Title: Proopiomelanocortin, Agouti-Related Protein and Leptin in Human Cerebrospinal Fluid: Correlations with Body Weight and Adiposity

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Abbreviated Title: Relationship of CSF Leptin, POMC and AgRP to BMI

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Abstract

Leptin and its neuronal targets, that produce proopiomelanocortin (POMC) and agouti-related protein (AgRP), regulate energy balance. This study characterized leptin, POMC and AgRP in cerebrospinal fluid (CSF) of 47 healthy human subjects, 23 lean, 24 overweight/obese (OW/OB), as related to BMI, adiposity, plasma leptin, soluble leptin receptor (s-OB-R) and insulin. POMC was measured as the POMC prohormone is the predominant POMC peptide in CSF and correlates with hypothalamic POMC in rodents. Plasma AgRP was similarly characterized. CSF leptin was 83-fold lower than in plasma and correlated strongly with BMI, body fat and insulin. The relative amount of leptin transported into CSF declined with increasing BMI, ranging from 4.5 to 0.52%, consistent with a saturable transport mechanism. CSF sOB-R was 78-fold lower than in plasma and correlated negatively with plasma and CSF leptin. CSF POMC was higher in lean vs OW/OB subjects (p<0.001) and correlated negatively with CSF leptin (r= -0.60; p<0.001) and with plasma leptin, insulin, BMI and adiposity. CSF AgRP was not different in lean vs OW/OB, however plasma AgRP was higher in lean subjects (p=0.001) and correlated negatively with BMI, adiposity, leptin, insulin and HOMA (p<0.005). Thus, CSF measurements may provide useful biomarkers for brain leptin and POMC activity. The striking negative correlation between CSF leptin and POMC could be secondary to leptin resistance and/or neuronal changes associated with obesity, but may also indicate that POMC plays a primary role in regulating body weight and adiposity. The role of plasma AgRP as a neuroendocrine biomarker deserves further study.
**Introduction**

The adipocyte-derived hormone leptin communicates levels of energy stores to key brain regions and elicits a host of neuronal responses that regulate energy balance (6, 14). Leptin enters the brain by a saturable transport mechanism that involves, at least in part, a short isoform (Ob-Ra) of the leptin receptor found in the choroid plexus and cerebral microvessels (1, 9). Another circulating leptin receptor isoform, the soluble leptin receptor (sOB-R or Ob-Re), can also impact leptin transport into the brain. sOB-R may be derived from ectodomain shedding of the long form of the leptin receptor OB-Rb that is essential for leptin signaling (7). The physiological role of sOB-R is not yet completely understood. There is evidence that sOB-R functions as a leptin binding protein that can inhibit leptin transport into brain but can also delay leptin clearance from the circulation (11, 33). High levels of sOB-R can block leptin’s actions (26) but sOB-R overexpression results in a lean phenotype in mice (16). In humans, plasma sOB-R levels correlate negatively with BMI and increase with fasting (4, 34).

The hypothalamic melanocortin system plays a critical role in responding to leptin and disruption of this system at multiple levels causes obesity in humans and animals. This system consists of proopiomelanocortin (POMC) and agouti-related protein (AgRP) neurons and the brain melanocortin receptors (MC-Rs). The POMC-derived peptide α-MSH inhibits food intake and stimulates energy expenditure while AgRP is an MC-R antagonist that stimulates food intake and decreases energy expenditure. Leptin stimulates POMC gene expression and peptide release and inhibits AgRP gene expression and peptide release (13). The physiology of this system has
been well studied in rodents but such studies are not possible in humans unless reliable biomarkers for brain leptin, POMC and AgRP can be found. We have previously shown during pregnancy, despite hyperleptinemia, that elevated sOB-R may serve to decrease leptin transport into brain and that CSF levels of target neuropeptides are consistent with leptin resistance (21). However little is known about CSF leptin and its relationship to sOB-R and to appropriate target neuropeptides in normal human subjects as a function of body weight and adiposity. The purpose of this study was to examine concentrations of leptin, POMC and AgRP in the CSF of normal lean and obese subjects, as related to BMI and adiposity and plasma hormone levels, in order to determine if CSF measurements can provide useful biomarkers of brain leptin and melanocortin activity. The relationships between CSF leptin and plasma leptin and sOB-R were studied and sOB-R was measured for the first time in CSF. POMC was measured by specific ELISA that detects the POMC prohormone, which is the predominant POMC peptide in human CSF, with levels up to 50-fold higher than its processed peptide products (32, 37). Although it is the POMC-derived peptide, \( \alpha \)-MSH, that engages brain MC-Rs, CSF levels of \( \alpha \)-MSH are quite low. Furthermore previous studies in the rodent have shown that CSF POMC levels, rather then CSF \( \alpha \)-MSH levels, reflect hypothalamic POMC activity under a variety of conditions (24). AgRP was measured in CSF by ELISA and RIA with relative specificities for full-length AgRP and AgRP \(_{83-132}\) which are both detected in human CSF. Finally AgRP was also measured in plasma as a potential indicator of hypothalamic AgRP activity. While it is clear that circulating POMC is of pituitary origin and does not reflect hypothalamic POMC activity, the origin of circulating AgRP is at present unknown. Our results support the use of CSF measurements as biomarkers of brain leptin and melanocortin activity that could be useful in studying the physiology and pathophysiology of human energy balance.
Materials and Methods

