Increased circadian prolactin release is blunted after body weight loss in obese premenopausal women

Petra Kok, Ferdinand Roelfsema, Janneke G. Langendonk, Caroline C. de Wit, Marijke Frölich, Jacobus Burggraaf, A. Edo Meinders, and Hanno Pijl

Departments of 1General Internal Medicine, 2Endocrinology and Metabolic Diseases, and 3Clinical Chemistry, Leiden University Medical Center, and 4Center for Human Drug Research, Leiden University Medical Center, Leiden, The Netherlands

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WE RECENTLY SHOWED that spontaneous diurnal prolactin (PRL) secretion is considerably enhanced in proportion to the size of the visceral fat mass in obese premenopausal women. Am J Physiol Endocrinol Metab 290: E218–E224, 2006. First published September 6, 2005; doi:10.1152/ajpendo.00156.2005.—We recently showed that prolactin (PRL) release is considerably enhanced in obese women in proportion to the size of their visceral fat mass. PRL release is inhibited by dopamine 2 receptor (D2R) activation, and dietary restriction/weight loss are associated with increased dopaminergic signaling in animals. Therefore, we hypothesized that enhanced PRL release in obese humans would be reversed by weight loss. To evaluate this postulate, we measured 24-h plasma PRL concentrations at 10-min intervals in 21 obese premenopausal women (BMI 33.3 ± 0.7 kg/m²) before and after weight loss (50% reduction of overweight/50% of overweight). We speculate that amelioration of deficit D2R-mediated neurotransmission and/or diminutions of circulating leptin/estrogen levels might be involved in the physiology of this phenomenon.

caloric restriction; estrogen; dopamine; leptin

WE RECENTLY SHOWED that spontaneous diurnal prolactin (PRL) secretion is considerably enhanced in proportion to the size of the visceral fat mass in obese premenopausal women compared with lean controls of similar age and sex (19). Because PRL has been reported to possess potent lipogenic and diabetogenic effects (for review see Ref. 3), hyperprolactinemia may modulate glucose and lipid metabolism to promote fat accrual in obese humans.

Although dietary restriction is consistently associated with low circulating plasma PRL concentrations in several animal species (10), the results of studies evaluating the effects of caloric restriction and body weight loss on plasma PRL concentrations in humans have been contradictory. Indeed, some studies suggest that the serum PRL response to TRH injection is blunted after a 4-wk period of caloric restriction (320 kcal/day) or a 36-h fast in obese subjects (20, 34), whereas others found no impact of a 3- to 9-wk period of total fasting on TRH-induced PRL release in seven hospitalized obese males (5a). Finally, prolonged fasting (no caloric intake) for 12 days significantly increased hourly integrated (spontaneous) PRL concentrations in six obese women compared with normal controls (6 women, 1 man) (8), whereas no changes in basal serum PRL levels were found during calorie restriction in another study of obese females (20). As far as we are aware, the effect of body weight loss per se on spontaneous PRL release, as calculated by deconvolution analysis from frequently sampled plasma hormone time series data, has not been quantified in obese humans before.

PRL synthesis and secretion is inhibited by dopamine (DA) through dopamine 2 receptor (D2R) activation at the lactotroph cell membrane (1). Studies in rats showed that caloric restriction increases hypothalamic DA levels (13) and retards age-associated loss of central dopamine receptors (22). Furthermore, it has been reported that obese humans are refractory to stimulation of PRL release by metoclopramide (MET), which normally increases PRL release by blockade of the D2R at the pituitary level, whereas short-term fasting increased the MET-induced PRL response (28). These data suggest that food restriction and body weight loss restore central dopaminergic tone in obese humans, at least to a certain extent. Therefore, we hypothesized that spontaneous PRL release would be reduced after weight loss in obese individuals. To test this postulate, we evaluated 24-h plasma PRL concentrations, measured at 10-min intervals, in 11 obese premenopausal women before and after 50% reduction of their overweight (15% absolute weight loss) by means of a very low-calorie diet (500 kcal/day).

SUBJECTS AND METHODS

Subjects

Subjects’ characteristics. Eleven healthy obese premenopausal women (BMI 33.1 ± 1.2 kg/m²) were enrolled in the study, after given written acknowledgement of informed consent for participation. A historical control group of 10 lean controls (BMI 21.4 ± 0.8 kg/m², P < 0.05 vs. obese) of similar sex and age (obese 35.8 ± 2.3 vs. lean 36.7 ± 2.4 yr, P = 0.80) was included for comparison of PRL secretion data with those in the obese women after weight loss.

