

Relationship Between Cerebrospinal Fluid Visfatin (PBEF/Nampt) Levels and Adiposity in Humans

Manfred Hallschmid,¹ Harpal Randeva,² Bee K. Tan,² Werner Kern,³ and Hendrik Lehnert^{2,3}

OBJECTIVE—Observations of elevated circulating concentrations of visfatin (PBEF/Nampt) in obesity and diabetes suggest that this recently described adipokine is involved in the regulation of body weight and metabolism. We examined in humans whether visfatin is found in cerebrospinal fluid (CSF) and, if so, how CSF visfatin concentrations relate to adiposity and metabolic parameters.

RESEARCH DESIGN AND METHODS—We measured visfatin concentrations in the plasma and CSF of 38 subjects (18 men and 20 women; age 19–80 years) with a wide range of body weight (BMI 16.24–38.10 kg/m²). In addition, anthropometric parameters and endocrine markers were assessed. Bivariate correlation coefficients were determined and stepwise multiple regression analyses were performed to detect associations of CSF and plasma visfatin levels with relevant parameters.

RESULTS—Plasma visfatin levels increased with rising BMI ($P < 0.0001$) and body fat mass ($P < 0.0001$). In contrast, CSF visfatin levels decreased with increasing plasma visfatin concentrations ($P < 0.03$), BMI ($P < 0.001$), body fat mass ($P < 0.0001$), and insulin resistance ($P < 0.05$). Body fat was the only factor independently associated with CSF visfatin, explaining 58% of the variation of CSF visfatin levels ($P < 0.0001$). Neither plasma ($P > 0.13$) nor CSF ($P > 0.61$) visfatin concentrations differed between men and women.

CONCLUSIONS—Our data indicate that visfatin concentrations in human CSF decrease with rising body fat, supporting the assumption that visfatin transport across the blood-brain barrier is impaired in obesity and that central nervous visfatin insufficiency or resistance are linked to pathogenetic mechanisms of obesity. *Diabetes* 58:637–640, 2009

Body weight regulation critically depends on the interplay between the central nervous system and endocrine messengers from the periphery, adipokines like leptin and adiponectin in particular (1). Visfatin is a recently described peptide, previously identified as pre-B-cell colony-enhancing factor (PBEF) or nicotinamide phosphoribosyltransferase (Nampt), that is produced by adipose tissue as well as skeletal muscle, liver, and immune cells (2). The initial assumption that visfatin acts directly on the insulin receptor and displays

insulin-mimetic properties has been recently cast into doubt, and still little is known about the role of this adipokine in energy homeostasis (3–5). Observations of acutely increased plasma visfatin concentrations during hyperglycemia have linked visfatin release with regulation of glucose metabolism (6). Moreover, circulating visfatin levels have been reported to be elevated in obesity (7,8) and type 2 diabetes (9). These findings suggest that visfatin may be actively involved in the control of weight regulatory networks. On this background, we investigated the presence of visfatin in human cerebrospinal fluid (CSF). More importantly, we also determined CSF concentrations of this adipokine in relation to corresponding plasma levels as well as to body weight and adiposity.

RESEARCH DESIGN AND METHODS

Thirty-eight subjects participated in the experiments (18 men and 20 women; age 19–80 years; BMI 16.24–38.10 kg/m²). Fourteen participants had normal body weight, 14 subjects were overweight (BMI 25 to <30 kg/m²) and 10 subjects were obese (BMI ≥30 kg/m²). Exclusion criteria were a history of diabetes, congestive heart failure, liver or kidney disease, malignancy, signs of inflammation, pregnancy, and any drugs influencing body weight like corticoids or contraceptives. During the study, three subjects (two men and one woman) were newly diagnosed with type 2 diabetes as indicated by elevated fasting plasma glucose levels >7 mmol/l according to the criteria of the American Diabetes Association. After an overnight fast, subjects reported to the laboratory for simultaneous sampling of blood and cerebrospinal fluid (1 ml) via lumbar puncture after local anesthesia (2 ml mepivacain-HCL 1%). In addition, body weight, body height, and waist and hip circumferences were assessed. Body composition was measured by standard bioelectrical impedance analysis (BIA 2000-mol/l; Data Input, Frankfurt, Germany). Frequencies of 1, 5, 50, and 100 Hz were employed, and Eurobody software (Data Input) was used to examine body fat mass. The study was approved by the local ethics committee, and all subjects gave written informed consent.

