# CONCENTRATION OF SOLUBLE LEPTIN RECEPTOR IN POPULATION

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Examinations were performed in 568 probands (217 men, 351 women) selected randomly from healthy population according to the following criterion: no treatment in the last six weeks for any disease. All probands were subjected to determination of BMI, cholesterol, triacylglycerols, LDL-cholesterol, intact proinsulin, insulin, uric acid, ALT, AST, GMT, LD, CRP, aterogenic plasma index, leptin, and soluble leptin receptor. We determined concentration of soluble leptin receptor in population sample of 560 probands. A positive correlation between leptinemia and BMI value was confirmed. It was found that Ob-Re concentration did not correlate with common anthropometrical parameters nor leptin concentration in the serum, but correlated negatively only with insulin and plasma aterogenity. This finding indicates an increased presence of free leptin in patients with signs of insulin (therefore also leptin) resistance.

## INTRODUCTION

As early as the 1950's, the existence of a signal of hormone nature, which gives information to the hypothalamus about food intake and quantity of adipose tissue, had been discussed. Forty years later on, the method of position cloning identified the Ob gene (responsible for leptin expression) and then the Db gene (responsible for expression of leptin receptor).

Discovery of these genes and subsequent investigation of their role in pathophysiology raised great expectations. It was found that leptin is produced mainly by adipocytes and then secreted into circulation where it binds on proteins. Then it passes through the hematoencephalic barrier and influences energy centers of the central nervous system (CNS). Simply said, the major effect of leptin in the CNS (in cooperation with other neuropeptides) consists of a decreased appetite and increased energy output. Moreover, the available information indicate that leptin influences many other functions of peripheral target tissues, namely liver and pancreas (involvement in the effect of insulin, gluconeogenesis, lipogenesis), kidney (natriuria), bone marrow (blockage of osteoresorption, stimulation of oesteogenesis, maturation of hematogenous cells), venous structures (angiogenesis), ovaries, uterus and testicles (maturation of germ cells, maturation of the fetus), adrenals (effect on production of catecholamines), and the lung. Leptin is involved in many hormone regulations and also influences production and effect of cytokines.

Leptin receptor (Ob-R) was cloned from DNA of mouse choriodeal plexus about one year later than leptin. Its structure is similar to the unit of the 1st subgroup of cytokine receptors; the Ob-R exists at least in five isoforms with the same extracellular domain and variable length of the cytoplasmic part.

The soluble form of Ob-R (Ob-Re) has neither intracellular nor transmembrane residues, consists only of the extracellular ligand binding the receptor domain. Other four isoforms of human Ob-R are not soluble and differ in sequence of cytoplasmic domains (they are designed as Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd).

Only the long form of the receptor (Ob-Rb) seems to be fully biologically active; other isoforms have mainly transport functions, are responsible for leptin degradation and may display a partial biological activity[3].

Leptin receptors were found in most tissues; particularly in CNS, pancreas, kidney, liver, skeletal muscles, adrenal marrow and cortex, endothelia, reproduction organs and hematopoetic structures (Ob-R occurs in many tissues even in early phases of the embryonal development[1, 2, 4, 5].

As almost no literary data are available about examination of concentration of soluble leptin receptor in population (only one paper deals with examination of Ob-Re in patients with manifested atherosclerosis and some papers estimated concentration of leptin receptor from leptin binding capacity[6, 7, 10], we decided to determine Ob-Re concentrations in common population.

## EXPERIMENTAL PART

We examined 568 probands, 217 men and 351 women. They underwent the preventive screening in the health service area covered by the Hospital Šternberk and were comprised in the study only under condition not to be treated by their physicians for any disorders in the last six weeks.

