Neuroendocrine perturbations in human obesity

Kok, P.

Chapter 6

Acipimox Enhances Spontaneous Growth Hormone Secretion in Obese Women

Petra Kok, Madelon M. Buijs, Simon W. Kok, Inge H.A.P. van Ierssel, Marijke Frölich, Ferdinand Roelfsema, Peter J. Voshol, A. Edo Meinders, Hanno Pijl

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Abstract

We hypothesized that a high circulating FFA concentration is involved in the pathogenesis of hyposomatotropism associated with obesity. To evaluate this hypothesis, ten healthy premenopausal women (BMI 33.8 ± 1.0 kg/m^2) were studied in the follicular phase of their menstrual cycle at two occasions with a time interval of at least 8 weeks, where body weight remained stable. Subjects were randomly assigned to treatment with either Acipimox (an 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 GH concentrations and GH secretion was estimated by deconvolution analysis. Identical methodology was used to study GH secretion in a historical control group of age-matched normal weight women. GH secretion, was clearly blunted in obese women (total daily release 66 ± 10 vs. lean controls: 201 ± 23 mU/V$_{dl}$/24 h, P = 0.005). Acipimox considerably enhanced total (113 ± 50 vs. 66 ± 10 mU/V$_{dl}$/24 h, P = 0.02) and pulsatile GH secretion (109 ± 49 vs. 62 ± 30 mU/V$_{dl}$/24 h, P = 0.02), but GH output remained lower compared to lean controls. Further analysis did not show any relationship between the effects of Acipimox on GH secretion and regional body fat distribution.

In conclusion, Acipimox unleashes spontaneous GH secretion in obese women. It specifically enhances GH secretory burst mass. This might mean that lowering of systemic FFA concentrations by Acipimox modulates neuroendocrine mechanisms that orchestrate the activity of the somatotropic ensemble.

Introduction

Spontaneous pulsatile GH release (36,49) and GH secretion in response to various provocative exogenous stimuli (1,7,14) are markedly blunted in obese patients. The mechanism underlying this neuroendocrine feature of obese humans remains elusive.

Obesity is associated with high circulating free fatty acid (FFA) concentrations (11,21) and FFA have been shown to suppress GH release in humans and animals (5,6,13,19,27,41). Thus, hyposomatotropism in obese individuals may be brought about by elevated plasma FFAs. Indeed, reduction of circulating FFA levels with Acipimox, a powerful anti-lipolytic drug, considerably enhances GH secretion in response to various secretagogues in obese humans (10,25,26,33,40). It remains to be established if Acipimox also unleashes spontaneous GH release in obese individuals.

Excess fat can be stored in various adipose depots. It appears that neuroendocrine alterations particularly occur in viscerally obese patients (28,36). Visceral fat is morphologically and functionally distinct from subcutaneous fat, in that cellularity and FFA turnover are higher per unit adipose tissue (22,29,42,47). Also, venous output of visceral fat drains directly into the portal system of the liver, while FFAs from subcutaneous fat enter the systemic circulation. FFA infusion into the portal vein enhances pituitary-adrenal axis and sympathetic nervous system activity, whereas systemic FFA infusion does not exert appreciable effects on these neuroendocrine ensembles (2,15). Thus, a high portal FFA flux, brought about by excess visceral fat, may particularly inhibit GH release.

We hypothesized that circulating FFAs are involved in the pathogenesis of hyposomatotropism in obese humans. Therefore, we measured 24 h spontaneous GH release in response to administration of Acipimox, a powerful inhibitor of lipolysis, in obese women. To further clarify the role of FFA released by visceral fat, we sought to determine the relationship between the effects of Acipimox and the size of various adipose depots.

Subjects and Methods

Subjects

Ten healthy, obese premenopausal women were enrolled in our study. Subjects were recruited taking body fat distribution into account. Conditions for participation were verified through medical screening, including medical history, physical examination, standard laboratory haematology, blood chemistry, urine and pregnancy tests and anthropometric measurements. A historical control group of lean women matched for age was included for comparison of GH secretion data with those in obese women. All obese subjects and the age-matched lean controls had an unremarkable medical history. Subjects were non-alcoholic, non-smoking and were not taking any medication, including hormonal contraception. All subjects gave written acknowledgement of informed consent.

Body composition

Total body fat mass (TBFM) was quantified on a separate day preceding the first study occasion using dual energy X-ray absorptiometry (DEXA)(3). Visceral and subcutaneous adipose tissue areas were assessed by MRI as described before (24), 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 independently by two observers.

