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Interleukin-6 Levels in the Central Nervous System Are Negatively Correlated with Fat Mass in Overweight/Obese Subjects

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Recently, we demonstrated that intracerebroventricular injection of IL-6 increases energy expenditure and decreases body fat in rodents. Therefore, IL-6 may play a role in appetite and body weight control in the central nervous system. In the present study we evaluated cerebrospinal fluid (CSF) and serum IL-6 levels in humans in relation to body fat content and to CSF and serum levels of leptin. Thirty-two healthy overweight/obese male subjects with a body mass index range of 29.3–36.0 kg/m^2 were studied. Total and sc body fat were measured by dual energy x-ray absorptiometry and computed tomography, respectively. CSF IL-6 levels were in some individuals higher than serum IL-6 levels and correlated negatively with total body weight, sc and total body fat. In contrast, CSF leptin levels were 30–60 times lower than serum leptin levels and correlated positively with serum leptin, body weight, sc and total body fat. Furthermore, there was a negative correlation between CSF IL-6 and leptin. In conclusion, CSF IL-6 differs in many ways from CSF leptin. CSF IL-6 may be locally produced rather than serum derived, and body fat-regulating regions in the central nervous system may be exposed to insufficient IL-6 levels in more severe obesity. (*J Clin Endocrinol Metab* 88: 4379–4383, 2003)

WITH ITS INCREASING prevalence and its severe consequences, obesity has emerged as a leading public health issue in most western countries (1, 2). For the development of treatment of obesity it is important to identify genes of importance for the regulation of body fat in humans. Although findings in mutant mouse models have been pivotal for the progress made to date (3, 4), it is important to verify the significance of these findings in humans.

IL-6 is a pleiotropic cytokine that is produced by the immune system as well as by other tissues (5–9). For instance, IL-6 is produced and released by adipose tissue, and the levels of IL-6 in serum are positively correlated to adipose tissue mass (5, 6, 10). Serum IL-6 levels have also been reported to correlate with metabolic disturbances and cardiovascular morbidity (11, 12), but it has been difficult to establish a cause-effect relationship in humans.

Recently, we have demonstrated that IL-6-deficient mice develop mature-onset obesity and several associated metabolic perturbations, and that this effect may be due to lack of central actions of IL-6 (13, 14). Several lines of evidence indicate that IL-6 is expressed in hypothalamic nuclei that are involved in the regulation of appetite and other metabolic functions, although IL-6 is also produced by several other parts of the brain (15–18). Moreover, it has been reported that IL-6 is released from the brain into the blood circulation during prolonged physical exercise, a condition associated with marked metabolic alterations (19). Therefore, we hypothesized that altered central levels of IL-6 may contribute to obesity in humans. In the present study we have investigated the association between cerebrospinal fluid (CSF) IL-6 levels and body fat content in healthy overweight/obese male subjects. As leptin is a prototype fat-derived cytokine acting on the brain (4), we also measured leptin levels in serum and CSF.

Abbreviations: BMI, Body mass index; BW, body weight; CNS, central nervous system; CSF, cerebrospinal fluid; DXA, dual energy x-ray absorptiometry.

Materials and Methods

Patients and study settings

This study was performed by the use of baseline examinations of subjects participating in a clinical weight loss trial. The study subjects were recruited in response to local advertisements. The inclusion criteria for the subjects were: male sex, age more than 18 yr, body mass index (BMI) between 27.5 and 37.0 kg/m^2, and weight stability. The exclusion criteria were reported weight change of more than 3 kg in the month before examination, diabetes mellitus requiring drug or insulin treatment, cardiovascular disease, unstable smoking, history or presence of eating disorder, and pharmacological treatment with weight loss agents, antidepressants, steroids, antiinflammatory drugs, or anti-convulsants. In total, 32 subjects with a BMI range of 29.3–36.0 kg/m^2, aged 26–68 yr, were recruited. Seven of the study subjects were current smokers.

