0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(3):1129–1136

doi: 10.1210/jc.2006-1841

Adiponectin and Resistin in Human Cerebrospinal Fluid and Expression of Adiponectin Receptors in the Human Hypothalamus

Katarina Kos, Alison L. Harte, Nancy F. da Silva, Anton Tonchev, Georgi Chaldakov, Sean James, David R. Snead, Barbara Hoggart, Joseph P. O'Hare, Philip G. McTernan, and Sudhesh Kumar

University of Warwick (K.K., A.L.H., N.F.d.S., J.P.O., P.G.M., S.K.), Unit for Diabetes and Metabolism, Clinical Sciences Research Institute, Coventry CV2 2DX, United Kingdom; Department of Forensic Medicine and Department of Clinical Pathology (A.T., G.C.), Varna Medical University, Varna 9002, Bulgaria; Department of Histopathology (S.J., D.R.S.), University Hospital of Coventry and Warwickshire National Health Service (NHS) Trust, Coventry CV2 2DX, United Kingdom; and Department of Anaesthetics (B.H.), Birmingham Heartlands Hospital, Heart of England NHS Foundation Trust, Birmingham B9 5SS, United Kingdom

Context: The adipokine leptin has critical importance in central appetite regulation. In contrast to some suggestion of adiponectin influencing energy homeostasis in rodents, there is no evidence for adiponectin or resistin entering the human blood-brain barrier.

Objective: The objective was to establish the presence of adiponectin or resistin in human cerebrospinal fluid (CSF) and to compare their distribution with leptin. Furthermore, we wished to examine the expression of the adiponectin receptors 1 and 2 (AdipR1, AdipR2) in the human hypothalamus.

Methods: For this purpose, serum and CSF samples were collected from 20 men and 19 women matched for age [men, 69.8 ± 8.6 yr (mean ± SD); women, 69.4 ± 4.3 yr] and BMI (men, 29.4 ± 3.4 kg/m^2; women, 27.3 ± 4.8 kg/m^2) undergoing elective surgery under spinal anesthesia.

Results: Adiponectin was identified in CSF with levels 1000-fold less than serum, whereas resistin and leptin levels were 100-fold less. Unlike their serum levels, adiponectin CSF levels showed no gender difference or correlation with insulin resistance, which is similar to resistin CSF levels. The adiponectin and leptin CSF/serum ratios in our study exhibit the same pattern of gender-specific BMI association with inverse correlation in women ($r = -0.61$; $P = 0.02$) and no correlation in men ($r = 0.026$; P = not significant). Furthermore, immunostaining established AdipR1 and -2 in the hypothalamus and increased AdipR2 expression in the paraventricular nucleus, which is involved in energy regulation.

Conclusion: In summary, our findings show both the presence of adiponectin and resistin in human CSF, with no effect of insulin resistance on CSF levels. The CSF entry of adiponectin and leptin in women appears to be impaired in obesity. **(*J Clin Endocrinol Metab* 92: 1129–1136, 2007)**

PREVIOUS STUDIES OF the adipokine leptin have highlighted its role as a mediator in the cross-talk between adipose tissue and the brain in regulating energy homeostasis. Recent studies also show that adiponectin appears to be involved in altering energy expenditure and thermogenesis (1) and resistin could affect hypothalamic feeding circuits (2). Adiponectin is a multimeric and multifunctional protein, the levels of which are reduced in states of obesity and type 2 diabetes mellitus (T2DM); its effects appear to be mediated by adiponectin receptors (3–5). In contrast, some reports have shown increased resistin in obesity and T2DM, which remains controversial (6), whilst its receptors remain unknown. To date, however, little is known about the potential central action of either of these adipokines in humans. Many studies have investigated leptin, its pleiotropic functions, including its role in central energy homeostasis, and its passage through the human blood-brain barrier (BBB). It can activate numerous signaling pathways (7) and, in particular, mediates its anorexigenic action through the AMP kinase pathway in the liver as well as the hypothalamus (8). Furthermore, it has been shown that leptin can stimulate the sympathetic system centrally to produce feedback of fat mass by induction of lipolysis (9). Leptin is a hormone that is predominantly secreted by adipocytes and also a key messenger to central energy homeostasis, so it is likely that other adipokines contribute to the cross-talk between adipose tissue and the brain.

The aim of this study was to examine the potential central role of resistin and adiponectin in humans. We examined matched paired serum and human cerebrospinal fluid (CSF) from male and female patients to explore the relationship between CSF and the corresponding serum levels and to determine the CSF/serum ratio as a measure of efficient uptake into the CSF. This study also considered possible correlations between adiponectin, resistin, and leptin in CSF with age, gender, BMI, and homeostasis assessment model (HOMA) index. Finally, we determined the expression and distribution of adiponectin receptors in the human hypothalamus. These issues have become particularly important

First Published Online January 9, 2007

Abbreviations: AdipR, Adiponectin receptor; BBB, blood-brain barrier; BMI, body mass index; CSF, cerebrospinal fluid; CRP, C-reactive protein; CV, coefficient of variation; HMW, higher molecular weight; HOMA, homeostasis assessment model; HOMA-IR, HOMA for insulin resistance; LWM, lower molecular weight; NPY, neuropeptide Y; NS, not significant; PVN, paraventricular nucleus; rh, recombinant human; T2DM, type 2 diabetes.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

in the light of recent reports that adiponectin is not present in human CSF (10) and exogenous adiponectin does not enter the BBB by endocytosis of rat brain endothelium 4 cerebral microvessel endothelial cells (11).