Drugs

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

Diet

To limit confounding by nutritional factors, a dietician prescribed a eucaloric diet for each patient, taking basal energy requirement (calculated by the Harris-Benedict Formula) and physical activity into account. The macronutrient composition of the diet was exactly the same for each patient 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.

Clinical Protocol

The Medical Ethics Committee of the Leiden University Medical Center approved the protocol for both study groups. Apart from the fact that controls did not receive Acipimox treatment, the procedures and the clinical set-up of the experiments were exactly the same in obese subjects and controls. Subjects were admitted to the Clinical Research Unit of the Department of General Internal Medicine in the early follicular stage of their menstrual cycle at two separate occasions at 1600 h with an interval of at least eight weeks. A cannula for blood sampling was inserted into an ante cubital vein and blood samples for basal parameters were withdrawn. 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). Twenty four hour blood sampling started at 1800h and blood was collected at 10 minute intervals for determination of GH concentrations. Plasma FFA levels were measured every 6 hours. Plasma FFA concentrations were not measured in the historical control group. Subjects remained recumbent, except for bathroom visits. Lights were switched of at 2300 h. Vital signs were recorded at regular time intervals. The clinical set-up was the same during both occasions apart from the subject receiving the alternative treatment (Acipimox or placebo).

Assays

Blood sample handling and GH assays were performed using the same methodology in obese subjects and controls. Each tube, except the serum tubes, was immediately chilled on ice. Samples were centrifuged at 4000r/min at 4 °C during 20 minutes, within 60 min of sampling. Subsequently, plasma was divided into separate aliquots and frozen at -80 °C until assays were performed. GH concentrations were measured with a sensitive time-resolved fluoro immunoassay (Wallac, Turku, Finland) specific for the 22 kDa GH protein. The assay uses rhGH as standard (Genotropin, Pharmacia & Upjohn, Uppsala, Sweden), which is calibrated against WHO First International Reference Preparation (80-505). The limit of detection is 0.03 mU/L. Intra-assay coefficients of variation (CV) were 1.6-8.4% in the concentration range 0.26-47 mU/L, with corresponding inter-assay CV's of 2.0-9.9%.

The total serum IGF-I concentration was determined by RIA after extraction and purification on ODS-silica columns (Incstar Corp., Stillwater, MN). The inter assay CV was less than 11.8 %. The detection limit was 1.5 nmol/L. Age-related normative data were determined in the same laboratory. FFA levels were determined using a NEFA-C Free Fatty acid kit (Wako Chemicals GmbH, Neuss, Germany). The detection limit was 0.03 mMol/L and the inter- and intra-assay coefficients of variation were 1.1% and 2.6% respectively.

Calculations and statistics

Deconvolution Analysis

Multi parameter deconvolution analysis was used to determine kinetic and secretory parameters of 24 h spontaneous GH secretion, calculated from GH plasma concentrations. An initial guess of the secretion profile for waveform-independent estimates of GH secretion, was 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, a second component half life of 20.8 min and a relative contribution of the slow component to the total elimination of 0.68 (50,51). 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 GH secretion is the product of secretory burst frequency and mean mass of GH released per event. Total GH secretion is the sum of basal and pulsatile secretion.

Approximate entropy

Approximate entropy (ApEn) is a scale and model independent statistic, applicable to a wide variety of physiological and clinical time-series data (16,38,39). ApEn quantities the orderliness or regularity of serial GH concentrations over 24 h. Normalized ApEn parameters of m = 1 (test range) and r = 20% (threshold) of the intra series SD were used, as described previously (37). 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 cosinor and deconvolution (pulse) analyses (53). Higher absolute ApEn values denote greater relative randomness of hormone patterns. Data are presented as absolute ApEn values and normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1000 randomly shuffled versions of the same series (54).

Statistics

TBFM is presented as a percentage of total body weight. Subcutaneous fat mass (SFM) and visceral fat mass (VFM) were expressed as a percentage of total fat mass. To determine the effect of Acipimox on daily GH secretion, numeric outcomes of deconvolution analysis and the ApEn metric were statistically analysed using one way ANOVA.

Differences between GH kinetic parameters between lean controls and obese women were analysed using Student's t-test for unpaired samples. Multiple regression analysis, using TBFM, VFM and SFM as independent variables, was done to determine specific correlations between measures of body fat distribution and GH secretory and kinetic parameters. All data are given as mean ± SEM and significance level was set at 0.05

Results

Subjects

Ten obese women (age 35.8 ± 2.0 yr, BMI 33.8 ± 1.0 kg/m^2) and 7 lean controls (age 35.1 ± 3.0 yr, BMI 21.5 ± 0.5 kg/m^2) were included. Body weight remained stable from 3 months before until the end of the study period.

