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Proopiomelanocortin, agouti-related protein, and leptin in human cerebrospinal fluid: correlations with body weight and adiposity

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1Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York; 2Faculties of Life Sciences and Medical and Human Sciences, University of Manchester, Manchester, United Kingdom; 3Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York; and 4Department of Anesthesiology, Columbia University College of Physicians and Surgeons, New York, New York

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Page-Wilson G, Meece K, White A, Rosenbaum M, Leibel RL, Smiley R, Wardlaw SL. Proopiomelanocortin, agouti-related protein, and leptin in human cerebrospinal fluid: correlations with body weight and adiposity. Am J Physiol Endocrinol Metab 309: E458–E465, 2015.—Leptin and its neuronal targets, which produce proopiomelanocortin (POMC) and agouti-related protein (AgRP), regulate energy balance. This study characterized leptin, POMC, and AgRP in the cerebrospinal fluid (CSF) of 47 healthy human subjects, 23 lean and 24 overweight/obese (OW/OB), as related to BMI, adiposity, plasma leptin, soluble leptin receptor (s-OB-R), and insulin. POMC was measured since 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.

Proopiomelanocortin; agouti-related protein; leptin; cerebrospinal fluid
engages brain MC-Rs, CSF levels of α-MSH are quite low. Furthermore, previous studies in the rodent have shown that CSF POMC levels, rather than CSF α-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 AgRP83–132, both of which are detected in human CSF. Finally, AgRP was also measured in plasma as a potential indicator of hypothalamic AgRP activity. Although 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 (23 males, 24 females) aged 19–50 yr; 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 nonsmokers, were taking no medications other than vitamins, and had no history of major psychiatric disorder, alcoholism, or neurological disease. Additionally, subjects were excluded for any other clinically significant medical condition or eating disorder, for recent weight change >5%, and for use of weight loss products or dieting during the 6 mo 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 800 and 1000 after an overnight fast (from 2100). A lumbar puncture (LP) was performed using a 25-G Whitacre needle; the first 0.5 ml 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 dual-energy...
X-ray absorptiometry (QDR 4500 A & Delphi W model) in 31 subjects; pregnancy was ruled out by human chorionic gonadotropin 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 -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 was 8 fmol/ml. ACTH was measured by RIA with an antiserum directed against ACTH (7–18) (IgG, Nashville, TN) (23). AgRP was measured by both ELISA and RIA with relative sensitivities for full-length AgRP and AgRP83–132, respectively. The ELISA (R & D Systems) uses recombinant full-length human AgRP standard. There is 17% cross-reactivity with AgRP83–132 (21). Assay sensitivity is 7 pg/ml. The RIA was performed as described previously using an antibody provided by Dr. Gregory Barsh and human

Fig. 2. Mean (± SE) CSF proopiomelanocortin (POMC) levels were significantly higher in lean vs. OW/OB subjects and in males vs. females (top left). There were strong negative correlations between CSF POMC and CSF leptin (top right) and between CSF POMC and BMI (bottom left). There was a strong positive correlation between CSF POMC and CSF sOB-R (bottom right).

Fig. 3. Correlations of CSF agouti-related protein (AgRP) with other variables. CSF AgRP did not correlate significantly with CSF leptin (top left) or plasma ghrelin (bottom right). Significant positive correlations were noted between CSF AgRP and CSF sOB-R (top right) and between CSF AgRP and CSF POMC (bottom left).
Table 2. Pearson correlations between CSF POMC and other measurements

<table>
<thead>
<tr>
<th>Measurement</th>
<th>r Value</th>
<th>P Value</th>
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<tbody>
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<td>BMI, kg/m²</td>
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<td>Body fat, %</td>
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<td>Plasma POMC</td>
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<td>CSF ACTH</td>
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<td>CSF AgRP</td>
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<td>0.003</td>
</tr>
<tr>
<td>Plasma AgRP</td>
<td>0.40</td>
<td>0.007</td>
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</table>

POMC, proopiomelanocortin; HOMA, homeostasis model assessment; sOB-R, soluble leptin receptor; AgRP, agouti-related peptide.

