Variations in glucocorticoid levels within the physiological range affect plasma leptin levels

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Abstract
Objective: Leptin, the obese gene product, is thought to regulate body fat through its action on hypothalamic receptors that influence satiety. The hormonal regulation of leptin is important, since it might affect adiposity. Leptin regulation in man is poorly understood. We studied the relation between endogenous cortisol and leptin levels as well as the acute and chronic effects of a low dose of dexamethasone (DEX) on plasma leptin levels in healthy male volunteers.

Subjects and experimental protocol: The correlation between basal plasma levels of leptin and cortisol and the chronic effect of DEX treatment were studied in 12 subjects. Plasma leptin and cortisol levels were determined every other hour for 24 h, before and after 2 weeks of oral administration of 0.1 mg DEX twice daily. The acute effect was studied in 20 subjects, who received 1 mg DEX at 2300 h. Fasting blood samples were taken at 0800 h on the same day (i.e. before DEX) and on the day after.

Results: Under basal conditions, we found a correlation between mean plasma levels of leptin and cortisol ($r = 0.7$, $P < 0.02$). Mean plasma leptin levels had increased by 50% after 2 weeks of DEX treatment ($P < 0.05$). The circadian rhythm of leptin was preserved, but the night peak occurred 2.5 h earlier ($P < 0.05$). Fasting plasma leptin levels were 20% higher 9 h after 1 mg DEX orally than at the same time on the day before ($P < 0.002$).

Conclusion: Physiological variations in cortisol are involved in the regulation of leptin.

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Introduction
Leptin is a hormone secreted by fat cells, which is thought to act on the hypothalamus and thus affect food intake and energy expenditure (1–3). It is reported to affect sexual behaviour and fertility in mice (4). In cell culture, leptin affects the process of proliferation and differentiation of haemopoietic tissue (5). Leptin has been shown to reduce food intake in mice, but no such effect has yet been seen in man (6).

Leptin is strongly related to the body mass index (BMI) and fat content (7, 8). However, it seems to be not only a passive indicator of fat mass, since serum levels vary greatly with the same fat content or with BMI (9). Furthermore, a circadian rhythm of circulating leptin, with a nocturnal peak that is not related to food intake, has also been shown (10, 11). The expression of leptin mRNA in fat cells is not constant and increased expression seems to occur in fat cells obtained from obese subjects (12, 13).

Hormonal regulation of leptin secretion and degradation is not fully understood. Animal and in vitro studies show that leptin is regulated by insulin both acutely and chronically (14–16). On the other hand, most studies have failed to note an acute effect of insulin on leptin in humans (17–21). In most in vitro and in vivo studies, glucocorticoids seem to upregulate leptin secretion and serum leptin levels (22–24). A minor inhibitory effect of dexamethasone (DEX) in a mouse cell line (14), as well as unchanged serum leptin levels after high doses of prednisolone given to healthy volunteers, have also been reported (25). However, it has been questioned if the effects of glucocorticoids on leptin in humans are restricted to acute pharmacological dosing (26). It is not known whether changes in glucocorticoid levels in the physiological range affect leptin levels, since high levels of glucocorticoids have been used in previous studies.

The aim of this study was to investigate the relation between plasma leptin and endogenous cortisol levels at baseline as well as the effect of the addition of a low dose of glucocorticoid on plasma leptin and its circadian rhythm.

Methods and research design

Basal studies and the chronic effect of DEX The relation between basal plasma leptin and cortisol levels and the chronic effect of DEX treatment on plasma
leptin were studied in 12 healthy men of mean ± s.d. age 30 ± 4.6 (range 23–38) years and mean ± s.d. BMI 22.9 ± 1.8 (range 20–26) kg/m². Subjects within a narrow range of BMI were included because this facilitated the study of BMI-independent factors affecting serum leptin levels. The subjects had no known diseases, took no medication and did not smoke.

Blood samples were taken over a period of 24 h on two occasions. In seven subjects, the samples were withdrawn before DEX treatment and after 2 weeks of oral treatment with DEX 0.1 mg twice daily. This dose corresponds to approximately 40% of a normal replacement dose (27). The subjects had been admitted to the ward at 1800 h on the day before. A venous line was established and next morning blood samples were collected every other hour from 0800 h until 24 h later. During this period, the subjects continued normal indoor physical activities and ate their meals at 0800, 1130 and 1600 h and had snacks at 2000 h. In five subjects, the study was carried out in reverse: first, the leptin levels were determined at the end of 2 weeks of DEX treatment. Then, after 6 months without treatment, the baseline curve was determined as described above. This was done to exclude stress as the cause of the difference in cortisol levels the first time the experiment was performed. A long period was left between the measurements because of our previous finding that the hypothalamic–pituitary–adrenal axis is affected for a long time after exposure to low doses of glucocorticoids (28).

