

# Soluble Leptin Receptor Levels in Patients with Chronic Renal Failure

J. KRÍŽOVÁ<sup>1</sup>, S. SULKOVÁ<sup>2</sup>, V. BEDNÁŘOVÁ<sup>2</sup>, J. PAŘÍZKOVÁ<sup>1</sup>, E. KOTRLÍKOVÁ<sup>1</sup>, M. HALUZÍK<sup>1</sup>

<sup>1</sup>Third Department of Medicine and <sup>2</sup>Department of Internal Medicine Strahov, First Medical Faculty, Charles University, Prague, Czech Republic

Received March 25, 2002

Accepted June 25, 2002

---

## Summary

Soluble leptin receptor (SLR) is the extracellular part of the leptin receptor. This protein is released into circulation and constitutes the main circulating leptin-binding protein. The aim of our study was to measure SLR concentrations in patients with chronic renal failure (CRF) and healthy subjects and to explore the relationship of SLR to other hormones and cytokines. The patients with CRF had significantly higher serum leptin, TNF- $\alpha$  and insulin levels than healthy subjects ( $25.1 \pm 23.5$  vs.  $9.4 \pm 7.6$  ng.ml<sup>-1</sup> (S.D.);  $14.2 \pm 4.2$  vs.  $4.55 \pm 2.5$  ng.ml<sup>-1</sup>;  $39.8 \pm 36.1$  vs.  $20.3 \pm 11.1$  mU.l<sup>-1</sup>). Serum soluble leptin receptor levels did not differ between these groups ( $19.1 \pm 11.3$  vs.  $19.6 \pm 6.1$  U.ml<sup>-1</sup>). An inverse relationship between serum SLR and leptin levels was found in both groups. In patients with CRF the inverse relationship between SLR and insulin, body fat content and total protein levels were also found, while in healthy subjects only inverse relationship of SLR with insulin and albumin concentrations were detected. We conclude that soluble leptin receptor levels in patients with chronic renal failure do not differ from those of healthy subjects despite higher serum leptin levels in CRF patients. The physiological consequences of this finding require further investigation.

---

## Key words

Soluble leptin receptor • Leptin • Anorexia • Chronic renal failure

## Introduction

Leptin is a protein hormone produced mainly by adipocytes (Zhang *et al.* 1994). Serum leptin concentrations positively correlate with body mass index (BMI) and body fat content. Malnourished patients or lean individuals have decreased leptin levels proportionally to the loss of body fat (Cederholm *et al.* 1997, Haluzik *et al.* 1999a,b). The only known exception are patients with renal failure that have higher leptin concentrations compared to healthy subjects (Iida *et al.* 1996, Merabet *et al.* 1997) despite a reduced body fat

content. There are several explanations for elevated leptin levels in these patients, especially lower clearance of leptin by the kidney and increased insulin and proinflammatory cytokine stimulation of leptin synthesis (Stenvinkel *et al.* 1999, Svobodová *et al.* 2001).

Leptin represents the main central satiety signal informing the hypothalamus about the status of body fat stores. Many other functions including regulation of energy expenditure, blood pressure, hematopoiesis etc. have also been demonstrated mostly in rodents (Mantzoros *et al.* 1997). In humans the regulation of appetite, food intake and energy expenditure seems to be

more complex. The effects of leptin are mediated through specific transmembrane receptors in the hypothalamus (Ob-Rb) (Tartaglia *et al.* 1995). Leptin receptors belong to class I of the cytokine receptor family (Devos *et al.* 1997). Hypothalamic leptin receptors have a long intracellular domain capable of signal transduction by interaction with Jak kinase and STAT proteins (Lee *et al.* 1996). There are several types of peripheral receptors expressed abundantly in different tissues (kidney, pancreas, liver, hematopoietic cells and others). The soluble leptin receptor (Ob-Re) is an extracellular domain of leptin receptor released into the blood (Tartaglia *et al.* 1995).

