Leptin in Cerebrospinal Fluid from Children: Correlation with Plasma Leptin, Sexual Dimorphism, and Lack of Protein Binding

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Background: Previous studies in adults have established that leptin is present at very low concentrations in cerebrospinal fluid (CSF), but few data exist concerning CSF leptin in children. Current evidence suggests that CSF leptin concentrations interact with hypothalamic centers controlling food intake. Serum leptin concentrations manifest a sexual dimorphism that arises during puberty.

Methods: Leptin concentrations were determined in CSF from 42 pre- and postpubertal children who had been objectively classified into non-neurological disease or aseptic meningitis groups. Multivariate analysis of the dependence of CSF leptin on gender, pubertal state, body mass index (BMI), presence of aseptic meningitis, and CSF protein concentration was performed.

Results: CSF leptin concentrations correlated with log-transformed plasma leptin concentrations in concomitantly collected samples ($r = 0.582; P = 0.029$). BMI and gender were significant determinants of CSF leptin in postpubertal children, but only BMI was significant in prepubertal children. Analysis with HPLC to separate protein-bound and free forms of leptin found only free leptin in CSF.

Conclusions: CSF leptin concentrations in children reflect plasma leptin concentrations, including the advent of sexual dimorphism at puberty. Only free leptin is detectable in CSF, suggesting that it is the biologically active form.

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The hormone leptin, produced by adipose tissue (1) and secreted into the blood (2–4), has been proposed to function as an important regulator of body composition by controlling food intake and metabolic rate (5, 6). Specific receptors have been identified for leptin (7, 8), and nuclei of the hypothalamus have high concentrations of the receptor (9). Most actions of leptin are thought to result from interaction with hypothalamic receptors (10, 11). Intracerebroventricular infusion of minute amounts of leptin is highly effective in suppressing food intake in rodents (12, 13). Mice with a mutation that produces a truncated receptor are resistant to intracerebroventricular leptin (12).

Leptin concentrations have been measured extensively in cerebrospinal fluid (CSF) from adults (14–19) and found to be at concentrations much lower than those in serum/plasma (14–16). A requirement for transport of leptin into the brain interstitial fluid or CSF as a means to cross the blood-brain barrier and gain access to the hypothalamus has been proposed (7, 14). Generally, CSF concentrations correlate with serum concentrations, but the steep increase in serum leptin seen with increasing adiposity is muted in CSF. The sexual dimorphism in serum leptin concentrations, which is evident even after taking into account the differences in adiposity between men and women at the same weight, has been reported to be reflected in the CSF as well (14, 16), but another study found no difference in CSF concentrations between men and women after taking into account adiposity (19).

A single study has examined CSF leptin concentrations in children, who were primarily prepubertal (20). Although children might be expected to have similar trends in CSF leptin as adults, the effects of puberty and growth development on CSF leptin concentrations have not been assessed. We studied CSF leptin concentrations in both pre- and postpubertal children who were without preexisting neurological or systemic disease and were ultimately judged to have either mild non-neurological disease or aseptic meningitis. With this approach, it was possible to discern the effect of pubertal development on CSF leptin concentrations and to determine the contribution of neurological disease to leptin concentrations.
Subjects
Children 1–18 years of age who came to St. Louis Children’s Hospital between June and December 1998 and had a lumbar puncture done for diagnostic reasons were eligible for study. All patients were classified into diagnostic groups, based on clinical information and laboratory data obtained from medical charts. Patients with evidence of an acute or chronic neurological disorder (e.g., epilepsy or hydrocephalus) or systemic disease (e.g., malignancy or sepsis) were excluded from the study. In addition, CSF specimens with evidence of contamination with blood (report of blood in the CSF by the physician, or \( >200 \times 10^6 \) erythrocytes/L, or CSF protein \( >800 \) mg/L) were also excluded. The remaining patients (21 males, 21 females) were grouped into aseptic meningitis (n = 26) or non-neurological disease (n = 16) groups. Aseptic meningitis was defined as (a) appropriate clinical presentation, and (b) the presence of CSF pleocytosis or positive CSF viral culture. In children with non-neurological disease, the final diagnoses included otitis media/upper respiratory infection, gastroenteritis, skin infection, benign headache syndrome, and non-specific febrile illness. This study was conducted in accordance with a protocol approved by the Human Studies Committee of Washington University.

