretion rate denoted as ACTH or a cortisol time profile. We assume a basal

We consider the following model applicable to either an ACTH–cortisol pulses. Random effects on cortisol secretory-burst mass and the S.D. of residual model recovery (downregulated) time windows within paired ACTH–cortisol pulses. Random effects on cortisol secretory-burst mass and the S.D. of residual model regression is defined here as an allowable decrease in hormone concentrations as a train of variable-amplitude secretory bursts superimposed upon basal (time-invariant) nonpulsatile secretion with hormone elimination proceeding via a biexponential function (20). The fast component of cortisol was 2.41 min and for ACTH 3.5 min and they were fixed, as well as the fractional component of cortisol secretion rate denoted as

We assume that there are fast and slow rates of elimination $\alpha^{(1)}$ and $\alpha^{(2)}$ to be estimated with their fractions (a and $1-a$) taken as literature-based population values. The (true) concentrations are then given as:

$$X(t) = (ae^{-\alpha^{(1)}t} + (1-a)e^{-\alpha^{(2)}t})X(0) + \int_0^t (ae^{-\alpha^{(1)}(t-s)} + (1-a)e^{-\alpha^{(2)}(t-s)})Z(s)ds \quad (2)$$

What are then observed, at a discrete time sampling, are the concentrations with measurement error:

$$Y_i = X(t_i) + \epsilon(i), \quad i = 1, \ldots, n,$$

where the errors are assumed to be IID normal with mean zero and variance $\sigma^2$.

We denote the collection of secretion and elimination parameters by $\theta$. The secretion rate function $Z$ is predicated on release occurring at the (unobserved) pulse times, and the estimation of the pulse times is intertwined with the estimation of $\theta$. For a given hormone concentration profile, a previously published pulse-time detection algorithm was applied (50, 51).

The algorithm is a nonlinear diffusion equation that selectively smoothes the observed concentrations locally, the diffusion coefficient being inversely related to the degree of positivity of the gradient of the concentration profile. The end result is a collection of putative pulse sets, $T_m = (T^{(1)}_m, \ldots, T^{(m)}_m)$, $1 \leq m \leq M$. There are $M$ such sets. Parameter estimation proceeds by penalized maximum-likelihood estimation, where the penalization is on the number of pulse times $m$. Pulse times get removed and added. Both AIC and BIC-penalizations are used, but AIC is emphasized.

The results of the penalized maximum likelihood estimation are $\hat{\theta}$, $\hat{m}$, and the AIC-optimal pulse-time set $T^{(\hat{m})} = (T^{(1)}_m, \ldots, T^{(\hat{m})-1}_m, T^{(\hat{m})})$. Based upon these estimates, for each subject, we have their fitted (reconvolved) ACTH (A) concentrations: $Y_{A,i}$, $i = 1, \ldots, n$, which will be the basis for the ACTH feedforward signal on cortisol (C) secretion; and (2) the estimated cortisol (C) secretion rate: $\hat{Z}_{C,i}$, $i = 1, \ldots, n$. Our objective is the estimation of the waveform of mass release (mass per distribution volume per unit time) via a three-parameter generalized Gamma density:

$$\psi(s) \propto s^{(\theta_1) - 1} \exp\left(-s^{(\theta_2)}\right), \quad s \geq 0.$$
dose–response mechanism of ACTH on C secretion. Two considerations which present themselves are: i) the fact that there is a varying time-delay between the onset of an ACTH pulse and the observed C secretory response; and ii) there is often a variable loss of responsiveness of C to the ACTH feedforward signal. If one hopes to accurately recover the dose–response structure, both of these considerations must be resolved. The solution is described below.

Utilizing the fitted (reconvolved) ACTH concentrations: \( \hat{Y}_{Ai}, i = 1, \ldots, n \), the ACTH feedforward signal \( F_A(t_i) \) was constructed piecewise, from one C pulse onset time to the next; this allowed one to account for the varying time lag between an ACTH pulse onset and a cortisol pulse onset. For each C pulse \( T_C^{(k)} \) time the ACTH pulse \( T_A^{(k)} \) nearest within the allowable time (−60, 10 min) was identified. If such an ACTH pulse exists, then it is shifted to the C onset point so that the two onset points are aligned. If no such pulse existed within the time interval, then a time lag of 40 min was applied to the ACTH concentrations, starting at the C pulse onset time. We also allow for the possibility that the ACTH pulse might slightly (10 min) follow the C pulse, due to neural innervations. This adaptation of the ACTH feedforward signal might slightly follow the C pulse onset. For each C pulse \( T_C^{(k)} \), due to neural innervations. This adaptation of the ACTH feedforward signal might slightly follow the C pulse onset. For each C pulse \( T_C^{(k)} \), due to neural innervations. This adaptation of the ACTH feedforward signal might slightly follow the C pulse onset.