The sampling of CSF was performed in the morning (at about 0600 h) according to standardized procedures with the examined subject in a lateral recumbent position and lumbar puncture at the L3–L4 or L4–L5 interspace with a standard needle (Sprotte standard needle with introducer, 0.7 mm, 22 gauge, 90/120 mm; Rusch Inc., Duluth, GA). The serum samples were taken shortly after the CSF sampling, at about 0800 h. The subjects were fasting overnight until the last serum sample had been taken. The samples of CSF or serum were immediately placed on ice and centrifuged at 4 C. Samples were then frozen in separate containers at −80 C pending analysis. All subjects gave their written informed consent, and the study protocol was approved by the ethics committee of the medical faculty of Goteborg University.

Body weight (BW) and body composition

BW was measured to the nearest 0.1 kg with the subject in light clothing on a calibrated balance scale. Body mass index (BMI) was

calculated as body mass/(height)^2 (kilograms per meter squared). Total body fat was assessed using dual energy x-ray absorptiometry (DXA) with a Lunar DPX-L scanner (Lunar Corp., Madison, WI) using software version 1.31. Body composition was determined using a four-scan computed tomography technique (GE Hi-Speed Advantage, General Electric, Fairfield, CT) to measure skeletal muscle and sc and visceral adipose tissue. The following settings were used: 20 kV; 250 mA; slice thickness, 10 mm. Scans were taken of the abdomen at the level of the L4–L5 discs and of the thigh midway between the iliac crest and the knee. The effective dose equivalent per examination was 0.4–0.8 mSv. The tissue areas and anatomical boundaries were determined as described previously (20). The precision (coefficient of variation) for the determination of sc adipose tissue was 0.5%.

Biochemical analyses

For measurements of CSF and serum IL-6 levels, the Quantikine High Sensitivity human IL-6 ELISA with a detection limit of 0.156 pg/ml was used (R&D Systems, Minneapolis, MN). To validate the Quantikine High Sensitivity human IL-6 ELISA for measurements in CSF, a control experiment was performed. CSF and serum samples were divided into two aliquots. One aliquot of 450 μl was spiked by the addition of 50 μl kit standard IL-6 concentrate (20 pg/ml), and the other aliquot was analyzed without spiking. The same amount of IL-6 was added to Calibrator Diluent as a control. To evaluate parallelism of CSF in the assay, serial doubling dilution was performed with the spiked samples. Two kinds of calculations, spike recovery and parallelism, were made. Spike recovery was determined as: % recovery = 100(IL-6 concentration of spiked sample − IL-6 concentration of unspiked sample)/IL-6 concentration of spiked calibrator diluent. Parallelism of the diluted samples to the standard curve was determined as: % recovery$_{1:2}$ = 100(observed IL-6 concentration of 1:2 diluted spiked sample)/(IL-6 concentration of spiked sample/2). Percent recovery$_{1:4}$ and percent recovery$_{1:8}$ were determined by similar calculations. The percent recovery was in the proper range (80–120%) for both serum and CSF samples. Moreover, there were good correlations between calculated and measured values for the serial dilutions of both the serum and CSF samples (r = 1.00 and r = 0.99, respectively). These results clearly indicate that the Quantikine High Sensitivity human IL-6 ELISA is valid for analyses of CSF samples. Moreover, this ELISA is very specific and does not cross-react with several tested peptides (R&D assay manual), and the capture antibody has been a part of other assays (R&D personal communication) used for measurements of CSF IL-6 since the mid 1990s (21, 22). The assay was used according to the manufacturer's instructions. Serum and CSF leptin concentrations were determined at Amgen, Inc. (Thousand Oaks, CA), by ELISA with a detection limit of 0.04 ng/ml. CSF levels of CRH, β-endorphin, and neuropeptide Y were determined according to standardized procedures. Measurements of serum and CSF levels of albumin were performed at Clinical Neurochemistry Laboratory, Molndal, Sahlgrenska University Hospital (23).

Leaner control group

Ten male control subjects with a BMI range of 21.0–27.5 kg/m^2, aged 24–45 yr, were recruited for sampling of blood and CSF. In these subjects, serum and CSF levels of IL-6, leptin, and albumin were measured. All subjects gave their written informed consent, and the study protocol was approved by the ethics committee at the medical faculty of Goteborg University.