In all probands, BMI index was calculated and venous blood was collected (according to principles of Good Laboratory Practice) for analysis of cholesterol (analyzer ILAB600, BioVendor), cholesterol HDL (analyzer ILAB600, BioVendor), triacylglycerols (analyzer ILAB600, BioVendor), LDL cholesterol (analyzer ILAB600, BioVendor), intact proinsulin (ELISA line MARC-MAX, DAKO), insulin (analyzer Immulite, DPC), glucose (analyzer ILAB600, BioVendor), uric acid (analyzer ILAB600, BioVendor), ALT (analyzer ILAB600, BioVendor), AST (analyzer ILAB600, BioVendor), GMT (analyzer ILAB600, BioVendor), LD (analyzer ILAB600, BioVendor), and CRP (analyzer ILAB600, BioVendor). The atherogenic index of plasma was calculated in all probands. Leptin was determined by double sandwich ELISA (set BioVendor) using human IgG-Fc fragments with recombinant Ob-R (dimeric chimera) as standards. Analytical characteristics of Ob-Re measurement are given in Table 1.

## RESULTS

We examined 568 probands of mean age of 43.4 years (minimal age: 0, maximal age: 91 years). In general, probands had slight hypercholesterolemia, limit glycemia and obesity (Table 2).

Investigation of relationship between Ob-Re concentration and other measured parameters showed negative correlations of medium significance between Ob-Re concentration and insulin, and between Ob-Re and atherogenic plasma index (AIP) (Table 4).

However, Ob-Re accounts only for 19% variability of AIP and thus cannot be used for prediction (Table 5).

When the group of probands was divided according to sex, no significant differences in Ob-Re concentration were observed (chi-square test).

Then probands were stratified according to age. It was found that after birth the values of Ob-Re were very low, increased significantly to peak between the 5$^{th}$ to 10$^{th}$ year of age, then decreased without statistical significance (Table 3).

**Table 1.** Analytical characteristics of Ob-Re measurement

| | |
|---|---|
| **Sensitivity:** | limit of detection –4 U/ml. |
| **Precision:** | Intra-assay (n = 8): <5.6% |
| | Inter-assay (n = 8): <7.4% |
| **Linearity (recovery):** | 94,9–110,5% |
| **Comparison:** | not available |

**Table 2.** Measured parameters in the whole group (n = 568 probands)

| Parameters | Units | X | Median | S | Min | Max |
|---|---|---|---|---|---|---|
| Man | | 217 | | | | |
| Woman | | 351 | | | | |
| Age | Years | 43.4 | 54 | 23.5 | 0 | 91 |
| Ob-Re | U/ml | 50.3 | 26.1 | 64 | 2.8 | 498.1 |
| Leptin | pg/ml | 14.7 | 11.1 | 12.8 | 0.5 | 57 |
| Leptin/ Ob-Re | U/ml | 0.5 | 0.2 | 0.86 | 0.004 | 8.6 |
| BMI | units | 27.9 | 27.6 | 4.3 | 17.6 | 39.1 |
| Insulin | mIU/l | 9.36 | 7.45 | 6.05 | 0.6 | 27.2 |
| Proinsulin | pmol/l | 4.56 | 3.68 | 2.96 | 1.7 | 12.1 |
| Glycemia | mmol/l | 6.38 | 5.7 | 2.15 | 4 | 16.8 |
| Chol | mmol/l | 5.3 | 5.1 | 1.9 | 3.9 | 6.9 |
| HDL | mmol/l | 1.4 | 1.32 | 0.36 | 0.64 | 3 |
| Triglycerids | mmol/l | 1.83 | 1.65 | 0.89 | 0.54 | 5.4 |
| LDL dir | mmol/l | 3.5 | 3.4 | 1 | 1 | 6.8 |
| AIP | | 0.77 | 0.48 | 0.86 | -0.49 | 4.35 |
| Uricemia | umol/l | 300 | 285 | 78.6 | 115 | 588 |
| ALT | ukat/l | 0.55 | 0.44 | 0.41 | 0.13 | 3.8 |
| AST | ukat/l | 0.49 | 0.45 | 0.22 | 0.2 | 1.96 |
| GMT | ukat/l | 1.27 | 0.51 | 2.03 | 0.17 | 13.9 |
| CRP | mg/l | 13.7 | 4.9 | 25.9 | 1 | 172.8 |
| LD | ukat/l | 5.47 | 5.4 | 1.14 | 2.5 | 9.8 |

