Neuroendocrine perturbations in human obesity

Kok, P.

Chapter 9

Activation of Dopamine D2 Receptors Lowers Circadian Leptin Concentrations in Obese Women

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Abstract

1. Context. Leptin release is regulated by factors other than fat mass alone. Previous observations provide indirect evidence for an inhibitory effect of dopaminergic neurotransmission on leptin secretion. We hypothesized that short term bromocriptine treatment would lower circadian plasma leptin concentrations in obese humans.
2. Objective. To study the acute effects of bromocriptine (a D2R agonist) on circadian leptin levels in obese women, while body weight and caloric intake remained constant.
3. Design. Prospective, single blind, cross-over study (2004).
4. Setting. Clinical Research Center
5. Participants. Eighteen healthy obese women (BMI 33.2 ± 0.6 kg/m^2) were studied twice in the early follicular phase of their menstrual cycle.
6. Intervention(s). Treatment with bromocriptine (B) or placebo (Pl) for eight days
7. Main Outcome Measure(s). Blood was collected during 24 hours at 20-minute intervals for determination of leptin concentrations. Blood samples for the measurements of plasma insulin and glucose concentrations were taken at 10-minute intervals and hourly for the assessment of plasma free fatty acid and triglyceride levels.
8. Results. We here show that short-term treatment with bromocriptine significantly reduces circulating leptin levels in obese women (Pl 33.6 ± 2.5 vs. B 30.5 ± 2.5 µg/L, P = 0.03). FFA concentrations were increased by bromocriptine treatment and the increase of circulating FFA's during bromocriptine treatment was inversely related with the decline of leptin levels. The decline of glucose, insulin or prolactin concentration in response to bromocriptine was not correlated with the reduction of circulating leptin in the present study.
9. Conclusion. Activation of dopamine D2 receptors by bromocriptine lowers circulating leptin levels in obese women, which suggests that dopaminergic neurotransmission is involved in the control of leptin release in humans.

Introduction

Leptin is produced by adipocytes and serves as an endocrine signal to inform the brain about the size of body adipose tissue stores (1-3). Although circulating leptin levels are positively related to fat mass in groups of obese individuals (4), individual concentrations vary considerably for a given measure of adiposity (3). Circulating leptin levels are characterised by diurnal rhythm. The fact that plasma leptin concentrations acutely change in response to fasting (5;6), refeeding (6;7) and increased food intake (7), even without any measurable alteration of body fat content, also supports the contention that leptin release and/or clearance is regulated by factors other than fat mass alone. Indeed, corticosteroids, insulin, prolactin, various cytokines, nutrient flux through adipocytes and the sympathetic nervous system have all been shown to modulate leptin release by adipocytes (8).

In this context, previous observations provide indirect evidence for an inhibitory effect of dopaminergic neurotransmission on leptin secretion. In particular, it has been reported that treatment with bromocriptine, a dopamine D2 receptor agonist, significantly lowers the plasma leptin concentration in a single blood sample of humans with prolactinoma, even without affecting body weight (9). Furthermore, a single iv bolus injection of bromocriptine significantly reduced both basal and

lipopolysaccharide (LPS)-induced leptin release in rats (10). These data led us to hypothesize that short term bromocriptine treatment would lower circadian plasma leptin concentrations in obese humans. To test this postulate, we measured plasma leptin concentrations in obese women who were treated with bromocriptine or placebo for 8 days. Since plasma leptin levels clearly exhibit circadian fluctuation, concentrations were measured over 24 hours. As bromocriptine significantly affected various metabolic and endocrine parameters that may impact on leptin secretion, including circulating insulin, glucose and prolactin levels (Kok et al, to be published in a separate manuscript), we also report the statistical correlation between these parameters and leptin concentrations.

Subjects and Methods

Subjects

Eighteen healthy obese premenopausal women (BMI 30-35 kg/m^2, mean age 37.5 ± 1.7 yr, range 22-51 yr) were enrolled. Before participation, all subjects underwent medical screening, including medical history taking, physical examination, standard laboratory haematology, blood chemistry and urine tests. Acute or chronic disease, depression (present or in history), head trauma, smoking, alcohol abuse, recent trans meridian flights, night-shift work, weight change prior to the study (> 5 kg in 3 months), recent blood donation or participation in another clinical trial (< 3 months) and use of medication (including oral contraceptives) were exclusion criteria for participation. All participants were required to have regular menstrual cycles. All studies were performed in the early follicular phase of the menstrual cycle.