The clinical significance of the soluble leptin receptor has not yet been clarified completely. Analogically to functions of soluble receptors of some cytokines it can regulate effects of leptin in target tissues. Some soluble receptors of cytokines act as binding proteins that prolong half-life or disable the peripheral action of a particular ligand (Baumann 1995, Peters *et al.* 1996).

The etiology of reduced appetite and malnutrition in patients with chronic renal failure is very complex and hyperleptinemia is one of its possible causes. Since SLR serves as a leptin binding protein and can influence its pharmacokinetics and binding to peripheral tissues, we measured the levels of this protein in patients with CRF and control group of healthy subjects. The relationships of SLR to leptin and selected hormones and cytokines were also studied.

## Methods

Sixty-eight patients with chronic renal failure on hemodialysis without any signs of acute infections and 17 age-matched healthy subjects were included in the study. The study protocol was approved by the local Ethical Committee. The subjects were informed about the purpose of the study and provided informed consent.

Subjects were measured and weighed. The total body fat content was predicted from the skinfold thickness (four skinfolds) measured by a Best calliper. The blood samples for all measurements were withdrawn after an overnight fast between 08:00 and 09:00h. Serum leptin and tumor necrosis alpha (TNF- $\alpha$ ) levels were measured by commercial ELISA kits (BioVendor, CR and R&DS, USA, respectively), insulin levels by RIA kits (Immunotech, CR). Serum nutritional parameters, total protein and albumin were measured at the

Department of Clinical Biochemistry of the University Hospital, Prague, by standard laboratory methods.

The serum soluble leptin receptor levels were assessed by a commercial human specific sandwich ELISA kit with antibody raised against the human leptin receptor (BioVendor, CR). The human leptin receptor dimeric chimera was used as standard: one unit of soluble native human leptin receptor measured was equivalent to 2 ng of the recombinant standard. The sensitivity of the kit is 0.4 unit of soluble leptin receptor per ml of serum. The kit was tested for cross-reactivity with human leptin, erythropoietin, interleukin-1 receptor antagonist, soluble transpherrine receptor, TNF- $\alpha$  etc. No cross-reactivity was found with any of these substances.

The statistical analysis was performed using SigmaStat software (Jandel Scientific, USA). The results are expressed as means  $\pm$  S.D. T-test was used to compare the values of CFR patients and control group. The relationship between the variables was explored by Pearson correlation test.

## Results

The body fat content tended to be lower in patients with chronic renal failure (CRF) than in healthy subjects, but the difference did not reach statistical significance. Patients with CRF had significantly lower serum total protein and albumin levels, while their TNF- $\alpha$  and insulin levels were significantly higher than in control group. Serum leptin concentrations were significantly higher in patients with CRF, whereas serum soluble leptin receptor levels did not differ between groups (Table 1).

**Table 1.** Biochemical and hormonal parameters in patients with chronic renal failure (CRF) and control group (C).

	CRF	C
<i>Body fat content [%]</i>	24.2 $\pm$ 8.9	28.2 $\pm$ 7.1
<i>Total protein [g.l<sup>-1</sup>]</i>	68.3 $\pm$ 5.8*	75.5 $\pm$ 3.4
<i>Albumin [g.l<sup>-1</sup>]</i>	34.0 $\pm$ 3.1*	48.4 $\pm$ 3.4
<i>TNF-<math>\alpha</math> [ng.ml<sup>-1</sup>]</i>	14.2 $\pm$ 4.2*	4.6 $\pm$ 2.5
<i>Insulin [mU.l<sup>-1</sup>]</i>	39.8 $\pm$ 36.1*	20.3 $\pm$ 11.1
<i>Leptin [ng.ml<sup>-1</sup>]</i>	25.1 $\pm$ 23.5*	9.4 $\pm$ 7.6
<i>SLR [U.ml<sup>-1</sup>]</i>	19.1 $\pm$ 11.3	19.9 $\pm$ 6.1

Expressed as means  $\pm$  S.D.; \* significant difference ( $p < 0.05$ ) from controls; SLR: soluble leptin receptor

Serum soluble leptin receptor concentrations correlated negatively with leptin and insulin levels in both groups. In CRF group, there was a negative correlation with body fat content and total protein levels, whereas in the control group, negative correlation with albumin levels was found (Table 2).