X..arithmet. mean S..standard deviation Min..minimum Max..maximum

**Table 3.** Concentration of Ob-Re, age groups, 568 probands (U/ml)

| Age | No. of probands | X | Median | S | Min | Max |
|---|---|---|---|---|---|---|
| 0-newborns | 14 | 17.8 | 11.98 | 14.7 | 7.13 | 51.36 |
| 1–5 yrs | 25 | 60.98 | 47.2 | 37.7 | 9.8 | 139.2 |
| 5–10 yrs | 25 | 36.1 | 32.3 | 15.8 | 15.7 | 81.96 |
| 10–15 yrs | 16 | 32.7 | 30.8 | 16.3 | 9.68 | 65 |
| 15–25 yrs | 40 | 47.6 | 24.9 | 60.2 | 6.6 | 265.5 |
| 25–35 yrs | 44 | 39.4 | 30.1 | 31.3 | 10.1 | 162.4 |
| 35–45 yrs | 36 | 75.6 | 41.1 | 90.6 | 6.96 | 450.3 |
| 45–55 yrs | 94 | 61.8 | 23.9 | 87.3 | 9.3 | 498.1 |
| 55–65 yrs | 113 | 52.3 | 25.7 | 65.8 | 6.66 | 374.7 |
| 65–75 yrs | 99 | 43.1 | 23.1 | 51.2 | 9.5 | 324.6 |
| 75–85 yrs | 48 | 50.4 | 23.1 | 71.2 | 2.8 | 419.6 |
| 85 yrs and over | 5 | 22.4 | 21.4 | 4.8 | 17.99 | 30.4 |

X..arithmet. mean S..standard deviation Min..minimum Max..maximum

**Table 4.** Significant correlations between leptin receptor, leptin and other measured parameters

| Parameter | Low | Mean | High | Very signific. |
|---|---|---|---|---|
| Ob-Re | | Insulin (cc –0.43)<br>AIP (cc – 0.49) | | |
| Leptin | Age (cc 0.20)<br>Glyc (cc 0.21) | BMI (cc 0.45) | Insulin (cc 0.64) | |
| Leptin/Ob-Re | AST (cc –0.22) | BMI (cc 0.36) | Insulin (cc 0.66) | |

cc..correlation coefficient

**Table 5.** Correlation between Ob-Re and AIP without effect of other parameters

| Parameter | Combination with: | Correlation coefficient |
|---|---|---|
| AIP | Insulin | -0.24 |
| AIP | BMI | 0.57 |
| AIP | Age | 0.42 |
| AIP | Age+insulin | -0.39 |
| AIP | Age+BMI | 0.58 |
| **Ob-Re accounts only for 19% variability of AIP** | | |

## DISCUSSION

Concentration of soluble leptin receptor can be measured either directly by ELISA or by LIFA method. Literary data on analysis of the presence of leptin receptors using separation methods are sporadical; no papers report on measurements using ELISA or LIFA technique in larger groups of patients[6, 7, 10].

Determination of Ob-Re concentration is supposed to contribute to better understanding of pathophysiological mechanisms as well as hormone regulations. Moreover, it could be a valuable diagnostic tool and maybe of therapeutical importance for many medical branches (e.g. osteology, diabetology, gastroenterology, obesitology, lipidology, cardiology, gynaecology, pediatrics, intensive care).

We believe that our findings could indicate a negative relationship between parameters of insulin resistance and Ob-Re concentration in persons who have a positive correlation between BMI and leptinemia. This suggests an increased occurrence of free, biologically active leptin in probands with hyperinsulinemia (insulin resistance and thus leptin resistance). Highly interesting is the fact that in normal population no correlation can be found between Ob-Re concentration and leptinemia nor between BMI and Ob-Re. Thus, these findings remain ambiguous and should be explained by further studies.

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