Subjects and Methods

Subjects

Serum- and CSF-matched samples were obtained from age- and BMI-matched subjects [men, age, 69.8 ± 8.6 yr (mean ± SD); BMI, 29.4 ± 3.4 kg/m^2, n = 20; women, age, 69.4 ± 4.3 yr; BMI, 27.3 ± 4.8 kg/m^2, n = 19] undergoing elective surgery, under spinal anesthesia, fasted for at least 8 h (Table 1). Exclusion criteria were malignancy, acute or chronic renal or liver disease, neurological disorders, and/or the use of immunosuppressants, current or recent use of systemic high-dose corticosteroids, antibiotics, or weight-modifying medication. Patients with C-reactive protein (CRP) levels above 10 mg/dl were also excluded. All samples were collected with the approval of the local ethics committees and with the informed written consent of study subjects. A fasting blood sample (5 ml) was taken at the time of venopuncture, and a clear CSF sample (2 ml) was collected before the spinal anesthetic agent was injected. Six patients had T2DM (two on insulin treatment), and three had impaired glucose tolerance. The T2DM subjects were on glucose/insulin infusion at the time of cannulation and could not be considered for HOMA calculation. Serum and plasma EDTA samples were immediately centrifuged, snap-frozen in liquid nitrogen, and stored at −80 C. CSF samples were passed through a 0.2-μm syringe filter (Schleicher & Schuell, Middlesex, UK), flash-frozen, and stored at −80 C.

ELISA assessment of serum levels

The serum samples were analyzed for the determination of adiponectin (1:500 dilution; Linco Research Inc., St. Charles, MO) with the assay limit of 0.78 ng/ml; intraassay percent coefficient of variation (%CV) was 7.4%, and interassay variability was 2.4–8.4%. Serum resistin levels were analyzed by ELISA (1:5 dilution; Quantikine, R&D Systems, Abingdon, UK). Assay limits were noted between 0.01 and 0.055 ng/ml, with intraassay %CV noted as between 3.8 and 5.3% and interassay variability being 7.8–9.2%. We have previously assessed the human resistin R&D Systems ELISA to examine resistin binding specificity. The recovery of resistin in serum and cross-reactivity with resistin-like molecules was assessed as previously described (13).

TABLE 1. Patient characteristics and mean adipokine levels

Variable	All subjects	Men	Women
No. of subjects	39	20	19
Age (yr)	69.4 ± 9	69.8 ± 8.6	69.4 ± 4.3
BMI (kg/m^2)	28 ± 4.6	29.4 ± 3.4	27.3 ± 4.8
Insulin (mU/liter)	5.5 ± 3.4	6.2 ± 4	4.8 ± 2.6
HOMA-IR	1.34 ± 1	1.5 ± 1.2	1.2 ± 0.8
Leptin			
Serum (ng/ml)	17.4 ± 12.5	13 ± 11	22.5 ± 13.7[a]
CSF (pg/ml)	67 ± 13	56 ± 9	79 ± 9
Adiponectin			
Serum (μg/ml)	26.6 ± 12	22.2 ± 11	31.5 ± 12[a]
CSF (ng/ml)	16.3 ± 6	16.7 ± 8.3	15.9 ± 3.6
Resistin			
Serum (ng/ml)	14.8 ± 8	14.1 ± 7	15 ± 8
CSF (ng/ml)	0.2 ± 0.13	0.2 ± 0.15	0.19 ± 0.12

Baseline characteristics of the study population are shown as well as distribution of adipokines levels (mean ± SD) between men and women.

[a] Significant gender differences are expressed as $P < 0.05$.

The leptin ELISA (1:100 dilution; Quantikine, R&D Systems) has a minimum detectable value of 7.8 pg/ml with an intraassay precision of 3–3.3% CV and interassay variability of 3.5–5.4% CV. The high-sensitive CRP ELISA (Life Diagnostics Inc., West Chester, PA) has a functional sensitivity of 0.1 mg/dl, intraassay precision of 2–7.5% CV, and interassay variability of 2.5–4.1% CV. An insulin ELISA (Linco Research Inc.) was used with the sensitivity of 2 μU/ml, intraassay %CV of 5.96, and interassay variability of 10.3% CV, in accordance with the manufacturer's protocol. Glucose levels were analyzed using the YSI-2300 STAT PLUS (YSI, Inc., Yellow Springs, OH), according to the manufacturer's instructions.

Assessment of CSF levels

CSF samples were used undiluted for assessment of adiponectin (20 μl), resistin (100 μl), and (10 μl) leptin. The serial dilution of CSF detected adiponectin levels up to a 1-in-4 dilution (86% of value expected 1-in-2 dilution and 80% of value expected 1-in-4 dilution). A similar serial dilution was documented for resistin, with serial dilution ranging from undiluted to 1 in 8. The use of neat CSF sample produced results with a recovery of 100%; therefore, they were deemed most acceptable in this system.

We further assessed potential sources of cross-reactivity in the adiponectin ELISA with C1q (Abcam, Cambridge, UK), collagen type III (Sigma, Poole, UK), and collagen type IV (Sigma). For this recombinant human (rh) C1q, rh collagen type III and rh type IV were analyzed at two concentrations (10 and 100 ng/ml) either in the presence of PBS pH 7.6 (Sigma) or in combination with CSF. No detectable cross-reactivity occurred between rh C1q, rh collagen type III, and rh type IV alone or in combination with CSF samples and adiponectin in this ELISA. The interassay and intraassay coefficients were evaluated for samples ranging from 12.1–15.9 ng/ml CSF adiponectin. The within percentage CV was 2.5%, and the between CVs were 6.7, 2.4, and 5.3%. The means between variations were calculated as described in the manufacturer's instructions.