The estimation then proceeds by using the estimated cortisol secretion rate \( \hat{Z}_C, i = 1, \ldots, n \), assumed to be \( Z_C + \text{error} \). Random effects (R’s) in efficacy are included to accommodate pulse-by-pulse variation. To allow for the loss of responsiveness (desensitization, habituation) to ACTH drive, a mid-pulse shift in the dose–response is included. The shift is assumed to occur at time \( \tau \) (to be estimated) following the pulse onset.

Hence, a hysteresis–like phenomenon occurs, with the system resetting, given sufficient time, to the initial curve, ready for the next secretory pulse. Such a change we denote as hysteresis. Two models are considered.

Four-parameter logistic: no hysteresis:

\[
\hat{Z}_C(t_i) = \left\{ \eta_0 + \frac{\eta_3 + R^{(k)}}{1 + \exp\left(-\eta_1 + \eta_2 \times F_A(t_i)\right)} \right\},
\]

\[
T_C^{(k)} \leq t_i < T_C^{(k+1)} + \varepsilon_i, \quad i = 1, \ldots, n
\]

The parameter \( \eta_2 \) is the sensitivity parameter, which acts much like the slope (locally), describing responsiveness to feedforward drive. The parameter \( \eta_1 \) is the potency term, in that the \( EC_{50} = -\eta_1/\eta_2 \), the value at which half-maximal response occurs. The efficacy term is \( \eta_3 \) and denotes maximal response.

Five-parameter logistic: hysteresis (two potency terms), plus a sixth parameter \( \tau \), referred to as the inflection time.

\[
\hat{Z}_C(t_i) = \left\{ \eta_0 + \frac{\eta_3 + R^{(k)}}{1 + \exp\left(-\eta_1 \uparrow + \eta_2 \times F_A(t_i)\right)} \right\},
\]

\[
T_C^{(k)} \leq t_i < T_C^{(k+1)} + \tau + \varepsilon_i, \quad i = 1, \ldots, n
\]

Because the random effects enter linearly, we again have Gaussian likelihoods, and the parameters are estimated by maximum likelihood. One can test whether the additional two parameters are statistically important through the application of a generalized likelihood ratio test. This test is that mentioned in the Materials and methods section, thus rejecting the one-potency model in favor of that of the two-potency model.

Because there are two potencies, one onset potency \( \eta_1^{(o)} \) and a recovery potency \( \eta_1^{(r)} \), there will be two \( EC_{50} \)s, for onset and recovery. The random effects enter linearly and we again have Gaussian likelihoods, and the parameters are estimated by maximum likelihood. One can test whether the additional two parameters are statistically important through the application of a generalized likelihood ratio test. Because of the independence across subjects, one can pool the values, and obtain an overall \( P \) value. This overall test, utilizing all subjects, is the test that is mentioned in the Materials and methods section, thus rejecting the one-potency model in favor of that of the two-potency model. Hence, the two-potency model is applied.

Statistical analysis

Data are presented in tables as mean and S.E.M. Statistical comparisons between groups were performed by ANOVA, after logarithmic transformation of the data, when required. \( P \) values <0.05 were considered significant. Statistical calculations were done with Systat, version 11 (Systat Software, Richmond, CA, USA).

Results

Clinical characteristics are depicted in Table 1. Age was slightly, but not statistically, higher in control subjects. As expected, fasting insulin, leptin, but not estradiol, and IGF1 levels were higher in obese subjects. Serum-free thyroxine was slightly higher in control subjects, but levels were within normal limits, and no subject had suppressed TSH levels (data not shown). Table 2 shows the secretion rates for the ACTH and cortisol profiles. Basal ACTH secretion tended to be larger in obese women, and pulsatile and total daily ACTH output were
Age (years) 36.9
BMI (kg/m²) 33.2
Estradiol (pmol/l) 172
Free thyroxine (pmol/l) 14.8
IGF1 (nmol/l) 20.5
Leptin (ng/l) 30.1
Insulin (mU/l) 23.5
Total secretion (ng/l per 24 h) 1615
Basal secretion (ng/l per 24 h) 796
Pulsatile secretion (ng/l per 24 h) 818

