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Serum Leptin Levels in Patients With Anorexia Nervosa Before and After Partial Refeeding, Relationships to Serum Lipids and Biochemical Nutritional Parameters

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Summary

Leptin is a protein hormone produced by adipocytes that provide information about the body fat content. It was previously reported that serum leptin levels were decreased in patients with anorexia nervosa in comparison with healthy control subjects. The aim of our study was to compare serum leptin levels in patients with anorexia nervosa (n=11, initial mean BMI=15.4 kg/m^2) before and after partial recovery with control age-matched subjects (n=11, mean BMI= 20.3 kg/m^2) and to study the relationships of leptin levels, serum lipids and biochemical nutritional parameters. We found that serum leptin concentrations in patients with anorexia nervosa were significantly reduced in comparison with control subjects (3.61 vs 9.37 ng.ml^{-1}, $p<0.01$). Serum cholesterol, triglycerides, total protein and albumin in patients with anorexia nervosa either before or after partial recovery did not differ from the control group. After partial recovery, a significant increase in serum leptin was observed (4.83 vs 3.61 ng.ml^{-1}, $p<0.05$), but the values still remained significantly lower than in the control group ($p<0.01$) Leptin levels correlated positively with the body mass index in the control group and anorexia nervosa group before recovery. The correlation with BMI in the anorexia nervosa group after refeeding was not significant. No significant correlation was found between leptin concentrations and serum lipids, total protein, albumin and prealbumin, respectively. Serum leptin thus represents a sensitive parameter that reflects the nutritional status in patients with anorexia nervosa suitable for long-term follow up during refeeding therapy.

Key words

Leptin • Anorexia nervosa • Body mass index • Refeeding

Introduction

Leptin is a protein hormone produced by adipocytes, that was identified by *ob gene* cloning in mice (Zhang *et al.* 1994). Serum leptin levels usually reflect the total body fat content, i.e. positive correlation was found between body fat content and body mass index respectively in both genders (Maffei *et al.* 1995). Serum leptin levels are increased in obese people in comparison with lean subjects (Lönnqvist *et al.* 1997, Ostlund *et al.* 1996). Serum leptin concentrations in females are two or three times higher than in males, the difference remains

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statistically significant even after adjustment for the body fat content (Couillard *et al.* 1997). This difference is probably not estrogen-dependent, since recently we showed that serum leptin values after estrogen treatment are lowered in male mice (Nedvídková *et al.* 1997).

Anorexia nervosa is a chronic eating disorder characterized by decreased food intake, chronically low body weight and resistance to efforts to regain weight. Multiple abnormalities of the neuroendocrine system, including suppression of gonadal and thyroid axes have been reported in patients with anorexia nervosa (Pomeroy and Mitchell 1989). Grinspoon *et al.* (1996) reported lower leptin concentrations in patients with anorexia nervosa in comparison with a control group that correlated with body weight, the body mass index as well as IGF- I levels. Ferron *et al.* (1997) studied serum leptin levels in patients with anorexia nervosa, bulimia nervosa and compared the values with age-matched control subjects. He found a significant reduction in serum leptin levels in patients with anorexia nervosa in comparison with the controls and this decrease ran in paralell with the body mass index. On the contrary, the body mass index as well as serum leptin concentrations in patients with bulimia nervosa did not significantly differ from the control group. Eckert *et al.* (1997) reported significantly decreased leptin levels in untreated patients with anorexia nervosa in comparison with healthy subjects. Serum leptin concentrations increased after refeeding, but remained still significantly below those of the controls with the normal body weight. Significant correlation with the body mass index was found in controls as well as in patients with anorexia nervosa after weight gain but not in untreated patients. Köpp *et al.* (1997) studied the relationship between serum leptin concentrations and the maintenance of normal menstrual cycles in patients with anorexia nervosa. He reported that leptin level of 1.85 μg/ml predicted a lifetime occurrence of amenorrhea in underweight females.

