Neuroendocrine perturbations in human obesity

Kok, P.

Chapter 7

Enhanced Circadian ACTH Release in obese Premenopausal Women: Reversal by Short-term Acipimox Treatment

Petra Kok, Simon W. Kok, Madelon M. Buijs, Jos J. M. Westenberg, Ferdinand Roelfsema, Marijke Frölich, Marcel P. M. Stokkel, A. Edo Meinders, Hanno Pijl

Am J Physiol Endocrinol Metab. 2004 Nov;287(5):E848-56. Epub 2004 Jul 27.

Abstract

Several studies suggest that 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 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 (BMI: obese 33.5 ± 0.9 vs. lean 21.2 ± 0.6 kg/m^2, 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 cross-over design, starting one day prior to 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 multi parameter deconvolution analysis. Daily ACTH secretion was substantially higher in obese than in lean women (7950 ± 1212 vs. 2808 ± 329 ng/24 h, P = 0.002), whereas cortisol was not altered (obese 36 362 ± 5639 vs. lean 37 187 ± 4239 nmol/24 h, P = 0.912). Acipimox significantly reduced ACTH secretion in the obese subjects (Acipimox 5850 ± 769 ng/24 h, P = 0.039 vs. placebo), while cortisol release did not change (Acipimox 33 542 ± 3436 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 FFA's are involved in the pathophysiology of this neuroendocrine anomaly.

Introduction

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

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 to 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), while 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 anti-lipolytic action is probably mediated through suppression of intracellular cyclic AMP levels, which inhibits cyclic AMP-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 free fatty acids (13).
We hypothesized that the spontaneous secretory activity of the HPA axis is elevated and that lowering of circulating FFAs by Acipimox would reduce HPA axis activity in obese humans.
To test these postulates, we measured 24 h spontaneous ACTH and cortisol release in lean and obese premenopausal women in the early follicular phase of their menstrual cycle. Obese women were studied twice, randomly assigned to short-term treatment with either Acipimox (250 mg orally four times daily) or placebo in a double blind crossover design.

Subjects and methods

Subjects
Eleven healthy obese premenopausal women (BMI > 30 kg/m^2) and 9 lean (BMI < 25 kg/m^2) controls with similar age and sex were recruited. All subjects enrolled in our study underwent medical screening, including medical history taking, physical examination, standard laboratory haematology, 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, while their BMI was required to fall within a relatively narrow range to be able to specifically judge the effect of regional body fat distribution on hormone release. The total amount and location of excess body fat was determined in the obese women only. Percentage total body fat mass (fraction of total body weight) was quantified using dual energy X-ray absorptiometry (DEXA, Hologic QDR4500)(7). Visceral and subcutaneous adipose tissue areas were assessed by MRI as described before (41), using a multi slice fast spin echo sequence (Gyroscan –T5 whole body scanner 0.5 Tesla, Philips Medical Systems, Best, The Netherlands). MRI images were analysed by two observers independently.

Drugs
The obese subjects were randomly assigned to 250 mg Acipimox or placebo in a double blind crossover design by an independent investigator. Drug and placebo were taken four times daily (total 10 tablets) at 0700 h, 1300 h, 1900 h, and 0100 h starting the day prior to admission until the end of the blood-sampling period.

Diet
To limit nutritional confounding, a dietician prescribed a personal eucaloric diet for each obese woman, taking basal energy requirements (calculated by the Harris-Benedict Formula) and physical activity into account. The macronutrient composition of the diet was exactly the same for each obese woman at both study occasions. The diet consisted of bread meals, prepared and supplied by the research center. Meals were served according to a fixed time schedule (breakfast at 0730 h, lunch at 1300 h, and dinner at 1900 h) and were consumed within limited time periods. Lean women received a standardized eucaloric diet as well. No dietary restrictions were imposed on the obese women just before or between both study occasions.

Clinical Protocol

The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center. All subjects were admitted at 1600 h to the Clinical Research Unit of the Department of General Internal Medicine in the early follicular stage of their menstrual cycle. Obese subjects were studied at two separate occasions with an interval of at least eight weeks and apart from the subject receiving Acipimox or placebo treatment, the clinical set-up was exactly the same during both study occasions. Identical methodology was used to study HPA hormonal secretion in obese and normal weight women. A cannula for blood sampling was inserted into an antecubital vein. The cannula was attached to a 3-way stopcock and kept patent by a continuous saline infusion. Blood samples were taken with S-monovetten (Sarstedt, Etten-Leur, The Netherlands). One hour after admission 24 h blood sampling started. 1.2 ml Blood was collected at 10-minute intervals for determination of plasma ACTH and cortisol concentrations. Blood samples (1.2 ml) for the measurement of plasma FFA levels were taken every 6 hours in the obese subjects only. The total amount of blood withdrawn for the measurement of ACTH, cortisol and FFA levels during each occasion was 187.5 ml. All subjects remained recumbent during the blood-sampling period, except for bathroom visits. Meals were served according to a fixed time schedule. Lights were switched off at 2300 h and subjects were not disturbed by withdrawal of blood samples during their sleep (sleep monitoring by EEG was not performed). Subjects were awakened at 0100 h 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 4000r/min at 4 °C during 20 minutes, 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 with a detection limit of 3 ng/L (Nichols Institute Diagnostics, San Juan Capistrano, California, USA). The intra-assay coefficient of variation ranged from 2.8-7.5%. The ACTH IRMA was calibrated against the standard obtained from the National Pituitary Agency (University of Maryland School of Medicine) and the National Institute of Arthritis, Metabolism and Digestive Disease. Plasma cortisol concentrations were measured by Radioimmunoassay (RIA) with a detection limit of 25 nmol/L (DiaSorin, Stillwater, Minnesota, USA). The intra-assay coefficient of variation ranged from 2.0-4.0%. The cortisol RIA was calibrated against the U. S. P. Cortisol Reference standard.

FFA levels were determined using a NEFA-C Free Fatty acid kit (Wako Chemicals GmbH, 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 inter- and intra-assay coefficients of variation were 6.8% and 15.8% respectively.