Abdominal subcutaneous fat was obtained by needle biopsy at the end of the 24 h measurement between 0800 and 1000 h, after an overnight fast, and was immediately frozen in liquid nitrogen and stored at −80 °C, pending analysis.

Acute effect of DEX The acute effect of DEX was studied in 20 healthy men with a mean age of 30.9 ± 4.4 (range 25–41) years and a mean BMI of 22.9 ± 1.8 (range 19.6–25.8) kg/m². They received 1 mg DEX orally at 1100 h. Fasting blood samples were taken at 0800 h on the same day (i.e. before DEX administration) and on the day after.

All the volunteers gave their informed consent and the protocol was approved by the ethics committee of Karolinska Institute.

Hormone measurement

Plasma was prepared by centriputing the EDTA blood at 1800 r.p.m. for 10 min and then freezing it immediately at −80 °C, pending analysis.

Plasma leptin levels were determined with a commercially available human leptin RIA kit (Linco Research Inc., St Charles, MO, USA) (29). The antibody was raised against highly purified human leptin and both the standard and tracer were prepared with human leptin. The samples were run in duplicate. They were all within the detection limit of the kit (0.5–100 ng leptin/ml). Control samples of known leptin concentrations (1, 2.5, 6.5 and 14 ng/ml) were run in duplicate at the beginning and end of each assay. The intra-assay and interassay coefficient of variation were 5.3 and 8.3% respectively at the low leptin concentrations (1 and 2.5 ng/ml) and 3.5 and 2.5% respectively at the higher concentrations (6.5 and 14 ng/ml).

Plasma insulin levels were measured with an RIA kit (Pharmacia, Uppsala, Sweden). Plasma cortisol levels were determined with a time-resolved fluoroimmunoassay kit (Wallac, Oy, Finland).

RNA isolation, cDNA synthesis and analysis of ob gene expression

Total RNA was isolated from adipose tissue, essentially as described by Chomczynski & Sacchi (30), with the following modifications: chloroform was increased during the extraction to a ratio of 1:2 (chloroform/phenol), and an additional final ethanol precipitation was included. Professor B Carlsson kindly performed the cDNA synthesis and semiquantitative reverse transcriptase PCR assays, as described previously (31).

Statistical analysis

Data are presented as means ± s.d. except in Fig. 1 and Fig. 3 where they are means ± S.E. Since the mean leptin levels did not show a normal distribution, leptin levels are presented as mean, median and quartiles (25th and 75th). After log transformation of the data, parametric statistical tests were used. Paired Student’s t-test was used to compare the levels before and after treatment. Relations between the basal levels of plasma leptin and cortisol, insulin and BMI were evaluated by Pearson’s correlation coefficient test. To demonstrate diurnal variations, ANOVA for repeated measurements was used. Since the sampling interval was constant and there were no missing values, analysis of the area under the curve of the circadian profiles was replaced by the mean levels as they yield identical information under these conditions.

Results

Under basal conditions, we recorded a circadian rhythm for leptin, with peak levels at 0200 h and a nadir at 1000 h (Fig. 1), with a mean difference of around 60% (P < 0.05). A correlation was found between mean cortisol and leptin levels in each subject (r = 0.7, P < 0.02, Fig. 2). No significant correlation was found between fasting insulin and mean leptin levels (r = 0.5, P = 0.08). Likewise, no correlation was observed between BMI and leptin levels (r = 0.3, P = 0.3), probably because all the BMI values were clustered within a narrow range. Instead, in the subjects with a similar BMI, the leptin levels varied by 250%.
After 2 weeks of DEX treatment, mean plasma cortisol levels were lower than at baseline in all subjects (221 ± 48 and 154 ± 39 nmol/l respectively, \( P < 0.0001 \)). However, the diurnal rhythm of cortisol was preserved (Fig. 3). Thus, a DEX dose corresponding to approximately 40% of a normal replacement dose (27) resulted in an approximately 30% reduction in circulating cortisol. The mean plasma leptin level in each subject was higher after DEX treatment, 3.6 ng/ml (median 3.5, quartiles 2.4 and 4.5 ng/ml) and 5.2 ng/ml (median 4.2, quartiles 3.2 and 6.0 ng/ml) respectively (\( P < 0.05 \)). The rhythm was the same. The peak was reached earlier, at 2330 h compared with 0200 h (\( P < 0.05 \)) (Fig. 1). There was no significant change in the time when the nadir occurred. BMI did not change significantly.