Statistical analyses

Relationships between continuous variables were analyzed using linear regression models. In some cases a logarithmic transformation was applied to the dependent variable to obtain a linear regression relationship. Due to heteroscedasticity, robust SEs for the regression estimates were calculated according to White's correction (24). For CSF leptin with censored observations, further analyses of association were made using the Tobit model (24). The data were analyzed using the Stata statistics package (25).

Results

In the present study CSF levels of IL-6 and leptin were correlated to different measures of body composition. CSF IL-6 levels were negatively correlated with BW (r = −0.354; $P = 0.021$), whereas there was, as expected, a positive association between CSF leptin levels and BW (r = 0.555; $P < 0.001$). CSF IL-6 also tended to be negatively correlated with BMI (r = −0.343; $P = 0.054$), whereas CSF leptin was positively correlated with BMI (r = 0.442; $P = 0.013$).

There was a negative correlation between CSF IL-6 levels and total body fat measured by DXA (Fig. 1A). In contrast, there was a positive correlation between CSF leptin levels and total body fat (Fig. 1B). CSF IL-6 levels were found to correlate negatively with both thigh and abdominal (L4 level) sc fat measured by CT (Fig. 2, A and C). However, CSF leptin levels correlated positively with thigh and abdominal (L4 level) sc fat (Fig. 2, B and D). CSF IL-6 levels tended to correlate positively with visceral fat the L4 level (r = 0.348; $P = 0.051$), whereas CSF leptin levels were negatively correlated with visceral fat (r = −0.379; $P = 0.036$). The association between CSF IL-6 levels and body fat was not affected by smoking (data not shown).

CSF IL-6 levels did not correlate with age (r = 0.125; $P = 0.50$), lean body mass (r = −0.033; $P = 0.86$), abdominal muscle mass (r = 0.035; $P = 0.85$), thigh muscle mass (r = −0.295; $P = 0.10$) measured by computed tomography, or CSF levels of CRH, β-endorphin, and neuropeptide Y (data not shown).

To elucidate whether IL-6 is produced locally in the brain or is filtrated from serum to CSF, we compared the CSF and

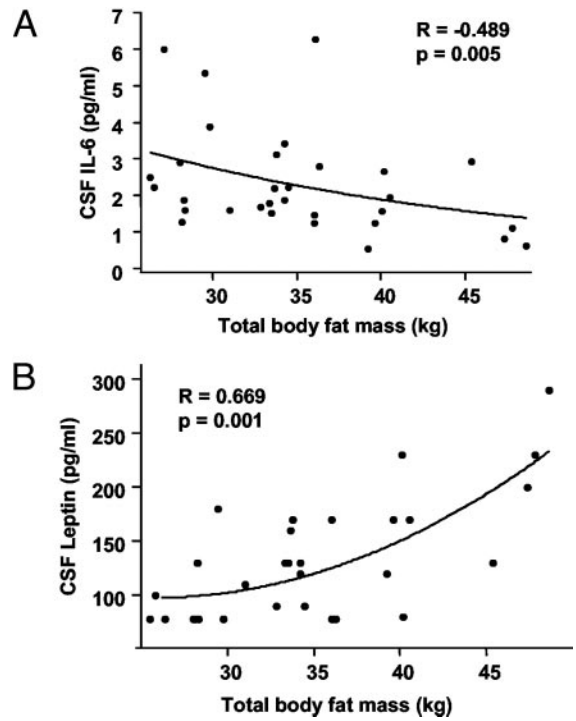

FIG. 1. CSF IL-6 levels correlated negatively with total body fat. Log CSF IL-6 *vs.* total body fat: r = −0.489; $P = 0.005$ (A); log CSF leptin *vs.* total body fat: r = 0.669; $P < 0.001$ (B). Total body fat was measured by DXA (n = 32).