Serum leptin levels correlated negatively with serum soluble leptin receptor concentrations and positively with serum insulin levels and the body fat content in both groups, in CRF group even with total protein (Table 3).

**Table 2.** Correlation between serum soluble leptin receptor levels and selected parameters in patients with chronic renal failure (CRF) and in the control group (C).

	CRF	C
<i>Leptin</i>	$r = -0.53^{**}$	$r = -0.45^*$
<i>Insulin [mU.l<sup>-1</sup>]</i>	$r = -0.36^*$	$r = -0.42^*$
<i>TNF-<math>\alpha</math> [ng.ml<sup>-1</sup>]</i>	NS	NS
<i>Body fat content [%]</i>	$r = -0.51^{**}$	NS
<i>Total protein [g.l<sup>-1</sup>]</i>	$r = -0.27^*$	NS
<i>Albumin [g.l<sup>-1</sup>]</i>	NS	$r = -0.55^*$

*r*: correlation coefficient; *p*: significance level, \*  $p < 0.05$ , \*\*  $p < 0.001$ .

**Table 3.** Correlation between serum leptin levels and selected parameters in patients with chronic renal failure (CRF) and in the control group (C).

	CRF	C
<i>SLR [U.ml<sup>-1</sup>]</i>	$r = -0.53^{**}$	$r = -0.45^*$
<i>Insulin [mU.l<sup>-1</sup>]</i>	$r = 0.52^*$	$r = 0.55^*$
<i>Body fat content [%]</i>	$r = 0.68^{**}$	$r = 0.80^*$
<i>TNF-<math>\alpha</math> [ng.ml<sup>-1</sup>]</i>	NS	NS
<i>Total protein [g.l<sup>-1</sup>]</i>	$r = 0.33^*$	NS
<i>Albumin [g.l<sup>-1</sup>]</i>	NS	NS

*r*: correlation coefficient; *p*: significance level; SLR: serum soluble leptin receptor, \*  $p < 0.05$ , \*\*  $p < 0.001$ .

## Discussion

The aim of our study was to compare soluble leptin receptor levels in patients with CRF on

hemodialysis and healthy subjects. We found that SLR concentrations in dialyzed patients did not differ while serum leptin levels were significantly higher compared to healthy subjects

There are only a few inconsistent studies exploring the function of soluble leptin receptor. It is suggested that soluble leptin receptor serves as a binding protein and modulates the bioavailability of leptin or its half-life and tissue effects (Liu *et al.* 1997, Lammert *et al.* 2001). Lee *et al.* (1996) demonstrated that just one tenth of bound leptin could be immunoprecipitated with a polyclonal antibody against leptin receptor and concluded that only 10 % of circulating leptin is bound by SLR. In contrast, the most recent study showed that the soluble leptin receptor represents the major leptin binding protein in human blood (Lammert *et al.* 2001). It has also been shown that hyperleptinemia in leptin-unresponsive Zucker diabetic fatty rats is at least in part the result of delayed clearance of leptin from circulation due to binding to its soluble receptor (Huang *et al.* 2001).

The clinical significance of free/bound leptin fractions has not yet been completely clarified. In lean subjects, 60-98 % of leptin circulates in a bound form (Houseknecht *et al.* 1996). Obese subjects have higher free leptin levels compared to healthy subjects. After weight loss due to gastric restrictive surgery in obese patients free leptin fraction decreases rapidly (van Dielen *et al.* 2002). It is suggested that food intake in obese subjects is not suppressed because of resistance to free leptin, its insufficient transport through hematoencephalic barrier or defects of receptor or postreceptor functions (Sinha *et al.* 1998). The free leptin fraction decreases during fasting, while the bound leptin fraction does not change. This effect is more pronounced in lean subjects (Sinha *et al.* 1998). The most direct evidence of physiological role of soluble leptin receptor in the modulation of leptin effects was reported by Huang *et al.* (2001) who demonstrated that adenovirus-mediated overexpression of soluble leptin receptor enhanced the weight reducing effects of exogenous leptin in leptin-deficient *ob/ob* mice.

