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ORIGINAL ARTICLE

Plasma leptin concentrations are highly correlated to emotional states throughout the day

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Previous work has shown that leptin appears to regulate the plasma levels of hormones such as adrenocorticotropic hormone (ACTH) and cortisol in humans and that it has antidepressant effects in animals. It is unknown whether fluctuations in circulating leptin levels are correlated to changes in human emotions. This study was conducted to determine whether minute-to-minute fluctuations in the plasma concentrations of human leptin were associated with psychological variables. Leptin was sampled every 7 min throughout the day in 10 healthy subjects (five men and five women) studied in a clinical research center, and visual analog scales were applied every hour. We found highly significant correlations between fluctuations in plasma leptin concentrations and three psychological variables: sadness, carbohydrate craving and social withdrawal. We showed that during the course of the day increases in leptin levels are associated with decreased search for starchy foods, decreased feelings of sadness and increased social withdrawal. Our findings support the hypothesis that during the course of the day as leptin levels increase individuals subjectively feel happier (less sad) and have less inclination to interact socially. Conversely, when leptin levels decrease, we show increases in sadness and social cooperation, which might facilitate the search for food. We suggest that increased human leptin levels may promote positive feelings and that decreased leptin levels might modulate inner states that motivate and facilitate the search for nutrients.

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INTRODUCTION

It has been widely observed that food intake, body weight and mood are associated in humans. Alterations in food intake and body weight are cardinal diagnostic features of mood disorders such as major depression.[1] The mechanisms underlying the interactions between body weight, food intake and mood have not been elucidated. Leptin, an adipose tissue hormone, acts on hypothalamic receptors to inhibit food intake and stimulate thermogenesis, thereby regulating energy balance and body weight.[2–4] Plasma leptin concentrations reflect fat mass; however, within individuals leptin levels fluctuate throughout the day in a pulsatile and circadian manner.[5–8] We hypothesized that leptin might affect mood acting as a link between the body's adipose mass and the brain. Here we show that during the course of the day fluctuations in the plasma leptin level are highly correlated with psychological measures of mood, social stress and food (carbohydrate) craving.

Despite the well-established effects of leptin administration in animals to reduce food consumption and decrease body weight, meals do not immediately influence leptin levels in humans. In a study investigating the circadian patterns of secretion of leptin, meal ingestion and meal-induced increases in plasma insulin and glucose did not influence changes in leptin concentrations during the 24-h period.[9] Furthermore, when appetite ratings and leptin levels were compared, subjective feelings of hunger were not affected by postprandial plasma leptin concentrations in healthy volunteers.[10] Those data indicate that leptin influences energy metabolism but that it does not act as an immediate satiety factor. In addition to its effect on eating behavior, leptin may have a role in the body's hormonal response to stress. We have shown that human leptin levels are highly and inversely correlated to the stress hormones cortisol and adrenocorticotropic hormone (ACTH).[5] Our group and others have conducted studies supporting the concept that leptin might modulate the bioactivity of neuropeptides, such as corticotropin-releasing hormone (CRH), a neurohormone that regulates the biological and behavioral responses to stress.[11–16]

Whereas several studies have included single hormone or cytokine measures as they relate to symptoms, including leptin[17,18] and interleukin-6 (IL-6),[19] we have previously shown that both leptin and IL-6 fluctuate substantially throughout the day.[6–8,20,21] Clinical symptoms likewise fluctuate from hour to hour. Single time point measurements are therefore very limited, as they cannot be temporally related to diurnal fluctuations in psychometric variables.

The experiment described here tests the hypothesis that fluctuations in plasma leptin concentrations throughout the day are highly associated with simultaneous fluctuations in psychometric variables such as mood, stress-related behaviors and appetite.

MATERIALS AND METHODS

Ten healthy normal-weight subjects (five male and five female subjects; body mass index = 23.4 ± 0.9 kg/m^2, age = 29.5 ± 2.2 years) were admitted to a clinical research unit at the Clinical Center at the National Institutes of Health. The research was approved by the Institutional Review Board of the National Institutes of Health Clinical Center (Bethesda, MD, USA). Informed consent was obtained before all procedures in writing. We started the

