



Effects of dietary restriction on serum leptin concentration in obese women

F Cella¹, GF Adami², G Giordano¹ and R Cordera^{1*}

¹Dipartimento di Scienze Endocrinologiche e Metaboliche, University of Genova, Genova, Italy and ²Istituto di Clinica Chirurgica, University of Genova, Genova, Italy

OBJECTIVE: To investigate the short- and long-term effects of dietary restriction on serum leptin in obese women and the role of the gastrointestinal system in the short-term regulation of leptin production.

DESIGN: Clinical longitudinal study of anthropometric and serum leptin changes induced in obese women by a balanced 300 kcal/d very low calorie diet (VLCD), administered either orally or parenterally for 5 d, and by a balanced 900 kcal/d low calorie diet (LCD) lasting six months.

SUBJECTS: 20 obese women (age: 38.1 ± 12.7 y; body mass index (BMI): 40.2 ± 8.3 kg/m²).

RESULTS: Five days following VLCD, a modest, even if significant ($P < 0.0001$), fall of both body weight (BW) and BMI was observed, along with a dramatic (> 50%) highly significant ($P < 0.0001$) reduction of circulating serum leptin. Baseline and five-day anthropometric and biochemical findings were closely similar in the group of orally fed subjects, when compared with those of their parenterally fed counterparts. The baseline positive correlation between serum leptin and BMI ($\rho = 0.533$) increased ($P < 0.05$) at the end of the five day VLCD ($\rho = 0.849$). A further fall of BW and BMI was observed at day 30 ($P < 0.001$) and day 180 ($P < 0.01$) during the 900 kcal/d LCD, while the serum leptin concentration gradually increased until day 180 when it was only slightly but non significantly lower than at baseline. At the end of the study, the correlation between serum leptin and BMI was similar to the baseline ($\rho = 0.562$).

CONCLUSIONS: Energy restriction causes a fall of serum leptin apparently not mediated by gastrointestinal signals and it seems not to affect the long-term regulatory pathways of circulating leptin.

Keywords: leptin; obesity; energy intake

Introduction

Leptin is recognized to be a cardinal peripheral feedback signal to the central nervous system (CNS), notably to the hypothalamus, taking part in the regulatory loop of feeding behavior and energy homeostasis.^{1–3} Since leptin, with the exception of pregnancy,⁴ is essentially produced by the adipocytes,^{5,6} a linear positive correlation between serum leptin concentration and body fat mass exists. However, the positive regression is characterized by a great deal of variability.^{7,8} As leptin is involved in the control of energy intake, and a positive relationship between serum leptin concentrations on one hand and serum insulin concentration and insulin-resistance on the other hand was reported,^{9–11} several studies have attempted to show whether food intake and insulinaemia changes would affect serum leptin concentrations.^{12–14} Sharp variations of serum leptin concentrations as an effect of short-term energy restriction were observed:^{15–18} these investigations have shown a considerable decrease of leptin

concentrations following a very low calorie diet (VLCD) in obese patients or following a complete starvation both in obese and in normal weight individuals. Longitudinal studies on long-term changes in serum leptin concentrations, due to chronic energy restriction, have been carried out, reporting, however, conflicting results.^{19–21}

With the aim of investigating the role of the gastrointestinal system in the short term regulation of leptin production, serum leptin concentrations were determined in obese patients submitted to VLCD, and data of the individuals fed by an enteral nutrition formula were compared with those obtained from individuals exclusively fed by parenteral infusions. Furthermore, the patients were evaluated longitudinally at longer term under a balanced hypoenergetic diet (low calorie diet (LCD)).

Material and methods

The study was carried out in 20 obese women, ranging in age from 28–45 y (mean 38 y), who, except from obesity, did not suffer from major diseases and did not take any medication known to influence the metabolic parameters. Informed consent was obtained and the trial was approved by the University of Genova

*Correspondence: Renzo Cordera, Dipartimento di Scienze Endocrinologiche e Metaboliche, University of Genova, viale Benedetto XV-6, 16132 Genova, Italy.
E-mail: record@unige.it
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Table 1 Anthropometric parameters and serum leptin concentrations from the baseline values (day 1) to the end of the 300 kcal/d very low calorie diet (VLCD, day 5), and after 1 month and 6 months (day 30 and day 180) of the 900 kcal/d low calorie diet (LCD). All data are expressed as mean \pm s.d.