Table 1 Clinical characteristics of obese women and lean controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese women</th>
<th>Lean controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.9 ± 1.5</td>
<td>41.4 ± 2.4</td>
<td>0.12</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.2 ± 0.5</td>
<td>23.2 ± 2.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>30.1 ± 1.8</td>
<td>9.6 ± 0.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>23.5 ± 3.9</td>
<td>7.4 ± 0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>172 ± 16</td>
<td>140 ± 20</td>
<td>0.54</td>
</tr>
<tr>
<td>Free thyroxine (pmol/l)</td>
<td>14.8 ± 0.3</td>
<td>16.5 ± 0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>IGF1 (nmol/l)</td>
<td>20.5 ± 1.5</td>
<td>20.0 ± 1.8</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Table 2 Deconvolution analysis of plasma ACTH and cortisol profiles in obese women and normal-weight controls. Data are shown as mean ± S.E.M. Statistical comparisons between obese and normal-weight women were done with the two-tailed Student’s t-test for unpaired data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese women</th>
<th>Control women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal secretion (ng/l per 24 h)</td>
<td>796 ± 79</td>
<td>582 ± 60</td>
<td>0.08</td>
</tr>
<tr>
<td>Pulsatile secretion (ng/l per 24 h)</td>
<td>818 ± 86</td>
<td>497 ± 41</td>
<td>0.005</td>
</tr>
<tr>
<td>Total secretion (ng/l per 24 h)</td>
<td>1615 ± 134</td>
<td>1079 ± 88</td>
<td>0.007</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal secretion (nmol/l per 24 h)</td>
<td>683 ± 97</td>
<td>562 ± 143</td>
<td>0.26</td>
</tr>
<tr>
<td>Pulsatile secretion (nmol/l per 24 h)</td>
<td>3960 ± 226</td>
<td>4528 ± 440</td>
<td>0.05</td>
</tr>
<tr>
<td>Total secretion (nmol/l per 24 h)</td>
<td>4245 ± 290</td>
<td>5090 ± 433</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Discussions

In this study we demonstrate that the ACTH–cortisol concentration–effect relationship is altered in obese premenopausal women compared with normal-weight women and characterized by decreased efficacy (maximal cortisol secretion rate) and sensitivity (slope of the curve), with a possible compensatory increase in potency of the recovery phase. In addition, lowering ACTH secretion by short-term treatment with acipimox did not change the concentration–effect curve. Finally, an effort to improve the metabolic condition of obese women using bromocriptine had no influence on ACTH and cortisol secretion and the mutual relationship.

In accordance with previous reports the cortisol secretion rate was not increased in obesity, when expressed as mass per liter distribution volume, while ACTH secretion was enhanced also when expressed as mass per liter distribution volume (4, 22). The relative increase of ACTH secretion was larger than the increase of cortisol production in obese women, suggesting that the adrenals gland is less responsive to ACTH stimulation in these individuals, which we previously inferred in a smaller group by regressing ACTH and cortisol pulse masses in a logistic four parameter equation (10). In the present larger study, a more precise and physiologically grounded model comparing circulating ACTH levels and the subsequent cortisol secretion rate per minute was used. This approach gives much more data per individual for construction of the dose–response relationships than comparing pulse masses. The depressed dose–response curve in obesity, with a less steep slope and decreased maximal level, would explain diminished cortisol secretion under physiological ACTH levels.

The most striking finding in this study was the decreased efficacy and sensitivity of ACTH, which might be caused by the direct inhibitory action of leptin on glucocorticoid synthesis by the normal human and rat adrenal glands (23, 24). We have previously demonstrated that 5 mg bromocriptine administration for 10 days to obese women improves various metabolic parameters, including insulin sensitivity, mean 24-h insulin concentration, mean glucose levels, and resting energy expenditure. In addition, leptin levels decreased by 11% (14, 15). However, ACTH and cortisol secretion and the endogenous dose–response relationship remained unchanged after bromocriptine administration. This finding indicates that leptin is probably not responsible for this altered relationship. On the other hand, it is also possible that the magnitude of the
greater (32). Finally, insulin-induced hypoglycemia with lean controls, but cortisol secretion was not syndrome resulted in amplified ACTH release compared to obese men with or without sleep apnea similar in the obese and controls. Administration of the cortisol increases to Cortrosyn or insulin were controls (31). The same study also demonstrated that the ACTH response to ovine CRH was similar to that in humans and mammals (12). Therefore, it is interesting to note that short-term treatment with acipimox, which decreased serum FFA and reduced ACTH secretion in the obese cohort, did not impact the dose–response relationship. Therefore, FFAs may not be the key mechanistic explanation for decreased efficacy and sensitivity of the adrenal gland for ACTH.