Spiking and recovery of human adiponectin in CSF was further examined. rh Adiponectin (20 and 50 ng/ml) was added to known concentrations of adiponectin in pooled CSF samples (12.3 and 15.9 ng/ml, respectively), and adiponectin content was determined in three separate assays. The percentage of recovery was determined in accordance with manufacturer's instructions. We observed that recovery ranged from 93–97% in CSF, which was within a comparable range as noted for serum according to the manufacturer's detailed assessments.

Spiking and recovery of human resistin in CSF was also addressed. For these studies, two concentrations of rh resistin (0.5 and 2 ng/ml) were added to known concentrations of resistin in pooled CSF samples (0.03 and 0.08 ng/ml,

respectively), and resistin concentration was determined in three separate ELISA assays. The percentage of resistin recovery ranged from 81–99%, with a mean of 88% in CSF; a similar recovery was noted for serum findings by the manufacturer as well as in our previous publications (13, 14).

Blood contamination was excluded with use of the micropore filter described. The cell count in CSF samples with the hemocytometer did not show any nucleus-bearing cells. Further screening of the CSF samples with the hemoccult test (Hema-screen; Immunostics Inc., Ocean, NJ) was negative, yet the test remained positive with spiking of full blood at a concentration of 1:10,000.

HOMA index analysis

The HOMA for insulin resistance (HOMA-IR) index (12) was calculated using the HOMA calculator available at www.dtu.ox.ac.uk/index.php?maindoc=/homa/index.php.

Immunohistochemistry

Human adipose tissue was obtained from Medical Solutions (Peterborough, UK). Human hypothalamic postautopsy sections were obtained with the approval of the Varna Medical Research Ethics Committee from Varna University, Bulgaria. Tissue was incubated with primary polyclonal adiponectin receptor (AdipR)1 and AdipR2 antibody (Phoenix Pharmaceuticals, Burlingame, CA) in a dilution of 1:2500 and 1:200, respectively, and dual-stained with neuropeptide Y (NPY) in a dilution of 1:400. Sections were developed using peroxidase substrate kit VIP (Vector Laboratories Ltd., Peterborough, UK) for AdipR1 and -2 and diaminobenzidine (BioGenex, San Ramon, CA) for NPY. To demonstrate specific binding, the primary antibody was omitted for negative control for both AdipR antibodies, independently.

Statistics

Statistics were performed using SPSS version 12 (SPSS UK, Surrey, UK). P values of less than 0.05 were considered as statistically significant. Analysis of normally distributed variables was by linear regression analysis and two-tailed Student's t test. For nonparametric distributions, *e.g.* HOMA, the Spearman correlation was used. Values are expressed as mean ± SD, unless otherwise stated.

Results

Serum and CSF adiponectin

Serum adiponectin was significantly higher in women than in men (31.5 ± 1.2 *vs.* 22.2 ± 1.1 μg/ml; $P = 0.016$). Adiponectin correlated positively with age in men (r = 0.56; $P = 0.01$) but not significantly in women [r = 0.36; P = not significant (NS)]. Serum adiponectin showed a negative association with BMI in males (r = −0.46; $P = 0.04$), whereas only a trend was noted in women (r = −0.27; P = NS). In agreement with previous studies, the HOMA index and serum adiponectin were inversely correlated for the total study population (r = −0.48; $P < 0.001$), and the adiponectin levels were significantly lower in the T2DM and insulin-resistant subjects compared with nondiabetic subjects (19.2 ± 3 μg/ml, n = 9, *vs.* 28.8 ± 2.9 μg/ml, n = 30; $P = 0.020$).

Mean CSF adiponectin was 16.3 ± 6 ng/ml, with a range of 7.2–41.6 ng/ml; therefore, the CSF levels were approximately 1000-fold lower than the serum adiponectin levels (Fig. 1). Unlike the serum, CSF adiponectin did not show sexual dimorphism (men, 16.7 ± 8.3 ng/ml; women, 15.9 ± 3.6 ng/ml; $P = 0.7$) (Table 1). In the total population, a positive age correlation was noted (r = 0.36; $P = 0.036$; n = 35), which was maintained when men and women were analyzed individually (r = 0.55, $P = 0.035$; r = 0.5, $P = 0.036$, respectively). CSF adiponectin was inversely correlated with BMI in men (r = −0.62; $P = 0.01$) but not in women (r = −0.26; P = NS). HOMA-IR and CSF adiponectin levels showed no correlation, and no change was noted due to diabetic status.

The CSF adiponectin levels exhibited a correlation with serum levels (r = 0.34; $P = 0.047$; n = 34) as described in Fig. 2A. The CSF levels failed to rise with high serum levels and demonstrate a threshold effect, suggesting a saturated transport. CSF/serum adiponectin ratios were significantly higher in men [1.0 ± 0.11 (∗10^{-3})] than women [0.65 ± 0.06 (∗10^{-3})] ($P < 0.01$). The CSF/serum ratio was inversely correlated with BMI in women (r = −0.61, $P = 0.02$) and did not show a correlation in men (r = 0.026; P = NS) (Fig. 3A).