Casanueva *et al.* (1997) found a decreased body mass index, serum leptin and IGF-I levels in untreated patients with anorexia nervosa, while IGFPB-3 concentrations did not differ from the control group. Partial recovery elevated the body mass index, IGF-I and leptin levels. While the IGF-I levels completely returned to normal, the serum leptin concentrations remained still below values of the control group. Mantzoros *et al.* (1997) examined the cerebrospinal fluid and plasma leptin concentrations in subjects with anorexia nervosa before and after refeeding and in a healthy control group. Patients with anorexia nervosa had lower plasma and cerebrospinal fluid leptin concentrations in comparison with healthy subjects. However, the cerebrospinal fluid to plasma ratio was higher in patients than in the controls. The normalization of serum as well as cerebrospinal fluid leptin concentrations appeared after refeeding, although the patients had not yet reached normal body weight.

Relatively scarce information is available about the relationship of serum leptin with serum lipids and the routinely used biochemical nutritional parameters (albumin, total protein, prealbumin) in patients with anorexia nervosa. We therefore performed our study in which we have followed the above mentioned parameters in 11 patients with anorexia nervosa before and after partial recovery and 11 age-matched healthy control females.

Subjects and Methods

Eleven female patients with DSM-IV diagnosed anorexia nervosa and 11 healthy age- and sex-matched volunteers were included in this study. All subjects were informed about the purpose of the study and gave their informed consent. Blood was withdrawn by venipuncture once in the controls and twice in the anorectics (at the beginning and the end of the refeeding period). The mean duration of the refeeding period was 30 days. Neither the control nor anorectic subjects suffered from thyroid disease or diabetes mellitus, nor were they taking any medication known to influence fat metabolism or the nutritional state. The blood samples were obtained after overnight fasting generally between 08:00–09:00 h.

Serum leptin concentrations were assessed by a commercially available radioimmunoassay kit (WAK Chemie, GMbH, Germany). Serum lipids and biochemical nutritional parameters were measured in the Department of Clinical Biochemistry of the General Faculty Hospital, Prague by standard laboratory methods.

Statistical analysis was performed by SigmaStat statistical software (Jandel scientific, USA). Means ± standard deviations were computed. ANOVA followed by Dunn's test was used to compare samples from the controls and patients with anorexia nervosa. Anorectic data before and after refeeding were compared by the paired T-test. The correlation between values was calculated by linear regression analysis and Pearson correlation test.

Table 1. *Age, body weight, body mass index (BMI), serum leptin, cholesterol, triglycerides, total protein, albumin and prealbumin in patients with anorexia nervosa before and after partial recovery (n=11) and healthy controls (n=11).*

	Anorexia nervosa untreated	Anorexia nervosa after partial recovery	Control group
Age (years)	23.7±3.1	23.7±3.1	24.3±3.2
Body weight (kg)	43.1±8.5*	47.9±8.4*$^{+}$	57.7±10.1
BMI (kg/m^2)	15.4±3.2*	17.2±3.0*$^{+}$	20.3±1.7
Leptin (ng.ml^{-1})	3.6±1.9*	4.83±2.1*$^{+}$	9.4±2.8
Cholesterol (mmol.l^{-1})	5.3±0.9	5.6±1.5	5.1±0.8
Triglycerides (mmol.l^{-1})	1.17±0.42	1.28±0.75	1.28±0.45
Total protein (g.l^{-1})	77.8±6.8	78.9±9.16	83.6±3.13
Albumin (g.l^{-1})	49.3±2.8	50.4±6.24	50.7±1.35
Prealbumin (g.l^{-1})	0.28±0.07	0.33±0.09	0.31±0.04

*Expressed as means ± standard deviations. * significant difference from control group (ANOVA, Dunn's test) $^{+}$ statistically significant difference from untreated patients with anorexia nervosa ($p<0.05$, paired T test, anorexia nervosa before vs after partial recovery) and (ANOVA, Dunn's test, anorexia nervosa before and after partial recovery respectively vs controls)*