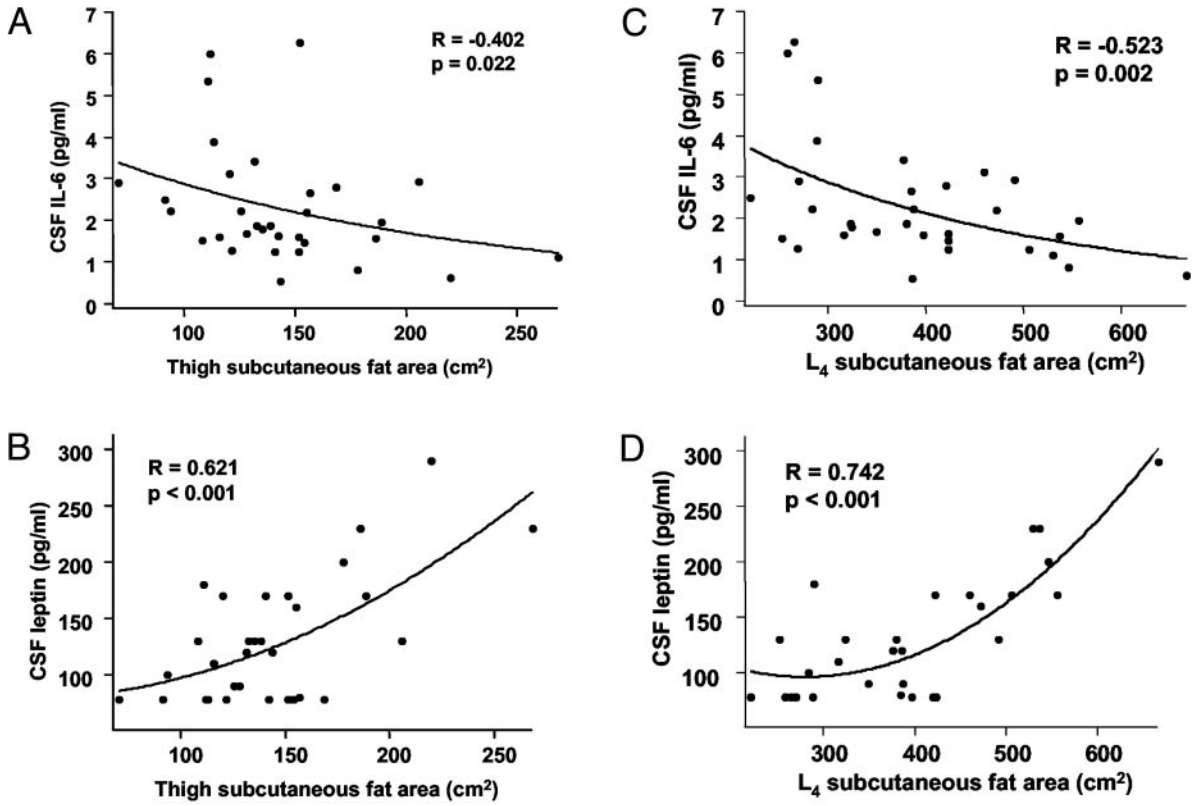

FIG. 2. CSF IL-6 levels correlated negatively with sc fat. IL-6 and leptin levels in CSF are plotted against different measures of sc adipose tissue (n = 32). Log CSF IL-6 *vs.* thigh AT: r = −0.402; $P = 0.022$ (A); log CSF leptin *vs.* thigh AT: r = 0.621; $P < 0.001$ (B); log CSF IL-6 *vs.* L4 sc AT: r = −0.523; $P = 0.002$ (C); log CSF leptin *vs.* L4 sc AT: r = 0.742; $P < 0.001$ (D).

serum levels of IL-6, leptin, and albumin. Albumin was used a control, as it is released to the circulation from the liver and is only filtered passively to the CSF (23). To study a wider range of BMI, CSF and serum samples were obtained from 10 control subjects with a BMI less than 27.5 kg/m^2, *i.e.* lower than those of the study group. We found, as expected, a positive correlation between serum leptin and CSF leptin (r = 0.778; $P < 0.001$; Fig. 3A), but there was no correlation between serum IL-6 and CSF IL-6 (r = 0.150; $P = 0.360$; Fig. 3B). Although CSF leptin levels were considerably lower than serum leptin levels (Fig 3A), CSF IL-6 levels were in some cases higher than serum IL-6 levels, especially in the leaner control group (Fig. 3B). Moreover, IL-6 and leptin levels in CSF were negatively correlated (Fig. 4).

