Neuroendocrine perturbations in human obesity

Kok, P.

Chapter 3

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, Hanno Pijl

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Abstract

We recently showed that 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 is associated with increased dopaminergic signalling 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 eleven obese premenopausal women (BMI 33.3 ± 0.7 kg/m^2) before and after weight loss (50% reduction of overweight/15% absolute weight loss, using a very low calorie diet) in the follicular phase of their menstrual cycle.

The 24 h PRL concentration profiles were analysed by a peak detection program (Cluster) and a waveform-independent deconvolution technique (Pulse). Spontaneous 24 h PRL secretion was significantly reduced in obese women (mean daily release before 128 ± 24 vs. after weight loss 110 ± 17 µg/V$_{dl}$ x 24 h, P = 0.05). Body weight loss particularly blunted PRL secretory burst mass (Pulse area before 230 ± 28 vs. after weight loss 221 ± 31 µg/V$_{dl}$ x min, P = 0.03), whereas burst frequency was unaffected (Number of pulses before 11 ± 1 vs. after weight loss 12 ± 1 n/24 h, P = 0.69). Thus, elevated PRL secretion rate in obese women is significantly reduced after loss of 50% of overweight. We speculate that amelioration of deficit dopamine D2 receptor mediated neurotransmission and/or diminutions of circulating leptin/estrogen levels might be involved in the physiology of this phenomenon.

Introduction

We recently showed that spontaneous diurnal PRL secretion is considerably enhanced in proportion to the size of the visceral fat mass in obese premenopausal women compared to lean controls of similar age and sex (19). Since prolactin (PRL) has been reported to possess potent lipogenic and diabetogenic effects (for review see (2)), 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 four week period of caloric restriction (320 kCal/day) or a 36 hour fast in obese subjects (20; 34), whereas others found no impact of a 3-9 week period of total fasting on TRH induced PRL release in seven hospitalized obese males (5). Finally, prolonged fasting (no caloric intake) during twelve days significantly increased hourly integrated (spontaneous) PRL concentrations in six obese women compared to normal controls (six women, one man)(8), whereas no changes in basal serum PRL levels were found during caloric 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 never been quantified in obese humans before.

PRL synthesis and secretion is inhibited by dopamine (DA) through dopamine 2 receptor (D2R) activation at the lactotoroph 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 dopamine

2 receptor 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 eleven 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

11 healthy obese premenopausal women (BMI 33.1 ± 1.2 kg/m^2) 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/m^2, $P < 0.05$ 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 (published data ref (19)). All subjects underwent medical screening, including medical history, physical examination, standard laboratory haematology, blood chemistry and urine tests. Acute or chronic disease, smoking, recent transmeridional flights, night-shift work, weight change prior to the study (> 3 kg in 3 months) and use of medication were exclusion criteria for participation. All participants were required to have regular menstrual cycles and not using oral contraceptives.

Body fat distribution

Specific body fat measurements were obtained in the obese subjects before and after weight loss. Total body fat mass was quantified using bioelectrical impedance analysis (Bodystat 1500, Bodystat Ltd., UK) and was expressed as a percentage of total body weight (23). Visceral and subcutaneous adipose tissue areas were assessed by MRI as described before, using a multi slice fast spin echo sequence (Gyro scan –T5 whole body scanner 0.5 Tesla, Philips Medical Systems, Best, The Netherlands)(21).

Weight Loss Program

Obese subjects were prescribed a liquid very low calorie diet (2MJ/day; 43% proteins, 15% fat, 42% carbohydrates; Modifast, Novartis, Veenendaal, The Netherlands) after the first study occasion, in order to reduce 50% of their overweight. Ideal body weight for height was determined according to the Metropolitan Life Insurance tables (1983). The subjects were instructed to keep their physical activity level constant. All subjects weekly visited the research center for medical screening (medical examination and blood chemistry tests if necessary) by the research physician. Obese subjects reduced their overweight within a mean time period of 4 months.