Subjects

Subjects were 47 healthy males and females (23M, 24F) aged 19-50 years; 23 were lean and 24 were overweight/obese (OW/OB). Female subjects were studied in the early follicular phase of the menstrual cycle. Subjects were screened by medical history and all subjects were non-smokers, were taking no medications other than vitamins and had no history of major psychiatric disorder, alcoholism, or neurologic disease. Additionally, subjects were excluded for any other clinically significant medical condition or eating disorder, for recent weight change ± 5%, for use of weight loss products or dieting during the 6 months prior to the study. Details on BMI, body fat percentage and leptin and insulin levels are provided in Table 1. This study was approved by the Columbia University Institutional Review Board and written informed consent was obtained from all subjects prior to their participation.

Protocol

Subjects were studied in the outpatient clinical research center between 8:00 and 10:00h after an overnight fast (from 21:00h). A lumbar puncture (LP) was performed using a 25 G Whitacre needle; the first 0.5ml of CSF was discarded before collection of the 10 ml study sample. CSF samples were immediately transferred to polyethylene tubes and placed on ice. Peripheral venous EDTA plasma and serum samples were obtained immediately after the LP. CSF and blood samples were centrifuged and stored at -80 C until assays were performed. Body
composition was determined by DXA (QDR 4500 A & Delphi W model) in 31 subjects; pregnancy was ruled out by β-hCG test.

Assays

CSF leptin was measured by sensitive ELISA (R&D Systems, Minneapolis, MN) with a sensitivity of 7 pg/ml (21). Plasma leptin was measured using the same assay with appropriate dilution. Plasma and CSF sOB-R were measured by ELISA (R&D Systems). POMC was measured by two-site ELISA; the capture monoclonal antibody is directed at ACTH (10-18) and detection antibody is directed against γ-MSH (21, 30). There is 100% crossreactivity with 22K pro-ACTH but none with ACTH, α-MSH, γ-MSH or β-endorphin. Affinity purified human 31K POMC was used for standards. Assay sensitivity is 8 fmol/ml. ACTH was measured by RIA with an antiserum directed against ACTH (7-18) (IgG Corporation, Nashville, TN) (23). AgRP was measured by both ELISA and RIA with relative sensitivities for full-length AgRP and AgRP

83-132 respectively. The ELISA (R&D Systems) uses recombinant full-length human AgRP standard. There is 17% cross-reactivity with AgRP

83-132 (21). Assay sensitivity is 7 pg/ml. The RIA was performed as previously described using an antibody provided by Dr. Gregory Barsh and human AgRP

83-132 for the standard (Phoenix Pharmaceuticals Inc., Burlingame, CA) (2). There is 20% crossreactivity with full-length AgRP. Assay sensitivity is 25 pg/ml. Serum insulin was measured by solid-phase enzyme-labeled chemiluminescent immunometric assay, Immulite1000 (Siemens Healthcare Diagnostics). Ghrelin was measured by ELISA (Millipore, Billerica, MA). Serum glucose was measured by the hexokinase method.
Characterization of the POMC and AgRP Immunoactivity in CSF

POMC immunoactivity was characterized by gel filtration of extracted CSF pooled from 12 subjects. CSF (12 ml) was extracted using a Sep-Pak C-18 cartridge (Waters Associates, Milford, MA) as previously described except that peptides were eluted with acetonitrile/0.1% trifluoroacetic acid (60:40) (38). The extract was chromatographed on a Sephadex G-75 column and fractions were assayed for POMC by ELISA and ACTH by RIA (21). The column was calibrated with affinity purified 31K human POMC and ACTH 1-39. AgRP immunoactivity was characterized in a 10 ml CSF pool from a male subject that was concentrated using an Amicon Ultra Centrifugal Filter (Millipore Corporation, Billerica, MA) and subjected to reverse phase HPLC. Samples were eluted with a gradient of 80% acetonitrile containing 0.1% TFA (39). Fractions were assayed for AgRP by RIA. The column was calibrated with 5ng of human AgRP (83-132) (Phoenix Pharmaceuticals Inc.) and with 5 ng human full-length AgRP (R&D Systems).

