Acipimox enhances spontaneous growth hormone secretion in obese women

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Kok, Petra, Madelon M. Buijs, Simon W. Kok, Inge H. A. P. van Ierssel, Marijke Frölich, Ferdinand Roelfsema, Peter J. Voshol, A. Edo Meinders, and Hanno Pijl. Acipimox enhances spontaneous growth hormone secretion in obese women. Am J Physiol Regul Integr Comp Physiol 286: R693–R698, 2004.—We hypothesized that a high circulating free fatty acid (FFA) concentration is involved in the pathogenesis of hyposomatotropism associated with obesity. To evaluate this hypothesis, 10 healthy premenopausal women (body mass index 33.8 ± 1.0 kg/m2) were studied in the follicular phase of their menstrual cycle at two occasions with a time interval of at least 8 wk, where body weight remained stable. Subjects were randomly assigned to treatment with either acipimox (an inhibitor of lipolysis, 250 mg orally 4 times daily) or placebo in a double-blind crossover design, starting 1 day before admission until the end of the blood sampling period. Blood samples were taken during 24 h with a sampling interval of 10 min for assessment of growth hormone (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·l−1·24 h−1, P = 0.005, where lVd is liter of distribution volume). Acipimox considerably enhanced total (113 ± 50 vs. 66 ± 10 mU·l−1·24 h−1, P = 0.02) and pulsatile GH secretion (109 ± 49 vs. 62 ± 30 mU·l−1·24 h−1, P = 0.02), but GH output remained lower compared with 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 unchains 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.

hyposomatotropism; growth hormone kinetics; somatotroph cell function; free fatty acid

SPONTANEOUS PULSATILE growth hormone (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 antilipolytic 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 hematologic, 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 nonalcoholic, nonsmoking, 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 (3). Visceral and subcutaneous adipose tissue areas were assessed by magnetic resonance imaging (MRI) as described before.

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(24), using a multislice fast spin echo sequence (Gyrosan-T5 whole body scanner, 0.5 T, Philips Medical Systems, Best, The Netherlands). MRI images were analyzed independently by two observers.

Drugs

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, 1300, 1900, and 0100 starting the day before admission until the end of the blood sampling period.

Diet

To limit confounding by nutritional factors, a dietitian 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, lunch at 1300, and dinner at 1900, 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 setup 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 with an interval of at least 8 wk. A cannula for blood sampling was inserted into an antecubital vein, and blood samples for basal parameters were withdrawn. The cannula was attached to a three-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 1800, and blood was collected at 10-min intervals for determination of GH concentrations. Plasma FFA levels were measured every 6 h. Plasma FFA concentrations were not measured in the historical control group. Subjects remained recumbent, except for bathroom visits. Lights were switched off at 2300. Vital signs were recorded at regular time intervals. The clinical setup 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 4,000 rpm at 4°C during 20 min, 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 fluoroimmunoassay (Wallac, Turku, Finland) specific for the 22-kDa GH protein. The assay uses recombinant human GH as standard (Genotropin, Pharmacia and Upjohn, Uppsala, Sweden), which is calibrated against World Health Organization 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 CVs of 2.0–9.9%.

The total serum insulin-like growth factor I (IGF-I) concentration was determined by RIA after extraction and purification on ODS-silica columns (Incstar, Stillwater, MN). The interassay CV was <11.8%. The detection limit was 1.5 mmol/l. Age-related normative data were determined in the same laboratory. FFA levels were determined using a NEFA-C FFA kit (Wako Chemicals, Neuss, Germany). The detection limit was 0.03 mmol/l, and the inter- and intra-assay CVs were 1.1 and 2.6%, respectively.

Calculations and Statistics

Deconvolution analysis. Multiparameter 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 multiparameter 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 quantitates 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 intraseries 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 (nonpulsatile) 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 1,000 randomly shuffled versions of the same series (54).

