Serum leptin and weight reduction in female obesity

R Geldszus, B Mayr, R Horn, F Geithövel, A von zur Mühlen and G Brabant

Abt. Klinische Endokrinologie der Medizinischen Hochschule Hannover, Germany; Institute for Gynaecological Endocrinology and Reproductive Medicine, Freiburg, Germany

Leptin, an adipocyte-derived hormone, induces a decrease in food intake and increases energy expenditure via hypothalamic interactions. In animal models obesity can be caused by leptin deficiency or by a dysfunction of the hypothalamic leptin receptor. Using a radioimmunoassay for the determination of leptin in human serum, we measured serum leptin levels in 227 otherwise healthy normal weight (N = 78; body mass index = 16.1–27.7 kg/m²) or obese women (N = 149; body mass index = 27.8–56.7 kg/m²). Fifty-three subjects were followed over a period of 12 weeks under weight reduction (800 kcal/day) and a subgroup of 33 for another 13 weeks after termination of the diet. Body mass index and serum leptin concentrations were measured longitudinally and compared to female controls not under diet. Under baseline conditions, log serum leptin levels were positively related to body mass index with a best fit using a non-linear regression (p < 0.001), indicating an attenuated increase in serum leptin levels with high body mass index. No subgroup with low serum leptin levels could be identified. Weight reduction induced a rapid decrease in serum leptin levels within the first 3 weeks to levels significantly lower than in body mass index-matched controls under normal diet (p < 0.001). This pattern was consistent after 6 and 12 weeks. Serum leptin levels increased again after the end of the diet but remained significantly lower than in the controls despite unrestricted calorie intake over 7 weeks. The rapid and persistent decrease in serum leptin to lower levels than expected from matched controls may explain the pertinent difficulties of obese subjects to cope with weight reduction.

G Brabant, Abt. Klinische Endokrinologie Medizinische Hochschule Hannover, Konstanty-Gutschowstr. 8, D-30623 Hannover, Germany

Body weight is kept remarkably constant during life (1). This has been explained hypothetically by the lipostat theory, proposing a feedback mechanism between central nervous centres regulating food intake and energy expenditure and a circulating factor secreted from adipose tissue (1–3). Using positional cloning, Friedman and co-workers recently characterized such a factor as the product of an adipocyte-specific obese gene (ob) coding for a secreted protein called leptin (5). Obesity in ob/ob mice, a well-characterized model for obesity, is caused by a nonsense mutation of the ob gene, inducing leptin deficiency. The increased food intake and the decreased energy expenditure of these animals can be corrected by treatment with recombinant leptin (5–11, 22). In contrast, obesity in another mouse model, the db/db mouse, is explained by an intrinsic mutation of the hypothalamic leptin receptors, resulting in a non-functional splice variant that renders the animals resistant against the effects of exogenous leptin and is associated with very high circulating leptin levels (8, 12, 13). In man, a positive correlation of both steady-state levels of ob mRNA in fat cells and circulating leptin levels to body fat mass was described recently (14–17, 26).

Data in a small group of obese subjects showed a decrease in serum leptin levels following weight reduction (17). The aim of the present study was to investigate the changes in serum leptin levels of obese but otherwise healthy female subjects under a balanced diet with 800 kcal/day in comparison to a large group of matched non-fasting controls.

Materials and methods

Patients and healthy subjects

The serum leptin levels were assessed in relation to body fat using body mass index (BMI) as a marker (21).

Blood samples were obtained from 78 lean (age 31.3 ± 10.2 years) and 149 obese otherwise healthy female subjects (age 30.7 ± 8.4 years). Obesity was defined as a BMI i.e. the weight in kilograms divided by the square of the height in meters) of > 27.8 kg/m² (24). The BMI was 22.9 kg/m² (range 16.1–27.7 kg/m²) in the lean and 35.9 kg/m² (range 27.8–56.7 kg/m²) in the obese subjects. All baseline blood samples were taken in the morning after an overnight fast and frozen immediately at −20°C until analysis.

