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## Brief report

## Differentiation between free and bound leptin in depressed patients



Maria Strauss<sup>a,\*</sup>, Roland Mergl<sup>a</sup>, Juergen Kratzsch<sup>b</sup>, Mathias Brügel<sup>c</sup>, Erik Strauss<sup>d</sup>,  
Ulrich Hegerl<sup>a</sup>, Peter Schoenknecht<sup>a</sup>

<sup>a</sup> Department of Psychiatry and Psychotherapy, University of Leipzig, Semmelweisstraße 10, 04103 Leipzig, Germany

<sup>b</sup> Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Germany

<sup>c</sup> Institute of Clinical Chemistry, Ludwig-Maximilians-University Munich, Germany

<sup>d</sup> Medical Practice for Neurology, Psychiatry and Psychotherapy, Kopfzentrum Leipzig<sup>®</sup>, Germany

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## ABSTRACT

The relationship between leptin and affective disorders is still unknown. We measured free and bound leptin in 13 drug naïve subjects. Leptin did not significantly differ between patients and controls. As part of future studies, it also appears useful to distinguish between free and bound leptin.

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## 1. Introduction

Leptin, the product of the *ob* gene, is mainly synthesized by adipose tissue in proportion to the percentage of body fat and may regulate food intake and energy balance (Zhang et al., 1994). The relationship between leptin and major depression (MD) is still unknown. However, when serum leptin concentrations were investigated in depressed patients inconsistent findings emerged: Deuschle et al. (1996) found normal leptin levels, compared to healthy controls (effect size in males: Cohen's  $d = -0.29$ ; 95% confidence interval (95% CI) =  $[-0.97; 0.41]$ ; females:  $d = -0.18$ ; 95% CI =  $[-1.01; 0.66]$ ). Others reported increased or decreased serum leptin levels in patients with depressive disorders (increased levels: e.g., Antonijevic et al. (1998): effect size in males:  $d = 0.83$ ; 95% CI =  $[-0.31; 1.86]$ ; females:  $d = 1.73$ ; 95% CI =  $[0.50; 2.77]$ ; decreased levels, e.g. Jow et al. (2006):  $d = -2.27$ ; 95% CI =  $[-2.72; -1.80]$ ). However, up to date, only in one study CSF leptin levels were investigated in patients with MDD. In this study, lower CSF leptin levels in female suicide attempters with MDD compared with other diagnoses such as substance abuse, anorexia nervosa or anxiety were found (Westling et al., 2004). These inconsistent findings prompted us to conduct this pilot study with the following purpose:

1. Do leptin concentrations in drug naïve depressed patients differ from those in healthy subjects?
2. How do the concentrations of free and bound leptin compare with those of a healthy control group?

Based on the previously reported changes in leptin concentration in serum under the influence of antidepressants (Kraus et al., 2002), we decided to include only drug-naïve depressed patients. Furthermore, instead of using a standard commercial radioimmunoassay (RAI) to determine leptin concentrations, we for the first time chose to differentiate between free and bound leptin. For this purpose, we decided to ascertain the soluble leptin receptor (sol-R) which is the main leptin-binding protein in the human circulatory system (Schaab et al., 2012; Lammert et al., 2001). In view of additional evidence that free leptin is more "active" and can cross the blood–brain barrier more easily (Land et al., 2000), we decided to also determine leptin levels in the patients' CSF.

## 2. Methods

## 2.1. Subjects

Out of a total of 30 initially screened MDD patients who had been admitted into the Department of Psychiatry and Psychotherapy, University Hospital Leipzig, seven underwent lumbar puncture for diagnostic reasons (seven inpatients (two males and five females) suffering from MDD). Unfortunately, only seven of the 30 initially screened, depressive patients gave their consent to carry out the investigation and thus also participate in the study after having been informed on indication and execution of lumbar puncture. Only seven patients could therefore ultimately be

\* Corresponding author. Tel.: +49 341 97 24304; fax: +49 341 97 24509.

E-mail address: [maria.strauss@medizin.uni-leipzig.de](mailto:maria.strauss@medizin.uni-leipzig.de) (M. Strauss).