	Day 1	Day 5	Day 30	Day 180
BW (kg)	104.2 \pm 22.1	98.1 \pm 20.7	96.2 \pm 20	88.1 \pm 17.9
BMI (kg/m ²)	40.2 \pm 8.3	38.1 \pm 7.9	37.3 \pm 7.7	34.6 \pm 7.1
Leptin (ng/ml)	59.1 \pm 21.3	30.6 \pm 12.2	40.3 \pm 15.3	46.1 \pm 15.1
Leptin-BMI (ρ)	0.533	0.849	0.673	0.562
Ketones (mg/l)	0	62.8 \pm 17.1	44.3 \pm 21.1	32.7 \pm 20.7

BW = body weight; BMI = body mass index.

Ethical Committee. Baseline anthropometric and biochemical data are shown in Table 1.

All patients were admitted to the endocrine-metabolic ward to be kept under medical observation during the initial part of the study. The patients underwent a five day VLCD period, randomly divided in two groups of ten subjects: the first group was given by mouth a 300 kcal/d enteral nutrition mixture (Pep-tamen, Nestlè & Baxter, Vevey, Switzerland), while the subjects of the other group were infused with a parenteral nutrition formula containing the same daily amount of energy (Table 2). No other food was allowed in this period, and liquid energy-free supplementation was *ad libitum*. By the sixth day, patients were discharged and were placed on a 900 kcal/d balanced oral LCD for six months. The compliance with the diet was monitored by a daily food self-record.

Anthropometric findings and serum leptin concentrations were measured prior to and at the end of the VLCD, and thereafter at the first and at the sixth month. Body weight was measured to the nearest 100 g. Serum leptin concentrations were determined in the morning after an overnight fast: measurements were made by a commercially available radioimmunoassay (RIA) kit (DRG Instrument, GmbH, Marburg Germany),²² the interassay and intra-assay coefficient of variation being 5.7% and 3.7%, respectively. Urinary

ketone bodies were measured by a semiquantitative method, in order to assess the compliance to dieting.

Due to the small number of cases, analysis was carried out by non-parametric statistics: the differences were evaluated by the Wilcoxon rank test for dependent comparison and by the U-Mann-Whitney test for independent data when appropriate, and the relationships between data were assessed by the Spearman coefficient. The differences between the correlation coefficients have been evaluated with the chi-square method, according to Fisher and Yates.²³

Results

The overall results of this study are reported in Table 1 and Table 3. At the end of the VLCD period, a modest but significant ($P < 0.0001$) fall of body weight (BW) and body mass index (BMI), which decreased by $5.8 \pm 1.3\%$ and $5.6 \pm 0.9\%$, respectively, was observed. Furthermore, a substantial highly significant ($P < 0.0001$) reduction of serum leptin concentration, which decreased by $52.5 \pm 4.7\%$, was found. Ketone bodies were detectable in the urine only after the VLCD and LCD.

When the group of the orally fed subjects is compared with that of their parenterally fed counterparts, baseline anthropometric and biochemical findings were similar (Table 3). At the end of the VLCD period, in both groups a highly significant ($P < 0.0001$) fall of BW, BMI and serum leptin concentrations, and the appearance of ketone bodies, were observed; in the two groups the five-day anthropometric and biochemical findings were very similar.

Serum leptin concentrations and BMI values were positively correlated at the beginning of the study

Table 2 Macronutrient composition of the diets administered in the very low calorie diet (VLCD) period and in the follow-up (low calorie diet, LCD) period.

	Parenteral VLCD	Oral VLCD	LCD
Proteins	22%	16%	20–25%
Glucose	56%	51%	55–60%
Lipids	22%	33%	20–25%

Table 3 Anthropometric parameters and serum leptin concentrations from the baseline values (day 1) to the end of the 300 kcal/d very low calorie diet (VLCD, day 5), in response to the oral and to the parenteral diet. All data are expressed as mean \pm s.d.

	Oral VLCD Day 1	Parenteral VLCD	Oral VLCD Day 5	Parenteral VLCD
BW (kg)	103.3 \pm 20.9	105.1 \pm 22.7	97.5 \pm 20.1	98.7 \pm 21.1
BMI (kg/m ²)	39.8 \pm 8.1	40.6 \pm 8.5	37.9 \pm 8.1	38.3 \pm 7.7
Leptin (ng/ml)	60.1 \pm 21.5	58.1 \pm 20	31.7 \pm 12.1	29.5 \pm 12.3
Leptin-BMI (ρ)	0.521	0.542	0.837	0.858
Ketones (mg/l)	0	0	64.9 \pm 16.9	60.7 \pm 18.7

BW = body weight; BMI = body mass index.