Serum and CSF resistin

Mean serum resistin levels were 14.8 ± 8 ng/ml, with slightly higher levels shown in women than in men (15.8 *vs.* 14.97 ng/ml) (Table 1), which did not attain significance ($P = 0.080$). Resistin levels correlated positively with increasing age (r = 0.45; $P < 0.01$); however, no correlation was observed with BMI (r = −0.21; P = NS) or HOMA-IR (r = −0.32; P = NS) index.

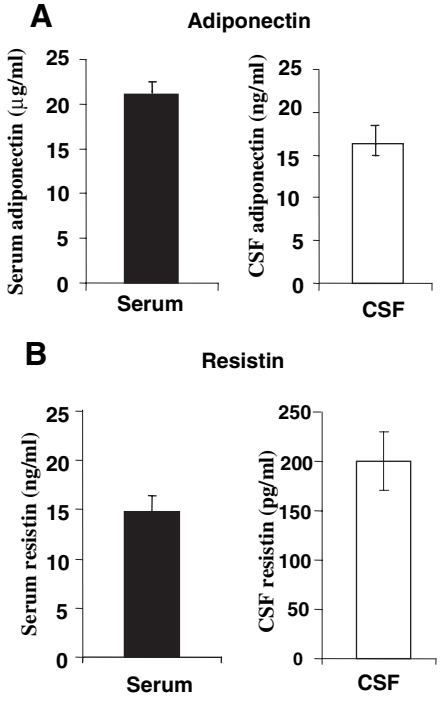

FIG. 1. Comparison between serum and CSF levels of adiponectin (A) and resistin (B). Serum adiponectin levels (μg/ml) are 1000 times lower in CSF (ng/ml), and serum resistin levels (ng/ml) are 100 times lower in CSF (pg/ml).

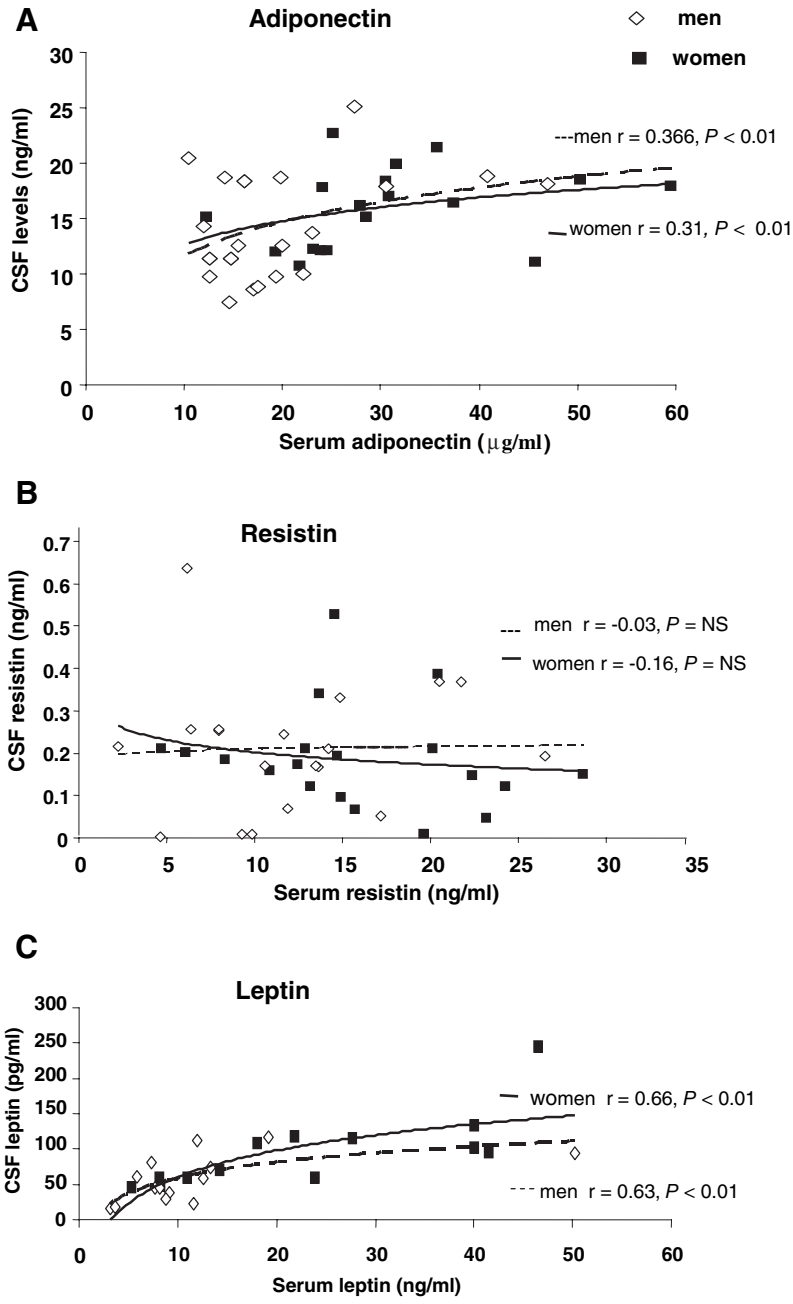

FIG. 2. Relationship between adipokine CSF and serum levels. This shows the association of the CSF with serum levels of the adipokines adiponectin (A), resistin (B), and leptin (C). With increasing serum levels, the trend lines for all adipokines show a flattening toward a threshold suggesting saturated transport into the CSF space similar to leptin (r represents the correlation coefficient of curve of best fit, with *P* values as shown).