Calculations and statistics

Deconvolution Analysis

Multi parameter 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 created with Pulse 2, an automated pulse detection program. Subsequent analysis with a waveform-dependent multi parameter deconvolution method was performed as described previously, using a first component half-life of 3.5 min, second component half life of 14 min and relative contribution of the slow component to the total elimination of 0.67 for ACTH and a first component half-life of 3.8 min, second component half life of 66 min and relative contribution of the slow component to the total elimination of 0.67 for cortisol (66). This technique thus estimates the rate of basal release, the number and mass of randomly ordered secretory bursts and the subject-specific half-life. The daily pulsatile secretion is the product of secretory burst frequency and mean secretory burst mass. Total secretion is the sum of basal and pulsatile secretion. Results were expressed per liter distribution volume. For the calculation of production rates per liter,

ACTH distribution volumes was estimated to amount to 40 ml/kg (65) and the distribution volume of cortisol was estimated to be 5.3 L/body surface area (m^2), which was calculated using the Dubois formula (37;71). The relationship between plasma ACTH and cortisol concentrations was determined by cross-correlation analysis.

Approximate Entropy

Approximate Entropy (ApEn) is a scale and model independent statistic that
assigns a non-negative number to time series data, reflecting regularity of these data (56). We used normalized ApEn parameters of m = 1, r = 20% and 1000 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 subordinate (non pulsatile) patterns in a time series, and thus yields information distinct from and complementary to deconvolution (pulse) analyses (67). Higher absolute 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 1000 randomly shuffled versions of the same series. Cross-ApEn was used to investigate joint regularity of the hormone pairs 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 daily ACTH and cortisol secretion in obese and normal weight women. Stepwise multiple regression analysis, including percentage body fat, subcutaneous fat area and visceral fat area as independent variables, was used to determine the relationship between the size 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 mean ± SEM, unless otherwise specified.

Results

Subjects

Eleven obese and 9 lean subjects were enrolled in this study. Mean age of both groups was similar (obese 36.6 ± 1.9 vs. lean 36.4 ± 2.0 yr., P = 0.971) while BMI was significantly different (obese 33.5 ± 0.9 vs. lean 21.2 ± 0.6 kg/m^2, 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 months 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, while basal secretion, half-life and secretory half-duration were not significantly different. Cortisol kinetic parameters were similar in the obese subjects compared to age-matched lean controls, except half-life, which was slightly prolonged in the obese women. A graphical 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 Figure 1. Data of ACTH kinetic parameters of the obese and lean subjects are presented in Table 1 and Figure 2. An overview of cortisol kinetics, as estimated by deconvolution analysis, is given in Table 2.

Effect 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 (Figure 2). In particular, peak frequency and pulsatile production were reduced, while 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, 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 resp., 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)(Figure 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) (Figure 3). Cross-ApEn statistics showed that the joint regularity of ACTH-cortisol hormones 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 minutes 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/m^2) vs. daily ACTH and cortisol production. A highly significant positive correlation was found for BMI vs. total ACTH production (R^2 = 0.39, P = 0.003, Figure 4). Also, BMI was positively related to peak frequency (R^2 = 0.39, P = 0.003) and peak burst mass (R^2 = 0.26, P = 0.022). Total cortisol secretion parameters were not related to BMI (Total cortisol vs. BMI: R^2 = 0.04, P = 0.413).

Body fat distribution vs. daily ACTH and cortisol secretion and the effect of Acipimox in the obese women
The obese subjects had a mean BMI of 33.5 (30.3-39.4) kg/m^2. The Mean of their percentage total body fat mass (% of total body weight) was 40.7 (36.9-46.3) %. Mean sizes of their visceral and subcutaneous fat area were 392 (274-539) cm^2 and 1326 (1106-1709) cm^2 respectively. Multiple regression analysis, with percentage total body fat mass, 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 the total daily ACTH production, 24 h cortisol production or the decrease of total daily ACTH production during Acipimox treatment. Additionally, regression analysis revealed that the reduction of FFA levels was not related tot the reduction of ACTH secretion after Acipimox treatment in the obese women (delta FFA (mmol/L) vs. delta ACTH (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, while 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, while 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).

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 FFA by intra lipid 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 contains a high density of both 1 and 2 adrenoreceptors and there is considerable evidence that these receptors are involved in facilitating the secretion of CRH/AVP into the hypophyseal 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 VHM in its turn down regulates pituitary adrenal activity (16). Thus, FFA 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 FFA enhance HPA output through effects on neuronal control systems in brain centres at the supra pituitary level and that circulating FFA 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 FFA through intravenous infusion of a lipid/heparin solution reduces plasma ACTH and cortisol levels in (normal weight) women (40). However, as the authors state in their discussion, the physiological relevance of their findings might be limited, because FFA plasma concentrations induced by intralipid infusion were 5-10 fold higher than those usually found in (obese) humans. Also, as various types of fatty acids (i.e. long-chain/short-chain, saturated/unsaturated) may have 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 (40) do not necessarily argue against the position that elevation of circulating FFA is involved in the pathophysiology of ACTH 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, which 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 if neural circuits involved in the control of pituitary-adrenal activity contain nicotinic acid receptors. Also, it is unclear if 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 to normal weight controls. Higher ApEn values denote greater irregularity (or higher process randomness). Regularity of hormonal secretion patterns mirrors the net result of feed forward signalling and feedback restraint (68). Since 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 feed forward 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 brain of obese rodents compared to their wild-type counterparts (5;6). There is experimental evidence that leptin receptors are abundant in these CRH-containing neurons in the paraventricular nuclei of the rat brain (28) and intracerebroventricular (icv) 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 papers 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 5- 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, due to 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 towards 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 desensitise adrenocortical cells to ACTH action. Since obesity appears to be associated with increased sympathetic activity (31), this might explain the occurrence of relatively low cortisol levels in face of elevated ACTH in the obese women enrolled in the present study. As a second alternative, it has also been described that leptin directly 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 adrenocortico-steroid 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 women. 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 suggest 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 gender and age effects on HPA activity (30), one has to take into account that these results are not necessarily applicable to men or post-menopausal 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 FFA are involved in the pathophysiology of this neuroendocrine perturbation associated with obesity.