Clinical Protocol

The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center. All subjects were studied in the early follicular stage of their menstrual cycle. Identical methodology was used to study spontaneous 24 h PRL secretion in obese and normal weight women. Obese women were studied twice, before and after body weight loss. All subjects used a standard eucalorie diet (1980 kCal/8.3 MJ per day), consisting of Nutridrink (Nutricia, Zoetermeer, The Netherlands) and Modifast, three days prior to each admission until the end of the blood sampling period. Subjects were admitted to the research center at 0800 h, after an overnight fast. A 20-gauge cannula for blood sampling was inserted into an antecubital vein. The cannula was attached to a constant withdrawal pump (Conflo, Carmeda AB, Taeby, Sweden) and tubes were switched every 10 minutes. The iv cannula was kept patent by a continuous 0.9% NaCl and heparin (1 U/ml) infusion (500ml/24 h). One hour after insertion of the iv cannula, blood sampling started. Each tube contained 1.2 ml of blood (totaling 174 ml blood). The plasma prolactin concentration was determined in every 10-minute sample, while leptin levels were measured every 20 minutes. Meals were served according to a fixed time schedule (0930 h breakfast, 1300 h lunch, 1830 h dinner). Vital signs were recorded at regular time intervals. No daytime naps were allowed. Lights were

switched off at 2300 h and switched on at 0730 h and great care was taken not to disturb patients while sampling blood during their sleep (no EEG sleep recording was performed).

Assays

Sampled tubes were immediately chilled on ice. Samples were centrifuged at 4000 r/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. Plasma PRL concentrations were measured with a sensitive time-resolved fluoro immunoassay with a detection limit of 0.04 µg/L (Delfia, Wallac Oy, Turku, Finland). The PRL IFMA was calibrated against the 3rd WHO standard: 84/500, 1 ng/ml = 36 mU/L. The intra-assay coefficient of variation varies from 3.0-5.2% and inter-assay coefficient of variation is 3.4-6.2%, in the concentration range from 0.1-250 µg/L. Plasma leptin concentrations were determined by RIA (Linco Research, St. Charles, MO) with a detection limit of 0.5 µg/L and the inter-assay coefficient ranged from 6-7%. Basal free thyroxine (T_4) concentrations were measured using an automated system (Elecsys 2010, Roche Diagnostics Nederland BV, Almere, Netherlands) with a detection limit of 2 pmol/L and the inter-assay coefficient ranged from 3.8-5.6%. Estrogen concentrations were determined by RIA (Orion Diagnostica, Espoo, Finland) with a detection limit of 6 pmol/L and an inter-assay coefficient of 6.8%.

Calculations and statistics

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 x 1 cluster configuration (2 samples in the test nadir and one 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 were 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 inter peak valley (nadir) and the total area under the curve.

Pulse

Deconvolution analysis estimates hormone secretion and clearance rates based on hormone concentration time-series. The Pulse algorithm is a waveform-independent deconvolution method, which can be used for calculation of hormonal secretion, without specifying shape, number and time of secretory events (17). The technique requires 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 distribution volume.

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 (27). Higher ApEn values denote greater relative randomness of hormone patterns. Normalized ApEn parameters of m = 1 (test range), r = 20% (threshold) and 1000 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 (non pulsatile) 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 1000 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 version 11 Systat Inc, Richmond, CA, (4; 7)). The acrophase is the clock time at which the fitted PRL concentration is maximal. The amplitude of the rhythm was defined as half the difference of the nocturnal zenith (maximum) and the day-time nadir (minimum). The relative amplitude was the maximal percentage increase of the mesor value.

Statistics

Data are presented as means ± SEM, unless otherwise specified. The means of PRL concentration and secretion parameters in obese subjects before and after weight loss were statistically analysed using non- parametric Wilcoxon signed-rank test. Means of PRL secretion and concentration parameters between groups (obese vs. lean) were compared using non-parametric Mann Whitney U- test. Non-parametric 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, percentage 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 and lean 190 ± 82 pmol/L, P = 0.84). All subjects were clinically euthyroid (Free thyroxine (free T_4) levels obese before weight loss 15.1 ± 0.5 and 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 total body fat as well as 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 subject 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 inter peak 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 (before 128 ± 24 vs. after weight loss 110 ± 17 $\mu g/V_{dl}$ x 24 h, P = 0.04). After weight loss, all PRL concentration and secretion parameters remained significantly enhanced in the obese women compared to those obtained in the lean historical controls. A graphical 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 Figure 1. The mean 24 h PRL secretion in obese women before and after weight loss and in lean controls is shown in Figure 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/m^2; percentage total body fat of 6.9 ± 0.9 (2.9-14.1) %; visceral fat area of 174 ± 29 (94-358) cm^2; and subcutaneous fat area of 697 ± 90 (214-1332) cm^2. Univariate analysis, including differences of body weight, BMI, percentage total body fat, visceral and subcutaneous fat areas as independent variables, revealed that there was a positive (but not significant) correlation between delta body weight, BMI, delta percentage total body fat, delta 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 37.4 ± 6.7 vs. after weight loss 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, percentage total body fat and mean 24 h leptin and estrogen concentrations as independent variables, revealed that differences in 24 h PRL secretion were significantly positively correlated to differences in mean 24 h leptin concentrations ($R^2 = 0.61$, $P < 0.01$, Figure 3), body weight change ($R^2 = 0.34$, $P = 0.01$) and BMI ($R^2 = 0.31$, $P = 0.02$).