Effect of Acipimox on spontaneous GH secretion

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). Under placebo conditions GH kinetic and secretory parameters were significantly lower in the obese subjects compared to the age-matched lean controls. Acipimox treatment significantly increased burst amplitude, burst mass, pulsatile and total daily GH production, while burst frequency, half-life, secretory half-duration and basal production were not significantly affected (Fig 1). However, Acipimox did not restore GH secretion to reference levels as determined in lean controls. Mean 24 h IGF-I levels were not affected by Acipimox (Table 1). An overview of GH secretory and kinetic parameters and reference values of GH secretory parameters in age matched premenopausal normal weight women, as determined in the control group, are given in Table 1. A graphical illustration of a representative 24 h GH concentration data set and corresponding secretory profile is shown in Fig 2.

Impact of body fat distribution

The obese subjects had a BMI of 33.8 ± 0.96 (range 31.0-39.4) kg/m^2, a WHR of 0.85 ± 0.01 (range 0.75-0.92) and their TBFM (% of total body weight) was 40.6 ± 1.1 (range 36.9-46.3) %. The seizes of their visceral and subcutaneous fat area were 392 ± 30 (range 274-539) cm^2 and 1348 ± 58 (range 1162-1709) cm^2 respectively.

Multiple regression analysis, including TBFM, VFM, and SFM as independent variables, showed no significant correlation (R^2 = 0.00, P = 0.48) between the size of visceral fat mass and the increase of total GH production during Acipimox treatment.

Approximate Entropy

ApEn ratios of plasma GH concentration time series were similar in obese and normal weight women. ApEn ratios were not affected by Acipimox (Table 1). Body fat distribution did not impact orderliness of the GH time series data either.

Discussion

Here we show that Acipimox unleashes spontaneous GH secretion in obese women. The drug particularly enhanced GH secretory burst mass, whereas burst frequency and basal GH secretion were largely unaffected. However, total daily GH production remained significantly lower than in normal weight controls. The distribution of excess fat over the various depots does not appear to impact the effect of Acipimox on GH secretion in obese individuals.

It has been repeatedly reported that the profound reduction of spontaneous GH secretion, that is invariably observed in obese humans, is primarily brought about by a diminution of secretory burst mass (20,52). The present data therefore suggest that Acipimox partially restores this primary neuroendocrine anomaly that underlies hyposomatotropism in obesity. GHRH input is a critical determinant of GH secretory burst mass, whereas other components of the somatotropic ensemble appear to control burst frequency and basal secretion (43). In vitro data show that incorporation of cis-unsaturated fatty acids into the plasma membrane of GH3 cells, disrupts signal transduction pathways that are pivotal for GHRH-induced GH release (34,35). Thus, Acipimox may enhance somatotroph sensitivity to GHRH feed forward inputs through lowering of circulating (cis-unsaturated) FFA and thereby specifically stimulate GH secretory burst mass, an inference that is in keeping with our observations.

A high portal FFA flux, released by excess visceral adipose tissue directly into the portal vein, could be responsible for the diminution of GH release in viscerally obese humans (3). In this scenario, one would expect Acipimox to exert its greatest effect on GH release in humans with large visceral fat stores. Our data are not in keeping with this postulate. However, the size of our study population was rather small, which considerably limits the statistical power to detect potential correlations in regression analyses.

Although Acipimox considerably enhanced GH secretion in obese subjects, it did not fully restore GH output to levels observed in normal weight controls. In this context, it seems prudent to emphasize that we used historical controls, which may limit the comparability of the data, although the clinical set-up, the applied assays and mathematical techniques for data analysis were identical in both groups.

Other physiological cues than FFA may also have affected GH release in our study. For example, insulin can blunt GH release (8,31). Acipimox can lower plasma insulin (48) and thereby enhance GH secretion. Also, high circulating insulin levels in our obese subjects might explain their persistently lower GH secretion rate with and without Acipimox treatment. Unfortunately, we did not measure insulin levels to further evaluate these possibilities. Another potential mechanistic explanation for the profound impact of Acipimox on GH secretion may relate to activation of dopaminergic neural circuits. Acipimox is a nicotinic acid derivative and nicotinic acid can activate dopaminergic neurons (9,17,18,23,30,46). Dopamine promotes (secretagogue-induced) GH release through activation of dopamine D2 receptors in rats and humans (4,12,32,44,45). Thus, Acipimox may also stimulate GH output through neural pathways.