Drugs

Subjects were assigned to bromocriptine or placebo treatment for a period of 8 days in a single (patient) blind crossover design, with a four weeks time interval between each study occasion. To avoid potential crossover effects of bromocriptine treatment, all subjects received placebo during the first intervention period. A dose of 2.5 mg of bromocriptine was prescribed on the first day. Thereafter, drug or placebo was taken twice daily (totalling 5.0 mg daily) at 0800 h and 2000 h for 7 days. The drug was well tolerated, although ten participants had gastro-intestinal complaints (nausea, vomiting) on the first day of bromocriptine treatment only.

Diet

To limit confounding by nutritional factors, all subjects were prescribed a standard eucaloric diet, as from one day prior to admission until the end of each study occasion. The caloric content and macronutrient composition of the diet was exactly the same at both study occasions. Intake of alcohol and caffeine/theine containing beverages were not allowed. Meals were served according to a fixed time schedule (breakfast 0930 h, lunch 1300 h, diner 1830 h) and were consumed within limited time periods. No dietary restrictions were imposed between both study occasions.

Clinical Protocol

The protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center and was performed according the Helsinki declaration. All subjects gave written acknowledgement of informed consent for participation and were admitted to the Clinical Research Unit of the Department of General Internal Medicine in the early follicular stage of their menstrual cycle. Subjects were studied twice with an interval of four weeks, where body weight remained stable and subjects were instructed to keep their physical activity level constant. The clinical set-up was the same during both occasions apart from the subject receiving the alternative treatment (Bromocriptine or placebo). Subjects were admitted to the research center at 0700 h. A cannula for blood sampling was inserted into an antecubital vein, which was attached to a 3-way stopcock and kept patent by a continuous 0.9% NaCl and heparin (1 U/ml) infusion (500ml/24 h). Blood samples for basal parameters were withdrawn and twenty four hour blood sampling started. Blood was collected with S-monovetten (Sarstedt, Etten-Leur, The Netherlands) at 20-minute intervals for determination of leptin concentrations. Blood samples for the measurements of plasma insulin and glucose concentrations were taken at 10-minute intervals and hourly for the assessment

of plasma FFA and TG levels. Subjects remained recumbent during the blood-sampling period, except for bathroom visits (24 h urine was collected). No daytime naps were allowed. Well-being and vital signs were recorded at regular time intervals (hourly). Meals were served according to a fixed time schedule (0930 h breakfast, 1300 h lunch, 1830 h diner) and consumed within limited time periods. Lights were switched off at 2300 h and great care was taken not to disturb and touch subjects during withdrawal of blood samples while they were sleeping. Lights were switched on and subjects were awakened at 0730 h in the morning.

Assays

Samples of each subject were determined in the same assay run. Plasma leptin concentrations were determined by RIA (Linco Research, St. Charles, MO). The detection limit was 0.5 µg/L and the inter-assay coefficients of variation (CV) was 3.6-6.8 %. Estradiol concentrations were determined by RIA (Diagnostic Systems Laboratory, Webster, TX). The detection limit was 10 pmol/L and the inter-assay CV was 5.1-8.1 %. Serum insulin was measured with IRMA (Biosource Europe, Nivelles, Belgium) with a detection limit of 2 µU/L and inter-assay CV of 4.4 to 5.9 %. Plasma FFA levels were determined using a NEFA-C Free Fatty acid kit (Wako Chemicals GmbH, Neuss, Germany) with a detection limit of 30 µmol/L and inter-assay CV of 2.6%. Plasma triglyceride concentrations were measured using an enzymatic colorimetric kit (Roche Diagnostics GmbH, Mannheim, Germany) with a detection limit of 50 µmol/L and inter-assay CV of 1.8%. Day long blood glucose concentrations were assessed using a blood glucose analyzer (Accutrend, Boehringer, Mannheim, Germany). Basal serum glucose was measured using a fully automated Modular P 800 (Hitachi, Tokyo, Japan) and Free thyroxine (fT_4) concentrations were estimated using electro chemo luminescence immunoassay (Elecsys 2010, Roche Diagnostics Nederland BV, Almere, Netherlands).

Urine Analysis

Urine was collected during the 24 h of blood sampling. Urinary epinephrine, nor-epinephrine and dopamine concentrations were assessed by high performance liquid chromatography with electron capture detection.