Statistical analysis

Data are expressed as mean ± SEM. Differences in parameters between the normal weight lean subjects and overweight/obese subjects were determined by unpaired t test; significance was set at p < 0.05. Correlations were determined by linear regression analysis with Pearson’s correlation. Analyses were performed with GraphPad Prism version 6.0b for Mac (Graphpad Software, San Diego, CA). Multiple regression analyses were performed using Statistical Analysis Systems version 9.4 (SAS Institute, Cary, NC). Insulin resistance was calculated in 37 subjects who had glucose measurements using the homeostasis model assessment (HOMA) (17).
Results

Plasma and CSF leptin and sOB-R levels

As expected, plasma leptin correlated strongly with BMI ($r=0.850$) and body fat % ($r=0.847$); ($p<0.0001$). The mean CSF leptin concentration was $225 \pm 23.4$ (SEM) pg/ml, 83-fold lower than the mean plasma leptin concentration of $18.7 \pm 3.5$ ng/ml. CSF leptin also correlated strongly with BMI ($r=0.709$) and body fat % ($r=0.943$); ($p<0.0001$). Plasma and CSF leptin concentrations in male and female lean and OW/OB subjects are depicted in Table 1. The relationship between plasma and CSF leptin is depicted in Fig.1. There was a strong positive linear correlation between plasma and CSF leptin ($r=0.847$; $p<0.0001$) but this relationship was best described by a nonlinear second order polynomial quadratic fit ($r=0.939$; $p<0.0001$). The relative amount of leptin measured in CSF, expressed as a percentage of plasma leptin, declined with increasing BMI, ranging from 4.5 to 0.52%, consistent with a saturable transport mechanism (Fig. 1).

Plasma and CSF sOB-R levels are depicted in Fig.1. Plasma sOB-R was higher in lean vs OW/OB subjects ($p<0.0001$). Mean CSF sOB-R was 78-fold lower than the mean plasma level and was also higher in lean vs OW/OB subjects ($p=0.012$). Plasma sOB-R correlated negatively with BMI ($r=-0.471$) and with leptin in plasma ($r=-0.438$) and CSF ($r=-0.422$); ($p<0.01$). Plasma sOB-R correlated positively with the CSF/plasma leptin % ($r=0.489$; $p<0.001$) (Fig.1); this correlation was not significant when the lean and OW/OB groups were analyzed separately.
CSF sOB-R correlated negatively with CSF leptin ($r = -0.504$; $p=0.0004$), plasma leptin ($r = -0.526$; $p=0.0002$) and BMI ($r = -0.411$; $p=0.004$). In contrast to the strong correlation between plasma and CSF leptin, plasma and CSF sOB-R were only weakly correlated ($r = 0.312$; $p=0.03$). A sex difference was noted for CSF sOB-R with significantly higher levels in males vs females ($0.287 \pm 0.02$ vs $0.229 \pm 0.02$ ng/ml; $p=0.038$); no sex difference was noted for plasma sOB-R. Positive correlations for CSF sOB-R (but not plasma sOB-R) were noted with CSF POMC ($r = 0.547$; $p<0.0001$) (Fig.2) and with CSF AgRP ($r=0.539$; $p=0.0001$) (Fig.3).