($\rho = 0.533$) and a significant ($P < 0.05$) increase of the correlation coefficient was observed at the end of the VLCD period ($\rho = 0.849$).

The longitudinal data at one month and at six months are only available for 17 patients. A further significant fall of BW and BMI was observed at day 30 ($P < 0.001$) and day 180 ($P < 0.01$) during the 900 kcal/d hypoenergetic diet, while serum leptin gradually increased. At day 180, serum leptin concentrations were higher than that found at the end of the VLCD ($P < 0.001$) and only slightly and not significantly lower than that observed at the beginning of the study, despite the significant BMI reduction. The increased energy intake, which was, however, lower than the energy expenditure, as indicated by the weight loss, was associated with an obvious progressive decrease in production of urinary ketone bodies. At the end of the study, the correlation coefficient of serum leptin concentration and BMI values was closely similar to that observed at the baseline ($\rho = 0.562$).

Discussion

This study suggests that the short-term regulation of serum leptin is accounted for, more by the daily dietary energy content than by changes in body mass and appears to be independent of the gastrointestinal system. It should be stressed, however, that the body weight loss after five days, even if statistically significant, is not biologically relevant ($< 6\%$). In the long term, adaptive mechanisms take place and body mass regains its main role in the regulation of serum leptin concentrations. Although serum leptin half-life is about 30 min, food intake does not affect circulating leptin concentrations.²⁴ On the contrary, prolonged fasting or a few-days reduction in food intake to very low energy levels, cause a sharp fall of circulating serum leptin, at which time only a slight reduction of body mass and a minimal loss of adipose tissue have occurred.^{12,16–18} Refeeding is accompanied by a complete return to baseline of serum leptin concentrations.

The results of this study are in complete agreement with these data, and confirm that in the short term regulatory networks, the severe and protracted reduction of energy substrates availability could be the sensor which mediates the fall of serum leptin concentrations.^{15,16,18–20} This finding agrees with the recent observation, that in rats, acute regulation of leptin production is dependent on either hyperglycaemia or hyperlipidaemia.²⁵ In our patients, having received an oral supplementation or having being fed exclusively by an intravenous route, the fall of serum leptin concentrations in response to the VLCD given, was essentially the same. This strongly suggests that in the short-term regulation of serum leptin,

the gastrointestinal tract has no role. However, it cannot be excluded that such a response is due substantially to the strongly negative energy balance, and when the subjects receive a supplementation matching their actual needs or the alimentary restriction was less severe, some differences between orally and parenterally fed patients could be observed. The increase of the correlation coefficient between serum leptin concentrations and BMI values, following severe energy restriction, observed in the patients of this investigation, confirms the results of previous investigations.^{21,26} It might be hypothesized that the strongly negative energy balance put all patients, regardless of baseline status, in nutritional conditions very similar to each other, thus minimizing interindividual differences which might be present in obese patients, and leaving adiposity as a principal component in the correlation.

In the long term, following a moderately hypoenergetic diet, a progressive fall of serum leptin concentration has been reported, the values closely paralleling the BW and body fat.^{19–21} In this study, very similar results were found. During the refeeding, the patients continued to lose weight, indicating that the actual energy intake was clearly lower than the total energy expenditure. The serum leptin, however, gradually increased, most likely for the sharp improvement of energy balance due to the increase of energy supplementation. Moreover, since the weight loss leads to a decrease of energy consumption caused by the loss of body mass, the difference between energy expenditure and intake attenuates and energy balance further improves. At six months, the decrease of body weight and BMI corresponds to a small decrease in serum leptin concentrations, which return to correlate with BMI with a correlation coefficient similar to that observed at the start of the dietary period. It is likely that patients react differently to the same dietary prescription, reflecting the variability of the baseline, which clearly affects the correlation. The progressive rise of leptin after the VLCD period and the positive correlation with BMI are in full agreement with previous longitudinal studies,^{19–21} and suggest that in the long term, serum leptin concentrations are no more dependent on energy intake or on energy balance, and continue to be substantially influenced by the body fat mass.

Conclusion

This study provides evidence that a strongly negative energy balance dramatically affects serum leptin concentrations, and that serum leptin is essentially influenced by the body mass only when the weight is stable or when the difference between the energy intake and expenditure is slight.

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