Tables and Figures

Table 1. 24 h ACTH secretory parameters

	Controls (N = 9)	Obese subjects (N = 11)		P-value [a]	P-value [b]
		Placebo	Acipimox		
Peak Frequency (number/24 h)	23 ± 2	32 ± 1 *	28 ± 2	0.001	0.054
Half-life (min)	16 ± 1	14 ± 1	16 ± 1	0.116	0.229
Secretory Half Duration (min)	20 ± 2	23 ± 2	18 ± 3	0.267	0.258
Peak Amplitude (ng/V_{dl})	1.3 ± 0.2	1.7 ± 0.1	2.0 ± 0.4	0.103	0.456
Burst Mass (ng/V_{dl}/peak)	27.9 ± 4.2	40.8 ± 4.7	32.9 ± 4.5	0.059	0.191
Basal Production (ng/V_{dl}/24 h)	533 ± 62	679 ± 170	603 ± 108	0.467	0.307
Pulse Production (ng/V_{dl}/24 h)	606 ± 89	1320 ± 181 *	878 ± 103	0.004	0.051
Total Production (ng/V_{dl}/24 h)	1139 ± 105	2000 ± 289 *	1481 ± 193†	0.019	0.043
Total Production (ng/24 h)[c]	2808 ± 329	7950 ± 1212 *	5850 ± 769†	0.002	0.039
ApEn	0.45 ± 0.04	0.56 ± 0.03	0.66 ± 0.05	0.033	0.092

Multi parameter deconvolution analysis was used to estimate various kinetic and secretory parameters of spontaneous 24 h ACTH concentration time series data. Data are presented as means ± SEM.

a) P-value obese vs. lean, statistical analysis was performed by independent Student's t-test b) P-value placebo vs. Acipimox, statistical analysis was performed by paired samples t-test c) Distribution Volume = 40 ml/kg (Ref.(65)

* $P < 0.05$ Obese vs. lean subjects

† $P < 0.05$ Placebo vs. Acipimox obese subjects

Table 2. 24 h Cortisol secretory parameters

	Controls (N = 9)	Obese subjects (N = 11)		P-value [a]	P-value [b]
		Placebo	Acipimox		
Peak Frequency (number/24 h)	24 ± 1	21 ± 1	20 ± 1	0.126	0.148
Half-life (min)	61 ± 2	73 ± 5 *	73 ± 5	0.045	0.995
Secretory Half Duration (min)	12 ± 1	14 ± 3	11 ± 2	0.668	0.337
Peak Amplitude (nmol/V_{dl})	13.5 ± 0.9	13.0 ± 1.6	24.3 ± 8.5	0.811	0.191
Burst Mass (nmol/V_{dl}/peak)	172 ± 16	154 ± 22	152 ± 13.4	0.550	0.899
Total Production (nmol/V_{dl}/24 h)	4134 ± 369	3305 ± 579	3027 ± 351	0.267	0.453
Total Production (nmol/24 h) [c]	37 186 ± 4239	36 362 ± 5639	33 542 ± 3436	0.912	0.484
ApEn	0.52 ± 0.02	0.51 ± 0.03	0.56 ± 0.03†	0.738	0.009

Multi parameter deconvolution analysis was used to estimate various kinetic and secretory parameters of spontaneous 24 h cortisol plasma concentration time series data. Data are presented as means ± SEM.

a) P-value obese vs. lean, statistical analysis was performed by independent Student's t-test

b) P-value placebo vs. Acipimox, statistical analysis was performed by paired samples t-test

c) Distribution Volume = 5.3L/BSA (m^2), BSA was calculated by the Dubois formula (Ref. (37;71)