**CSF POMC levels**

High levels of POMC were measured in CSF. Levels were 14-fold higher than in plasma; CSF and plasma POMC were not correlated. CSF POMC levels correlated with CSF ACTH levels ($r=0.676$; $p<0.0001$) but POMC was 20-fold higher than ACTH on a molar basis. CSF POMC was higher in lean (269 ± 16.0) vs OW/OB (192 ± 12) subjects ($p=0.0003$) (Fig. 2). There was a strong negative correlation between CSF POMC and CSF leptin ($r = -0.601$; $p<0.0001$) (Fig. 2). Significant negative correlations were also noted between CSF POMC and plasma leptin ($r = -0.531$; $p<0.0001$), insulin ($r = -0.328$; $p=0.03$) and BMI ($r = -0.492$; $p=0.0004$). When adjusted for BMI, there was no longer a significant correlation between CSF POMC and insulin. Correlations between CSF POMC and other measures are provided in Table 2. CSF POMC was higher in males than in females ($p=0.01$) (Fig. 2), however the negative correlations between CSF POMC and leptin remained when the genders were analyzed separately: $r=-0.478$; $p=0.02$ for males and $r=-0.634$; $p=0.0009$ for females. The negative correlations between CSF POMC and leptin also remained when the lean ($r=-0.437$) and OW/OB ($r=-0.421$) groups were
analyzed separately (p=0.04). However this correlation was no longer significant when the lean female group was analyzed separately (r= -0.446; p=0.16) and not seen at all when the lean male group was analyzed separately (r= 0.063; p=0.85).

CSF and plasma levels AgRP levels

AgRP was measured in CSF by both ELISA and RIA, and in plasma by ELISA only. Unless otherwise specified all values in the text and figures were obtained with the ELISA as it was more sensitive and had a lower inter-assay coefficient of variation. CSF AgRP was not significantly different in lean vs OW/OB subjects or in male vs female subjects when measured either by ELISA or RIA. Nonsignificant negative correlations were noted between CSF AgRP and leptin and BMI (Table 3). However significant positive correlations were noted between CSF AgRP and CSF POMC (r=0.421; p=0.003) and CSF sOB-R (r=0.539; p=0.0001) (Fig. 3).

In contrast to CSF, plasma AgRP was significantly higher in lean (87.4 ± 5.0 pg/ml) vs OB/OW (63.8 ± 4.5 pg/ml) subjects (p=0.001). No significant sex difference was noted. There were significant negative correlations between plasma AgRP and BMI as well as with adiposity, plasma and CSF leptin and insulin (Fig. 4; Table 4). After adjustment for BMI, the negative correlation between plasma AgRP and insulin persisted (p=0.055). Plasma AgRP also correlated negatively with HOMA (r= -0.580; p=0.0003). A highly significant positive correlation was noted between plasma AgRP and CSF POMC (r=0.403; p=0.007) (Fig. 4).
Characterization of the POMC and AgRP Immunoactivity in CSF

POMC and ACTH immunoactivity was characterized by gel filtration in an extracted 12 ml CSF pool. The majority of the POMC immunoactivity eluted in the same position as affinity purified 31K human POMC (Fig. 5). A much smaller amount of ACTH immunoactivity was detected with the ACTH RIA, with most of the immunoactivity eluting in the position of ACTH (1-39).

AgRP immunoactivity in the CSF was characterized by HPLC and fractions were assayed by AgRP RIA. There were two peaks of AgRP immunoactivity corresponding to the C-terminal and full length peptides (Fig. 5).

Discussion

This study provides novel information about levels of the melanocortin neuropeptides, POMC and AgRP, in human CSF as a function of BMI and adiposity. The results also confirm and extend previous studies that have examined the relationship between plasma and CSF leptin in lean and obese subjects and provide new data about the relationship between leptin and sOB-R in plasma and CSF. A strength of this study is that all subjects were healthy volunteers, in contrast to previous studies of leptin in CSF that included patients having LPs for clinical purposes with various diagnoses and degrees of illness.

CSF leptin was 83-fold lower than in plasma and correlated strongly with plasma leptin in a nonlinear manner over a wide spectrum of BMI. CSF leptin correlated strongly with BMI,
adiposity and plasma insulin. Leptin was measured with a sensitive ELISA that enabled detection of leptin in all CSF samples. CSF leptin, expressed as a percentage of plasma leptin, declined with increasing BMI, ranging from 4.5 to 0.52%, consistent with a saturable transport mechanism. This confirms and extends previous human studies showing that CSF leptin reflects adipocyte-derived plasma leptin levels (3, 28) and is consistent with studies in mice demonstrating that leptin enters the brain by a receptor mediated saturable transport mechanism (22).