Statistics. TBFM is presented as a percentage of total body weight. Subcutaneous fat mass (SMF) 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 analyzed using one-way ANOVA.

Differences between GH kinetic parameters between lean controls and obese women were analyzed using Student’s t-test for unpaired samples. Multiple regression analysis, using TBFM, VFM, and SMF 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 means ± SE, and significance level was set at 0.05

RESULTS

Subjects

Ten obese women [age 35.8 ± 2.0 yr, body mass index (BMI) 33.8 ± 1.0 kg/m²] and seven lean controls [age 35.1 ± 3.0 yr, BMI 21.5 ± 0.5 kg/m²] were included. Body weight remained stable from 3 mo 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 with the age-matched lean controls. Acipimox treatment significantly increased burst amplitude, burst mass, and 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, is given in Table 1. A graphical illustration of a representative 24-h GH concentration dataset 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/m² and a waist-to-hip ratio of 0.85 ± 0.01 (range 0.75–0.92) and their TBFM (% of total body wt) was 40.6 ± 1.1 (range 36.9–46.3)%. The sizes of their visceral and subcutaneous fat area were 392 ± 30 (range 274–539) cm² and 1,348 ± 58 (range 1,162–1,709) cm², 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.

ApEn

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. GH-releasing hormone (GHRH) input is a critical determinant of GH secretory burst mass, whereas other components of the somatotrophic 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 feedforward inputs through lowering of circulating (cis-unsaturated) FFA and thereby specifi
cally 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.

In conclusion, the present data show that acipimox acutely

Table 1. Effect of acipimox on 24-h GH secretory parameters in obese subjects

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo</th>
<th>Acipimox</th>
<th>P value†</th>
<th>Controls (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak frequency, number/24 h</td>
<td>23±2*</td>
<td>23±2*</td>
<td>0.97</td>
<td>16±2</td>
</tr>
<tr>
<td>Half-life, min</td>
<td>16.5±0.7</td>
<td>17.1±0.6*</td>
<td>0.42</td>
<td>14.7±0.7</td>
</tr>
<tr>
<td>Secretory half-duration, min</td>
<td>19.7±1.7*</td>
<td>22.1±1.7</td>
<td>0.06</td>
<td>26.8±1.9</td>
</tr>
<tr>
<td>Peak amplitude, mU/lVd</td>
<td>0.13±0.02*</td>
<td>0.20±0.02*</td>
<td>0.03</td>
<td>0.46±0.06</td>
</tr>
<tr>
<td>Burst mass, mU/lVd·peak⁻¹</td>
<td>2.7±0.4*</td>
<td>4.7±0.6*</td>
<td>0.008</td>
<td>13.3±2.1</td>
</tr>
<tr>
<td>Basal production, mU/lVd·24 h⁻¹</td>
<td>3±0.1*</td>
<td>5±1.2*</td>
<td>0.17</td>
<td>12±2.3</td>
</tr>
<tr>
<td>Pulse production, mU/lVd·24 h⁻¹</td>
<td>62±10*</td>
<td>109±15*</td>
<td>0.005</td>
<td>190±23</td>
</tr>
<tr>
<td>Total production, mU/lVd·24 h⁻¹</td>
<td>66±10*</td>
<td>113±16*</td>
<td>0.005</td>
<td>201±23</td>
</tr>
<tr>
<td>AptEn 24-h GH concentration</td>
<td>0.43±0.02</td>
<td>0.39±0.03</td>
<td>0.41</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>Mean 24-h IGF-I, nmol/l</td>
<td>17.9±0.8</td>
<td>18.9±1.2</td>
<td>0.27</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. GH, growth hormone; AptEn, approximate entropy; IGF-I, insulin-like growth factor I; lVd, liter of distribution volume; ND, not determined. *P < 0.05 vs. lean controls, using independent Student’s t-test. †P value, placebo vs. acipimox by 1-way ANOVA.
REFERENCES


