Physiol. Res. 49: 679-684, 2000

Serum Leptin Levels in Patients with Sideropenic and Pernicious Anemia: The Influence of Anemia Treatment

M. MARKOVÁ , M. HALUZÍK[1], J. SVOBODOVÁ[1], M. ROSICKÁ[1], J. NEDVÍDKOVÁ[2], T. HAAS[1]

Institute of Hematology and Blood Transfusion, [1]Third Department of Medicine, First Faculty of Medicine, Charles University and [2]Institute of Endocrinology, Prague, Czech Republic

Received January 10, 2000
Accepted March 10, 2000

Summary

Leptin is a 16 kDa protein hormone involved in food intake, energy expenditure regulation and numerous other physiological processes. Recently, leptin has been demonstrated to stimulate hematopoietic stem cells *in vitro*. The aim of our study was to measure serum leptin and erythropoietin levels in patients with sideropenic (n =18) and pernicious anemia (n=7) before and during anemia treatment. Blood samples for the blood count, leptin and erythropoietin determinations were obtained by venepunction at the time of the diagnosis of anemia and after partial and complete anemia recovery. The relationships of serum leptin levels to erythropoietin levels and blood count parameters were also studied. No significant differences in serum leptin levels between the groups studied were found. The serum leptin levels in none of groups were modified by treatment of anemia (basal levels, the levels during treatment and after anemia recovery were 13.1±14.5 vs 12.8±15.6 vs 12.0±14.8 ng/ml in patients with sideropenic anemia and 7.8±8.5 vs 9.5±10.0 vs 8.9±6.6 ng/ml in patients with pernicious anemia). The erythropoietin levels were higher at the time of anemia in both groups and decreased significantly after partial or complete recovery. Serum leptin levels in both groups correlated positively with the body mass index. No significant relationships were found between serum leptin levels and erythropoietin values or various parameters of the peripheral blood count. We conclude that serum leptin levels in patients with sideropenic and pernicious anemia positively correlate with the body mass index but are not influenced by the treatment of anemia.

Key words

Leptin • Hematopoiesis • Anemia • Body mass index • Blood count • Stem cells

Introduction

Leptin is a 16 kDa protein hormone produced predominantly by adipocytes. Its serum concentrations usually reflect the degree of body adiposity, i.e. they are significantly higher in obese compared to lean subjects (Ostlund *et al.* 1996, Haluzík *et al.* 1999a). Serum leptin levels are significantly higher in females than in males even after adjustment for the body fat content. This dimorphism is probably the result of differences in sexual hormone levels, although some experimental reports concerning the influence of estrogens on serum leptin do not completely support this hypothesis (Nedvídková *et al.* 1997, Elbers *et al.* 1999).

Fax+4202 24920590
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

Serum leptin levels are significantly decreased in subjects with a lower body fat content, e.g. highly trained endurance sportsmen, patients with malnutrition of various etiology and patients with anorexia nervosa (Eckert *et al.* 1997, Haluzík *et al.* 1999b,c). The only known group of malnourished patients with increased serum leptin levels are those with chronic renal failure (Sharma *et al.* 1997). The increased serum leptin levels in these patients are probably due to impaired renal leptin excretion.

Leptin is primarily involved in food intake and probably also in energy expenditure regulation. However, a series of other possible functions of leptin in the human body, e.g. blood pressure regulation, angiogenesis etc, has also been reported (for review see Dagogo-Jack 1999).

Multiple isoforms of leptin receptors have been identified: a long isoform capable of full signal transduction and five short isoforms with a shortened or missing intracellular domain which are probably incapable of full signal transduction (Tartaglia 1997). The primary structure of the long isoform of leptin receptor exhibits homologies to the signaling subunits of the interleukin-6 type cytokine receptors including gp130 and to receptors for the leukemia inhibitory factor and the granulocyte colony stimulating factor (Konopleva *et al.* 1999). Cioffi *et al.* (1996) first reported that the leptin receptor is expressed in hematopoietic stem cells. It was found that leptin stimulates the proliferation of murine myelocytic and primitive hematopoietic progenitor cells *in vitro* (Umemoto *et al.* 1997). Leptin was also shown to modulate the T-cell immune response and to reverse starvation-induced immunosuppression in *ob/ob* mice (Lord *et al.* 1998). Mikhail *et al.* (1997) demonstrated that leptin alone increases the number of macrophage and granulocyte colonies and acts synergistically with erythropoietin to increase erythroid development.

Direct evidence of leptin stimulating action on human hematopoietic cells has been reported by Konopleva *et al.* (1999). The authors demonstrated that recombinant human leptin alone induced proliferation of blasts from patients with acute myeloid leukemia and augmented the stimulating effect of human granulocyte colony-stimulating factor, interleukin-3 and stem cell factor in these cells.

There is a strong experimental evidence for functional leptin receptors on hematopoietic stem cells. However, the data concerning the possible changes of serum leptin concentration in patients with various hematological diseases are almost completely missing. Therefore, we performed a study in which we measured serum leptin levels in patients with sideropenic and pernicious anemia before and during standard anemia treatment and after normalization of anemic values.