Plasma levels of sOB-R were measured to determine if sOB-R impacted leptin transport into brain, as reflected by CSF leptin levels. We have previously shown that there is a diurnal rhythm for plasma sOB-R and that levels were lowest after midnight when CSF leptin levels peaked and postulated that this fall in sOB-R may facilitate leptin transport into brain at night (37). We have also shown that during pregnancy elevated plasma leptin levels are accompanied by elevated sOB-R levels without any change in CSF leptin levels, consistent with an inhibitory effect of sOB-R on leptin transport into brain (21). In contrast, in the current study, the relative amount of leptin in CSF was higher in the presence of high circulating sOB-R levels. Plasma sOB-R correlated negatively with BMI and plasma leptin as previously reported (4, 34). In addition we show for the first time that plasma sOB-R also correlated negatively with CSF leptin. However plasma sOB-R correlated positively with the CSF to plasma leptin ratio. This may in part be a consequence of the already saturated transport system in the very obese subjects. Of note no significant correlation between sOB-R and CSF to plasma leptin ratio was
noted amongst only the lean subjects. Thus under some conditions sOB-R may influence leptin transport into brain but this was not evident in the current study.

sOB-R was also measured for the first time in CSF and levels were 78-fold lower than plasma levels. In contrast to leptin, sOB-R is not known to be transported from the blood into the brain. It is possible that CSF sOB-R may be derived from blood but it may also be derived from brain and may reflect cellular expression of the long form of the leptin receptor in brain. CSF sOB-R and plasma sOB-R were similar in that both were significantly higher in lean vs OW/OB subjects but differences were noted when sOB-R levels in CSF and plasma were correlated with other variables, including CSF POMC which correlated strongly with CSF but not plasma sOB-R. A sex difference was also noted for CSF but not plasma sOB-R levels. These differences suggest that CSF sOB-R may derive from a central rather than a peripheral source.

High levels of POMC were detected in CSF as we have previously reported but we now show significant differences related to adiposity and gender. Characterization by gel filtration showed that the majority of the POMC immunoactivity in CSF eluted in the position of the 31K POMC standard. CSF POMC levels correlated with but were 20-fold higher than CSF levels of the POMC-derived peptide ACTH. No change in the ratio of POMC to ACTH was noted in lean vs OW/OB subjects. Thus there was no indication that high POMC levels resulted from decreased POMC processing which could affect energy balance (36). CSF POMC was 14-fold
higher than plasma POMC and plasma and CSF POMC were not correlated, consistent with prior studies showing that plasma and CSF POMC derive from the pituitary and brain respectively and are regulated differently (27, 32). Although it is the POMC-derived peptide α-MSH that engages brain MC-Rs, it is difficult to reproducibly measure the low levels of α-MSH in CSF. This may in part result from the inactivation of α-MSH by the enzyme prolylcarboxypeptidase (35). An earlier study in human subjects did not find any differences in CSF α-MSH levels related to BMI (19). However previous studies in the rodent have shown that levels of the intact POMC prohormone in CSF rather than ACTH or α-MSH can serve as a better measure of hypothalamic POMC activity (24). Of note, diurnal rhythms have been reported for POMC in human CSF and for POMC expression in the rodent hypothalamus (29, 37). In the current study CSF POMC was higher in lean vs OW/OB subjects and there were striking negative correlations with leptin and BMI. CSF POMC was higher in males than in females, but the negative correlation between CSF POMC and leptin and BMI remained when the genders were analyzed separately. This is similar to our study in female monkeys that showed negative correlations between CSF POMC and leptin and BMI (39). These results suggest that POMC plays a primary role in regulating body weight and adiposity. It is possible that the lower level of POMC in the CSF of obese subjects is secondary to leptin resistance that is well documented in POMC neurons in obese animals (5). Alternatively it could be a consequence of decreased neuronal remodeling that is caused by a high fat diet in animals or to hypothalamic inflammation and gliosis that been demonstrated in obese animals and humans. (10, 18, 31). However in our study, the fact that a negative correlation between CSF POMC and leptin persisted when the lean subjects were analyzed separately supports the interpretation that POMC plays a primary role in regulating body weight and adiposity.
AgRP was measured in CSF and plasma with the expectation that CSF AgRP would reflect hypothalamic AgRP activity as anatomical studies in the monkey and rat show dense AgRP staining along the third ventricle (8). CSF AgRP was not different in lean vs OW/OB individuals when measured by either ELISA or RIA. Only nonsignificant negative correlations were noted between CSF AgRP and leptin and BMI. However CSF AgRP was strongly correlated with CSF POMC and with CSF sOB-R. In contrast to CSF AgRP, there were highly significant negative correlations between plasma AgRP and BMI, adiposity, leptin, insulin and HOMA. Of note the negative correlation with insulin persisted when adjusted for BMI. This is consistent with the known role of AgRP neurons in responding to insulin and regulating glucose metabolism independently of changes in body weight (12, 25). Plasma AgRP also exhibited a strong positive correlation with CSF POMC. The origin of circulating AgRP is not entirely clear but there is evidence that circulating AgRP may reflect hypothalamic AgRP activity. Anatomical evidence shows heavy AgRP fiber staining in the monkey median eminence which could be secreted into blood (8). Furthermore we have found that AgRP increases in human plasma after fasting or diet-induced weight loss, consistent with changes in hypothalamic AgRP expression seen after fasting and weight loss in rodents (20). Thus our measurements of plasma AgRP may in fact reflect brain AgRP activity. If so, the negative correlations with BMI, adiposity and leptin are consistent with the known inhibitory effects of leptin on AgRP neurons. While the adrenals express significant amounts AgRP mRNA and may be another potential source for circulating AgRP, it is notable that plasma AgRP levels did not change significantly in rats after adrenalectomy (15). We have similarly found that AgRP persists in human plasma after bilateral adrenalectomy in 2 cases (unpublished observations). The differences noted in the current study
between CSF and plasma AgRP levels may relate to anatomical differences in AgRP fiber tracks that gain access to CSF and blood respectively. Plasma and CSF AgRP are both strongly positively correlated with CSF POMC. The explanation for this is at present unclear but it may be that the activities of both sets of neurons and the entire brain melanocortin circuit are increased in lean vs obese subjects. Of note, the reactive gliosis and suppression of neurogenesis in response to high fat feeding in rodents have been shown to affect both POMC and AgRP neurons (10, 18). More mechanistic studies will be required to elucidate the nature of this interesting relationship between POMC and AgRP in human subjects as related to body weight and adiposity.