**Table 1**  
Demographic and clinical characteristics of the sample.

Variable	MDD group (n=6)	Healthy controls (n=7)	p	Cohen's d (95% CI)
Age (years) (M ± s)	43.83 ± 9.01	38.43 ± 17.20	0.43 <sup>a</sup>	–
Sex (female (n (%)))	4 (66.7%)	5 (71.4%)	1 <sup>b</sup>	–
Body Mass Index (kg/m <sup>2</sup> ) (M ± s)	25.12 ± 2.76	27.79 ± 3.88	0.25 <sup>a</sup>	–
Overweight (Body Mass Index > 25.0) (n (%))	3 (50.0%)	5 (71.4%)	0.59 <sup>b</sup>	–
Family history of psychiatric disorder (n (%))	3 (50.0%)	–	–	–
ICD-10 diagnosis	–5 (83.3%)–1 (16.7%)	–	–	–
F32.1 (n (%))	–	–	–	–
F33.3 (n (%))	–	–	–	–
HAMD-17 total score (M ± s) (range)	21.83 ± 3.87 (17–26)	–	–	–
HAMD-17 total score (F32.1) (n=5) (M ± s)	21.00 ± 3.67	–	–	–
HAMD-17 total score (F31.3/F33.3) (n=2) (M ± s)	26.00	–	–	–
Leptin serum concentration (ng/ml) (M ± s) [median (range)]	12.20 ± 12.17 [9.10 (1.4–33.2)]	18.73 ± 16.16 [9.70 (5.8–43.8)]	0.48 <sup>a</sup> /0.61 <sup>c</sup>	–0.45 (–1.52; 0.68)
Leptin cerebrospinal fluid concentration (ng/ml) (M ± s) [median (range)]	0.20 ± 0.12 [0.21 (0.04–0.35)]	0.26 ± 0.16 [0.21 (0.05–0.50)]	0.43 <sup>a</sup> /0.57 <sup>c</sup>	–0.42 (–1.49; 0.71)
Soluble leptin receptor concentration (ng/ml) (M ± s) [median (range)]	20.98 ± 5.16 [19.85 (16.1–29.6)]	22.14 ± 5.68 [20.60 (15.3–33.3)]	0.78 <sup>a</sup> /0.74 <sup>c</sup>	–0.21 (–1.29; 0.90)
Cerebrospinal fluid leptin/serum leptin ratio (M ± s) [median (range)]	0.03 ± 0.01 [0.02 (0.01–0.05)]	0.02 ± 0.01 [0.02 (0.01–0.03)]	0.20 <sup>a</sup> /0.32 <sup>c</sup>	0.74 (–0.44; 1.81)
Soluble leptin receptor/ serum leptin ratio (M ± s) [median (range)]	5.75 ± 6.11 [3.10 (0.48–14.00)]	2.00 ± 1.15 [2.47 (0.46–3.55)]	0.39 <sup>a</sup> /0.16 <sup>c</sup>	0.89 (–0.31; 1.97)

<sup>a</sup> Mann–Whitney test.

<sup>b</sup> Exact test by Fisher.

<sup>c</sup> Analysis of covariance (ANCOVA) with body mass index as covariate.

included in the study. The CSF examination was conducted to exclude organic genesis of depression (such as central inflammatory genesis).

The diagnosis was based on the International Statistical Classification of Mental Disorders, 10th revision (ICD-10) criteria (Dilling et al., 2006). Six patients suffered from unipolar depression and one from bipolar depression. The latter patient had been excluded to reduce clinical heterogeneity of the sample. All patients were drug-free prior to the blood and CSF sampling. There were no other physical illnesses.

Subjects who showed evidence of any other psychiatric disorder were excluded. In addition, we studied seven healthy controls in a group comprising five females and two males. The healthy control group comprised patients from the neurological department who underwent the lumbar puncture to exclude any physical illness. The investigation yielded no pathological findings in these subjects. All control subjects were free of any psychotropic medication, and had no history of a psychiatric disorder or a suicide attempt. They were comparable with the MDD patients regarding age and gender. The general characteristics of the patients and the healthy controls are summarized in Table 1.

All the patients gave a written informed consent according to the guidelines laid down in the Declaration of Helsinki. A positive vote was obtained from the local ethics committee.