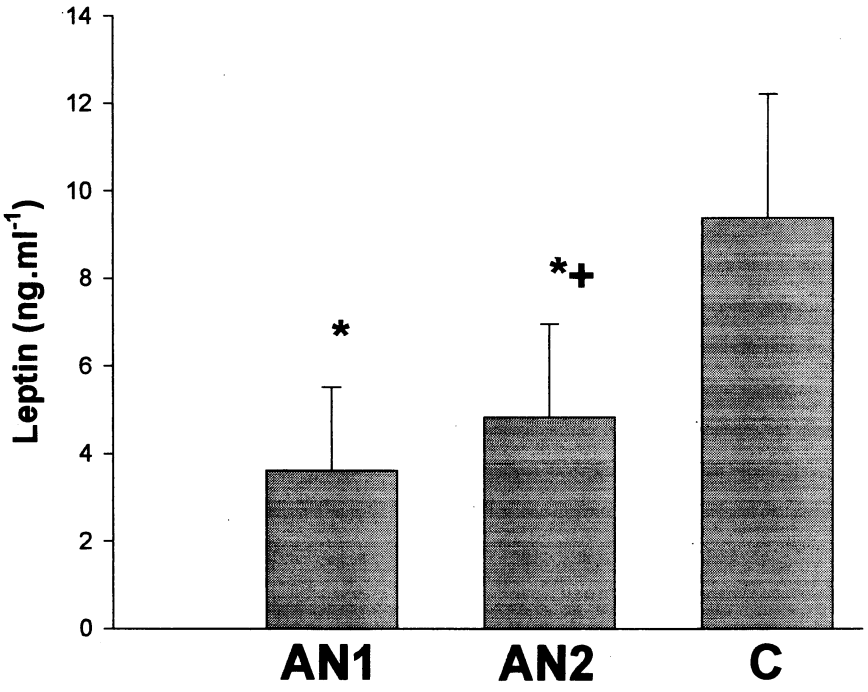

Fig. 1. *Serum leptin levels (ng.ml^{-1}) in patients with anorexia nervosa before (AN1) and after (AN2) partial recovery and control group C, expressed as means ± standard deviations. * Significant difference from control group, $^{+}$ significant difference from anorexia nervosa group before partial recovery ($p<0.05$, T-test),*

Results

Serum leptin levels, body weight, the body mass index in the anorexia nervosa group were significantly lower than in the controls (Table 1, Fig. 1). After the refeeding period, a significant elevation of leptin levels, body mass index and body weight was found, but the values after refeeding still remained significantly below those of the control group (Table 1, Fig. 1). No significant differences in serum lipids, total protein,

albumin and prealbumin concentrations were found either between anorexia nervosa patients before and after refeeding, or between controls vs anorectics before refeeding or controls vs anorectics after refeeding (Table 1).

Serum leptin levels correlated positively with the body mass index in the control group (r=0.79, p=0.004), the positive correlation with body mass index was less pronounced in anorectics before refeeding (r=0.64, p=0.032) and was not significant in anorectics after refeeding (Table 1, Fig. 2). No statistically significant correlation was found between leptin and the other parameters followed, either in the control group, or in the anorexia nervosa group before and after refeeding (Table 1).

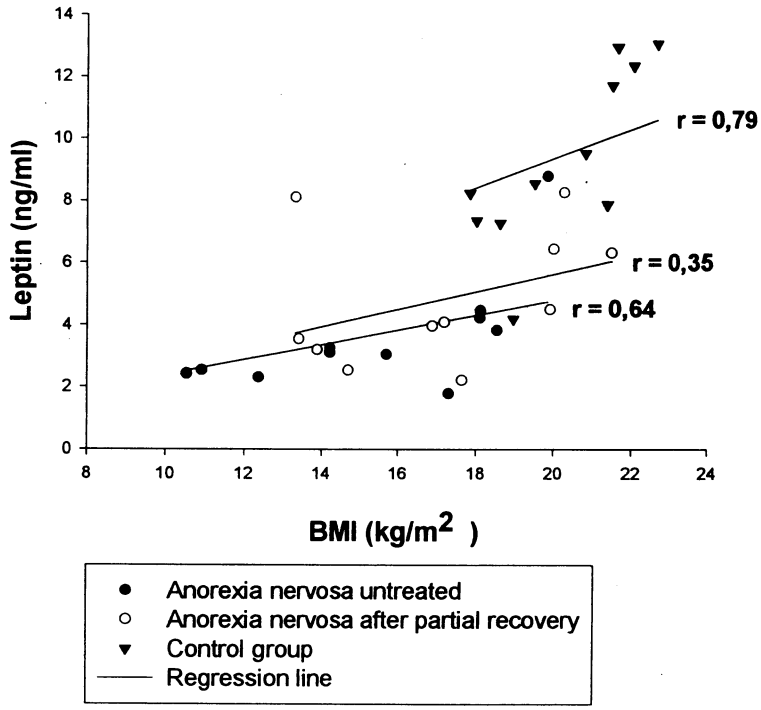

Fig. 2. *The correlation between serum leptin and body mass index in untreated patients with anorexia nervosa (lower regression line), patients with anorexia nervosa after partial recovery (middle regression line) and healthy controls (upper regression line). The correlation is significant (p<0.05) only for untreated patients with anorexia nervosa and control group.*