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experiment after 48 h of acclimatization to the research suite. Subjects had four standardized meals a day: breakfast, served at 0830; lunch, at 1230; dinner, at 1730, and an evening snack, at 2100 hours. Each subject received a total amount of calories per day that was calculated to keep each individual at their admission body weight, with 20% of calories served at breakfast, 35% at lunch, 35% at dinner and 10% at the evening snack. Subjects were exposed to light from 0700 to 2300 hours. The subjects were allowed to walk from their bed to the bathroom and to an adjacent hospital day room but were not allowed to exercise in order to maintain their level of physical activity at a comparable baseline. Blood samples for the determination of leptin were drawn via a venous catheter from each subject at 7-min intervals for 24 h starting at 0800 hours, as previously described.[5,6,8] Partial results that did not include psychometric data were previously reported.[6] Plasma leptin concentrations were determined using radioimmunoassay as described,[5,8] and values were averaged at 1-h intervals. Symptom ratings were obtained from 100-mm visual analog self-rating scales for eight emotional states: sadness, social withdrawal, appetite, carbohydrate craving, concentration, tiredness, self-esteem and physical discomfort.[22] Each subject completed the self-ratings hourly from 0900 to 2300 hours, simultaneously with the blood collection. Results were analyzed by linear regression (Pearson correlations), using the software program StatisticaTM (StatSoft, Tulsa, OK, USA). Bonferroni correction was used in the assessment of statistical significance because of multiple comparisons.

RESULTS

We collected a total of 2070 blood samples and 1200 psychometric variable data points. To assess fluctuation in the plasma leptin concentration, we calculated leptin variability, which has been previously defined as a percentage of individual 24-h average, using the formula: variability at time t = (hormone level at time t/24-h individual average level)x100.[5] A higher score in the visual analog scale measurement indicates more pronounced symptoms. Social withdrawal, concentration, self-esteem and physical discomfort were considered as measures analogous to behaviors observed in animal models of stress.[23] Using linear regression (Pearson's correlation) we found that craving for sugar, social withdrawal and sadness were highly and significantly correlated with leptin variability. The other subjective psychological states did not show significant correlation with leptin levels. Significance was calculated taking into consideration multiple comparisons (Bonferroni's correction). We found a highly significant negative correlation between leptin variability and craving, indicating that an increase in leptin levels was accompanied by similar decrease in the subjective feelings of carbohydrate craving (Figure 1a). A highly negative correlation was also found when sadness and leptin variability were compared (Figure 1b). On the other hand, social withdrawal had a highly positive correlation with plasma leptin concentrations (Figure 1c). There is a clear increase in leptin values throughout the day, which is followed by a similar pattern in social withdrawal scores. Craving for carbohydrates and sadness have a tendency to diminish towards the end of the day, indicating an hourly negative correlation between leptin and both scores.

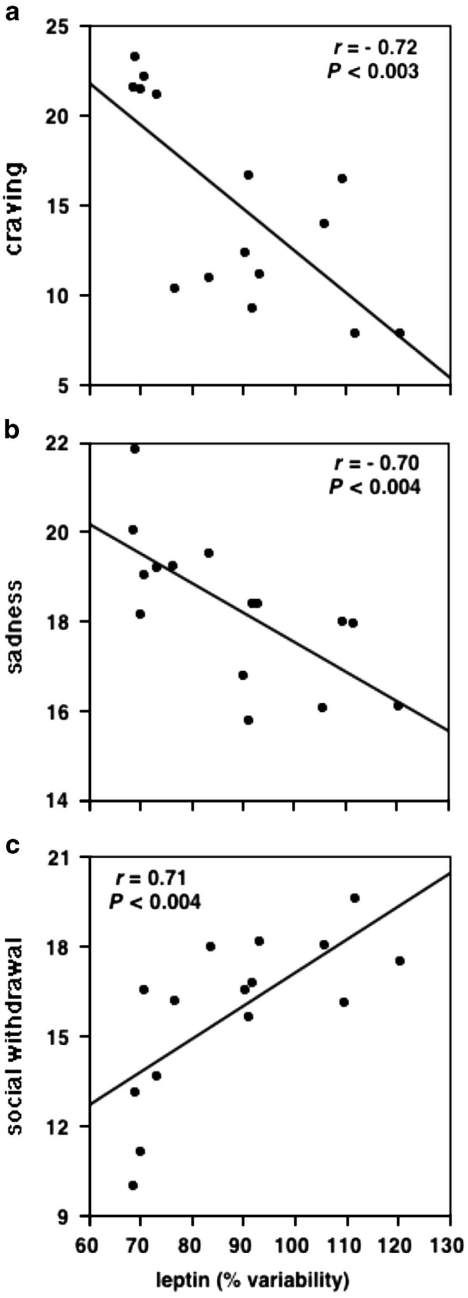

Figure 1. Variability in leptin concentrations is highly correlated with psychological states. (**a**) Leptin is inversely correlated with subjective feelings of craving ($r = 0.72$; $P < 0.003$). (**b**) Likewise, there is a highly significant correlation between the subjective ratings of sadness and leptin ($r = 0.70$; $P < 0.004$). (**c**) There is a positive correlation between ratings of social withdrawal and leptin ($r = 0.71$; $P < 0.003$).