To study the effect of weight loss, we investigated a subgroup of 53 otherwise healthy obese female subjects
Unrestricted supply revealed female subjects: (○) controls; (●) fasted subjects. Stepwise regression revealed a best fit using quadratic regression model (p < 0.001; upper left). Effects of weight reduction with 800 kcal/day at baseline, at 3, 6 and 12 weeks of weight reduction and at 6 weeks of increasing caloric supply (100 kcal/day with weekly increases) plus 7 weeks of unrestricted food intake (week 25).

(mean initial BMI = 37.2 kg/m², range 29.4–56.7 kg/m²) before and following 12 weeks of fasting under a liquid-protein diet providing 800 kcal/day (Optifast, Wander, Celle, Germany). All subjects were tested 3, 6 and 12 weeks after initiation of weight reduction. A subgroup of 33 subjects was followed for another 13 weeks. During weeks 13–19 daily allowance was increased by 100 kcal/day weekly and diet was unrestricted thereafter. Subjects were retested for serum leptin levels and BMI 25 weeks after initiation of diet and following 7 weeks of unrestricted food intake. Informed consent was obtained from each subject.

**Immunization and assay procedure**

The generation of antileptin antibodies and the assay procedure have been described in detail elsewhere (18). In brief, antibodies directed to a C-terminal peptide, leptin (126–140), were generated in rabbits and used in a final dilution of 1:1500. Leptin (126–140) standards and unknown samples (100 µl) and 100 µl of assay buffer were incubated with approximately 5000 cpm of radioactive leptin (126–140) (100 µl) and 100 µl of antibody for 16 h at 4°C and separated with 2% dextran–charcoal (Pharmacia, Freiburg, FRG; Serva, Heidelberg, FRG). Unspecific binding was less than 1%. Peptide tracer was prepared with the iodogen method purified by HPLC. The sensitivity of the assay was approximately 6 pmol/l and the inter- and intra-assay coefficients of variation were less than 8.3%. The peptide used had no sequence homology to any other known peptide in the Swiss-Prot database.

**Statistical analysis**

Stepwise regression analysis was performed from linear to higher order polynomial regression models. Serum leptin levels had a log distribution and were analysed after log transformation with paired and unpaired Student’s t-tests using Elch’s modification when appropriate.

All analyses were conducted with SAS software (SAS Institute, Cary, NC).

**Results**

Serum leptin levels were significantly higher in obese (388 ± 34 pmol/l) than in lean subjects (125 ± 18 pmol/l; p < 0.001). None of the obese subjects had unexpectedly low serum leptin concentrations. The BMI and serum leptin levels were positively related. Testing linear and polynomial models of regression, we found a significantly better fit with a quadratic than with a linear regression model (r² = 0.6; p < 0.001) whereas higher order regression models did not increase significantly the accuracy of
the fit. This non-linear increase in serum leptin concentrations in relation to BMI was still present when the 23 subjects with a BMI of >40 kg/m² were excluded (p < 0.001; Fig. 1).

Baseline serum leptin levels of the 53 subjects starting weight reduction with 800 kcal/day over 12 weeks did not differ from BMI-matched controls. Within the first 3 weeks a rapid and significant decrease in leptin serum levels was observed. The BMI decreased more slowly and the maximal decline was found between weeks 6 and 12 (Fig. 2). Compared to BMI-matched controls not under weight reduction, serum leptin levels were clearly lower (Fig. 1 and Table 1). This dissociation, which was significant 3 weeks after initiation of weight reduction, was still detectable another 13 weeks later after any food restriction had been terminated over the last 7 weeks when tested in a subgroup of 33 subjects (Table 1).

Discussion

The adipocyte derived hormone leptin plays an important role for the regulation of body fat mass both in animals (5–11, 22) and in humans (14–17, 26, 27). Our results in normal weight and obese women support and expand these assumptions. None of the obese subjects in our study had unexpectedly low serum leptin concentrations indicating a defective leptin synthesis, as shown in the ob/ob mouse (5–8, 11). This confirms the investigations of others on plasma leptin levels in obese subjects (17, 19) and fits in with the negative results of a recent screening for genetic alterations of the ob gene in obese and diabetic subjects (28).