Patients with chronic renal failure have higher serum leptin levels than healthy subjects due to decreased leptin clearance by the kidney and due to increased stimulation of leptin synthesis by elevated insulin and proinflammatory cytokine levels (Iida *et al.* 1996, Merabet *et al.* 1997, Haluzik *et al.* 2000). Here, we show that serum SLR concentrations in CRF patients do not differ from those of healthy subjects despite higher serum leptin levels in CRF patients. Since soluble leptin

receptor was demonstrated to be the main circulating leptin-binding protein, we suggest that this finding indicates increased free leptin fraction in circulation of CRF patients. This suggestion is in agreement with the published data showing that CRF patients have higher free but unchanged bound fraction of circulating leptin (Widjaja *et al.* 2000). The pathophysiological consequences of increased free circulating leptin levels and unchanged SLR concentrations in CRF patients as

well as the possibility of contribution of chronic hyperleptinemia to anorexia and malnutrition of CRF patients require further investigation.

### Acknowledgements

This study was supported by grant IGA MH 6663-3 and grant from the Institute Danone. We thank BioVendor company for kind providing ELISA kits for leptin and soluble leptin receptor determination.

### References

- BAUMANN G: Growth hormone binding to a circulating receptor fragment – the concept of receptor shedding and receptor splicing. *Exp Clin Endocrinol Diabetes* **103**: 2-6, 1995.
- CEDERHOLM T, ARNER T, PALMBLAD J: Low circulating leptin levels in protein energy malnourished chronically ill elderly patients. *J Int Med* **242**: 377-382, 1997.
- DEVOS R, GUISEZ Y, HEYDEN J, WHITE DW, KALAI M, FOUNTOULAKISIM, PLAETINCK G: Ligand-independent dimerization of the extracellular domain of the leptin receptor, and determination of the stoichiometry of leptin binding. *J Biol Chem* **272**: 18304-18310, 1997.
- HALUZÍK M., BOUDOVA L, NEDVÍDKOVÁ J, HALUZÍKOVÁ D, BARÁČKOVÁ M, BRANDEJSKÝ P, VILIKUS Z: Lower serum leptin concentrations in rugby players in comparison with healthy non sporting controls, relationships to anthropometric and biochemical parameters. *Eur J Appl Physiol* **79**: 58-61, 1998.
- HALUZÍK M, KÁBRT J, NEDVÍDKOVÁ J, SVOBODOVÁ J, KOTRLÍKOVÁ E, PAPEŽOVÁ H: The relationship of serum leptin levels and selected nutritional parameters in patients with protein-caloric malnutrition. *Nutrition*, **15**: 829-833, 1999a.
- HALUZÍK M, PAPEŽOVÁ M, NEDVÍDKOVÁ J, KÁBRT J: Serum leptin levels in patients with anorexia nervosa before and after partial refeeding, relationships to serum lipids and biochemical nutritional parameters. *Physiol Res* **48**: 197-202, 1999b.
- HALUZÍK M, SULKOVÁ S, SVOBODOVÁ J, BEDNÁŘOVÁ V, BODLÁKOVÁ B, MARKOVÁ M, TURKOVÁ M, HAAS T: Serum leptin levels in diabetic patients on hemodialysis: the relationship to parameters of diabetes compensation. *Endocrine Res* **26**: 303-317, 2000.
- HOUSEKNECHT KL, MANTZOROS CS, KULIWAT R, HADRO E, FLIER JS, KAHN BB: Evidence from leptin binding to proteins in serum of rodents and humans: modulation with obesity. *Diabetes* **45**: 1638-1643, 1996.
- HUANG L, WANG Z, LI C: Modulation of circulating leptin levels by its soluble receptor. *J Biol Chem* **276**: 6343-6349, 2001.
- IIDA M, MURAKAMI T, YAMADA M, SEI M, KUWAJIMA M, MIZUNO A, NOMA Y, AONO T, SHIMA K: Hyperleptinemia in chronic renal failure. *Horm Metab Res* **28**: 724-727, 1996.
- LAMMERT A, KIERSS W, BOTTNER A, GLASOW A, KRATZCH J: Soluble leptin receptor represents the main leptin binding activity in human blood. *Biochem Biophys Res Commun* **283**: 982-988, 2001.
- LEE GH, PROENCA R, MONTEZ JM, CARROL KM, PROENCA R, MOTEZ JM, DARVISHZADEH JG, LEE JI, FRIEDMAN JM: Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **279**: 632-635, 1996.
- LIU C, LIU X, BARRY G, LING A, MAKI R, SOUSA E: Expression and characterisation of putative high affinity human soluble leptin receptor. *Endocrinology* **138**: 3548-3554, 1997.
- MANTZOROS C, FLIER JS, LESEM MD, BREWERTON TD, JIMERSON DC: Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. *J Clin Endocrinol Metab* **82**: 1845-1851, 1997.
- MERABET E, DAGOGO-JACK S, COYNE DW, KLEIN S, SANTIAGO JV, HMIEL SP, LANDT M: Increased plasma leptin concentration in end-stage renal disease. *J Clin Endocrinol Metab* **82**: 847-850, 1997.