Analytical Procedures
Plasma leptin concentrations were measured as described previously (21), using a commercial assay from Linco Research Inc. CSF leptin concentrations are too low to be measured in the commercial assay for plasma leptin, but Linco Research has recently developed a more sensitive RIA, which detects concentrations as low as 0.05 \( \mu \)g/L (0.02 \( \mu \)g/L if sample volume is doubled); this RIA was used in this study. Briefly, 100–200 \( \mu \)L of CSF was incubated overnight with antibody at room temperature. Leptin labeled with \( ^{125}I \) was added, and incubation was continued for another night. Cold (4 °C) second antibody was added for a 20-min incubation, and centrifugation precipitated bound leptin for quantification by gamma counting. The specimen leptin concentration was calculated from a logit-log transformation of calibrators and unknowns. CVs for day-to-day analysis were 17% at 0.29 \( \mu \)g/L and 6% at 2.28 \( \mu \)g/L. CSF protein concentrations were measured with a Vitros 250 analyzer (Ortho-Clinical Diagnostics, Johnson and Johnson).

Analysis of Protein-Bound and Free Leptin
HPLC was used to separate the leptin forms in CSF, and the presence of protein-bound and free leptin species was determined by RIA of the chromatographic fractions as described previously (22).

Statistical Analysis
All results are presented as means \( \pm 1 \) SD unless otherwise stated. Relationships between continuous variables were evaluated by least-squares linear regression. ANOVA was used to assess statistical differences between groups, with a two-tailed \( P < 0.05 \) regarded as significant.

Results
Leptin concentrations in CSF of 42 children averaged 0.12 \( \pm 0.10 \) \( \mu \)g/L (range, 0.02–0.41 \( \mu \)g/L). Pairs of CSF and concomitantly collected plasma specimens were available for 14 children (11 males, 3 females); in this subset, CSF leptin averaged 0.12 \( \pm 0.07 \) \( \mu \)g/L, whereas plasma leptin was 4.36 \( \pm 7.42 \) \( \mu \)g/L. Thus, CSF leptin was \(<3\% \) of the corresponding plasma concentrations in these children; the much lower CSF concentrations have been noted previously (14–20). When the relationship of plasma and CSF leptin was analyzed by least-squares linear regression, a linear relationship was evident between the log10 plasma leptin and CSF leptin concentrations (\( r = 0.582; P = 0.029 \); Fig. 1). There was no statistically significant difference in this relationship between the seven subjects with aseptic meningitis and seven subjects with non-neurological disease (\( P = 0.923 \)).

A possible sexual dimorphism in CSF leptin concentrations was explored. The CSF leptin concentration in males (0.10 \( \pm 0.07 \) \( \mu \)g/L) was not significantly different from that of females (0.14 \( \pm 0.11 \) \( \mu \)g/L; \( P = 0.302 \)). Because serum leptin concentrations manifest sexual dimorphism only after puberty (23, 24), the subjects were divided by sex and into those younger and older than 12 years, and the data were analyzed by ANOVA. In addition, body mass index (BMI), disease category (non-neurological vs aseptic meningitis), and CSF protein concentrations were included as covariate predictors of CSF leptin concentrations. CSF protein concentrations for all subjects averaged 290 \( \pm 110 \) mg/L (range, 130–710 mg/L) and thus were mostly in the range for healthy subjects (25). CSF protein

![Figure 1](https://example.com/fig1.png)

Fig. 1. Correlation of CSF leptin concentration with log10 plasma leptin concentration.

Subjects with non-neurological disease (○) and aseptic meningitis (●) are plotted together. All 14 subjects were used to calculate the least-squares linear regression line shown.
concentrations were 314 ± 122 mg/L in the aseptic meningitis cohort and 252 ± 73 mg/L in the non-neurological cohort (P = 0.074); specimens with highly increased CSF protein concentrations had been excluded from the study out of concern that increased concentrations might represent contamination with plasma during collection. BMI was a significant determinant for both females and males, but neither disease category (P = 0.547) nor CSF protein concentration (P = 0.112) had significant predictive value for CSF leptin concentrations. After taking into account BMI, CSF leptin concentrations in males <12 years were not statistically different from those of females <12 years (P = 0.755), but the same comparison between males and females >12 years found a highly significant difference (P = 0.0002). The distinction is evident in plots of BMI vs CSF leptin, where the least-squares linear regression lines are similar for the subjects <12 years (Fig. 2A) but clearly divergent for children >12 (Fig. 2B). Examination of the slopes of the regression lines shows that males of any age have a similar relationship of CSF leptin to BMI, but that females >12 years have a much steeper increase in CSF leptin with BMI compared with males or prepubertal females.