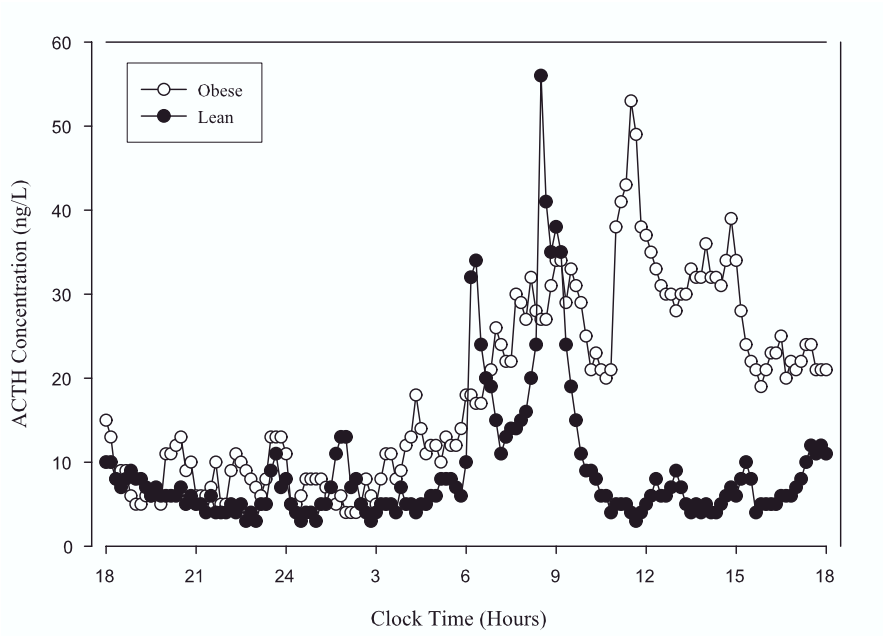
* $P < 0.05$ Obese vs. lean subjects

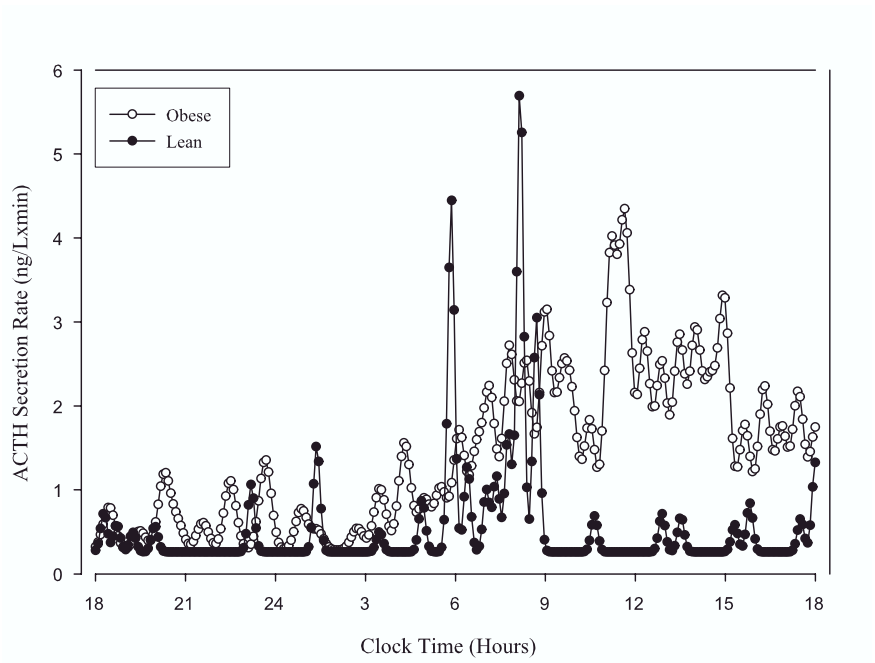
† $P < 0.05$ Placebo vs. Acipimox obese subjects

Figure 1.

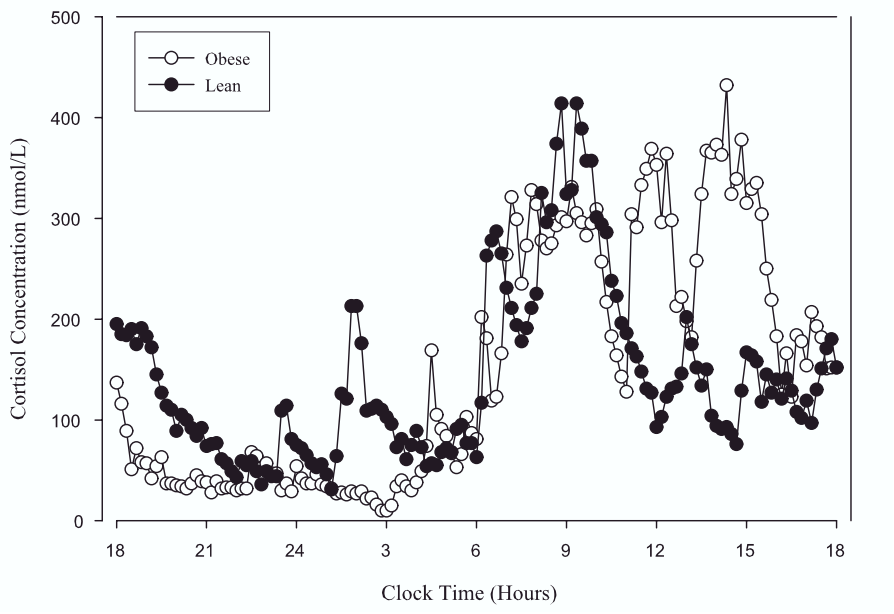
Representative 24 h ACTH (A) and cortisol (B) concentration profiles and corresponding diurnal secretion plots of one lean (-•-) and one obese woman (-o-). Lean woman Age = 33 yr, BMI = 18.3 (kg/m^2) and obese woman Age = 31 yr, BMI = 39.4 (kg/m^2)

A) 24 h ACTH concentration (ng/L) and corresponding secretion (ng/L x min) profiles





B) 24 h Cortisol concentration (nmol/L) and corresponding secretion (nmol/Lxmin) profiles



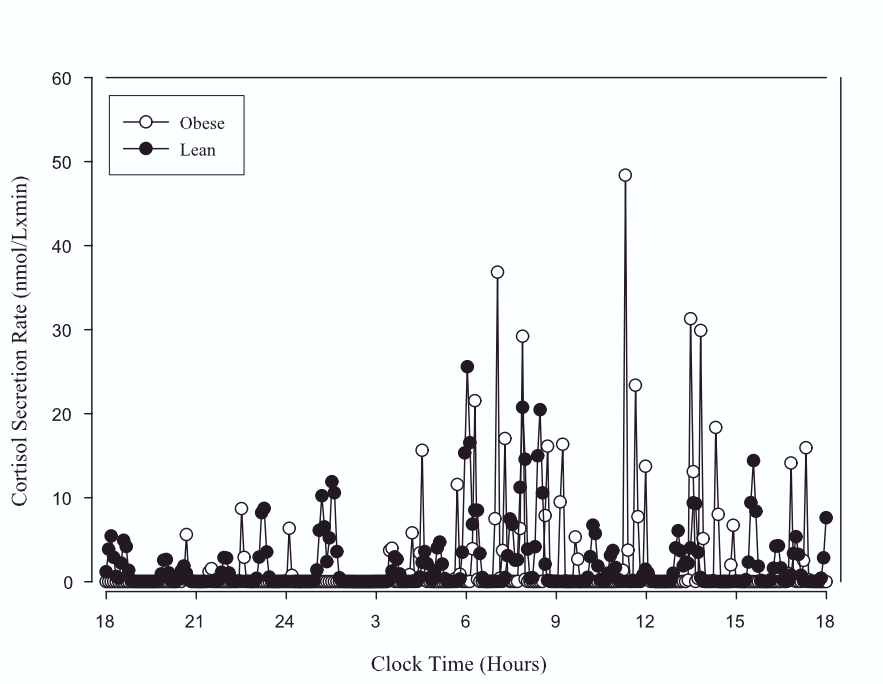


Figure 2.