Patients and Methods

Eighteen patients with sideropenic anemia (5 males and 13 females), mean age 50±16.9 years and seven patients with pernicious anemia (1 male and 6 females), mean age 73±14.5 years, were included in our study. None of the patients suffered from diabetes and/or acute infectious diseases, none were treated by drugs known to affect food intake. All the patients were examined to exclude malignancy as a cause of sideropenic anemia or as a consequence of atrophic gastritis in pernicious anemia. All of the patients were informed about the purpose of the study and gave their informed consent to participate.

All subjects were measured and weighed. The first blood sample for leptin, erythropoietin and blood count determinations was withdrawn by venipuncture after an overnight fasting before the onset of anemia treatment. The second sample was withdrawn at the time of maximally stimulated erythropoiesis defined as the highest reticulocyte count (approximately between the 7th and 14th day after the beginning of treatment in patients with pernicious anemia and between the 12th and 20th day after the onset of treatment in patients with sideropenic anemia). The third sample was withdrawn after normal values of the red blood cells count and hemoglobin levels had been attained. The patients with sideropenic anemia were treated by ferrum sulphate (Aktiferrin cps, Merckle, Germany), while patients with pernicious anemia were treated by vitamin B_{12} (Vitamin B_{12}, Léčiva, Czech Republic).

Serum leptin and erythropoietin levels were measured by commercial sandwich immunoassay kits (BioVendor, Czech Republic and R&D Systems, USA, respectively). The normal range for erythropoietin levels established in our laboratory was from 3.3 to 16.6 mIU/ml. The erythrocyte and leucocyte count and hemoglobin levels were measured on an automatic blood count analyzer (Abbott, USA).

The SigmaStat software (Jandel Scientific, USA) was used for statistical analysis. Means and standard

deviations were calculated. The data within or between the studied groups were compared by one-way ANOVA followed by the Student-Newman-Keuls test. The relationships between leptin and the other studied parameters were calculated by Pearson's correlation test.

Results

The body mass index and serum leptin levels in the sideropenic anemia group did not significantly differ from those of the pernicious anemia group (Table 1). Neither partial nor complete anemia recovery changed serum leptin levels significantly (Table 1). Erythrocyte counts and hemoglobin levels increased significantly after partial anemia recovery and further increased after complete recovery from anemia. The serum erythropoietin levels decreased after partial anemia recovery and further decreased after complete anemia recovery (Table 1).

Serum leptin levels in both groups correlated positively with the body mass index (Fig. 1). This correlation still persisted after partial or full anemia recovery. No additional statistically significant relationships were found between serum leptin levels and the other parameters studied.

Table 1. Body mas index (BMI), serum leptin, erythropoietin (EPO), hemoglobin (HGB), red (Ery) and white (Leu) blood cell counts in patients with sideropenic and pernicious anemia before treatment (I), after partial (II) and complete (III) anemia recovery.

	Sideropenic anemia (n = 18)			Pernicious anemia (n = 7)		
	I	II	III	I	II	III
BMI (kg/m²)	23.8±5.6			24.4±6.1		
Leptin (ng/ml)	13.1±14.5	12.8±15.6	12.0±14.8	7.8±8.5	9.5±10.0	8.9±6.6
Ery (10^{12}/l)	3.9±0.6	4.4±0.4*	4.7±0.4*	2.4±0.7	3.0±0.6*	4.1±0.6*
HGB (g/l)	83.4±22.8	105.8±11.9*	126.5±9.8*	91.1±17.5	105.9±18.2*	127.6±11.0*
Leu (10^9/l)	7.5±5.8	8.6±6.6*	8.8±8.1	5.7±2.5	6.6±1.4	7.9±2.9
EPO (mIU/ml)	52.4±39.5	25.6±14.9*	11.2±7.6*	116.9±134	54.3±83.5*	12.9±7.4*

Data are expressed as means ± S.D., * significant difference from values before treatment ($p < 0.05$, ANOVA, Student-Newman-Keuls test)

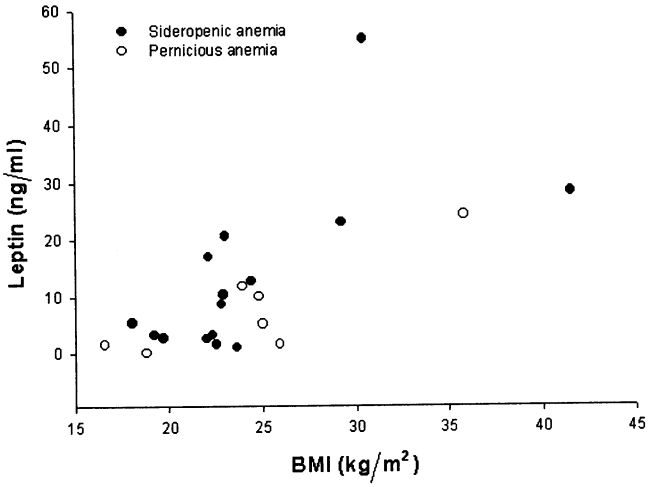

Fig. 1. *The relationship of serum leptin levels and the body mass index (BMI) in patients with sideropenic and pernicious anemia at the time of diagnosis ($p < 0.001$, $r = 0.66$, Pearson test)*

Discussion

The aim of this study was to measure serum leptin levels in patients with sideropenic and pernicious anemia at the time of diagnosis and after partial or complete anemia recovery. It was found that serum leptin levels in patients with sideropenic anemia do not significantly differ from those with pernicious anemia. The standard anemia treatment normalized the blood count and serum erythropoietin levels but did not change serum leptin levels in any of the groups studied.