Because serum leptin is known to circulate in protein-bound as well as free forms, the form of leptin in CSF was investigated to see whether protein binding could help explain the much lower concentrations in CSF compared with serum. A pool of CSF was concentrated 10-fold by ultrafiltration and chromatographed by HPLC to fractionate CSF protein species on the basis of molecular dimensions/weight (22). Leptin concentrations were assayed in the resulting fractions by RIA. Whereas fractionation of serum produces a broad peak of protein-bound leptin and a sharp peak of free leptin, only a single peak of leptin immunoreactivity was evident in CSF, which was increased at the position of free (unbound) leptin (Fig. 3).

**Discussion**

In children, like adults, CSF leptin concentrations are only a small percentage of the plasma concentrations, suggesting that the processes that determine CSF concentrations are similar in children and adults. As with adults, plasma concentrations predict CSF concentrations in children; this relationship has been part of a previously developed theory suggesting that specific transport of plasma leptin into the CSF by leptin receptors may have a role in regulating the effective concentrations of leptin that reach the hypothalamic centers, controlling food intake and metabolic rate (7, 14). The results presented here are consistent with this hypothesis.

Neurological disease, and specifically meningitis, increases CSF total protein concentrations; the mechanism of this increase is thought to be increased permeability of

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**Fig. 2.** Correlation of CSF leptin concentration with BMI. Data for males (●) and females (○) are plotted together; least-squares linear regression lines are shown for males (—) and females (— —) separately. (A), children <12 years of age; (B), children >12 years of age.

**Fig. 3.** Elution diagram of HPLC fractionation of CSF leptin. Two tracings are shown: leptin concentration in eluted fractions (●), and absorbance at 280 nm (thin line). The elution position of recombinant human leptin is marked for comparison.
the capillary endothelium and other structures of the brain. Because the blood concentrations of leptin are so much higher than CSF concentrations in healthy individuals, we anticipated that meningitis might increase CSF leptin concentrations relative to plasma concentrations. Such increases, if these occurred, could alter the physiological action of leptin by increasing leptin concentrations in the hypothalamus, and they potentially have a role in the anorexia of infection. No alteration of the relationship of plasma leptin to CSF leptin was discernable in the cohort with aseptic meningitis, suggesting that meningitis does not increase CSF leptin concentrations.

Sexual dimorphism in plasma leptin concentrations has been noted in many studies, and studies of children through puberty showed that the dimorphism was absent in prepubertal children but became evident after puberty (23, 24). Assessment of body fat content with BMI or direct measures such as dual-energy x-ray absorption is an important adjunct of such studies because it accounts for the large variation in leptin concentrations in individuals of the same sex attributable to variation in body fat content (3, 4). Two studies found sexual dimorphism in CSF leptin concentrations (14, 16), although another did not (19). A single study of CSF leptin in mostly prepubertal children found slightly higher CSF leptin concentrations in females, but did not address the effect of puberty (20). If CSF leptin concentrations are importantly determined by plasma concentrations, then CSF leptin concentrations should reflect the development of the dimorphism seen at puberty in plasma concentrations. Our results confirm the development of dimorphism at puberty and support the view that CSF concentrations are primarily determined by plasma leptin concentrations. Because our study utilized BMI as a surrogate measure of adiposity, and because females are known to have a somewhat higher percentage of body fat than males at the same BMI, part of the observed dimorphism may be attributable to the gender differences in body fat content that is not reflected by BMI.

Leptin is known to circulate in protein-bound as well as unbound forms in blood (22, 26), but there is no information currently available about the forms of leptin found in CSF or other body fluids. Such information could be useful in understanding the dynamic relationship of leptin between plasma and other fluid compartments. Protein-bound leptin may be less permeable or transportable into CSF because of increased molecular weight or decreased availability to receptor-mediated transport. In addition, the presence of binding proteins in a body fluid could readily influence total leptin concentrations in that fluid by acting as a buffer for leptin that would increase total leptin concentrations at regulated free-leptin concentrations. We found that all of the leptin in CSF is in the free form, suggesting that the binding proteins are not transported with leptin and that the CSF does not generate binding proteins. Thus, plasma binding proteins, if these have a role in modulating CSF leptin concentrations, may restrict the availability of plasma leptin for transport to the CSF. These results also offer evidence that it is the free form of leptin that is biologically active, assuming the hypothesis that leptin in CSF is the form that bathes the nuclei of the hypothalamus that regulate food intake and metabolic rate.

References