Serum leptin levels were significantly higher in the obese as compared to lean subjects, with a positive relation to body fat, reflected by the BMI. Quite obviously the high circulating leptin concentrations are ineffective for controlling body weight in the obese. A number of mechanisms have been discussed: Considine and co-workers suggested a central insensitivity to leptin in obesity (17). This assumption is supported by the results of chemical or mechanical destruction of the hypothalamus in animals. Either monosodium glutamate or GTG treatments, as well as direct destruction of the hypothalamus, induces a dramatic increase in ob mRNA (4, 19) and supports the importance of hypothalamic centres in the feedback regulation of leptin. Mutations of the leptin receptor lead to a comparable increase in ob gene expression in two animal models of obesity: In the db/db mouse an intronic mutation of the hypothalamic isoform of the receptor and in the fa/fa Zucker rat an exonic mutation have been identified, resulting in a non-functional splice variant of the hypothalamic leptin receptor (12, 12, 23, 25). Our results do not exclude such a mutation of the hypothalamic leptin receptor because excessively high serum leptin levels were found in some of the obese subjects.

However, the concept of leptin resistance in obesity due to a functionally altered hypothalamic leptin receptor have been challenged recently. Studies in normal weight and obese subjects revealed relatively lower levels of cerebrospinal leptin in relation to plasma leptin levels in obese than in normal weight subjects, indicating that the uptake of leptin into the brain is decreased in obesity (20). Alterations of the leptin transporter, identified as another splice variant of the leptin receptor expressed in the plexus chorioideus, may thus add another mechanism to the development of obesity in some subjects (20, 23).

Finally, the increase in leptin serum levels observed in obesity may simply reflect an insufficient attempt to counter-regulate the gain of body weight induced by environmental or genetic factors. Leptin in such a concept would play a less dominant role for the negative long-term regulation of food intake and energy expenditure in man, as expected from animal studies.

The control of leptin secretion is currently ill defined. Recent studies suggest a direct relation of leptin to fat cell size and number (29). This assumption is not supported by our finding of an attenuated increase in circulating leptin levels in a large number of grossly overweight subjects. The dramatic decrease in leptin concentrations following 3 weeks of weight reduction without a comparable alteration in BMI rather favors a more important control by circulating regulators such as nutritional or humoral factors. Nutrition itself seems unlikely to explain the differences because unrestricted diet following the weight reduction protocol did not abolish the difference in serum leptin compared to the

---

**Table 1. Decrease in serum leptin levels (pmol/l) and BMI (kg/m²) following weight reduction in comparison to a BMI-matched control group.**

<table>
<thead>
<tr>
<th>Subjects under weight reduction</th>
<th>Mean BMI</th>
<th>Range</th>
<th>Serum leptin</th>
<th>95% CI</th>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.2</td>
<td>29.4–56.7</td>
<td>416</td>
<td>352–492</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>35.4</td>
<td>27.8–53.5</td>
<td>191</td>
<td>153–237</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>33.6</td>
<td>26.3–51.2</td>
<td>141</td>
<td>112–177</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>31.1</td>
<td>25.0–46.5</td>
<td>109</td>
<td>88–135</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>29</td>
<td>23.4–41.3</td>
<td>125</td>
<td>100–155</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects under weight reduction</th>
<th>Mean BMI</th>
<th>Range</th>
<th>Serum leptin</th>
<th>95% CI</th>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.7</td>
<td>29.4–49.8</td>
<td>364</td>
<td>322–412</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>34.9</td>
<td>27.8–49.8</td>
<td>351</td>
<td>313–394</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>33.1</td>
<td>25.9–49.8</td>
<td>324</td>
<td>290–309</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>31</td>
<td>24.4–45.9</td>
<td>276</td>
<td>246–309</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>28.6</td>
<td>23.3–41.4</td>
<td>231</td>
<td>201–265</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*CI = confidence interval.
control subjects never losing weight. Humoral factors such as insulin or alterations in fatty acids have not been controlled in the present study. However, available data on the influence of insulin or lipids suggest only a small contribution to leptin regulation (26, 27). Regardless of which mechanism is operative, lower leptin levels under an unrestricted diet over almost 2 months following weight reduction may represent an important cofactor to explain the apparent difficulties of obese subjects to adhere to a weight reduction protocol and for the rapid gain of weight frequently observed after the end of a diet. Therapeutical application of leptin in this situation may be useful to correct the deficit and to help subjects to maintain their body weight after a period of weight reduction.

References


Received June 18th, 1996
Accepted July 23rd, 1996