Leptin is a 16 kDa protein hormone produced predominantly by adipocytes, which has been identified by mice *ob* gene cloning (Zhang *et al.* 1994). It is suggested to play an important role in food intake and energy expenditure regulation. Serum leptin levels usually correlate positively with body fat content and the body mass index, i.e. they are higher in obese compared to lean subjects (Ostlund *et al.* 1996). Besides the role of leptin in food intake regulation, its receptors have also been identified in a number of peripheral organs and tissues. Therefore, its role in other physiological processes such as angiogenesis, insulin secretion and action, lipolysis and blood pressure regulation etc. has also been suggested (for review see Dagogo-Jack 1999).

The primary structure of the leptin receptor shows close structural homology to the signaling subunits of interleukin-6 type cytokine receptors and receptors for the leukemia inhibitory factor and the granulocyte colony stimulating factor (Konopleva *et al.* 1999). The leptin receptor is expressed in hematopoietic stem cells (Cioffi *et al.* 1996). Leptin stimulates the *in vitro* proliferation of murine myelocytic and primitive hematopoietic progenitor cells (Umemoto *et al.* 1997) and modulates the T-cell immune response and starvation-induced immunosuppression in leptin-deficient *ob/ob* mice (Lord *et al.* 1998). It also increases the number of macrophage and granulocyte colonies and acts synergistically with erythropoietin to enhance erythroid development (Umemoto *et al.* 1997).

Although a number of experimental data implicate the possible role of leptin in hematopoiesis regulation, the clinical data concerning the possible changes of serum leptin levels in patients with various hematological diseases are very scarce. Kokot *et al.* (1998) studied the influence of long-term recombinant human erythropoietin therapy on plasma leptin levels in patients with chronic renal failure. These authors found that basal serum leptin levels in patients with chronic renal failure were significantly higher compared to healthy subjects. The recombinant human erythropoietin therapy significantly decreased serum leptin levels as compared to baseline values. The results of the study of Kokot *et al.* (1998) have suggested a possibility of the relationship between serum leptin and erythropoietin levels and/or leptin and erythropoiesis regulation. The patients in our study suffered from sideropenic or pernicious anemia, so that their serum erythropoietin levels at the beginning of the study were significantly elevated because of its compensatory renal hyperproduction. The anemia treatment led to the normalization of the blood count and to a return of elevated serum erythropoietin levels to normal values. However, serum leptin levels were not significantly affected by partial or complete anemia recovery.

In contrast to the above mentioned study of Kokot *et al.* (1998), we did not find any influence of alteration in serum erythropoietin levels and erythrocyte counts on serum leptin concentrations. Our results indicate that systemic serum leptin levels are not changed in patients with sideropenic or pernicious anemia and the anemia treatment did not affect serum leptin concentrations. However, this result does not exclude the possible interrelationship of serum leptin and erythropoietin levels in the control of erythropoiesis. The most likely explanation for the negative results of our study is the possibility that leptin rather regulates the erythropoiesis locally than systemically. Thus, although the local leptin production by bone marrow adipocytes could be significantly altered during anemia, no changes of serum leptin levels can be detected because the amount of leptin produced by bone marrow represents only a minor part of its total circulating pool.

Serum leptin levels usually positively correlate with body fat mass and the body mass index. It was previously shown that the white blood cell count is correlated with the amount of body fat in humans independently of age, gender and ethnicity (Pratley *et al.* 1995). Several reports have demonstrated that serum leptin levels positively correlate with the white blood cell count and suggested that leptin could play a role in the mediation of body fat influence on the white blood cell count (Wilson *et al.* 1997, Hirose *et al.* 1998). The relationship of serum leptin levels and the white blood cell count was also calculated in our study. However, in contrast to the above mentioned studies, we did not find

any significant relationship between serum leptin levels and the white blood cell count either at the time of anemia diagnosis or after its partial or complete recovery.

To our knowledge, this is the first study searching for changes of serum leptin levels in patients with anemia at the time of diagnosis and throughout its treatment. In conclusion, we have demonstrated here that serum leptin levels in patients with sideropenic and pernicious anemia positively correlate with the body mass index and are not changed after normalization of the red cell count by standard anemia treatment.

Acknowledgements

Supported by grant of IGA of MHCR No. 5455-3 and by the Danone Institute grant. We thank BioVendor company for kindly providing ELISA kits for the leptin measurements.

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Reprint requests

M. Haluzík, M.D., Ph.D., Third Department of Medicine, First Faculty of Medicine, Charles University, U nemocnice 1, Praha 2, 128 08, Czech Republic, e-mail: MHALU@LF1.CUNI.CZ