Address for reprint requests and other correspondence: H. Pijl, Leiden Univ. Medical Center, Dept. of Internal Medicine (C4-83), PO Box 9600, 2300 RC Leiden, The Netherlands (e-mail: h.pijl@lumc.nl).

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Plasma leptin concentrations were determined by RIA (Linco Research, St. Charles, MO) with a detection limit of 0.5 μg/l, and the interassay coefficient ranged from 6 to 7%. Basal free thyroxine (T4) concentrations were measured using an automated system (Elecsys 2010; Roche Diagnostics Nederland, Almere, Netherlands) with a detection limit of 2 pmol/l, and the interassay coefficient ranged from 3.8 to 5.6%. Estrogen concentrations were determined by RIA (Orion Diagnostica, Espoo, Finland) with a detection limit of 6 pmol/l and an interassay coefficient of 6.8%.

Calculations

**Cluster.** The Cluster program describes various characteristics of pulsatile hormone concentration profiles (32). A concentration peak is defined as a significant increase in the test peak cluster vs. the test nadir cluster. We used a 2 × 1 cluster configuration (2 samples in the test nadir and 1 in the test peak) and t-statistics of 2.0 for significant up- and downstrokes in PRL levels to constrain the false positive rate of peak identification to less than 5% of signal-free noise. The locations and durations of all significant plasma hormone peaks were identified and the following parameters determined: mean PRL concentration, peak frequency, peak width, mean peak height (maximum concentration attained within the peak), mean peak area (above the baseline), overall mean concentration of the interpeak valley (nadir), and total area under the curve.

**Pulse.** Deconvolution analysis estimates hormone secretion and clearance rates on the basis of hormone concentration time series. The Pulse algorithm is a waveform-independent deconvolution method that can be used for calculation of hormonal secretion without specifying shape, number, and time of secretory events (17). The technique requires an a priori specification of hormonal half-life in plasma. PRL disappearance from plasma is best described by a two-compartment model, characterized by a fast-component half-life of 18.4 min and a slow-component half-life of 139 min where the fractional contribution of the slow component to the overall decay amounts to 49.5% (29). Pulse was used to quantify mean 24-h PRL secretion. Secretion rates were calculated per liter of distribution volume.

**Approximate entropy.** Approximate entropy (ApEn) is a scale- and model-independent statistic that assigns a nonnegative number to time series data, reflecting regularity of these data (27). Higher ApEn values denote greater relative randomness of hormone profiles. Normalized ApEn parameters of m = 1 (test range), r = 20% (threshold), and 1,000 for the number of runs were used, as described previously (15). Hence, this member of the ApEn family is designated (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 deconvolution (Pulse) analyses (33). Data are presented as normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1,000 randomly shuffled versions of the same series. ApEn ratios close to 1.0 express highly irregular (maximum randomness) secretory patterns.

**Circadian rhythmicity.** Nyctohemeral characteristics of PRL concentration patterns were determined using a robust curve-fitting algorithm [LOWESS analysis, SYSTAT v. 11; Systat, Richmond, CA (5, 7)]. The acrophase is the clock time at which the fitted PRL concentration is maximal. The amplitude of the rhythm was defined as one-half the difference of the nocturnal zenith (maximum) and the daytime nadir (minimum). The relative amplitude was the maximal percentage increase of the mesor value.

**Statistics**

Data are presented as means ± SE unless otherwise specified. The means of PRL concentration and secretion parameters in obese subjects before and after weight loss were statistically analyzed using the nonparametric Wilcoxon signed rank test. Means of PRL secretion and concentration parameters between groups (obese vs. lean) were compared using the nonparametric Mann Whitney U-test. Nonpara-
metric tests were used because the distribution of data was not normal. Significance level was set at 0.05. Regression analysis was used to determine the correlation between differences of 24-h PRL secretion (before and after weight loss) and changes of body composition parameters in obese subjects. Multiple regression analysis, using body weight, BMI, percent total body fat, visceral and subcutaneous fat areas, and mean 24-h leptin concentrations as independent variables, was performed to estimate the correlation between differences of 24-h PRL secretion vs. mean 24-h leptin concentrations and different features of body composition in the obese subjects.

RESULTS

Subjects

Both obese and lean historical subjects were studied in the early follicular phase of their menstrual cycle [estrogen (E2) levels: obese before weight loss 203 ± 22, lean 190 ± 82 pmol/l, P = 0.84]. All subjects were clinically euthyroid (free T3) levels: obese before weight loss (15.1 ± 0.5; lean 16.5 ± 0.6 pmol/l, P = 0.10). Body composition parameters and baseline serum measurements were obtained at each study occasion in the obese subjects. BMI, percentage of total body fat, and sizes of visceral and subcutaneous fat areas were significantly reduced at the end of the weight loss period. No significant loss of lean body mass was seen at the end of the weight loss period. An overview of the subjects’ characteristics and baseline serum measurements is given in Table 1.