Features of diurnal ACTH secretion in obese women during placebo (white bars) and Acipimox treatment (grey bars) and in lean controls (black bars). Error bars of the box plot represent SEM.

* 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

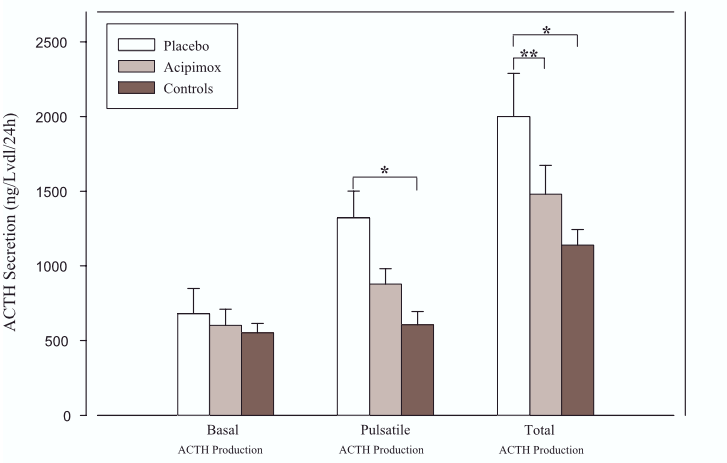


Figure 3.

Regularity of plasma ACTH and cortisol concentration- time series in obese women during placebo (open symbols) and Acipimox treatment (grey symbols) and in lean controls (closed symbols). Higher absolute 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 1000 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

** P < 0.05 Placebo vs. Acipimox obese women, statistical analysis was performed using paired samples t-test

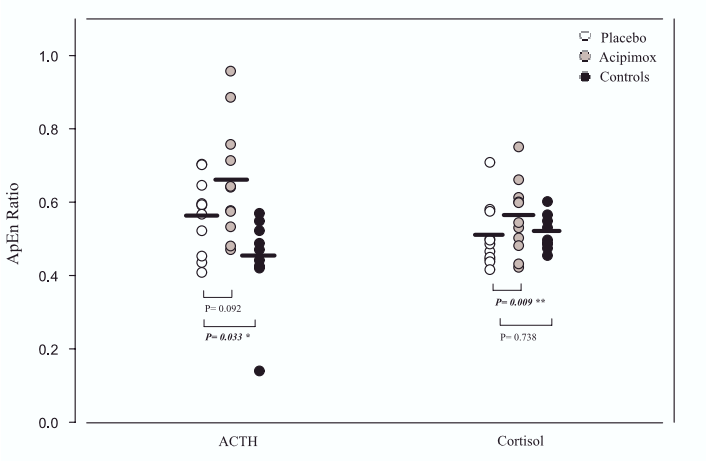
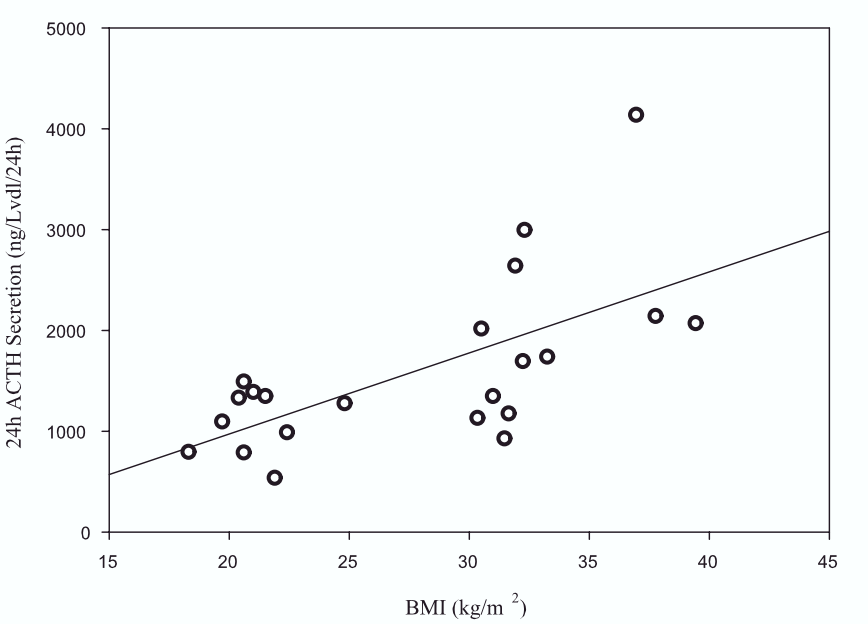


Figure 4.

Correlation 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/m^2) vs. daily ACTH production. 24 h Total ACTH production is calculated per litter distribution volume.