In conclusion, the present data show that Acipimox acutely promotes spontaneous GH output in obese humans. It specifically enhances GH secretory burst mass, which might support the notion that Acipimox improves GHRH's ability to induce GH release by pituitary somatotrophs. The mechanism through which Acipimox exerts its effect on GH secretion in obese humans remains elusive.

Tables and Figures

Table 1. Effect of Acipimox on 24 h GH secretory Parameters in Obese Subjects

Treatment	Obese subjects (N = 10)			Controls (N = 7)
	Placebo	Acipimox	P-value [a]	
Peak Frequency (number/24 h)	23 ± 2*	23 ± 2 *	0.97	16 ± 2
Half life (min)	16.5 ± 0.7	17.1 ± 0.6 *	0.42	14.7 ± 0.7
Secretory Half Duration (min)	19.7 ± 1.7*	22.1 ± 1.7	0.06	26.8 ± 1.9
Peak Amplitude (mU/V_{dl})	0.13 ± 0.02*	0.20 ± 0.02 *	0.03	0.46 ± 0.06
Burst Mass (mU/V_{dl}/peak)	2.7 ± 0.4*	4.7 ± 0.6 *	0.008	13.3 ± 2.1
Basal production (mU/V_{dl}/24 h)	3 ± 0.1*	5 ± 1.2 *	0.17	12 ± 2.3
Pulse Production (mU/V_{dl}/24 h)	62 ± 10*	109 ± 15 *	0.005	190 ± 23
Total Production (mU/V_{dl}/24 h)	66 ± 10*	113 ± 16 *	0.005	201 ± 23
ApEn 24 h GH concentration	0.43 ± 0.02	0.39 ± 0.03	0.41	0.39 ± 0.01
Mean 24 h IGF-1 (nmol/L)	17.9 ± 0.8	18.9 ± 1.2	0.27	ND

Data are presented as means ± SEM

ND = not determined

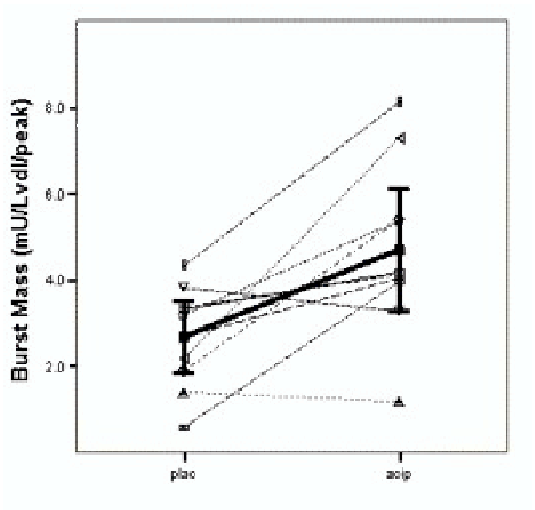
a) p-value placebo vs. Acipimox by one way ANOVA

* $p < 0.05$ vs. lean controls, using independent Student's t-test

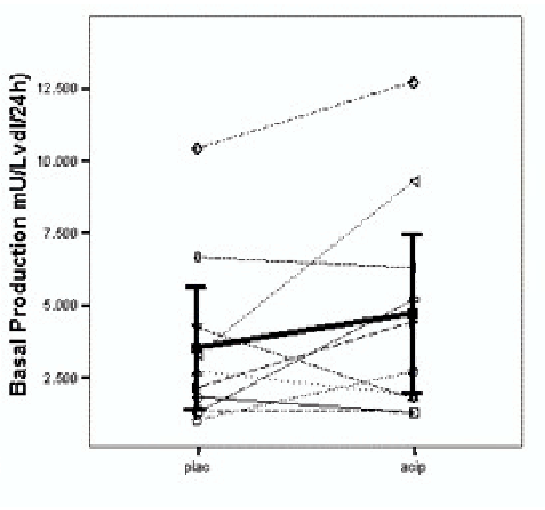
Figure 1.

Effect Acipimox on GH secretion parameters in obese subjects various parameters of spontaneous pulsatile GH release during placebo (plac) and Acipimox (acip) in obese women. P-values of the difference between means during Placebo vs. Acipimox treatment determined by one way ANOVA are presented.