Discussion

It has been demonstrated in our study that serum leptin levels, body weight and the body mass index are significantly reduced in patients with anorexia nervosa in comparison with healthy controls. After the refeeding period, these values increased significantly, however, they still remained lower than those in the control group. Our results are in agreement with previously published reports (Grinspoon *et al.* 1996, Ferron *et al.* 1997, Eckert *et al.* 1997, Köpp *et al.* 1997, Casanueva *et al.* 1997).

The present study is the first to follow the relationships between leptin, serum lipids and routinely used parameters of nutritional status (total serum protein, albumin, prealbumin) in patients with anorexia nervosa before and after refeeding. In our study we did not find any alterations in serum cholesterol or triglyceride levels (previously reported in anorectic patients). Similar results concerned total serum protein, albumin and prealbumin which did not significantly differ in the control and anorexia nervosa group before and after partial recovery. The normal values of biochemical nutritional parameters observed in our study can be explained by the relatively high body mass index of the anorectic group at the beginning of our study. We suggest that the most pronounced changes in body composition in the initial stages of anorexia nervosa are related to the decrease of body fat content, while lean body mass remains relatively unaffected. This may be the reason why the changes in serum leptin levels after the refeeding period are so marked, while serum protein, albumin and prealbumin levels remain unchanged.

The observed positive correlation between serum leptin levels and the body mass index in anorexia nervosa group before refeeding is in agreement with the results published by Grinspoon *et al.* (1996), but not with those

of Eckert *et al.* (1997). However, in Eckert's group of anorectic patients there were several patients with the body mass index lower than 13 kg/m^2, while only one such patient was in our group and none in Grinspoon's group. We assume that there is a certain threshold beyond which leptin cannot further decrease physiologically, as was suggested by Eckert *et al.* (1997). This may explain the lack of significant correlation in his study between the body mass index and serum leptin values before recovery.

In contradiction to Eckert *et al.* (1997), we observed a dissociation between serum leptin levels and body mass index after recovery in our group of anorectics (the positive correlation was very weak and non-significant). We do not have any clear interpretation of these results. There is a number of factors which could potentially change serum leptin concentrations without direct relation alterations in body fat. It has been reported that insulin (Kolaczynski *et al.* 1996), proinflammatory cytokines (Sarraf *et al.* 1997) as well as progesterone (Hardie *et al.* 1997) are able to increase the synthesis of leptin. In the present study, we did not measure the concentrations of these hormones. Hence, the explanation of the dissociation between serum leptin and the body mass index can be purely speculative.

We suggest that the degree of correlation between serum leptin and the body mass index depends on the initial value of this index. In patients with a lower initial body mass index, proteosynthesis is probably preferred to fat storage during realimentation, so that the elevation of body weight and the body mass index is predominantly the result of increased lean body mass. On the contrary, the increase of the body mass index in anorectics with higher initial body weight is accompanied by a relatively higher increase of body fat content. Therefore, the increase in serum leptin levels in these patients is more closely related to the body mass index. This hypothesis is supported if our group of patients is divided into two subgroups according their body mass index. In the group with a higher body mass index (above 17.5 after realimentation, n=5) the correlation of leptin with the body mass index was relatively stronger, although still non-significant ($r = 0.79$, $p=0.12$), than in the group with body mass index below 17.5 after realimentation (n=6). In this subgroup, the correlation of leptin to body mass index is much less pronounced and tends even to be negative ($r = -0.28$, $p=0.60$).

We conclude that serum leptin levels represent a sensitive parameter of nutritional status in patients with anorexia nervosa which reflects changes of the body fat content before and after realimentation. The changes of serum leptin concentration appear earlier in the initial stages of the disease, at the time when serum concentrations of total protein, albumin and prealbumin are still normal.

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