Reference List

1. Abdel-Malek, Z. A. Melanocortin receptors: their functions and regulation by physiological agonists and antagonists. Cell Mol.Life Sci. 58: 434-441, 2001.
2. Al Damluji, S., T. Iveson, J. M. Thomas, D. J. Pendlebury, L. H. Rees, and G. M. Besser. Food-induced cortisol secretion is mediated by central alpha-1 adrenoceptor modulation of pituitary ACTH secretion. Clin.Endocrinol.(Oxf) 26: 629-636, 1987.
3. Al Damluji, S., L. Perry, S. Tomlin, P. Bouloux, A. Grossman, L. H. Rees, and G. M. Besser. Alpha-adrenergic stimulation of corticotropin secretion by a specific central mechanism in man. Neuroendocrinology 45: 68-76, 1987.
4. Benthem, L., K. Keizer, C. H. Wiegman, S. F. de Boer, J. H. Strubbe, A. B. Steffens, F. Kuipers, and A. J. Scheurink. Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation. Am.J.Physiol Endocrinol.Metab 279: E1286-E1293, 2000.
5. Bestetti, G. E., F. Abramo, C. Guillaume-Gentil, F. Rohner-Jeanrenaud, B. Jeanrenaud, and G. L. Rossi. Changes in the hypothalamo-pituitary-adrenal axis of genetically obese fa/fa rats: a structural, immunocytochemical, and morphometrical study. Endocrinology 126: 1880-1887, 1990.
6. Bina, K. G. and A. H. Cincotta. Dopaminergic agonists normalize elevated hypothalamic neuropeptide Y and corticotropin-releasing hormone, body weight gain, and hyperglycemia in ob/ob mice. Neuroendocrinology 71: 68-78, 2000.
7. Blake, G. M. and I. Fogelman. Technical principles of dual energy x-ray absorptiometry. Semin.Nucl.Med. 27: 210-228, 1997.
8. Bornstein, S. R. Is leptin a stress related peptide? Nat.Med. 3: 937, 1997.
9. Bornstein, S. R., K. Uhlmann, A. Haidan, M. Ehrhart-Bornstein, and W. A. Scherbaum. Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. Diabetes 46: 1235-1238, 1997.
10. Boston, B. A. The role of melanocortins in adipocyte function. Ann.N.Y.Acad.Sci. 885: 75-84, 1999.
11. Buijs, R. M., J. Wortel, J. J. Van Heerikhuize, M. G. Feenstra, G. J. Ter Horst, H. J. Romijn, and A. Kalsbeek. Anatomical and functional demonstration of a multisynaptic suprachiasmatic nucleus adrenal (cortex) pathway. Eur.J.Neurosci. 11: 1535-1544, 1999.
12. Castonguay, T. W., M. F. Dallman, and J. S. Stern. Some metabolic and behavioral effects of adrenalectomy on obese Zucker rats. Am.J.Physiol 251: R923-R933, 1986.
13. Christie, A. W., D. K. McCormick, N. Emmison, F. B. Kraemer, K. G. Alberti, and S. J. Yeaman. Mechanism of anti-lipolytic action of acipimox in isolated rat adipocytes. Diabetologia 39: 45-53, 1996.
14. Considine, R. V., M. K. Sinha, M. L. Heiman, A. Kriauciunas, T. W. Stephens, M. R. Nyce, J. P. Ohannesian, C. C. Marco, L. J. McKee, T. L. Bauer, and . Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N.Engl.J.Med. 334: 292-295, 1996.
15. Couillard, C., N. Bergeron, D. Prud'homme, J. Bergeron, A. Tremblay, C. Bouchard, P. Mauriege, and J. P. Despres. Postprandial triglyceride response in visceral obesity in men. Diabetes 47: 953-960, 1998.
16. Dallman, M. F. Viewing the ventromedial hypothalamus from the adrenal gland. Am.J.Physiol 246: R1-12, 1984.
17. Dani, J. A. Overview of nicotinic receptors and their roles in the central nervous system. Biol.Psychiatry 49: 166-174, 2001.
18. Deshaies, Y., A. Dagnault, J. Lalonde, and D. Richard. Interaction of corticosterone and gonadal position in the female rat. Am.J.Physiol 273: E355-E362, 1997.
19. Drazen, D. L., M. D. Wortman, M. W. Schwartz, D. J. Clegg, G. van Dijk, S. C. Woods, and R. J. Seeley. Adrenalectomy alters the sensitivity of the central nervous system melanocortin system. Diabetes 52: 2928-2934, 2003.
20. Epel, E. S., B. McEwen, T. Seeman, K. Matthews, G. Castellazzo, K. D. Brownell, J. Bell, and J. R. Ickovics. Stress and body shape: stress-induced cortisol secretion is consistently greater among women with central fat. Psychosom.Med. 62: 623-632, 2000.
21. Freedman, M. R., T. W. Castonguay, and J. S. Stern. Effect of adrenalectomy and corticosterone replacement on meal patterns of Zucker rats. Am.J.Physiol 249: R584-R594, 1985.
22. Freedman, M. R., B. A. Horwitz, and J. S. Stern. Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am.J.Physiol 250: R595-R607, 1986.
23. Gardner, J. D., N. J. Rothwell, and G. N. Luheshi. Leptin affects food intake via CRF-receptor-mediated pathways. Nat.Neurosci. 1: 103, 1998.
24. Glasow, A. and S. R. Bornstein. Leptin and the adrenal gland. Eur.J.Clin.Invest 30 Suppl 3: 39-45, 2000.
25. Gotti, C., D. Fornasari, and F. Clementi. Human neuronal nicotinic receptors. Prog.Neurobiol. 53: 199-237, 1997.
26. Green, P. K., C. W. Wilkinson, and S. C. Woods. Intraventricular corticosterone increases the rate of body weight gain in underweight adrenalectomized rats. Endocrinology 130: 269-275, 1992.