The CSF:serum ratios were determined for IL-6, leptin, and albumin. In both the study group and the leaner control group, the CSF:serum ratio for IL-6 was considerably higher than that for leptin, which, in turn, was higher than that for albumin. In leaner subjects the IL-6 CSF:serum ratio was more than 1, indicating higher levels of IL-6 in CSF than in serum (Table 1). The CSF:serum ratio for albumin did not differ between leaner and more obese individuals. In contrast, the CSF:serum ratios for leptin and IL-6 were higher in leaner individuals than in more overweight/obese individuals (Table 1).

Discussion

The present results demonstrate that CSF IL-6 levels correlate negatively with both sc fat mass and total body fat mass measured with the sensitive DEXA and CT methods in overweight/obese subjects. Recently, we reported that IL-6-deficient mice become obese and that intracerebroventricular IL-6 treatment decreases body fat mass in rats via a central nervous system (CNS) effect (13, 14). Moreover, IL-6 and IL-6 receptors are expressed in neurons and glia cells in hypothalamic nuclei that regulate body fat (as well as in other parts of the brain) (15–18). Thus, available published data suggest an adipostatic effect of IL-6 in the CNS, and this study suggests that there is less IL-6 to exert this effect in more overweight/obese subjects. It would be of interest to investigate whether the negative correlation of CSF IL-6 levels and body fat mass remains when lean subjects are included in a similar study.

Recent reports on production and effects of IL-6 (5, 6, 13, 14) have so far shown similarities with the well known adipostatic and fat-derived cytokine leptin (4), but our results indicate that there are differences as well. In the present study there were positive correlations between CSF leptin levels and total body fat, between CSF leptin levels and sc fat, as well as between CSF leptin and serum leptin. These results confirm earlier studies (26–28) and are in line with the idea that CSF leptin originates from the transport of fat-derived leptin over the blood-brain barrier. In contrast, there was a negative correlation of CSF levels of IL-6 and leptin, and CSF IL-6 levels were also negatively correlated with total body fat and sc fat. Finally, CSF IL-6 did not correlate with serum IL-6. These results suggest that IL-6 and leptin are regulated dif-

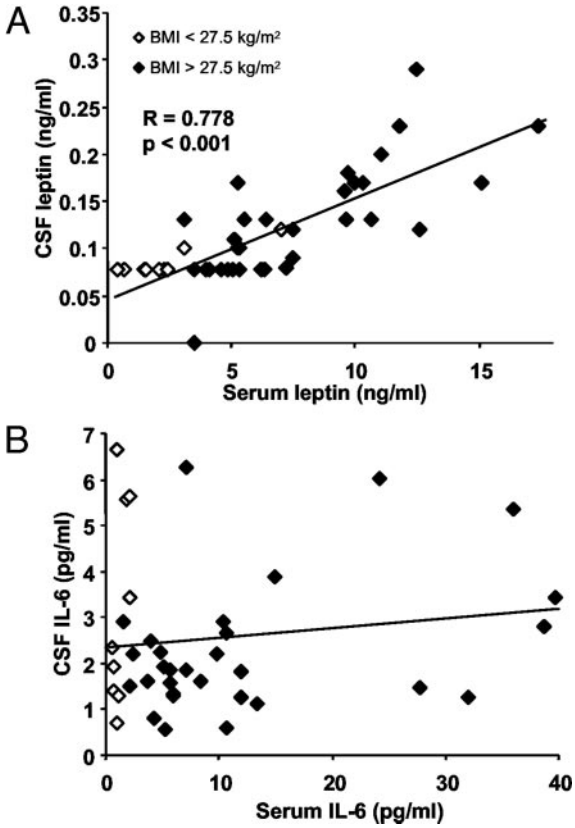

FIG. 3. CSF IL-6 levels were not correlated with serum IL-6 levels. CSF leptin *vs.* serum leptin: r = 0.778; $P < 0.001$ (A); CSF IL-6 *vs.* serum IL-6: r = 0.150; $P = 0.360$ (B; n = 40).