In summary, these studies provide evidence that CSF measurements may provide useful biomarkers for brain leptin, leptin receptor activity and POMC activity and could be useful in studying the physiology and pathophysiology of human energy balance. The results also suggest that plasma AgRP measurements may be a useful biomarker of brain AgRP activity.

Author Contributions


Acknowledgements
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**References**


Legends to Figures

**Figure 1.** Correlation between CSF and plasma leptin (*upper left panel*) and between BMI and the CSF/plasma leptin ratio (expressed as %) (*upper right panel*). Mean plasma and CSF sOB-R levels (± SEM) were significantly higher in lean vs OW/OB subjects (*lower left panel*). Plasma sOB-R correlated negatively with BMI and positively with the CSF/plasma leptin % (*lower middle and right panels*).

**Figure 2.** Mean CSF POMC levels (± SEM) were significantly higher in lean vs OW/OB subjects and in males vs females (*upper left panel*). There were strong negative correlations between CSF POMC and CSF leptin (*upper right panel*) and between CSF POMC and BMI (*lower left panel*). There was a strong positive correlation between CSF POMC and CSF sOB-R (*lower right panel*).

**Figure 3.** Correlations of CSF AgRP with other variables. CSF AgRP did not correlate significantly with CSF leptin (*upper left panel*) or plasma ghrelin (*lower right panel*). Significant
positive correlations were noted between CSF AgRP and CSF sOB-R (*upper right panel*) and between CSF AgRP and CSF POMC (*lower left panel*).

**Figure 4.** Correlations of plasma AgRP with other variables. There were significant negative correlations between plasma AgRP and BMI and CSF leptin (*upper panels*) and insulin (*lower left panel*). There was a significant positive correlation between plasma AgRP and CSF POMC (*lower right panel*).

**Figure 5.** Sephadex G-75 chromatography of pooled CSF extracts (*upper panel*). Fractions were assayed for POMC (black circles) by ELISA and ACTH (grey triangles) by RIA. Arrows indicate the void volume and the elution positions of POMC and ACTH standards. HPLC of a CSF pool (*lower panel*) with fractions assayed for AgRP by RIA. Arrows indicate the elution positions of full length AgRP and AgRP (83-132) standards.
Table 1

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Table 2

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<tr>
<td>CSF ACTH</td>
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<td>CSF AgRP (RIA)</td>
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<td>0.01</td>
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<tr>
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<td>0.32</td>
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<tr>
<td>Plasma Ghrelin</td>
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Table 4

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<th>Measures</th>
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<th>P-Value</th>
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<td>BMI (kg/m²)</td>
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<td>Body Fat (%)</td>
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<td>Ghrelin</td>
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