27. Grekin, R. J., A. P. Vollmer, and R. S. Sider. Pressor effects of portal venous oleate infusion. A proposed mechanism for obesity hypertension. Hypertension 26: 193-198, 1995.
28. Hakansson, M. L., H. Brown, N. Ghilardi, R. C. Skoda, and B. Meister. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J.Neurosci. 18: 559-572, 1998.
29. Hautanen, A. and H. Adlercreutz. Altered adrenocorticotropin and cortisol secretion in abdominal obesity: implications for the insulin resistance syndrome. J.Intern.Med. 234: 461-469, 1993.
30. Heuser, I. J., U. Gotthardt, U. Schweiger, J. Schmider, C. H. Lammers, M. Dettling, and F. Holsboer. Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. Neurobiol.Aging 15: 227-231, 1994.
31. Heuser, I. J., U. Gotthardt, U. Schweiger, J. Schmider, C. H. Lammers, M. Dettling, and F. Holsboer. Age-associated changes of pituitary-adrenocortical hormone regulation in humans: importance of gender. Neurobiol.Aging 15: 227-231, 1994.
32. Holt, S., D. A. York, and J. T. Fitzsimons. The effects of corticosterone, cold exposure and overfeeding with sucrose on brown adipose tissue of obese Zucker rats (fa/fa). Biochem.J. 214: 215-223, 1983.
33. Jang, M., A. Mistry, A. G. Swick, and D. R. Romsos. Leptin rapidly inhibits hypothalamic neuropeptide Y secretion and stimulates corticotropin-releasing hormone secretion in adrenalectomized mice. J.Nutr. 130: 2813-2820, 2000.
34. Jensen, M. D., M. W. Haymond, R. A. Rizza, P. E. Cryer, and J. M. Miles. Influence of body fat distribution on free fatty acid metabolism in obesity. J. Clin.Invest 83: 1168-1173, 1989.
35. Jessop, D. S., M. F. Dallman, D. Fleming, and S. L. Lightman. Resistance to glucocorticoid feedback in obesity. J.Clin.Endocrinol.Metab 86: 4109-4114, 2001.
36. Kang, J. S., J. D. Pilkington, D. Ferguson, H. K. Kim, and D. R. Romsos. Dietary glucose and fat attenuate effects of adrenalectomy on energy balance in ob/ob mice. J.Nutr. 122: 895-905, 1992.
37. Kerrigan, J. R., J. D. Veldhuis, S. A. Leyo, A. Iranmanesh, and A. D. Rogol. Estimation of daily cortisol production and clearance rates in normal pubertal males by deconvolution analysis. J.Clin.Endocrinol.Metab 76: 1505-1510, 1993.
38. Kok, S. W., A. E. Meinders, S. Overeem, G. J. Lammers, F. Roelfsema, M. Frolich, and H. Pijl. Reduction of plasma leptin levels and loss of its circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. J.Clin.Endocrinol.Metab 87: 805-809, 2002.
39. Kok, S. W., F. Roelfsema, S. Overeem, G. J. Lammers, R. L. Strijers, M. Frolich, A. E. Meinders, and H. Pijl. Dynamics of the pituitary-adrenal ensemble in hypocretin-deficient narcoleptic humans: blunted basal adrenocorticotropin release and evidence for normal time-keeping by the master pacemaker. J.Clin.Endocrinol.Metab 87: 5085-5091, 2002.
40. Lanfranco, F., R. Giordano, M. Pellegrino, L. Gianotti, J. Ramunni, A. Picu, M. Baldi, E. Ghigo, and E. Arvat. Free Fatty acids exert an inhibitory effect on adrenocorticotropin and cortisol secretion in humans. J.Clin.Endocrinol.Metab 89: 1385-1390, 2004.
41. Langendonk, J. G., H. Pijl, A. C. Toornvliet, J. Burggraaf, M. Frolich, R. C. Schoemaker, J. Doornbos, A. F. Cohen, and A. E. Meinders. Circadian rhythm of plasma leptin levels in upper and lower body obese women: influence of body fat distribution and weight loss. J.Clin.Endocrinol.Metab 83: 1706-1712, 1998.
42. Licinio, J., C. Mantzoros, A. B. Negrao, G. Cizza, M. L. Wong, P. B. Bongiorno, G. P. Chrousos, B. Karp, C. Allen, J. S. Flier, and P. W. Gold. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat.Med. 3: 575-579, 1997.
43. Ljung, T., B. Andersson, B. A. Bengtsson, P. Bjorntorp, and P. Marin. Inhibition of cortisol secretion by dexamethasone in relation to body fat distribution: a dose-response study. Obes.Res. 4: 277-282, 1996.
44. Ljung, T., G. Holm, P. Friberg, B. Andersson, B. A. Bengtsson, J. Svensson, M. Dallman, B. McEwen, and P. Bjorntorp. The activity of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in relation to waist/hip circumference ratio in men. Obes.Res. 8: 487-495, 2000.
45. Lonn, L., H. Kvist, I. Ernest, and L. Sjostrom. Changes in body composition and adipose tissue distribution after treatment of women with Cushing's syndrome. Metabolism 43: 1517-1522, 1994.

46. Love, J. A., W. R. Saum, and R. McGee, Jr. The effects of exposure to exogenous fatty acids and membrane fatty acid modification on the electrical properties of NG108-15 cells. Cell Mol.Neurobiol. 5: 333-352, 1985.
47. Maffei, M., J. Halaas, E. Ravussin, R. E. Pratley, G. H. Lee, Y. Zhang, H. Fei, S. Kim, R. Lallone, S. Ranganathan, and . Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat.Med. 1: 1155-1161, 1995.
48. Mantha, L., E. Palacios, and Y. Deshaies. Modulation of triglyceride metabolism by glucocorticoids in diet-induced obesity. Am.J.Physiol 277: R455-R464, 1999.