The mean CSF resistin level was measured as 0.20 ± 0.13 ng/ml, with a range of 0.01–0.63 ng/ml. CSF resistin levels were not influenced by gender ($P = 0.65$) (Table 1). No relationship between CSF resistin levels and serum was noted (Fig. 2B). Furthermore, CSF resistin levels did not correlate with age (r = 0.09; P = NS) or HOMA-IR index (r = 0.24; P = NS) and remained unaltered by diabetic status. The CSF/serum ratio did not differ significantly between men and women and bore no relationship with BMI (Fig. 3B).

Serum and CSF leptin

Mean serum leptin levels were 17.4 ± 2.5 ng/ml, with significantly higher levels in women (22.5 ± 3.7 ng/ml) *vs.* men (13.0 ± 3.0 ng/ml; $P = 0.03$) (Table 1). Serum leptin levels were inversely correlated with BMI (r = 0.52, $P < 0.001$; men, r = 0.75, $P = 0.001$; women, r = 0.82; $P < 0.001$) but showed no correlation with age.

The CSF levels showed a tendency toward higher leptin levels in women than men but did not attain significance (women, 79.0 ± 9.0 pg/ml *vs.* men, 56.0 ± 9.0 pg/ml; P = NS). CSF leptin was positively correlated with BMI in men and women (r = 0.42; $P = 0.028$) but not with age. The correlation of CSF with serum levels was best described by a logarithmic curve fit (r = 0.65; $P < 0.001$), similar to previous observations (15) (Fig. 2C). The CSF/serum ratio was inversely correlated with BMI in women (r = −0.81; P = 0.001) but showed no correlation with BMI in men (r = −0.17; P = NS) (Fig. 3C). Serum leptin was higher in T2DM than

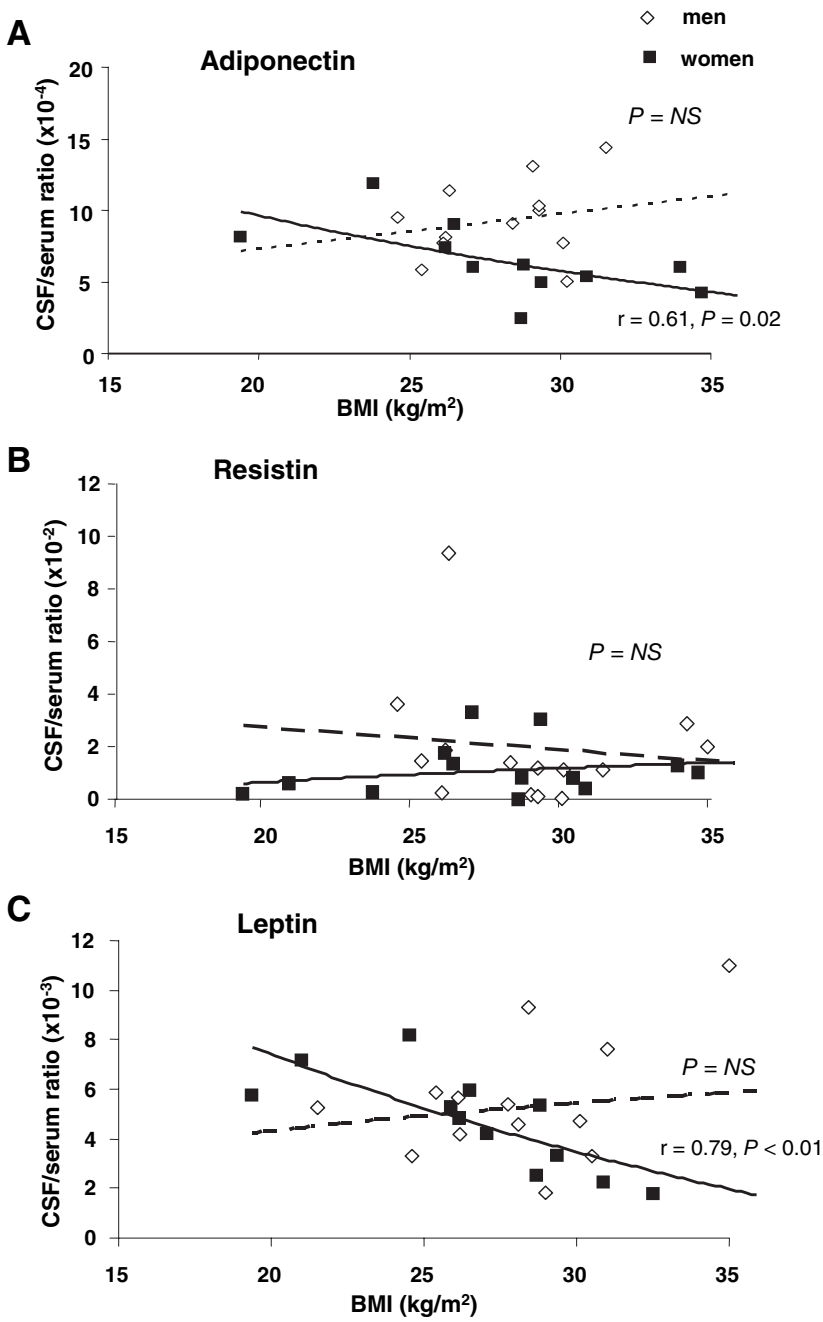

FIG. 3. CSF/serum ratio *vs.* BMI for men and women. This shows the association between CSF/serum ratio *vs.* BMI (kg/m^2) for men and women for adiponectin (A) (inverse correlation in women), resistin (B) (no significant correlation in men or women), and leptin (C) (inverse correlation in women) (r represents Pearson correlation, with *P* values as shown).

nondiabetic subjects (28.9 ± 5.8 ng/ml *vs.* 12.5 ± 8.7 ng/ml; $P = 0.02$), whereas CSF leptin was not significantly different in the T2DM subjects (T2DM subjects, 105 ± 2 pg/ml *vs.* nondiabetic subjects, 62 ± 7 pg/ml; P = NS).