Blood samples were immediately centrifuged, and plasma and CSF samples were frozen at –80°C until assay. Visfatin was determined using a commercially available enzyme immunoassay (Phoenix Pharmaceuticals, Burlingame, CA). Analyses were performed according to the manufacturer's protocol, with an intra-assay coefficient of variation (CV) <5% for determination in CSF and plasma. In addition, plasma and CSF glucose (CV <1.1%; Beckman glucose analyser II; Beckman Instruments, Fullerton, CA), insulin, and adiponectin concentrations were measured. Insulin was determined with a commercial competitive double-antibody radioimmunoassay (Pharmacia Insulin RIA 100; Pharmacia Diagnostics, Upsalla, Sweden) that was slightly modified as described previously (10); the assay limit of sensitivity was 1.8 pmol/l, and intra-assay variation was <4.5% for determination in CSF and plasma. Adiponectin was determined using a commercially available radioimmunoassay (HADP-61HK; Linco Research, St. Charles, MO) with a sensitivity limit of 0.78 ng/ml; intra-assay variation was <6.2%, and interassay variation was <9.3%. Insulin resistance was estimated employing the homeostatic model assessment formula, with high scores denoting a high degree of insulin resistance.

Where necessary, values were log-transformed to achieve normal distribution. Bivariate Pearson correlation coefficients were determined, and stepwise multiple regression analyses were performed to detect associations between CSF visfatin levels and relevant variables (sex, age, body weight, BMI, fat mass, plasma visfatin, homeostatic model assessment score, waist and hip circumferences, waist-to-hip ratio, and CSF levels of glucose, insulin, and adiponectin) as well as between the CSF-to-plasma visfatin ratio and corresponding variables. In addition, plasma and CSF visfatin concentrations were

From the ¹Department of Neuroendocrinology, University of Luebeck, Luebeck, Germany; ²Warwick Medical School, University of Warwick, Coventry, U.K.; and the ³Department of Internal Medicine I, University of Luebeck, Luebeck, Germany.

Corresponding author: Manfred Hallschmid, hallschmid@kfg.uni-luebeck.de. Received 27 August 2008 and accepted 12 December 2008

Published ahead of print at <http://diabetes.diabetesjournals.org> on 18 December 2008. DOI: 10.2337/db08-1176.

© 2009 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

TABLE 1
Subject characteristics and correlations with plasma and CSF visfatin concentrations and the CSF-to-plasma visfatin ratio

	Mean \pm SEM	Plasma visfatin	CSF visfatin	CSF-to-plasma visfatin ratio
Age (years)	53.26 \pm 2.58	0.23	-0.36*	-0.34*
BMI (kg/m ²)	26.67 \pm 0.76	0.69†	-0.52‡	-0.73‡
Body weight (kg)	77.99 \pm 2.81	0.71†	-0.47‡	-0.70‡
Fat mass (kg)	22.56 \pm 1.64	0.71†	-0.68‡	-0.80‡
Waist circumference (cm)	95.11 \pm 2.37	0.59†	-0.48‡	-0.65‡
Hip circumference (cm)	99.73 \pm 1.82	0.61†	-0.57‡	-0.72‡
Waist-to-hip ratio	0.95 \pm 0.01	0.32	-0.18	-0.30
Visfatin				
Plasma (ng/ml)	33.58 \pm 2.49	—	—	—
CSF (ng/ml)	3.41 \pm 0.12	-0.36*	—	—
CSF-to-plasma ratio	0.12 \pm 0.01	-0.86†	0.74†	—
Glucose				
Plasma (mmol/l)	5.14 \pm 0.12	0.20	-0.20	-0.24
CSF (mmol/l)	3.31 \pm 0.07	0.21	-0.16	-0.28
CSF-to-plasma ratio	0.65 \pm 0.01	0.03	0.00	-0.08
Insulin				
Plasma (pmol/l)	84.35 \pm 10.56	0.23	-0.32*	-0.33*
CSF (pmol/l)	2.23 \pm 0.19	-0.04	0.07	0.08
CSF-to-plasma ratio	0.037 \pm 0.004	-0.24	0.28	0.31
HOMA	3.36 \pm 0.51	0.25	-0.33*	-0.35*
Adiponectin				
Plasma (μ g/ml)	12.24 \pm 0.85	-0.22	-0.03	0.12
CSF (ng/ml)	6.02 \pm 1.19	-0.12	-0.20	-0.03
CSF-to-plasma ratio	0.00046 \pm 0.00004	0.11	-0.12	-0.12

Plasma levels of visfatin and insulin, CSF insulin concentrations, and HOMA values were log-transformed prior to correlational analyses to achieve normal distribution. $n = 38$. * $P < 0.05$, † $P < 0.001$, ‡ $P < 0.01$. CSF, cerebrospinal fluid; HOMA, insulin resistance as calculated by the homeostatic model assessment formula.

subjected to two-way ANOVA, including the factors sex and, respectively, body weight status (normal weight/overweight/obese) and body fat quartile, followed by single comparisons via unpaired Bonferroni corrected t tests. For analyses, SPSS 15.0 for Windows (SPSS, Chicago, IL) was used. A two-sided P value < 0.05 was considered significant.