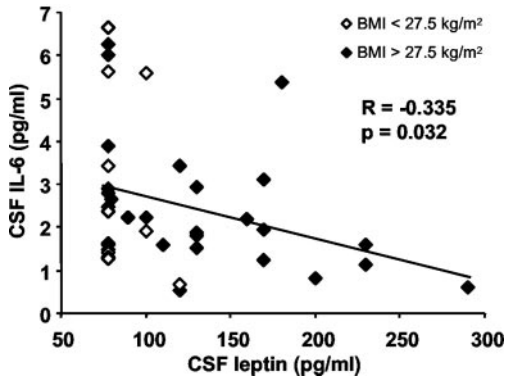

FIG. 4. CSF IL-6 levels correlated negatively with CSF leptin levels. CSF leptin *vs.* CSF IL-6: r = −0.335; $P = 0.032$ (n = 41).

ferently in the brain, although their production is regulated in a similar way in the adipose tissue.

At present, it is not known whether IL-6 in CSF is only derived from the blood circulation or is produced in the CNS. However, the results of the present study provide evidence that IL-6 in CSF is not only derived from the blood circulation. In line with previous results (21), we found that serum and CSF IL-6 levels are of the same magnitude. Moreover, IL-6 levels in CSF were often higher than those in serum, especially in leaner subjects. Actually we found that leaner subjects (BMI, <27.5 kg/m^2) had IL-6 levels that, on an average, were 2.5-fold higher in CSF than in serum. In contrast, the levels of leptin and albumin were considerably lower in CSF than in serum, as expected for peptides known to be transported from serum to CSF (23). Thus, if IL-6 in the CSF is produced centrally, it can at present only be a matter of speculation at which site(s) in the CNS and by which cell types it is produced. Neurons, microglia and endothelial cells can produce IL-6 *in vitro* (29), and neurons and glia cells in fat-regulating hypothalamic nuclei appear to produce IL-6 *in vivo* in rodents (15–18). However, it may seem more likely that functions of neurons, rather than microglia and endothelial cells, correlate with body fat mass.

TABLE 1. CSF:serum ratios for IL-6, leptin, and albumin in the study group (BMI > 27.5 kg/m^2) and in a leaner control group (BMI < 27.5 kg/m^2)

	CSF/serum		*P* values
	BMI >27.5 kg/m^2	BMI <27.5 kg/m^2	
IL-6	1:5.5 (0.51–25.6)	1:0.6 (0.15–4.52)	0.006
Leptin	1:60 (23.6–105)[a]	1:30 (5.13–58.2)[a]	<0.001
Albumin	1:152 (71.6–315)[a]	1:166 (94.8–325)[a]	0.45

In each individual, the relation between CSF and serum levels was normalized to give the CSF level the value of 1. Data are presented as mean of normalized CSF:serum ratios for each group. The range for the normalized serum levels in each group is presented within *parentheses*.

[a] $P < 0.001$, *vs.* the IL-6 CSF:serum ratio in the same group of subjects.

The CSF:serum ratio for albumin did not differ between leaner and more obese individuals in this study, as expected for substance filtered from serum to CSF (23). In contrast, the CSF:serum ratio for leptin was significantly higher in leaner individuals (1:30) than in more obese individuals (1:60). This could support the hypothesis put forward previously (27, 28) that leptin is not only filtered over the blood-brain barrier, but is actively transported via a mechanism that can get saturated in more obese individuals with higher serum leptin levels.

To summarize, we have found that CSF IL-6 levels correlate negatively with body fat mass in overweight and obese subjects. This finding is in line with the assumption that the target neurons for the antiobesity effects of IL-6 in CNS are exposed to insufficient levels of IL-6 in individuals with more severe obesity. It has been reported that IL-6 decreases body weight in nonhuman primates (30) and rodents (13, 14), and that injection of IL-6 enhances energy expenditure and lipid oxidation in humans (31, 32). These data open for the possibility that changes in IL-6 production can affect the risk of overweight/obesity in humans via effects at the CNS level.

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* K.S. and I.W. contributed equally to this study.

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