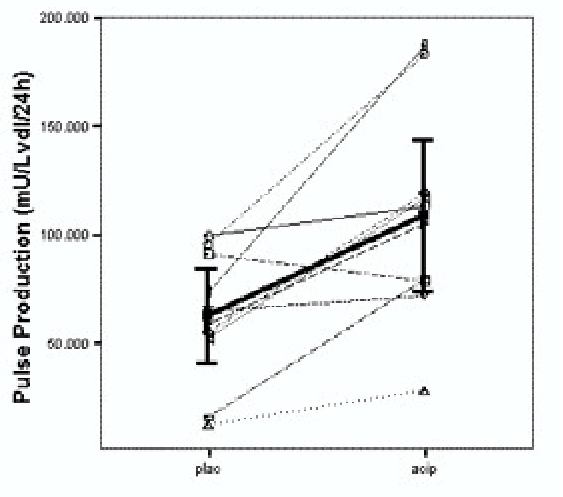
A) Burst Mass



B) Basal Production



C) Pulse Production



D) Total Production

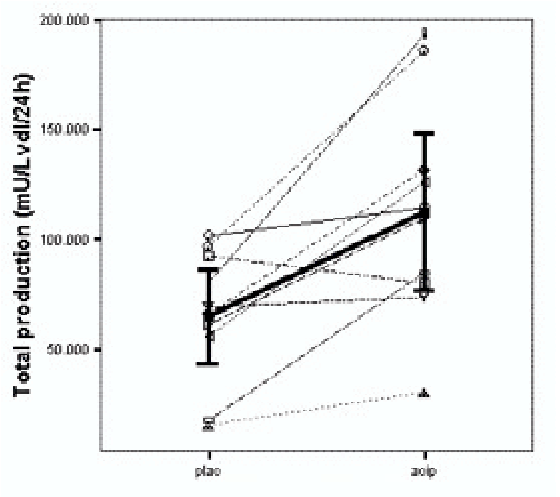
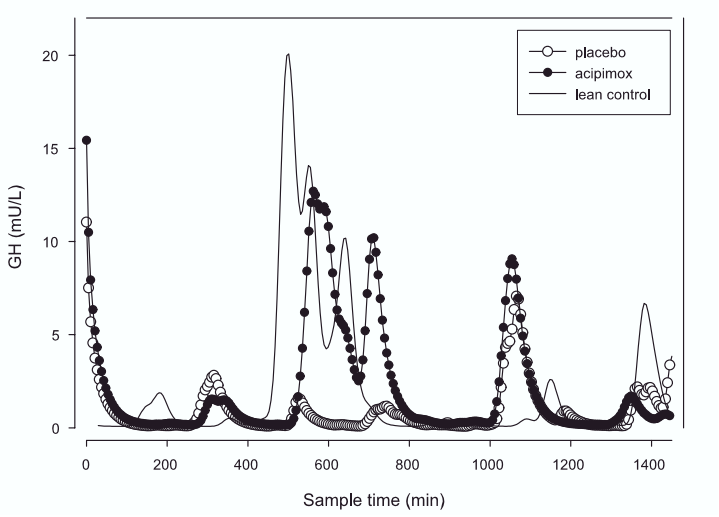


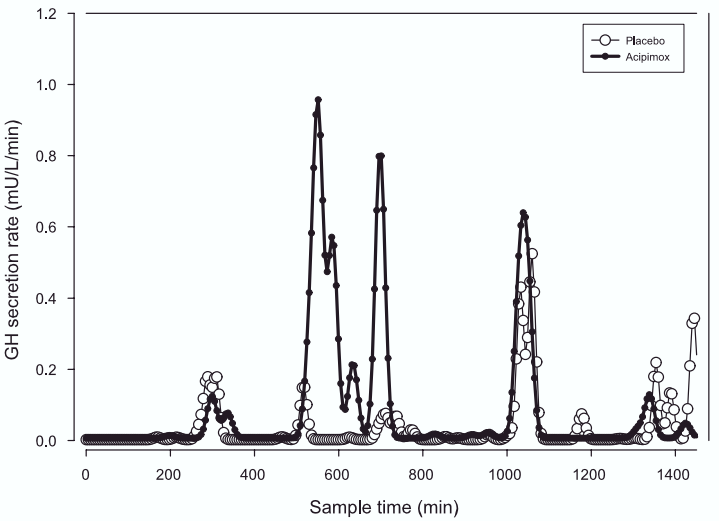
Figure 2.

24 h Plasma GH concentrations and corresponding secretion rates in 2 representative subjects.

A) Example of plasma GH concentration of one obese subject on placebo or Acipimox treatment and a lean control (age 36 resp. 34 yr)



B) Corresponding secretion rate profiles before and during Acipimox treatment in the obese woman



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