Calculations and statistics

Area under the Curves Leptin Profiles

Area under the curves of leptin concentration plots were calculated using the trapezoidal rule (Sigma Plot 2002 for Windows version 8.02).

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 (11). Higher ApEn values denote greater relative randomness of hormone patterns. Data are presented as normalized ApEn ratios, defined by the mean ratio of absolute ApEn to that of 1000 randomly shuffled versions of the same series. ApEn ratios close to 1.0 express high irregularity (maximum randomness) of pulsatile hormone patterns (12).

Circadian Rhythmicity

Circadian characteristics of leptin concentration patterns were determined using a robust curve fitting algorithm (LOWESS analysis, SYSTAT version 11 Systat Inc, Richmond, CA,(13;14)). The acrophase (clock time during 24 h at which leptin concentration is maximal) is the maximal value of the fitted curve. The mesor is the average value about which the diurnal rhythm oscillates. The amplitude of the rhythm was defined as half the difference of the nocturnal zenith and the day-time nadir. The relative amplitude is the maximal percentage increase of the mesor value.

Statistics

Data are presented as means ± SEM, unless otherwise specified. Data were logarithmically transformed before statistical computations when appropriate and statistically analysed using a parametric test (paired samples t-test). Significance level was set at 0.05. Multiple regression analysis was performed to estimate the correlation between changes in metabolic parameters (mean 24 h glucose, insulin, triglyceride and free fatty acid plasma concentrations) vs. changes of mean circadian leptin concentrations induced by bromocriptine treatment in the obese subjects. Differences were calculated subtracting values during bromocriptine treatment from values during placebo treatment. Negative differences reflect a decrease and positive differences reflect an increase induced by bromocriptine treatment of the parameter.

Results

Subjects

Eighteen obese subjects were enrolled in the present study. Body weight and BMI were similar after placebo and Bromocriptine treatment (Weight Pl 94.1 ± 2.5 vs. B 94.4 ± 2.5kg, P = 0.33 and BMI Pl 33.2 ± 0.6 vs. B 33.3 ± 0.6 kg/m^2, P = 0.35). All subjects were studied in the early follicular phase of their menstrual cycle (Estrogen Pl 163 ± 21 vs. B 209 ± 21 pmol/L, P = 0.10 and progesterone Pl 2.13 ± 0.64 vs. B 2.94 ± 1.13 nmol/L, P = 0.56). Subjects were clinically euthyroid (Thyroxine (free T_4) levels Pl 14.6 ± 0.4 vs. B 14.4 ± 0.4 pmol/L, P = 0.56).

Urine analysis

Urinary norepinephrine was significantly reduced after bromocriptine treatment (Pl 0.184 ± 0.020 vs. B 0.119 ± 0.015 µmol/24 h, P < 0.001). Urinary epinephrine (Pl 0.015 ± 0.005 vs. B 0.011 ± 0.004 µmol/24 h, P = 0.416) were not significantly different during placebo and bromocriptine treatment.

Leptin concentration parameters

Mean and AUC of 24 h leptin concentrations were significantly reduced by bromocriptine treatment (Table 1). A graphical illustration of mean 24 h plasma leptin concentrations during placebo and bromocriptine treatment vs. clock time is presented in Figure 1.

The Approximate Entropy (ApEn) ratio was not significantly affected by bromocriptine (Pl 0.88 ± 0.02 vs. B 0.87 ± 0.02, P = 0.81). Analysis of the circadian variation in plasma leptin concentrations revealed that the acrophase of the circadian leptin rhythm occurred at night at similar clock-times during placebo and bromocriptine treatment (Pl 0200 h ± 40 min and B 0100 h ± 40 min, P = 0.33). The mesor (Pl 33.1 ± 2.5 µg/L vs. B 30.0 ± 2.4 µg/L, P = 0.04) of the rhythm was significantly decreased by bromocriptine, whereas both the amplitude (Pl 8.0 ± 0.8 µg/L vs. B 7.0 ± 0.9 ng/L, P = 0.24) and the relative increase in leptin concentration (Pl 24.5 ± 1.7 % vs. B 22.5 ± 2.1 %, P = 0.39) were not significantly altered after bromocriptine treatment. An overview of the leptin concentration parameters is given in Table 1.