Diurnal variation 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 h ± 15 min and after 0430 h ± 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 h ± 01 h 16 min). The mesor (before 11.7 ± 1.9 vs. after 9.9 ± 1.3 µg/L respectively, $P = 0.03$) as well as the amplitude (before 5.4 ± 1.0 vs. after weight loss 4.4 ± 0.8 µg/L respectively, $P = 0.01$) of the rhythm were significantly decreased after weight reduction in obese subjects, whereas the relative increase in PRL concentration was not significantly altered after weight loss in the obese women (before 49.5 ± 4.6 vs. after weight loss 46.6 ± 4.9 % respectively, $P = 0.12$).

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 was unaffected. However, PRL secretion remained significantly higher than that in normal weight controls.

Only a few previous clinical studies evaluated the effect of calorie restriction and weight loss on PRL secretion in obese humans and conflicting results have been reported (5; 8; 20; 34). Although some studies suggest that TRH induced PRL release is not affected by severe calorie restriction, most papers show that the incremental peak of serum PRL in response to TRH is significantly reduced after a four week 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 calorie restriction since the obese women were studied after using a balanced eucaloric diet for three 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 and in the striatum of obese humans (35). Dietary restriction and weight loss are accompanied by increased dopaminergic signalling 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 directly assessed 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 estrogen 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). Estrogen concentrations were significantly reduced after reduction of overweight in the present study, a finding which 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 estrogen 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 estrogen levels.

Obesity predisposes to the metabolic syndrome, which is a major risk factor for cardiovascular disease and diabetes mellitus type 2. 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 diabetes mellitus type 2, whereas D2R activation ameliorates the metabolic profile in obese nondiabetic and diabetic humans (for review see (26)). Caloric 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 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 (2)). 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 favourable 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 prolactin secretion rate in response to weight loss must be determined.

PRL levels remained higher after weight loss in the obese women compared to normal controls. Our study design does not allow for definitive conclusion as to why this is. 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 weight did not completely normalize in response to calorie restriction.

It is important to note, that all obese subjects took a standard liquid, eucalorie diet for 3 days prior to 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 wash out period is needed 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. 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.

Tables and Figures

Table 1. Subject characteristics and fasting basal serum measurements

Parameter	Obese (N = 11)		P-value [a]
	Before Weight loss	After Weight loss	
Weight (kg)	92.7 ± 4.1	79.2 ± 3.2	< 0.01
BMI (kg/m^2)	33.1 ± 1.2	28.2 ± 0.8	< 0.01
WHR	0.85 ± 0.03	0.81 ± 0.02	0.03
Lean Body Mass (kg)	53.0 ± 1.6	50.5 ± 1.6	0.08
Body Fat (%)	41.2 ± 1.8	35.1 ± 1.3	< 0.01
Visceral Fat Mass (cm^2)	432 ± 8	258 ± 5	< 0.01
Subcutaneous Fat Mass (cm^2)	2659 ± 18	1961 ± 13	< 0.01
Estrogen (E2) (pmol/L)	203 ± 22	163 ± 25	< 0.01

Data are presented as means ± SEM

a) P-values were determined by non- parametric Wilcoxon signed-rank test, before vs. after weight loss in obese women

Percentage body fat was estimated by bioelectrical impedance analysis and was calculated as a fraction of total body weight. Visceral and subcutaneous fat areas were determined using MRI.