RESULTS

Table 1 summarizes anthropometric and endocrine parameters of our subjects and respective correlations with visfatin levels in plasma and CSF and with the CSF-to-plasma visfatin ratio. Plasma visfatin levels increased with rising body fat mass (Fig. 1A), BMI, and waist and hip circumferences. In CSF, visfatin was detectable at concentrations approximately 10% of plasma levels. Plasma and CSF visfatin levels were negatively correlated as CSF visfatin levels decreased with increasing body fat mass (Fig. 1B), BMI, and waist and hip circumferences, as well as with increasing insulin resistance and age. Accordingly, the CSF-to-plasma visfatin ratio was also negatively associated with fat mass (Fig. 1C), BMI, and waist and hip circumferences, as well as with the degree of insulin resistance, plasma insulin levels, and age. Supporting analyses relying on Spearman's rank correlations and partial correlations including the control variables age and sex yielded essentially the same results.

In the stepwise multiple linear regression analyses, body fat mass was the only factor independently associated with CSF visfatin, explaining 58% of the variation of CSF visfatin levels ($R^2 = 0.58$, $B = -0.066$, $SE = 0.009$ [95% CI -0.085 to -0.047], $\beta = -0.768$, $P < 0.0001$), whereas none of the other variables, including sex, age, and CSF levels of glucose, insulin, and adiponectin, were independently related to CSF visfatin (all $P > 0.11$). With our sample size, we had 99% power to detect an increment to the model R^2

of 0.58 (at $\alpha = 0.05$). Body fat was also the only factor independently associated with the CSF-to-plasma visfatin ratio, explaining 64% of the ratio's variation ($R^2 = 0.64$, $B = -0.004$, $SE = 0.001$ [95% CI -0.006 to -0.003], $\beta = -0.806$, $P < 0.0001$). None of the other parameters, including sex and age, was independently related to the CSF-to-plasma visfatin ratio (all $P > 0.12$). Exclusion of the three subjects newly diagnosed with type 2 diabetes did not essentially alter the results. Again, body fat mass was the only factor independently correlated with CSF visfatin levels ($n = 35$; $R^2 = 0.59$, $B = -0.067$, $SE = 0.010$ [95% CI -0.087 to -0.047], $\beta = -0.773$, $P < 0.0001$) and with the CSF-to-plasma visfatin ratio ($R^2 = 0.61$, $B = -0.004$, $SE = 0.001$ [95% CI 0.006 to -0.003], $\beta = -0.789$, $P < 0.0001$).

Strong associations between adiposity and plasma and CSF visfatin concentrations were confirmed by comparisons across body fat quartiles (Table 2). Plasma visfatin levels of subjects in the highest quartile significantly exceeded those of subjects in the other quartiles [$F(3,30) = 8.72$, $P < 0.0001$, for main effect of body fat quartile] and a reverse pattern was observed for CSF visfatin levels [$F(3,30) = 16.38$, $P < 0.0001$]. Corresponding results were obtained when obese subjects were compared with normal-weight and overweight subjects [plasma visfatin $F(2,32) = 8.64$, $P < 0.001$; CSF visfatin $F(2,32) = 6.42$, $P < 0.005$, for main effects of body weight status]. Visfatin concentrations were comparable between men and women in plasma [$F(1,30) = 2.46$, $P > 0.13$, for main effect of sex] and CSF [$F(1,30) = 0.27$, $P > 0.61$]. Also, the factor sex did not statistically interact with measures of body fat (all $P > 0.10$), excluding pronounced effects of sex on visfatin concentrations in plasma and CSF.