Correlations between leptin concentrations and metabolic parameters

The impact of bromocriptine treatment on metabolic parameters is reported in a separate paper (P. Kok et al unpublished). Multiple regression analysis, including differences (Δ) of mean 24 h glucose, insulin, free fatty acid and triglyceride concentrations as independent variables, revealed that differences in mean 24 h FFA concentrations (range Δ FFA-0.04 to 0.48 mmol/L) were significantly correlated with differences in mean 24 h leptin concentrations in response to bromocriptine treatment (partial correlation R^2 = 0.46, range Δ leptin-14.7 to 8.2 µg/L, P = 0.03, Figure 2). Changes in mean 24 h circulating glucose (range Δ glucose-1.22 to 0.72 mmol/L, P = 0.23), insulin (range Δ insulin-34.16 to 6.97 mU/L, P = 0.40) and triglyceride concentrations (range Δ triglyceride-0.41 to 0.63 mmol/L, P = 0.23) were not related to changes in leptin concentrations.

Discussion

We here show that short-term treatment with bromocriptine significantly reduces circulating leptin levels in obese women, while caloric intake was standardized and body weight remained stable. The increase of circulating FFA's during bromocriptine treatment was inversely related with the decline of leptin levels. Our finding is in keeping with a few previous reports documenting an inhibitory effect of bromocriptine on leptin release in rodents and humans (9;10).

Circulating leptin concentrations are the net result of concerted influences of prior and ongoing hormone secretion, distribution and elimination. As there is no evidence indicating that bromocriptine alters leptin clearance from the circulation, our observations suggest that activation of dopamine 2 receptors, directly or indirectly modulates leptin release by adipocytes. The brain is involved in the control of (circadian) leptin levels (15) perhaps via modulation of adipocyte metabolism by autonomic nerves (16), where sympathetic input inhibits leptin synthesis (8) Bromocriptine acts on presynaptic D2 receptors to inhibit (sympathetic) norepinephrine release (17;18). The fact that urinary norepinephrine excretion was reduced during bromocriptine treatment in our study corroborates this data. Thus, as sympathetic signals reduce leptin release by adipocytes (8), the reduction of circulating leptin levels we observe here, was not due to the inhibitory effects of bromocriptine on sympathetic activity. Alternatively, the decline of leptin during bromocriptine treatment was brought about via effects on other metabolic parameters that modulate leptin release. Glucose, insulin and prolactin all stimulate leptin synthesis (8;19-21). However, the decline of the concentration of either glucose or these hormones in response to bromocriptine was not correlated with the reduction of circulating leptin in the present study, which does not support the possibility that these factors are involved in bromocriptine's effect.

Interestingly, the decline of leptin in response to bromocriptine was correlated with the concomitant increase of circulating FFA's. Fuel flux through adipocytes is instrumental in the control of leptin synthesis, where net influx promotes, and net efflux inhibits leptin gene transcription (22). The rise of FFA levels is probably due to inhibition of the net influx of FFA in adipocytes by bromocriptine (23). Thus, the fact that changes of FFA and leptin in response to bromocriptine were inversely related supports the position that the drug reduces circulating leptin concentration via modulation of FFA flux in adipocytes.

Leptin levels are clearly increased in obese humans in proportion to fat mass, whereas dopamine D2 receptor availability in the brain is reduced in obese humans in proportion to body adiposity (24). The present findings allow for the postulate that these phenomena are related.

In conclusion, short-term bromocriptine treatment lowers circulating leptin levels in obese women, which suggests that dopaminergic neurotransmission is involved in the control of leptin release in humans.

Tables and Figures

Table 1. Features of 24 h plasma leptin concentration profiles

Parameter	Obese (N = 18)		P-value [a)]
	Placebo	Bromocriptine	
Mean 24 h plasma concentration (ng/L)	33.6 ± 2.5	30.5 ± 2.5	0.03
AUC Leptin (ng/Lx24 h)	48 423 ± 3615	44 046 ± 3604	0.03
ApEn	0.88 ± 0.02	0.87 ± 0.02	0.81
Acrophase (hours)	0200 ± 40	0100 ± 40	0.33
Mesor (ng/L)	33.1 ± 2.5	30.0 ± 2.4	0.04
Amplitude (ng/L)	8.0 ± 0.8	7.0 ± 0.9	0.24

Data are presented as means ± SEM.

a) P-values placebo vs. bromocriptine obese women, as determined by paired samples t-test

* P-value < 0.05 placebo vs. bromocriptine

Figure 1.

