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.
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 affect-
ing 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 with-
drawn before DEX treatment and after 2 weeks of oral
treatment with DEX 0.1 mg twice daily. This dose
consists of approximately 40% of a normal replace-
dment 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 treat-
ment, 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 experi-
ment was performed. A long period was left be-
tween 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 administra-
tion) 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 centrifuging the EDTA blood at
1800 h.p.m. for 10 min and then freezing it immediately
at −80 °C, pending analysis.

Plasma leptin levels were determined with a com-
mercially 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, Upssala, Sweden). Plasma cortisol levels
were determined with a time-resolved fluoroimmunoas-
say 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 transcrip-
tase 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

![Figure 1](image1.png)

**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](image2.png)

**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 emphasised 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 leptin 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.

Acknowledgements

The authors thank Professor Björn Carlsson for measuring leptin mRNA and Carina Ankerberg for expert technical assistance. This study was supported by grants from the Wera Ekström Foundation, Fimrurare Barnhuset Foundation, the Swedish Medical Research Council 9941, 7509, 9522 and Eli Lilly Sweden AB.

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**Received 10 August 1998**

**Accepted 16 September 1998**