**Figure 1** Twenty four hour profiles of mean plasma leptin levels, expressed as percentage change from fasting value (0800 h) in 12 healthy men. ■, Before treatment; ○, after 2 weeks of oral treatment with 0.1 mg DEX twice daily. Data are expressed as means ± s.e.

**Figure 2** Correlation between mean plasma cortisol and leptin levels during 24 h in 12 healthy men (\( r = 0.7, P < 0.02 \)). The broken lines are the 95% confidence intervals.
not change during the experiment (22.9 ± 1.8 and 22.9 ± 1.7 kg/m²). There was no significant change in fasting insulin levels (7.9 ± 2.2 and 9.1 ± 3.4 pmol/l respectively).

Leptin mRNA levels in the adipose tissue biopsy specimens were measured by semiquantitative reverse transcriptase PCR. We found no significant difference in the levels after 2 weeks of DEX treatment from those found under basal conditions (0.12 ± 0.02 and 0.12 ± 0.06 comparative units).

The acute effect of 1 mg DEX on leptin levels was also studied. Mean fasting serum leptin concentration was significantly higher 9 h after DEX treatment than the mean fasting level on the day before (3.3 ng/ml (median 3.0, quartiles 2.0 and 4.3 ng/ml) and 3.9 ng/ml (median 3.0, quartiles 2.4 and 4.7 ng/ml) respectively; P < 0.002) (Fig. 4).

Discussion

Body fat is not the only determinant of leptin, since at a given BMI or percentage body fat, these levels vary considerably. This finding was confirmed in the present study. Thus circulating leptin is under hormonal and metabolic regulation and glucocorticoids are probably of importance (32). This study shows that glucocorticoids, also within the physiological range, regulate plasma leptin levels and that cortisol is probably an important regulator of leptin under normal conditions in man. First, there was a significant correlation between the mean levels of cortisol and leptin over 24 h. Secondly, we found an increase in the level of plasma leptin during low-dose DEX treatment. DEX
treatment equivalent to 40% of the normal replacement dose (27) resulted in a 30% decrease in endogenous cortisol. Thus the net effect is a mild increase in the total amount of plasma glucocorticoids. This is emphasized by the absence of a significant change in serum insulin levels during treatment. Nevertheless, circulating leptin levels increased by 50%, without any change in BMI. Thirdly, a single dose of 1 mg DEX significantly increased plasma leptin levels 9 h later.

The present findings are in line with previous observations that the effects of glucocorticoids, previously described as pharmacological, can also be seen within the physiological range of cortisol. The late-phase response to an antigenic challenge has been shown to be more pronounced in the evening, when cortisol levels are at a nadir, than early in the morning (33), and changes in cortisol within the physiological range affect both proteolysis and glucose levels (34, 35).

On the other hand, there are marked variations in cortisol levels between individuals, which are not related to any differences in growth rate or body composition (36). Thus our finding of a basal correlation between plasma levels of cortisol and leptin may indicate that circulating leptin is a sensitive indicator of cortisol changes in man.

We found marked diurnal changes in plasma leptin levels, with a nocturnal peak at 0200 h which was 60% higher than the lowest value at 1000 h. These results agree with those from previous studies (10, 11, 37). DEX treatment had only a minor effect on the diurnal variation in plasma leptin levels, which could be due to the fact that the diurnal rhythm of cortisol was maintained during treatment or that other unknown factors may be a major cause of the diurnal variation of plasma leptin.

Leptin mRNA levels did not change after DEX treatment, despite the significant change in plasma levels. This could be explained either by insensitivity of the method (reverse transcriptase PCR) in detecting small changes in leptin mRNA expression or by the 50% increase in plasma leptin levels not being associated with a significant change in leptin mRNA. There may also be regional differences. Adipose tissue from different parts of the body may respond differently to hormonal stimulation of leptin synthesis. Finally, the increase in plasma leptin levels could also partly be due to changes in metabolism or excretion and not to production.

In conclusion, variations in glucocorticoid tonus within the physiological range affect leptin levels in healthy men. These results suggest that cortisol is an important regulator of circulating leptin levels under normal conditions.

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