## 2.2. Laboratory procedures

Venous blood was collected from all patients and control subjects in the fasting state around 8:00 a.m. Lumbar puncture was performed in both (patients and healthy control group) in a sitting position between 8:00 and 9:00 a.m.

All analyses were performed in the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig. Leptin was measured by the “sensitive Leptin ELISA” method of Mediagnost™ (Reutlingen, Germany). The sol-R determination in serum was performed as described recently (Kratsch et al., 2002).

## 2.3. Statistical analysis

Statistical analyses were performed with the Predictive Analytics Software (PASW) Version 18 from SPSS™ (Statistical Package for the Social Sciences). In view of the small sample size, non-parametric Mann–Whitney tests were applied to address the questions of whether depressed patients and healthy controls significantly differed in several biochemical parameters related to leptin (leptin serum concentration, leptin cerebrospinal fluid (CSF) concentration, soluble leptin receptor (SLR) concentration; CSF leptin/serum leptin ratio, SLR/serum leptin ratio). Moreover, ANCOVA with BMI as covariate was used for this purpose. The significance level was set at  $\alpha=0.05$ . All statistical tests were two-sided. Because of the exploratory approach no correction for multiple testing was done.

## 3. Results

Neither leptin serum nor serum sol-R levels did differ significantly between patients with MDD and the healthy controls.

Regarding group differences in the leptin serum concentrations, an effect size between small and medium (Cohen's  $d=0.45$ ) was found which would be statistically significant in a larger study. For an effect size of  $d=0.45$  a sample of 73 patients and 85 controls would have a statistical power of 80% for a two-tailed  $p < 0.05$  according to a calculation by using G\*Power (Version 3.0.5.).

Likewise, no statistically significant difference was found when comparing the CSF leptin concentration between patients and the controls.

The results in terms of leptin concentrations do not differ (ANCOVA:  $p=0.67$  (serum leptin);  $p=0.63$  (CSF leptin)) if two patients without a single moderate episode of MDD were excluded from analysis (descriptive statistics not shown).

See Table 1 for an overview of the results.

## 4. Discussion

According to our knowledge in the pilot study presented here, three different leptin concentrations were first measured, and comparisons subsequently performed between drug naïve, depressed patients and healthy control subjects. There were no significant differences between diseased and healthy subjects in this sampling. This is in contrast to most previously published investigations of leptin in depressed patients. As a major limitation of the most previously published studies, leptin levels were investigated when the patients had already received antidepressant medication or following a wash-out phase ranging from 1 week to 3 months (Deuschle et al. 1996; Antonijevic et al., 1998; Kraus et al., 2001). An effect of psychotropic medication on the leptin concentration has to be considered since changes in leptin levels under antidepressant medication have been reported (Kraus et al., 2002). Perhaps the changes in leptin concentration reported in other studies were triggered by the antidepressants

usually taken beforehand or after a short wash-out phase (Deuschle et al. 1996; Antonijevic et al., 1998; Kraus et al., 2001).

Due to the very small scale of the sampling, however, our results should be interpreted cautiously, and substantiated by larger samples. Apart from the small sampling scale, the following limitation on results must be critically noted:

First, the proportion of overweight people is higher at 71.43% in the control group, compared with 50.00% in the patient group. However, this difference has no statistical significance (Table 1).

The ANCOVA with the BMI as a covariate revealed no significant differences between the two groups, though the results can be interpreted only to a limited extent due to the small sample. Second, it would have been interesting to consider the values of glucose, triglycerides and HDL-cholesterol in view of known relations of leptin to these analytes; however, these values were not available in the context of our study. Third, in female patients and controls, there might have been a significant association between the leptin concentration and the hormonal milieu (menopausal/premenopausal status; time of cycle) which has to be investigated in further studies.

We conclude that wherever possible, future studies should include drug-naïve patients, and differentiate between bound and free leptin in the concentrations.

#### Conflict of interest

Ulrich Hegerl is an advisory board member for Lilly and Lundbeck; a consultant for Nycomed; and a speaker for Bristol-Myers Squibb. All other authors declare that they have no conflicts of interest.

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