Expression of adiponectin receptors in the human hypothalamus

Immunohistochemistry showed the expression of AdipR1 and AdipR2 in the neuronal cells in the human hypothalamus. AdipR1 was diffusely expressed in both the anterior and posterior hypothalamus, whereas AdipR2 was concentrated in the paraventricular nucleus (PVN) along with neurons that were dual-stained with NPY. Human adipose human tissue was also observed to express AdipR1 (Fig. 4).

Discussion

This is the first study to detect adiponectin and resistin in human CSF. Unlike the recently published findings by Spranger *et al.* (10), we were able to detect and confirm low adiponectin concentrations that were approximately 1000-fold lower in the CSF than serum. The same 1:1000 ratio of CSF to serum adiponectin has also been noted in rats (16), whereas a higher ratio (1:100) has been observed in mice (1). Taken together, these results suggest a species-specific vari-

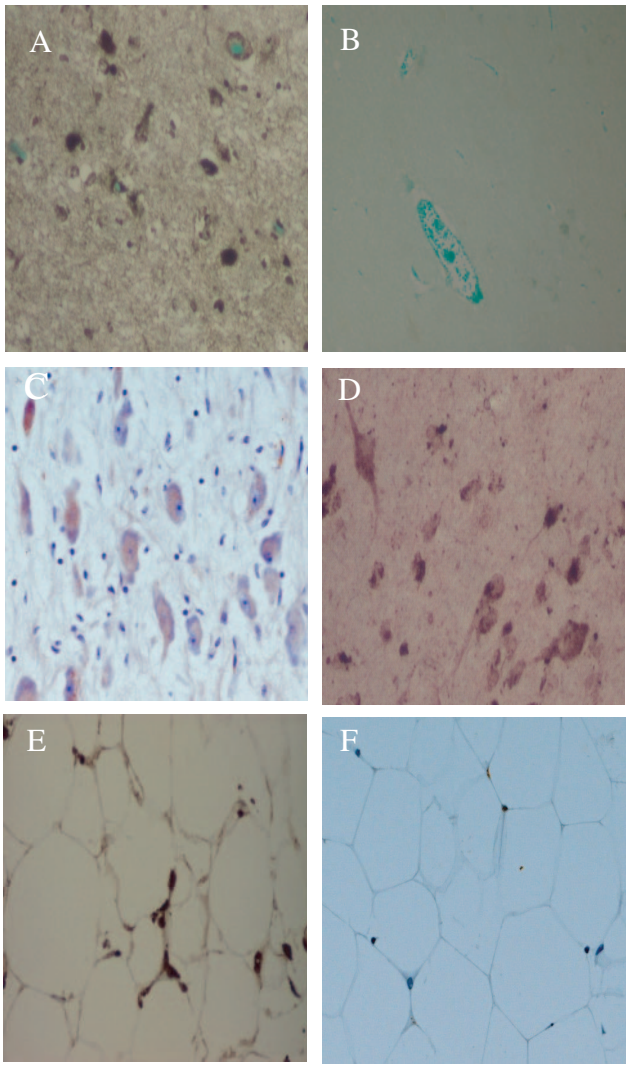

FIG. 4. Immunohistological staining of human hypothalamus with positive staining for AdipR1 (×1000 magnification) (A); negative staining for human hypothalamus (*blue*) (B); and human hypothalamus with positive staining for AdipR2 (*brown*; ×1000 magnification), which highlights the area of the PVN (C). D, Dual staining of AdipR2 (*red*) and NPY (*brown*). Note the dendritic projections of the neural cells and cytoplasmatic expression of AdipR2. E, Human adipose tissue with positive staining for AdipR1 (*red*). F, Negative staining for human adipose tissue (*blue*; ×2000 magnification).

ation of adiponectin in CSF. We established the presence of resistin in human CSF at approximately 100 times lower levels than serum. In contrast, no resistin has been identified in the CSF of rodents (17). The relationship between CSF and serum levels varied between patients dependent on variables, including BMI and the magnitude of the serum levels of the adipokine, which, if high, could exceed the threshold of saturation in CSF. This is also the first study to identify the presence of AdipR1 and AdipR2, as observed not only in the rat brain (5) but also in the human hypothalamus, in which a concentrated expression of AdipR2 was noted in neuronal cells of the PVN, which also express NPY. A previous study has documented mRNA expression of AdipR1 and -2 on human endothelial cells of the choroid plexus (10), which is relevant to the controlled entry of proteins through the BBB and where functional leptin receptors have been identified (18). Adiponectin could thus be transported from blood to CSF by receptor-mediated transcytosis in a similar manner to leptin and may have a role in energy homeostasis as implicated in studies by Qi *et al.* (1), as well as Fry *et al.* (19). These data suggest that adiponectin enters the CSF with potential action via neuronal pathways in the PVN, a hypothalamic area that receives input from the arcuate nucleus, a region of the brain containing key appetite-regulating pathways via NPY neurons (20). The function of adiponectin may thus be dependent on different neuronal networks compared with leptin, for which cFOS immunoreactivity was reported highest within the arcuate nucleus (1).