We also explored whether diminishing ACTH secretion might change this relation. There is an experimental evidence that FFAs, which are increased in obesity, enhance HPA output through their effects on neuronal control systems in brain centers at the suprapituitary level, and that circulating FFAs are involved in the activation of the HPA axis in obese humans and mammals (12). Therefore, it is interesting to note that short-term treatment with acipimox, which decreased serum FFA and reduced ACTH secretion in the obese cohort, did not impact the dose–response relationship. Therefore, FFAs may not be the key mechanistic explanation for decreased efficacy and sensitivity of the adrenal gland for ACTH.

Some studies, which used pharmacological amounts of ACTH(1–29) (Cortrosyn, Synachten) or CRH–vasopressin, showed, however, an increased cortisol response, suggesting that the adrenal gland in obesity is hyperresponsive, in contrast to the present finding of diminished efficacy (29, 30). However, in another study the ACTH response to ovine CRH was similar to that in controls, whereas cortisol increased less than that in controls (31). The same study also demonstrated that the cortisol increases to Cortrosyn or insulin were similar in the obese and controls. Administration of CRH to obese men with or without sleep apnea syndrome resulted in amplified ACTH release compared with lean controls, but cortisol secretion was not greater (32). Finally, insulin-induced hypoglycemia caused a larger ACTH increase in obese subjects than that in controls, both in the morning and evening, but the cortisol response in obese subjects was diminished in obesity (33). Comparable effects were noted by Weaver et al (34). Collectively, these data are not unequivocal and the discrepancies are difficult to explain, although the studies which used insulin showed an increased ACTH, but diminished cortisol response, compatible with decreased adrenal efficacy. Therefore, one could postulate that in obesity, adrenal autonomic (splanchnic) drive might be reduced, thus decreasing (unstimulated) efficacy of cortisol pulses, but that of stimulatory neuropeptides augments autonomic drive to adrenal cortisol synthesis and release in obesity (35).

In obesity, the ACTH–adrenal system differs in several respects from that in lean subjects. First, isotope-based techniques have invariably demonstrated increased cortisol production in obesity, but urinary cortisol metabolite excretion data, when corrected for urinary creatinine excretion, BMI or body surface, were not increased compared with controls (22). In combination with the observed normal or even slightly diminished serum cortisol concentration in obese

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese women</th>
<th>Control women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potency (initial, ng/l)</td>
<td>−7.83 ± 0.75</td>
<td>−10.14 ± 1.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Potency (recovery, ng/l)</td>
<td>−26.62 ± 2.21</td>
<td>−36.67 ± 1.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Sensitivity (slope units)</td>
<td>0.468 ± 0.05</td>
<td>0.784 ± 0.09</td>
<td>0.004</td>
</tr>
<tr>
<td>Efficacy (nmol/l per min)</td>
<td>17.6 ± 4.9</td>
<td>26.3 ± 3.8</td>
<td>0.009</td>
</tr>
<tr>
<td>Basal (nmol/l per min)</td>
<td>1.31 ± 0.15</td>
<td>1.52 ± 0.22</td>
<td>0.67</td>
</tr>
<tr>
<td>Inflection point (min)</td>
<td>24.0 ± 2.7</td>
<td>20.0 ± 3.0</td>
<td>0.39</td>
</tr>
<tr>
<td>Initial EC50 (ng/l)</td>
<td>18.8 ± 1.9</td>
<td>14.2 ± 1.4</td>
<td>0.09</td>
</tr>
<tr>
<td>Recovery EC50 (ng/l)</td>
<td>57.2 ± 4.0</td>
<td>46.8 ± 5.6</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Figure 1 Mean ACTH–cortisol dose–response curves of 25 obese premenopausal women (continuous lines) and 16 normal-weight controls (dashed lines). Note the different slopes (sensitivity) and height (efficacy) of the curves. The left two curves represent the dose–response during the initial phase of the secretory cortisol pulse, and the right two curves that during the recovery phase, i.e. the decreasing part of the pulse, displaying the downregulation.
subjects, these data suggest increased hormone clearance and/or increased hormone distribution space (8). Increased clearance of cortisol might be caused by enhanced 5α- or 5β-reduction of the A-ring of cortisol in the liver, or changed activity of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) decreasing the conversion of cortisone into cortisol (36, 37). A drawback of these studies is that locally produced cortisol from cortisone and vice versa by 11β-HSD1, facilitating oxidation or reduction determined by local cell conditions, and subsequent release from fat, muscle, or splanchnic organs into the circulation, was not quantitated. However, recently a new stable isotope method in which 1,2-[2H]2-cortisone and 9,11,12,12-[H2]4-cortisol were infused has revealed that in human subjects active recycling between cortisol and cortisone occurs in subcutaneous fat, muscle, and splanchnic organs (38). Comparable data in obesity and diabetes mellitus are not yet available. The consequence for this study is that cortisol influx in the circulation is not only adrenal gland-derived but also from many other tissues as well, thus from local inter conversion, but the magnitude is unknown in obese subjects. This influx will contribute to the basal (non-pulsatile) component of secretion. In this study basal cortisol secretion was indeed somewhat larger, but not significantly, and it is unlikely that this could have influenced the dose–response calculations.