Mean diurnal serum leptin concentration time series the obese subjects (N = 18) during placebo (-•-) and bromocriptine treatment (-○-). Data reflect sampling of blood every 20 min for 24 h. Blood sampling starts at 0900 h. Lights were switched off and subjects went to sleep at 2300 h until 0730 h next morning, when lights were switched on (grey horizontal bar indicates sleeping period).

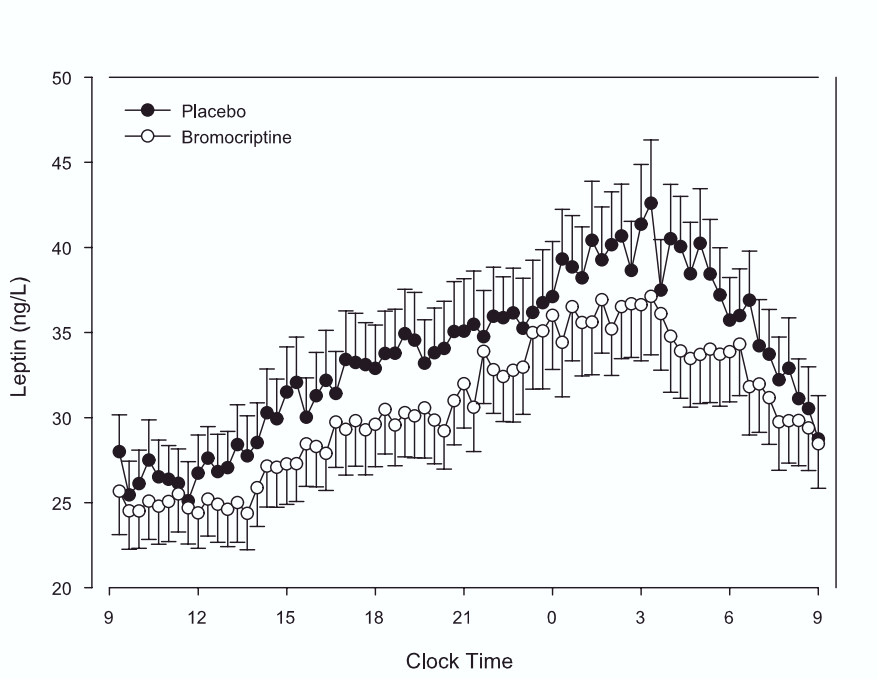
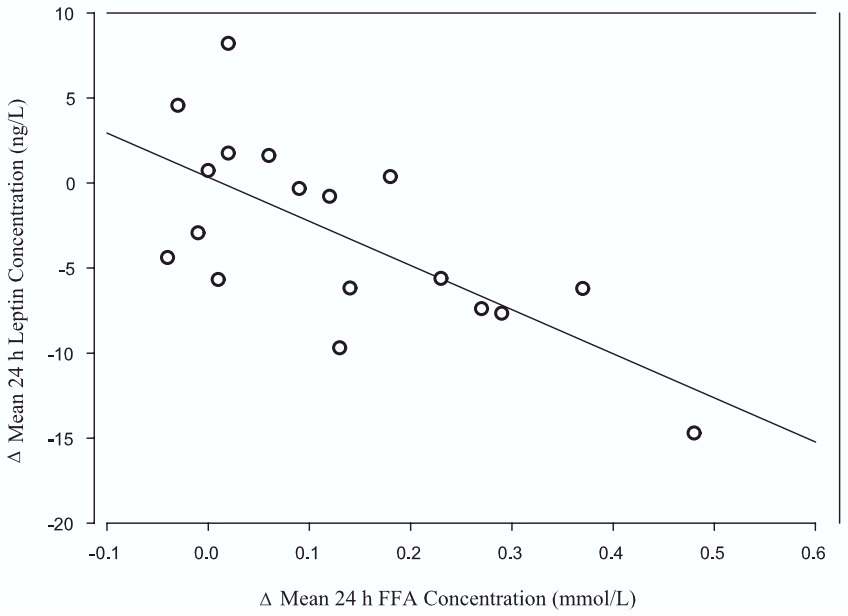


Figure 2.

Differences in mean FFA concentrations were significantly inversely related to differences in mean 24 h leptin concentrations ($R^2 = 0.46$, P = 0.03) during placebo and bromocriptine in obese women. The range of differences mean 24 h leptin concentrations was-14.7 to 8.2 µg/L.



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