Although there is some confirmation of the role of adiponectin in CSF through receptor expression studies in the brain and cFos activity with adiponectin (1), other studies have failed to establish the presence of adiponectin in human CSF (10) or to verify the passage of adiponectin through the BBB (11). This may be due to the need for high-sensitivity assays to be used for its detection. Furthermore, entry of additional adiponectin, when administered as radioactive-labeled iv adiponectin, may not lead to observable differences due to potentially already saturated levels of adiponectin in the CSF.

Leptin is a known potent central appetite regulator (21), and its serum levels directly correlate with fat mass (21, 22). Comparison of leptin CSF levels within our and other studies (15, 23–25) has highlighted variation (15). The average leptin CSF levels in our findings were relatively low in comparison with some previously reported data (15, 23) but similar to levels identified in other studies (21, 24). In comparison, mean CSF fasting levels of leptin are much smaller than those of adiponectin and resistin, suggesting that their levels are within the range to activate central nervous system effects. Much lower concentrations than in the periphery are sufficient for activation of neural pathways and signaling in the hypothalamic nuclei for proteins like leptin (25, 26).

AdipR1 and AdipR2 can be activated by both globular and full-length adiponectin (3, 5). AdipR1 shows a high affinity for globular adiponectin, but data for binding affinities only exist for the muscle receptors in mice (5) and not for neuronal cells in humans. Adiponectin receptors mediate action through signaling molecules such as peroxisome proliferated activated receptor-α, MAPK, and AMP kinase (27, 28). AMP kinase pathways that can be activated by globular adiponectin, as well as trimers, are involved in hormonal and nutrient signaling in the hypothalamus, including the regulation of food intake by leptin and insulin (8, 28). Hexamers and higher molecular weight (HMW) isoforms of adiponectin can also bind to T-cadherin (29) with unclear biological function. Given that HMW isoforms do not induce AMP kinase activation but mediate their action through nuclear factor-κB in the periphery (30), T-cadherin is unlikely to play a role in central appetite regulation.

In concordance with previous observations, serum circulating levels of adiponectin were found at higher levels in women (31). Similar to other studies, serum and CSF adiponectin were inversely correlated with BMI in men but not in women (32). Although the serum adiponectin levels showed sexual dimorphism, intrathecal adiponectin levels

showed no gender difference. Higher circulating serum levels in women and the lack of a CSF gender difference have previously been observed with leptin levels (33) and reaffirmed within our study population.

Our current study does not allow us to determine which isoforms of adiponectin enter the CSF directly. However, we note that higher serum HMW adiponectin was reported in women (31), but we found no gender difference in CSF. Therefore, it is likely that HMW structures do not enter the CSF. Additionally, due to the size of the HMW structures (>500 kDa), these are unlikely to pass through the BBB.

Resistin appears to have a role in obesity-associated inflammation and insulin resistance, which remains controversial in the human context (13, 14, 34). In this study, raised circulating resistin levels do not correspond with higher CSF levels, suggesting limited uptake into the CSF. The wide variation in resistin concentration requires further studies to examine entry of resistin across the BBB. No correlation between serum and CSF resistin levels was observed with BMI, and neither of the adipokines' CSF levels were noted to be affected by insulin resistance. T2DM is also associated with reduced HMW isoforms (27) and impaired multimerization of adiponectin (35). Thus adiponectin CSF levels may predominantly be made up of LMW isoforms for reasons explained above and, as such, may not be affected by diabetes.

In summary, adiponectin and resistin are present in human CSF with levels unaffected by gender. AdipR1 and AdipR2 are present in the human hypothalamus, especially in the PVN. These findings highlight the potential involvement of adipokines in central energy homeostasis in humans. Based on our data, we postulate an active transport mechanism for both adiponectin and resistin across the BBB.

Acknowledgments

We thank research nurses Jacqueline Farmer and Amelia Williamson and all the anesthetists at Heartlands and Solihull NHS Trust Hospital for their support.

Received August 22, 2006. Accepted December 28, 2006.

Address all correspondence and requests for reprints to: Professor Sudhesh Kumar, University of Warwick, Clinical Sciences Research Institute, University Hospital Coventry and Warwickshire, Clifford Bridge Road, Coventry CV2 2DX, United Kingdom. E-mail: Sudhesh.Kumar@warwick.ac.uk.

Disclosure Statement: The authors have nothing to disclose.