PRL Concentration and Secretion Parameters

An overview of PRL concentration and secretion parameters is shown in Table 2. Different characteristics of 24-h PRL hormone concentration profiles were determined using Cluster. Mean 24-h PRL concentration, mean peak amplitude (maximum concentration attained within the peak), peak area, and interpeak valley (nadir) were significantly lower, whereas peak frequency and peak width were unaltered in obese subjects after weight loss. Pulse analysis revealed that mean 24-h PRL secretion was significantly reduced by weight loss in the obese women (weight loss before 128 ± 24 vs. after 110 ± 17 µg/liter distribution volume–1 × 24 h, P = 0.04). After weight loss, all PRL concentration and secretion parameters remained significantly enhanced in the obese women compared with those obtained in the lean historical controls. A graphic illustration of the mean 24-h plasma PRL concentrations in the obese subjects before and after weight loss and those in age-matched lean historical controls vs. clock time is presented in Fig. 1. The mean 24-h PRL secretion in obese women before and after weight loss and in lean controls is shown in Fig. 2.

Regularity of Plasma PRL Concentration Time Series

ApEn ratios of PRL concentration time series data were significantly affected by weight loss in the obese subjects (before 0.46 ± 0.05 vs. after weight loss 0.50 ± 0.05 respectively, P = 0.01) and were similar after weight loss in the obese and normal weight premenopausal women (lean 0.50 ± 0.05, P = 0.97 vs. obese).

Differences of 24-h PRL Secretion and Body Composition

Pearson’s correlations between differences in PRL secretion before and after weight loss vs. differences of body fat mass and distribution were estimated in the obese subjects only. PRL secretion was not related to body composition parameters before body weight loss in the obese women. Obese subjects had mean changes of body weight of 13.5 ± 1.7 (4.6–25.2) kg; BMI of 4.8 ± 0.6 (1.4–8.4) kg/m2; percent total body fat of 6.9 ± 0.9 (2.9–14.1)%; visceral fat area of 174 ± 29 (94–358) cm2.

Table 2. PRL concentration parameters and secretion rates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Obese (n = 11)</th>
<th>Before Weight Loss</th>
<th>After Weight Loss</th>
<th>P Value*</th>
<th>Controls (n = 10)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 24-h concentration, µg/l</td>
<td>10.0±1.8</td>
<td>8.6±1.3</td>
<td>0.03</td>
<td>5.1±0.5</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>No. of pulses, n/24 h</td>
<td>11±1</td>
<td>12±1</td>
<td>0.69</td>
<td>17±1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Pulse width, min</td>
<td>83±7</td>
<td>80±5</td>
<td>0.72</td>
<td>56±4</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Pulse amplitude, µg/l</td>
<td>12.2±2.2</td>
<td>10.2±1.4</td>
<td>0.01</td>
<td>5.8±0.5</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Pulse area, µg/l × min</td>
<td>230±28</td>
<td>221±31</td>
<td>0.03</td>
<td>91±13</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Nadir concentration, µg/l</td>
<td>8.4±1.8</td>
<td>7.8±1.1</td>
<td>0.05</td>
<td>4.0±0.4</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Total area, µg/l × 24 h</td>
<td>14,474 ± 2,666</td>
<td>12,354 ± 190</td>
<td>0.02</td>
<td>7,276 ± 644</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Mean 24-h secretion, µg/Vdl × 24 h</td>
<td>128±24</td>
<td>110±17</td>
<td>0.04</td>
<td>67±6</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>ApEn ratios</td>
<td>0.46±0.05</td>
<td>0.50±0.05</td>
<td>0.01</td>
<td>0.50±0.05</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. ApEn, approximate entropy. Concentration parameters were calculated from 24-h prolactin (PRL) concentration profiles using Cluster analysis. Mean PRL secretion was calculated from 24-h PRL concentration profiles by use of the Pulse algorithm, which is a waveform-independent deconvolution method. Secretion rates are calculated per liter distribution volume (Vdl). *P values were determined by nonparametric Wilcoxon signed rank test, before vs. after weight loss in obese women. †P values were determined by nonparametric Mann Whitney U-test, obese after weight loss vs. lean historical women (19).
cm²; and subcutaneous fat area of 697 ± 90 (214–1,332) cm². Univariate analysis, including differences of body weight, BMI, percent total body fat, and visceral and subcutaneous fat areas as independent variables, revealed that there was a positive (but not significant) correlation between body weight, BMI, percent total body fat, and subcutaneous fat area but not visceral fat area vs. differences in PRL secretion rates before and after weight loss (Table 3).