- PETERS M, JACOBS S, EHLERS M, VOLLMER P, MULLBERG J, WOLF E, BREM G, MEYER ZUM BUSCHENFELDE KH, ROSE-JOHN S.: The function of the soluble interleukin 6 (IL-6) receptor in vivo: sensitization of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6. *J Exp Med* **183**: 1399-1406, 1996.
- SINHA M, OPENTANOVA I, OHANNESIAN JP, KOLACZYNSKI JW, HEIMAN ML, HALE J, BECKER GW, BOWSHER RR, STEPHENS TW, CARO JF: Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. *J Clin Invest* **98**: 1277-1282, 1998.
- STENVINKEL P, LÖNNQVIST F, SCHALLING M: Molecular studies of leptin: implications for renal disease. *Nephrol Dial Transplant* **14**: 1103-1112, 1999.
- SVOBODOVÁ J, HALUZÍK M, BEDNÁŘOVÁ V, SULKOVÁ S, JISKRA J, NEDVÍDKOVÁ J, KOTRLÍKOVÁ E, KÁBRT J: Relation between serum leptin levels and selected nutritional parameters in hemodialyzed patients.(in Czech) *Vnitr Lek* **47**: 594-598, 2001.
- TARTAGLIA LA, DEMBSKI M, WENG X, DENG N, CULPEPPER J, DEVOS R, RICHARDS GJ, CAMPFIELD LA, CLARK FT, DEEDS J: Identification and expression cloning of a leptin receptor, Ob-R. *Cell* **83**: 1263-1271, 1995.
- VAN DIELEN FM, VAN 'T VEER C, BUURMAN WA, GREVE JW: Leptin and soluble leptin receptor levels in obese and weight-losing individuals. *J Clin Endocrinol Metab* **87**: 1708-1716, 2002.
- WIDJAJA A, KIELSTEIN JT, HORN R, MUHLEN A, KLIEM V, BRABANT G: Free serum leptin but not bound leptin concentrations are elevated in patients with end-stage renal disease. *Nephrol Dial Transplant* **15**: 846-850, 2000.
- ZHANG Y, PROENCA R, MAFFEI M, BARONE M, LEOPOLD L, FRIEDMAN JM: Positional cloning of the mouse ob gene and its human homologue. *Nature* **372**: 425-443, 1994.

---

**Reprint requests**

Jarmila Křížová, MD, Third Medical Department, First Faculty of Medicine, Charles University, U nemocnice 1, 120 00 Prague 2, Czech Republic, e-mail: jsvob@email.cz