References

1. **Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS** 2004 Adiponectin acts in the brain to decrease body weight. Nat Med 10:524–529
2. **Brunetti L, Orlando G, Recinella L, Michelotto B, Ferrante C, Vacca M** 2004 Resistin, but not adiponectin, inhibits dopamine and norepinephrine release in the hypothalamus. Eur J Pharmacol 493:41–44
3. **Wolfe BE, Jimerson D, Orlova C, Mantzoros C** 2004 Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers. Clin Endocrinol (Oxf) 61:332–338
4. **Berg AH, Scherer PE** 2005 Adipose tissue, inflammation, and cardiovascular disease. Circ Res 96:939–949
5. **Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T** 2003 Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762–769
6. **McTernan PG, Kusminski CM, Kumar S** 2006 Resistin. Curr Opin Lipidol 17:170–175
7. **Frühbeck G** 2006 Intracellular signalling pathways activated by leptin. Biochem J 393:7–20
8. **Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferre P, Birnbaum MJ, Stuck BJ, Kahn BB** 2004 AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428:569–574
9. **Rayner DV, Trayhurn P** 2001 Regulation of leptin production: sympathetic nervous system interactions. J Mol Med 79:8–20
10. **Spranger J, Verma S, Gohring I, Bobbert T, Seifert J, Sindler AL, Pfeiffer A, Hileman SM, Tschop M, Banks WA** 2006 Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 55:141–147
11. **Pan W, Tu H, Kastin AJ** 2006 Differential BBB interactions of three ingestive peptides: obestatin, ghrelin, and adiponectin. Peptides 27:911–916
12. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
13. **Kusminski CM, da Silva NF, Creely SJ, Fisher FM, Harte AL, Baker AR, Kumar S, McTernan PG** 2007 The *in vitro* effects of resistin on the innate immune signaling pathway in isolated human subcutaneous adipocytes. J Clin Endocrinol Metab 92:270–276
14. **McTernan PG, Fisher FM, Valsamakis G, McTernan CL, Chetty R, Harte AL, Smith SA, Barnett AH, Kumar S** 2003 Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. J Clin Endocrinol Metab 88:6098–6106
15. **Nam SY, Kratzsch J, Kim KW, Kim KR, Lim SK, Marcus C** 2001 Cerebrospinal fluid and plasma concentrations of leptin, NPY, and α-MSH in obese women and their relationship to negative energy balance. J Clin Endocrinol Metab 86:4849–4853
16. **Caja S, Torrente M, Martinez I, Abelenda M, Puerta M** 2005 Adiponectin values are unchanged during pregnancy in rats. J Endocrinol Invest 28:609–615
17. **Caja S, Martinez I, Abelenda M, Puerta M** 2005 Resistin expression and plasma concentration peak at different times during pregnancy in rats. J Endocrinol 185:551–559
18. **Merino B, Diez-Fernandez C, Ruiz-Gayo M, Somoza B** 2006 Choroid plexus epithelial cells co-express the long and short form of the leptin receptor. Neurosci Lett 393:269–272
19. **Fry M, Smith PM, Hoyda TD, Duncan M, Ahima RS, Sharkey KA, Ferguson AV** 2006 Area postrema neurons are modulated by the adipocyte hormone adiponectin. J Neurosci 26:9695–9702
20. **Cowley MA, Pronchuk N, Fan W, Dinulescu DM, Colmers WF, Cone RD** 1999 Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. Neuron 24:155–163
21. **Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte Jr D, Woods SC, Seeley RJ, Weigle DS** 1996 Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 45:531–535
22. **Zhang F, Basinski MB, Beals JM, Briggs SL, Churgay LM, Clawson DK, DiMarchi RD, Furman TC, Hale JE, Hsiung HM, Schoner BE, Smith DP, Zhang XY, Wery JP, Schevitz RW** 1997 Crystal structure of the obese protein leptin-E100. Nature 387:206–209
23. **Rodrigues AM, Radominski RB, de Lacerda Suplicy H, De Almeida SM, Niclewicz PA, Boguszewski CL** 2002 The cerebrospinal fluid/serum leptin ratio during pharmacological therapy for obesity. J Clin Endocrinol Metab 87:1621–1626
24. **Wong ML, Licinio J, Yildiz BO, Mantzoros CS, Prolo P, Kling M, Gold PW** 2004 Simultaneous and continuous 24-hour plasma and cerebrospinal fluid leptin measurements: dissociation of concentrations in central and peripheral compartments. J Clin Endocrinol Metab 89:258–265
25. **Corp ES, Conze DB, Smith F, Campfield AL** 1998 Regional localization of specific [^{125}I] leptin binding sites in rat forebrain. Brain Res 789:40–47
26. **Zlokovic BV, Jovanovic S, Miao W, Samara S, Verma S, Farrell CL** 2000 Differential regulation of leptin transport by the choroid plexus and blood-brain barrier and high affinity transport systems for entry into hypothalamus and across the blood-cerebrospinal fluid barrier. Endocrinology 141:1434–1441
27. **Fisher FF, Trujillo ME, Hanif W, Barnett AH, McTernan PG, Scherer PE, Kumar S** 2005 Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. Diabetologia 48:1084–1087
28. **Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang Cc C, Itani SI, Lodish HF, Ruderman NB** 2002 Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci USA 99:16309–16313
29. **Hug C, Wang J, Ahmad NS, Bogan JS, Tsao TS, Lodish HF** 2004 T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proc Natl Acad Sci USA 101:10308–10313
30. **Tsao TS, Tomas E, Murrey HE, Hug C, Lee DH, Ruderman NB, Heuser JE, Lodish HF** 2003 Role of disulfide bonds in Acrp30/adiponectin structure and

signaling specificity. Different oligomers activate different signal transduction pathways. J Biol Chem 278:50810–50817

31. **Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, Igura T, Inui Y, Kihara S, Nakamura T, Yamashita S, Miyagawa J, Funahashi T, Matsuzawa Y** 2000 An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res 32:47–50
32. **Vilarrasa N, Vendrell J, Maravall J, Broch M, Estepa A, Megia A, Soler J, Simon I, Richart C, Gomez JM** 2005 Distribution and determinants of adiponectin, resistin and ghrelin in a randomly selected healthy population. Clin Endocrinol (Oxf) 63:329–335
33. **Wiedenhoft A, Muller C, Stenger R, Blum WF, Fusch C** 1999 Lack of sex difference in cerebrospinal fluid (CSF) leptin levels and contribution of CSF/plasma ratios to variations in body mass index in children. J Clin Endocrinol Metab 84:3021–3024
34. **Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS** 2002 A central role for JNK in obesity and insulin resistance. Nature 420:333–336
35. **Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T** 2003 Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem 278:40352–40363

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.