Table 2. PRL concentration parameters and secretion rates

Parameter	Obese (N = 11)		P-value [a]	Controls (N = 10)	P-value [b]
	Before Weight Loss	After Weight Loss			
Mean 24 h concentration (µg/L)	10.0 ± 1.8	8.6 ± 1.3	0.03	5.1 ± 0.5	< 0.01
Number of pulses (n/24 h)	11 ± 1	12 ± 1	0.69	17 ± 1	0.01
Pulse width (min)	83 ± 7	80 ± 5	0.72	56 ± 4	< 0.01
Pulse amplitude (µg/L)	12.2 ± 2.2	10.2 ± 1.4	0.01	5.8 ± 0.5	< 0.01
Pulse area (µg/Lxmin)	230 ± 28	221 ± 31	0.03	91 ± 13	< 0.01
Nadir concentration (µg/L)	8.4 ± 1.8	6.8 ± 1.1	0.05	4.0 ± 0.4	< 0.01
Total Area (µg/Lx24 h)	14 474 ± 2666	12 354 ± 190	0.02	7 276 ± 644	< 0.01
Mean 24 h secretion (µg/V_{dl} x 24 h)	128 ± 24	110 ± 17	0.04	67 ± 6	< 0.01
ApEn ratios	0.46 ± 0.05	0.50 ± 0.05	0.01	0.50 ± 0.05	0.32

Data are presented as means ± SEM.

Concentration parameters were calculated from 24 h PRL concentration profiles using Cluster analysis. Mean PRL secretion was calculated from 24 h PRL concentration profiles using the Pulse algorithm, which is a waveform-independent deconvolution method. Secretion rates are calculated per liter distribution volume (V_{dl}).

a) P-values were determined by non- parametric Wilcoxon signed-rank test, before vs. after weight loss in obese women

b) P-values were determined by non parametric Mann Whitney U- test, obese after weight loss vs. lean historical women (19)

Table 3. Correlations between differences of 24 h PRL secretion (before and after weight loss) and changes of body composition parameters in obese subjects

Obese Subjects (N = 11)	Delta 24 h PRL secretion (Difference Before-After weight loss)	
Parameter (Difference Before-After weight loss)	R-square	P-value
Delta Body Weight (kg)	0.34	0.06
Delta BMI (kg/m^2)	0.31	0.07
Delta Percentage Total Body Fat[1)] (%)	0.55	0.08
Delta Subcutaneous Fat Area (cm^2)	0.33	0.07
Delta Visceral Fat Area[2)] (cm^2)	0.04	0.57

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

1) Percentage is the calculated fraction of the total body weight

2) Parameter was negatively correlated with Δ 24 h PRL secretion

Figure 1.

Mean serum 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 starts at 0900 h. Lights were switched off and subjects went to sleep (lights off) at 2300 h until 0730 h next morning (vertical grey bar). Sleep was not interrupted.

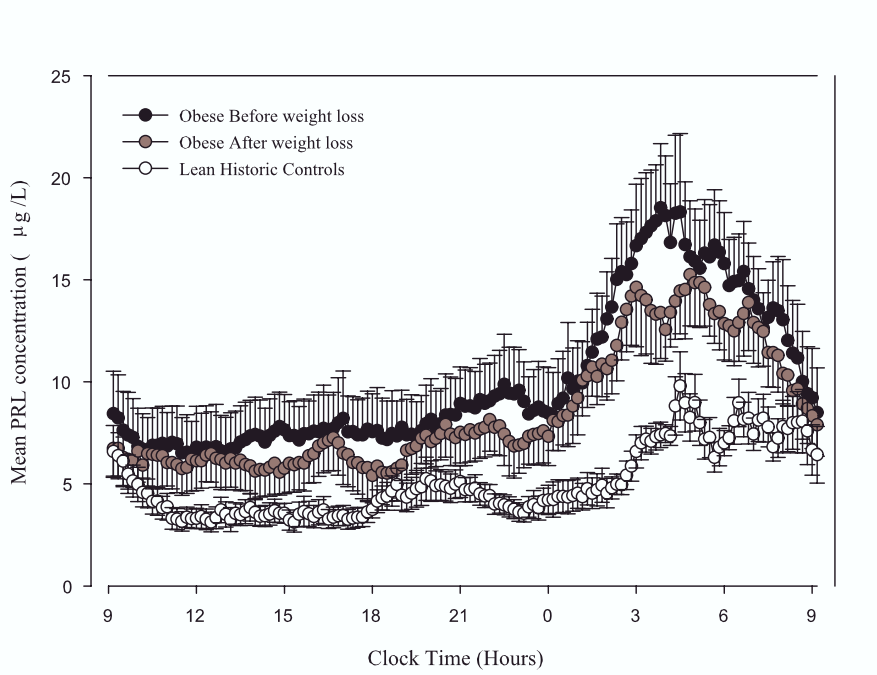


Figure 2.

Diurnal PRL secretion in obese women before (black bars) and after weight loss (grey bars) and in lean historical controls (white bars). Error bars of the box plot represent SEM.