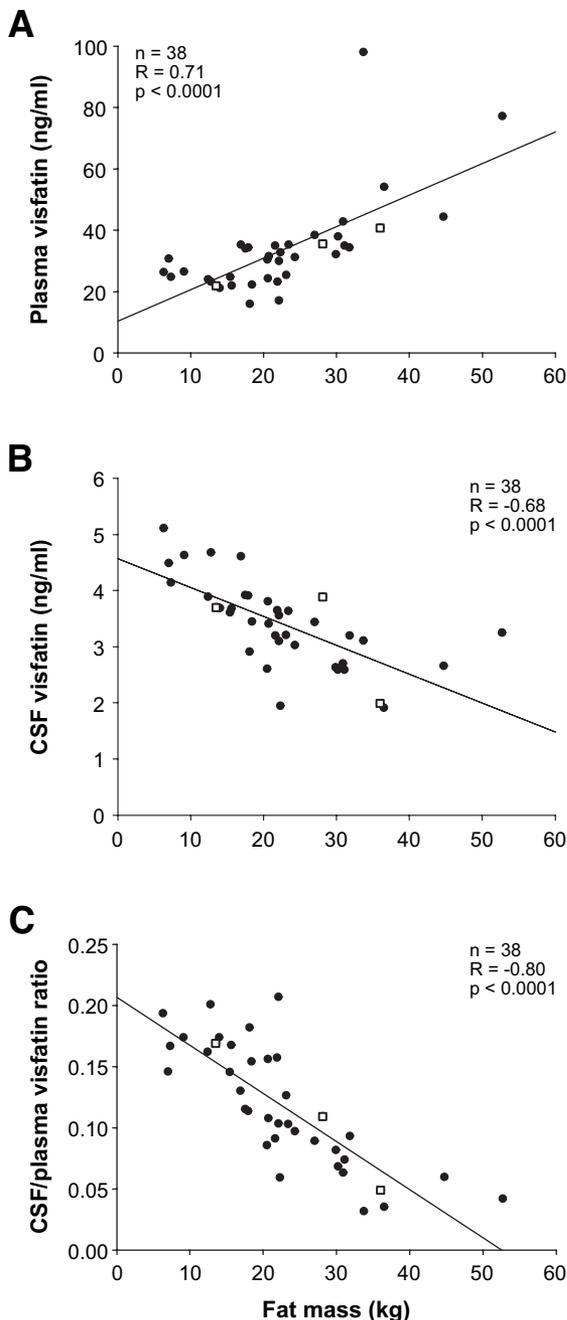


FIG. 1. Correlation of body fat mass with plasma visfatin concentration (A), CSF visfatin concentration (B), and CSF-to-plasma visfatin ratio (C). For statistical analyses, plasma visfatin levels were log-transformed to achieve a normal distribution. □, subjects newly diagnosed with type 2 diabetes.

DISCUSSION

Our results indicate that visfatin is present in human CSF and, furthermore, that CSF visfatin concentrations decrease in conjunction with increasing adiposity. We also found a strong positive association between plasma visfatin levels and body fat mass, body weight, and waist and hip circumferences. This observation confirms previous results indicating that obesity (7,8) and type 2 diabetes (9,11) are linked to elevated circulating visfatin concentrations. Accordingly, massive weight loss is accompanied by a drop in plasma visfatin (12). Other studies have indicated no association between body fat and plasma visfatin (13) or have even reported on reduced visfatin levels in obesity

TABLE 2

Plasma and CSF visfatin concentrations with respect to body fat, body weight status, and sex

	Plasma visfatin (ng/ml)	P*	CSF visfatin (ng/ml)	P*
Body fat quartile				
1	24.87 ± 0.96	0.001	4.22 ± 0.18†	0.0001
2	28.58 ± 2.17	0.001	3.55 ± 0.57	0.003
3	29.92 ± 2.28	0.01	3.27 ± 0.19	NS
4	49.71 ± 6.81		2.66 ± 0.14	
Body weight status				
Normal weight	26.43 ± 1.58	0.002	3.81 ± 0.24‡	0.005
Overweight	29.45 ± 1.75	0.004	3.32 ± 0.14	NS
Obese	49.37 ± 6.86		2.98 ± 0.20	
Sex				
Male	35.56 ± 4.19		3.50 ± 0.16	
Female	31.79 ± 2.89		3.33 ± 0.19	

Data are means ± SEM. n = 38: 18 men and 20 women; 14 normal-weight subjects, 14 overweight subjects (BMI 25 to <30 kg/m²), and 10 obese subjects (BMI ≥30 kg/m²). Differences between men and women were not significant. *For comparisons with body fat quartile 4 and obesity, respectively. †P = 0.015 and 0.002 for comparison with body fat quartiles 2 and 3, respectively. ‡P = 0.03 for comparison with overweight.