The CRH–ACTH system appears to be activated in obesity, although the number of studies in which spontaneous plasma ACTH secretion patterns were analyzed is rather limited (10, 12, 33, 39). Elevated CRH in obese subjects is likely responsible for the increased ACTH secretion. Indeed, it has been demonstrated that CRH levels are elevated in hypothalamic areas and neurons involved in the regulation of the HPA axis activity in the brain of obese rodents compared with their wild-type counterparts (40). Mechanistically, leptin might be involved in this regulatory axis. Leptin receptors are abundant in CRH-containing neurons in the paraventricular nucleus of the rat brain (41), and intracerebroventricular administration of leptin in animals increases the hypothalamic CRH content (42, 43, 44). Thus, these data demonstrate that leptin, which is abundant in obesity, stimulates CRH and hence ACTH production in obese animals. Alternatively, but mutually not exclusive, is diminished feedback by glucocorticoids. One large study used body weight-based low doses of dexamethasone in men and women, and found no evidence of diminished suppression of the ACTH–adrenal axis, although it was larger in women...
than in men (45). However, dexamethasone has limited access to the brain and binds to low-capacity glucocorticoid receptors in the pituitary in contrast to the natural glucocorticosteroid in humans, which binds to the glucocorticoid receptors in the brain (46, 47). Administration of physiological amounts of cortisol to obese and lean men showed that ACTH inhibition during the nocturnal period was diminished, but comparable with controls during waking hours (33). Because of the lack of physiological feedback studies of cortisol in obese women, it is presently not possible to determine whether diminished feedback sensitivity contributes to increased ACTH levels and secretion as observed here.

This study confirms and extends results of a large study on ACTH–cortisol dose responsiveness in 111 adults (48). Here the same techniques were used in 8-h blood sampling during the night. In this study not overlapping with the present one, BMI and age were independent significant factors in defining sensitivity and recovery potency.

A recent study revealed that the ACTH–cortisol dose–effect relationship in Cushing’s disease is also changed, essentially showing increased efficacy, but with a remarkable parallel right-shift of the curve, giving an increased EC50, so that ACTH levels are less effective and thereby limiting excessive cortisol secretion. After curative surgery this relation normalizes (49). In obesity the dose–response relation is different from that observed in Cushing’s disease and the adaptive mechanisms would seem different also.

This study has some limitations. We only investigated premenopausal women with uncomplicated central obesity, who all had regular periods and no signs of hyperandrogenism. Thus, our findings do not necessarily apply for men, other ages, obesity complicated by diabetes, polycystic ovary syndrome, or other forms of obesity.

In summary, an altered ACTH–cortisol concentration–secretion relationship exists in premenopausal, centrally obese women. Novel findings were decreased efficacy and decreased sensitivity in obese premenopausal women. Leptin and FFAs are not likely responsible for the decreased adrenal responsiveness. The results suggest that the changed dose–response relationship provides some protection of the body to the increased ACTH secretion and enhanced peripheral conversion of cortisone into cortisol in obesity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported in part via NIH R01DK73148 to J D Veldhuis.

References

15. Kok P, Roelfsema F, Frolich M, van Pelt J, Stokkel MP, Meinders AE & Pijl H. Activation of dopamine D2 receptors simultaneously


26 
dexamethasone in obesity and effects of sex, body fat distribution, and dexamethasone concentrations: a dose–response study. *Journal of Clinical Endocrinology and Metabolism* 2002 87 166–175. (doi:10.1210/jc.87.1.166)


Received 10 July 2012
Revised version received 14 August 2012
Accepted 21 August 2012