* P < 0.05 Before vs. after weight loss obese women, statistical analysis was performed using non- parametric Wilcoxon signed-rank test

# P < 0.05 Obese vs. lean historical women (19), statistical analysis was performed using non parametric Mann Whitney U- test

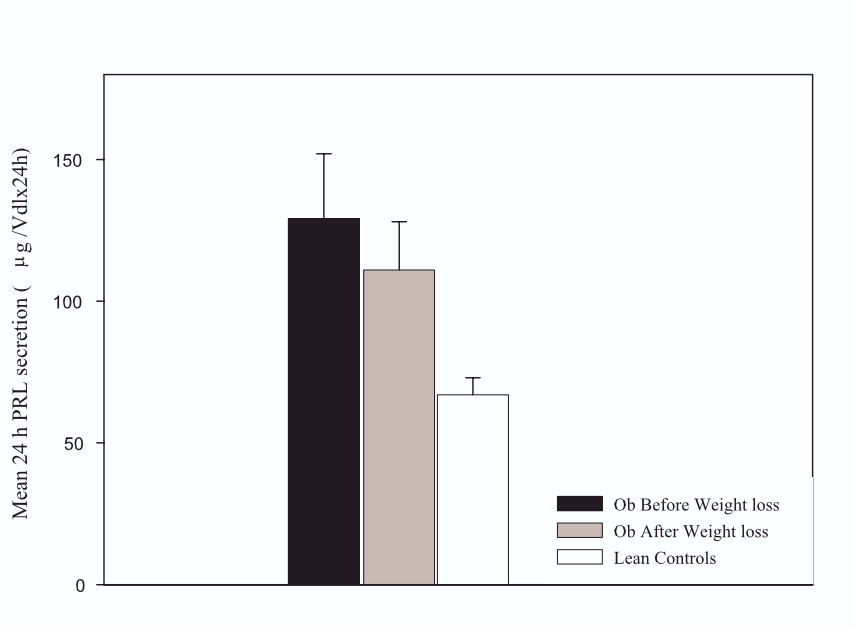
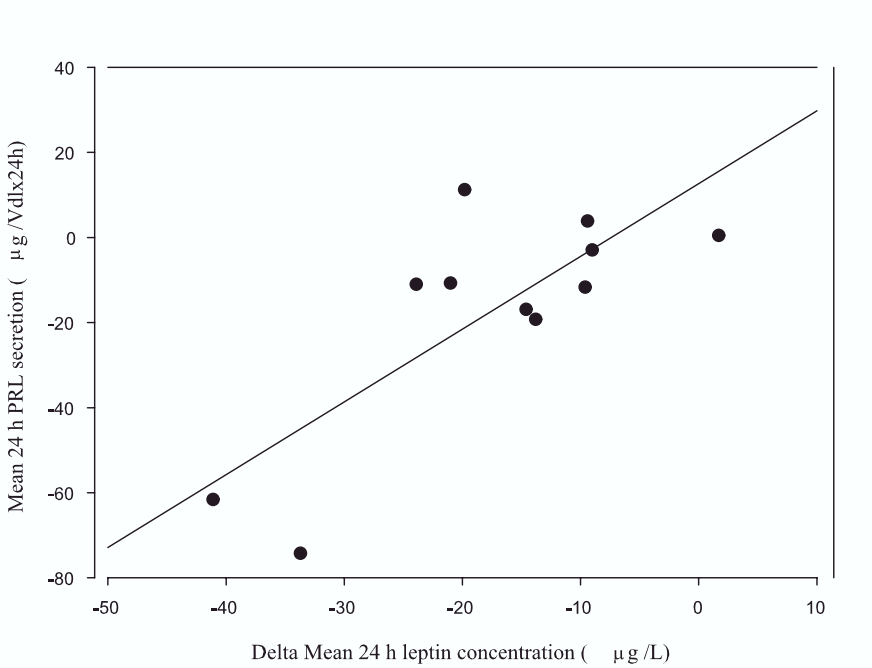


Figure 3.

Correlations between the decrease of 24 h PRL secretion and 24 h leptin concentrations after body weight loss in the obese women. Obese women were included in multiple regression analysis of mean 24 h leptin concentrations and different features of body composition vs. differences of 24 h PRL secretion before and after weight loss. PRL secretion is calculated per liter distribution volume. Differences in PRL secretion were significantly positively related to differences in mean 24 h leptin concentrations ($R^2 = 0.61$, $P < 0.01$).



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