Leptin and 24-h PRL Secretion

Mean 24-h leptin concentrations were significantly reduced in the obese subjects after weight loss (before vs. after weight loss: 37.4 ± 6.7 vs. 19.7 ± 4.0 µg/l, P < 0.01), but values were still significantly higher than those lean controls (lean 12.8 ± 2.5 µg/l, P < 0.01 vs. obese). Multiple regression analysis, including body weight, BMI, percent total body fat, and mean 24-h leptin and E2 concentrations as independent variables, revealed that differences in 24-h PRL secretion were significantly positively correlated with differences in mean 24-h leptin concentrations (r² = 0.61, P < 0.01; Fig. 3), body weight change (r² = 0.34, P = 0.01), and BMI (r² = 0.31, P = 0.02).

Diurnal Variation in 24-h PRL Concentration Profiles

Analysis of the diurnal variation in plasma PRL concentrations revealed that the acrophase of the nyctohemeral PRL rhythm occurred at night at similar clock times before and after weight loss in obese subjects (obese before 0400 ± 15 min and after 0430 ± 16 min, respectively, P = 0.46), and the time points of the acrophase before and after weight loss in the obese subjects were not significantly different from the lean subjects (0530 ± 1 h 16 min). The mesor (before vs. after: 11.7 ± 1.9 vs. 9.9 ± 1.3 µg/l, respectively, P = 0.03) as well as the amplitude (before vs. after weight loss: 5.4 ± 1.0 vs. 4.4 ± 0.8 µg/l, respectively, P = 0.01) of the rhythm were significantly decreased after weight reduction in obese sub-

Fig. 1. Mean serum prolactin (PRL) concentration time series of the obese subjects before and after weight loss and mean serum PRL concentration time series of the historical control subjects. Data reflect sampling of blood every 10 min for 24 h. Sampling started at 0900. Lights were switched off, and subjects went to sleep (lights off) at 2300 until 0730 next morning (horizontal gray bar). Sleep was not interrupted.

Fig. 2. Diurnal PRL secretion in obese women before and after weight loss and in lean historical controls. Error bars of the box plot represent SE. VdI, liter distribution volume. *P < 0.05, before vs. after weight loss obese women. Statistical analysis was performed using nonparametric Wilcoxon signed rank test. #P < 0.05, obese vs. lean historical women (19). Statistical analysis was performed using nonparametric Mann Whitney U-test.
Table 3. Correlations between differences of 24-h PRL secretion (before and after weight loss) and changes of body composition parameters in obese subjects

<table>
<thead>
<tr>
<th>Parameter (A before-after weight loss)</th>
<th>Δ24-h PRL secretion (A before-after weight loss)</th>
<th>r²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔBody weight, kg</td>
<td>0.34</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>ΔBMI, kg/m²</td>
<td>0.31</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>ΔPercent total body fat, %</td>
<td>0.55</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>ΔSubcutaneous fat area, cm²</td>
<td>0.33</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>ΔVisceral fat area, cm²</td>
<td>0.04</td>
<td>0.57</td>
<td></td>
</tr>
</tbody>
</table>

Pearson’s correlation analysis was used to determine the association between differences of 24-h PRL secretion (before and after weight loss) and changes of body composition parameters in the obese subjects. Percentage is the calculated fraction of total body weight. Parameter was negatively correlated with Δ24-h PRL secretion.

DISCUSSION

The present study shows that elevated PRL secretion rates in obese women are significantly reduced after loss of 50% of overweight (15% absolute weight loss). Body weight loss particularly blunted PRL secretory burst mass, whereas burst frequency remained unaffected. However, PRL secretion remained significantly higher than that in normal-weight controls.

Only a few previous clinical studies evaluated the effect of caloric restriction and weight loss on PRL secretion in obese humans, and conflicting results have been reported (5a, 8, 20, 34). Although some studies suggest that TRH-induced PRL release is not affected by severe caloric restriction, most studies show that the incremental peak of serum PRL in response to TRH is significantly reduced after a 4-wk period of caloric restriction (20, 34), which is in line with the results of the present study. Furthermore, low circulating PRL concentrations were found in food-restricted animals (10), which also corroborates our data. To our knowledge, this is the first study to evaluate the effect of body weight loss (and not the effect of the severe caloric restriction, as the obese women were studied after using a balanced eucaloric diet for 3 days after the weight loss period) per se on diurnal spontaneous PRL secretion rates (as estimated by deconvolution analysis) in obese humans.