49. Marin, P., B. Andersson, M. Ottosson, L. Olbe, B. Chowdhury, H. Kvist, G. Holm, L. Sjostrom, and P. Bjorntorp. The morphology and metabolism of intraabdominal adipose tissue in men. Metabolism 41: 1242-1248, 1992.
50. Marin, P., N. Darin, T. Amemiya, B. Andersson, S. Jern, and P. Bjorntorp. Cortisol secretion in relation to body fat distribution in obese premenopausal women. Metabolism 41: 882-886, 1992.
51. Pasquali, R., B. Anconetani, R. Chattat, M. Biscotti, G. Spinucci, F. Casimirri, V. Vicennati, A. Carcello, and A. M. Labate. Hypothalamic-pituitary-adrenal axis activity and its relationship to the autonomic nervous system in women with visceral and subcutaneous obesity: effects of the corticotropin-releasing factor/arginine-vasopressin test and of stress. Metabolism 45: 351-356, 1996.
52. Pasquali, R., D. Biscotti, G. Spinucci, V. Vicennati, A. D. Genazzani, L. Sgarbi, and F. Casimirri. Pulsatile secretion of ACTH and cortisol in premenopausal women: effect of obesity and body fat distribution. Clin.Endocrinol.(Oxf) 48: 603-612, 1998.
53. Pasquali, R., S. Cantobelli, F. Casimirri, M. Capelli, L. Bortoluzzi, R. Flamia, A. M. Labate, and L. Barbara. The hypothalamic-pituitary-adrenal axis in obese women with different patterns of body fat distribution. J.Clin.Endocrinol.Metab 77: 341-346, 1993.
54. Pasquali, R., L. Gagliardi, V. Vicennati, A. Gambineri, D. Colitta, L. Ceroni, and F. Casimirri. ACTH and cortisol response to combined corticotropin releasing hormone-arginine vasopressin stimulation in obese males and its relationship to body weight, fat distribution and parameters of the metabolic syndrome. Int.J.Obes.Relat Metab Disord. 23: 419-424, 1999.
55. Pincus, S. and B. H. Singer. Randomness and degrees of irregularity. Proc.Natl.Acad.Sci.U.S.A 93: 2083-2088, 1996.
56. Pincus, S. M. and D. L. Keefe. Quantification of hormone pulsatility via an approximate entropy algorithm. Am.J.Physiol 262: E741-E754, 1992.
57. Pralong, F. P., R. Roduit, G. Waeber, E. Castillo, F. Mosimann, B. Thorens, and R. C. Gaillard. Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. Endocrinology 139: 4264-4268, 1998.
58. Rapoport, S. I. In vivo fatty acid incorporation into brain phosholipids in relation to plasma availability, signal transduction and membrane remodeling. J.Mol.Neurosci. 16: 243-261, 2001.
59. Rosmond, R., M. F. Dallman, and P. Bjorntorp. Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. J.Clin.Endocrinol.Metab 83: 1853-1859, 1998.
60. Schwartz, M. W., R. J. Seeley, L. A. Campfield, P. Burn, and D. G. Baskin. Identification of targets of leptin action in rat hypothalamus. J.Clin.Invest 98: 1101-1106, 1996.
61. Shimomura, Y., G. A. Bray, and M. Lee. Adrenalectomy and steroid treatment in obese (ob/ob) and diabetic (db/db) mice. Horm.Metab Res. 19: 295-299, 1987.
62. Storlien, L. H., D. E. James, K. M. Burleigh, D. J. Chisholm, and E. W. Kraegen. Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. Am.J.Physiol 251: E576-E583, 1986.
63. Tannenbaum, B. M., D. N. Brindley, G. S. Tannenbaum, M. F. Dallman, M. D. McArthur, and M. J. Meaney. High-fat feeding alters both basal and stress-induced hypothalamic-pituitary-adrenal activity in the rat. Am.J.Physiol 273: E1168-E1177, 1997.
64. Uehara, Y., H. Shimizu, K. Ohtani, N. Sato, and M. Mori. Hypothalamic corticotropin-releasing hormone is a mediator of the anorexigenic effect of leptin. Diabetes 47: 890-893, 1998.
65. Veldhuis, J. D., A. Iranmanesh, M. L. Johnson, and G. Lizarralde. Amplitude, but not frequency, modulation of adrenocorticotropin secretory bursts gives rise to the nyctohemeral rhythm of the corticotropic axis in man. J.Clin.Endocrinol.Metab 71: 452-463, 1990.
66. Veldhuis, J. D. and M. L. Johnson. Deconvolution analysis of hormone data. Methods Enzymol. 210: 539-575, 1992.
67. Veldhuis, J. D. and S. M. Pincus. Orderliness of hormone release patterns: a complementary measure to conventional pulsatile and circadian analyses. Eur.J.Endocrinol. 138: 358-362, 1998.
68. Veldhuis, J. D., M. Straume, A. Iranmanesh, T. Mulligan, C. Jaffe, A. Barkan, M. L. Johnson, and S. Pincus. Secretory process regularity monitors neuroendocrine feedback and feedforward signaling strength in humans. Am.J.Physiol Regul.Integr.Comp Physiol 280: R721-R729, 2001.
69. Vicennati, V. and R. Pasquali. Abnormalities of the hypothalamic-pituitary-adrenal axis in nondepressed women with abdominal obesity and relations with insulin resistance: evidence for a central and a peripheral alteration. J.Clin.Endocrinol.Metab 85: 4093-4098, 2000.
70. Wajchenberg, B. L., A. Bosco, M. M. Marone, S. Levin, M. Rocha, A. C. Lerario, M. Nery, J. Goldman, and B. Liberman. Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. J.Clin. Endocrinol.Metab 80: 2791-2794, 1995.
71. Wang, Y., J. Moss, and R. Thisted. Predictors of body surface area. J.Clin.Anesth. 4: 4-10, 1992.

72. Westerbacka, J., H. Yki-Jarvinen, S. Vehkavaara, A. M. Hakkinen, R. Andrew, D. J. Wake, J. R. Seckl, and B. R. Walker. Body fat distribution and cortisol metabolism in healthy men: enhanced 5beta-reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver. J.Clin.Endocrinol. Metab 88: 4924-4931, 2003.
73. Widmaier, E. P., J. Margenthaler, and I. Sarel. Regulation of pituitary-adrenocortical activity by free fatty acids in vivo and in vitro. Prostaglandins Leukot.Essent.Fatty Acids 52: 179-183, 1995.
74. Widmaier, E. P., K. Rosen, and B. Abbott. Free fatty acids activate the hypothalamic-pituitary-adrenocortical axis in rats. Endocrinology 131: 2313-2318, 1992.
75. Yehuda, S., S. Rabinovitz, R. L. Carasso, and D. I. Mostofsky. The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. Neurobiol.Aging 23: 843-853, 2002.
76. Yukimura, Y., G. A. Bray, and A. R. Wolfsen. Some effects of adrenalectomy in the fatty rat. Endocrinology 103: 1924-1928, 1978.
77. Zakrzewska, K. E., I. Cusin, A. Sainsbury, F. Rohner-Jeanrenaud, and B. Jeanrenaud. Glucocorticoids as counterregulatory hormones of leptin: toward an understanding of leptin resistance. Diabetes 46: 717-719, 1997.