DISCUSSION

To our knowledge, this is the first report that fluctuations in longitudinally sampled human leptin concentrations are temporally correlated with psychometric variables.

A study by Larsson *et al.*[24] examined 64 healthy postmenopausal women and showed a correlation between plasma leptin concentrations and total carbohydrate intake. That study examined the correlation between leptin and carbohydrate intake in a group of subjects whose leptin levels were assessed once and related to actual food intake. In our study we standardized food intake and examined how fluctuations in rapidly sampled plasma leptin concentrations correlated to psychometric variables within subjects. In spite of these very distinct study designs, using group comparisons (Larsson *et al.*) and within subject variations (this study), it is noteworthy that the results of both studies are consistent with each other, indicating that the psychometric variables we studied may be relevant to actual patterns of *ad libitum* food intake, as documented by Larsson *et al.*[24]

Tsofliou *et al.*[18] used brisk walking and a chocolate-based snack, in an attempt to replicate typical physical activity and eating behaviors, to investigate the effects on appetite and on associations between serum leptin and appetite. Associations between circulating leptin and suppressed appetite or elevated satiety were found following a bout of moderate physical activity, but not at any time point during the snack or the control

conditions. The authors concluded that moderate physical activity and snack intake suppress the appetite of obese women acutely. They suggested that associations between circulating leptin and appetite satiety ratings might indicate the involvement of leptin in short-term appetite regulation in response to physical activity-induced factors. Moreover, two other studies looked at the correlation between leptin and subjective variables; however, those were based on single point measurements of leptin[17,18] and cannot establish a temporal correlation between variability in diurnal leptin levels and fluctuations in psychometric variables throughout the course of the day.

Despite our previous work showing that leptin replacement specifically affects macro- and micronutient intake in leptin-deficient patients,[25,26] in this study we could not detect any correlation between appetite and leptin, which further supports the concept that leptin might not be a short-term satiety factor, at least in baseline levels of energy expenditure. However, the effects of leptin on food intake might explain the negative correlation we found with craving. Other hormones may have a more pronounced role in short-term satiety. This includes gastrointestinal peoptides, such as glucagon-like peptide-1.[27]

Previous work from our group and others have shown that leptin affects brain function in food-related cognitive tasks.[28,29] Leptin also increases gray matter in the cerebellum, anterior cingulate gyrus and inferior parietal lobule.[30–33] In addition to these effects, leptin has potent pro-cognitive effects.[34,35] Leptin levels are inversely correlated to rates of dementia and Alzheimer's disease[36,37] and it acts on dopaminergic midbrain neurons to promote survival and to protect against apoptosis[38] via activation of mitogen-activated protein kinase and phosphoinositide 3-kinase/Akt/nuclear factor kappa B-dependent signaling cascades. Given the functional and structural effects of leptin on the human brain, it is noteworthy, but not unexpected, that leptin might be closely correlated with the diurnal regulation of emotional states, in a manner suggestive of a possible regulatory role by this signal of nutrional status on specific emotions, such as sadness and emotional withdrawal. The possible effects of leptin on human emotions might be understood in terms of evolution. We show that during the course of the day, increases in leptin concentrations are associated with decreased motivation to search for starchy foods, decreased feelings of sadness and increased social withdrawal. This is consistent with the hypothesis that as leptin levels increase individuals subjectively feel happier (less sad) and have less inclination to interact socially in a manner that might facilitate the search for food. Conversely, when leptin levels decrease there are increases in sadness and social cooperation (necessary to obtain food).

CONCLUSIONS

We showed that during the course of the day increases in leptin levels are associated with decreased search for starchy foods, decreased feelings of sadness and increased social withdrawal. Our results support the hypothesis that decreased circulating leptin levels might modulate inner states that facilitate the search for food. Sadness and social withdrawal are the key symptoms of major depression, a disorder that has been postulated to involve abnormal CRH activity.[1] The effects of leptin in the brain have been well studied in animals, including its potential role as an antidepressant[39] that can increase neurogenesis.[40,41] Moreover, deletion of leptin receptors in the adult hippocampus induces depression-related behaviors in mice.[42] However, the specific central mechanisms by which leptin, a peripherally secreted hormone, might regulate human emotional states throughout the course of the day have not yet been identified. Future studies should determine whether the effects of leptin on the regulation of higher brain functions in humans are directly regulated by leptin receptors or whether such effects are mediated by neuropeptides, such as CRH.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

The funders had no role in study design, data collection, analysis, and decision to publish or preparation of the manuscript.

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