Dopamine is the major inhibitor of PRL synthesis and secretion (1), and D2R expression is diminished in hypothalamic nuclei of obese Zucker rats (10a) and in the striatum of obese humans (35). Dietary restriction and weight loss are accompanied by increased dopaminergic signaling in animals (13, 22), and indirect evidence suggests that calorie restriction also reinforces central dopaminergic tone in obese humans (28). Although dopaminergic neuronal activity was not assessed directly in the present study, it is conceivable that body weight loss enhanced D2R-mediated neurotransmission to reduce diurnal PRL secretion rates in our obese subjects. Alternatively, other physiological cues, such as leptin or E2, might have changed PRL secretion after body weight loss in the present study. Exogenous estrogens raise basal serum PRL levels (11, 37), and estrogens enhance PRL release in response to several exogenous stimuli (3, 18). E2 concentrations were significantly reduced after reduction of overweight in the present study, a finding that has been reported previously by other authors (24, 30). However, changes of 24-h PRL secretion in response to weight loss were not related to the decrease of plasma E2 concentrations. Leptin is one of the various other cues apparently modulating PRL secretion. Leptin administration restores lactation in leptin-deficient ob/ob mice (6), and leptin infusion raises plasma PRL concentrations in fasted rats to levels similar to those in fed littermates (36). These findings suggest that leptin plays a role in the control of PRL release. Indeed, a direct stimulatory effect of leptin on PRL secretion has been observed in vitro in anterior pituitary cells (31, 38) and in vivo in rats (14, 16). The reduction of 24-h PRL secretion in response to weight loss in the present study was
closely associated with the mean decrease of plasma leptin concentrations, which supports the thesis that both phenomena are related. Thus the diminution of PRL secretion in response to weight loss may be due to changes in leptin and/or E2 levels.

Obesity predisposes to the metabolic syndrome, which is a major risk factor for cardiovascular disease and type 2 diabetes mellitus. A plethora of data from animal and clinical studies suggests that reduced dopaminergic neurotransmission is involved in the pathogenesis of syndrome X. Furthermore, treatment with D2R antagonists induces obesity and type 2 diabetes mellitus, whereas D2R activation ameliorates the metabolic profile in obese nondiabetic and diabetic humans (for review see Ref. 26). Calorie restriction and weight loss tend to restore the metabolic profile to normal in obese individuals (9). In a variety of animal species, PRL exerts potent lipogenic and diabetogenic effects. For example, PRL injections promote body fat storage in rats and birds, and PRL stimulates lipoprotein lipase activity both in the liver in rats and in adipose tissue in birds. Furthermore, PRL activates glycogen phosphorylase-a in hepatocytes and directly stimulates insulin release by the pancreas, thereby affecting carbohydrate metabolism (for review see Ref. 3). The data presented here support the notion that the beneficial effect of weight loss on metabolic parameters in obese individuals may be brought about by amelioration of deficit D2R-mediated dopaminergic transmission in hypothalamic nuclei and that PRL serves as a messenger mediating the favorable effects of dopamine on glucose and lipid metabolism in peripheral tissues. We did not measure the effect of weight loss on metabolic parameters (i.e., oral glucose tolerance test, stimulated area under the insulin curve, and androgen levels) and dopaminergic tone in the present study. Thus it clearly requires further investigation to test this postulate. For example, imaging studies assessing D2R availability in the brain of obese humans before and after weight loss are needed, and the impact of D2R antagonism on the metabolic benefits and PRL secretion rate in response to weight loss must be determined.

PRL levels remained higher after weight loss in the obese women compared with normal controls. Our study design did not allow for a definitive conclusion as to why this is so. Obese subjects may have intrinsic regulatory cues promoting PRL release that are at least partly independent of their weight. Alternatively, PRL levels remained higher because our subjects’ body weights did not completely normalize in response to calorie restriction.

It is important to note that all the obese subjects took a standard liquid eucaloric diet for 3 days before each study occasion to “wash out” any potential confounding effect of calorie restriction per se on the PRL secretion rate. Although it is unclear from the literature how long a washout period needs to be exactly to achieve that goal, the secretion rate and/or plasma concentration of various other hormones responds rather quickly (i.e., within hours to days) to changes in nutrient availability (2, 16a). Therefore it is reasonable to assume that the decline of PRL levels we report here is due to weight loss and not to calorie restriction.

In conclusion, body weight loss partly reverses elevated PRL secretion in obese women. Amelioration of deficit D2R dopaminergic transmission and/or reduction of circulating leptin and estrogen levels may all be involved in the physiology of this phenomenon.

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