AgRP<sub>83-132</sub> for the standard (Phoenix Pharmaceuticals, Burlingame, CA) (2). There is 20% cross-reactivity with full-length AgRP. Assay sensitivity is 25 pg/ml. Serum insulin was measured by the 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 described previously, 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) 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 5 ng of human AgRP<sub>83-132</sub> (Phoenix Pharmaceuticals) and with 5 ng of human full-length AgRP (R & D Systems).

Statistical analysis. Data are expressed as means ± SE. Differences in parameters between the normal-weight lean subjects and OW/OB 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 percentage (r = 0.847); (P < 0.0001). The mean CSF leptin concentration was 225 ± 23.4 (SE) pg/ml, 83-fold lower than the mean plasma leptin concentration of 18.7 ± 3.5 ng/ml. CSF leptin also correlated strongly with BMI (r = 0.709) and body fat percentage (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 0.5%, 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 percentage (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 ± 0.02 vs. 0.229 ± 0.02 ng/ml, P = 0.039); 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 sexes were analyzed separately: r = −0.478, P = 0.02 for males and r = −0.634, P = 0.0009 for females. The negative correlations

Table 3. Pearson correlations between CSF AgRP and other measurements

<table>
<thead>
<tr>
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<th>r Value</th>
<th>P Value</th>
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<td>Body fat, %</td>
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<td>Plasma insulin</td>
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<td>CSF POMC</td>
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<td>CSF ACTH</td>
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<td>CSF AgRP (RIA)</td>
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<td>Plasma ghrelin</td>
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<td>0.15</td>
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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 \( (r = 0.063, \ P = 0.85) \).

**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 relation-
ship 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 stud-
ies 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,

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<tbody>
<tr>
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<td>Plasma leptin</td>
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<tr>
<td>CSF leptin</td>
<td>-0.51</td>
<td>0.0004</td>
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<td>Plasma insulin</td>
<td>-0.51</td>
<td>0.0006</td>
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<tr>
<td>HOMA</td>
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<td>0.0003</td>
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<td>CSF POMC</td>
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<td>0.007</td>
</tr>
<tr>
<td>CSF AgRP-ELISA</td>
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<td>0.32</td>
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<td>CSF sOB-R</td>
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<td>0.05</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>0.01</td>
<td>0.96</td>
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**Table 4. Pearson correlations between plasma AgRP and other measurements**

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Fig. 4. Correlations of plasma AgRP with other variables. There were significant neg-
ative correlations between plasma AgRP and 
BMI and CSF leptin (top right and top left) 
and insulin (bottom left). There was a significant positive correlation between plasma 
AgRP and CSF POMC (bottom right).
In addition, we show for the first time that plasma sOB-R negatively with BMI and plasma leptin, as reported previously (4, 34) and is consistent with studies in mice demonstrating that sOB-R may derive from a central rather than a peripheral source. High levels of POMC were detected in CSF, as we have reported previously, but we now show significant differences related to adiposity and sex difference. Characterization by gel filtration showed that the majority of the POMC immunoactivity in CSF was 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 is that 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 sexes 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 the leptin resistance that is well documented in POMC neurons in obese animals (5).
Alternatively, it could be a consequence of the decreased neuronal remodeling that is caused by a high-fat diet in animals or the hypothalamic inflammation and gliosis that have 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 anatomic 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 is that 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. Anatomic 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. Although the adrenals express significant amounts of 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 two cases (Wardlaw SL and Page-Wilson G, unpublished observations).

The differences noted in the current study between CSF and plasma AgRP levels may relate to anatomic differences in AgRP fiber tracks that gain access to CSF and blood, respectively. Both plasma and CSF AgRP are 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 is that 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.

ACKNOWLEDGMENTS

We are grateful to our study subjects for their participation, to Shveta Dighe for help with recruitment and administration, and to Catherine Huang for help with data analysis.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