(14). The initial assumption that visceral fat is the determining factor in visfatin production (3) has likewise been challenged (4,7). However, increases in visfatin mRNA in visceral adipose tissue of obese subjects and a positive correlation between BMI and visceral visfatin mRNA have been observed even in the absence of an association between circulating visfatin concentrations and body fat (14). More recently, visfatin has been shown to be secreted by adipocytes through a nonclassical secretory pathway that probably represents a highly regulated process (5,15). Therefore, plasma in comparison with cellular levels of the adipokine may display greater variance, which, in conjunction with the fact that visfatin is abundantly produced by the stromal vascular fraction of adipose tissue (16) and that relatively low levels of circulating visfatin are found under physiological conditions, might explain the conflicting results on plasma visfatin in obesity. Our observations agree with the majority of previous studies (7–9,11,12) indicating that plasma visfatin concentrations increase with rising adiposity.

To our knowledge, this is the first study investigating the presence of visfatin in CSF. Our results indicate that in humans visfatin is found in CSF at concentrations that are ~10% of those in plasma. It is not yet known whether the peptide penetrates the central nervous system from the circulation. Leptin, an adipokine that is considerably smaller than visfatin (16 vs. 52 kDa; 167 vs. 473 amino acids), crosses the blood-brain barrier (BBB) via an active, saturable transport system (17) and displays a 5- to 10-fold less pronounced CSF-to-plasma ratio than that observed for visfatin in the present experiments (18). This pattern suggests that circulating visfatin can likewise enter the brain compartment, probably also via an active transport mechanism, although it cannot be ruled out that there are central nervous secretion sites for the peptide (19). In our study, CSF visfatin levels decreased with increasing plasma visfatin levels and adiposity, supporting the assumption that visfatin transport across the BBB is impaired when circulating visfatin increases as a result of excess body fat. However, this preliminary conclusion is in

need of corroboration by studies investigating the actual transport of visfatin across the BBB in normal weight and obesity. Moreover, although we could exclude a significant influence of sex and age on CSF visfatin levels in our study, the potential contribution of, e.g., sex-specific endocrine factors (20) to the observed pattern still has to be examined.

Adiposity turned out to be the only variable independently associated with the variance of CSF levels and the CSF-to-plasma ratio of visfatin, suggesting obesity to be closely connected with reduced CSF visfatin levels. With the physiological role of visfatin in energy homeostasis far from being elucidated, low CSF visfatin levels may be a mere by-product of increased body fat stores. However, remarkably similar patterns have been revealed for leptin (18,21) and insulin (10,22), two endocrine adiposity signals that provide the brain with negative feedback on the amount of body fat. Dysfunctional central nervous insulin and leptin signaling has been well established as a key feature of obesity (1), and preliminary findings in animals hint at the involvement of central nervous visfatin in the regulation of food intake (23). Plasma visfatin levels are reduced by diet-induced weight loss in obese nondiabetic patients (24) and by exercise in patients with type 1 diabetes (25), and it remains to be seen whether these clinical interventions in turn increase central nervous levels of the peptide. In sum, our results support the intriguing, albeit tentative, assumption that central nervous visfatin insufficiency or resistance might be linked to pathogenetic mechanisms of obesity.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (SFB-654/B3).

No potential conflicts of interest relevant to this article were reported.

The funding source had no input in the design and conduct of this study, the collection, analysis, and interpretation of the data, or the preparation, review, and approval of the manuscript.

REFERENCES

- Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW: Central nervous system control of food intake and body weight. *Nature* 443:289–295, 2006
- Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I: Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol* 14:1431–1437, 1994
- Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I: Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307:426–430, 2005
- Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I: Retraction. *Science* 318: 565, 2007
- Revollo JR, Komer A, Mills KF, Satoh A, Wang T, Garten A, Dasgupta B, Sasaki Y, Wolberger C, Townsend RR, Milbrandt J, Kiess W, Imai S: Namp1/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. *Cell Metab* 6:363–375, 2007
- Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M: The release of the adipocytokine visfatin is regulated by glucose and insulin. *Diabetologia* 49:1909–1914, 2006
- Berndt J, Klötting N, Kralisch S, Kovacs P, Fasshauer M, Schön MR, Stumvoll M, Bluher M: Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 54:2911–2916, 2005
- Filippatos TD, Derdemezis CS, Gazi IF, Lagos K, Kiortsis DN, Tselepis AD, Elisaf MS: Increased plasma visfatin levels in subjects with the metabolic syndrome. *Eur J Clin Invest* 38:71–72, 2008
- Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF, Shin SJ, Lee YJ: Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 91:295–299, 2006
- Kern W, Benedict C, Schultes B, Plohr F, Moser A, Born J, Fehm HL, Hallschmid M: Low cerebrospinal fluid insulin levels in obese humans. *Diabetologia* 49:2790–2792, 2006
- Hammarstedt A, Pihlajamäki J, Rotter Sopasakis V, Gogg S, Jansson PA, Laakso M, Smith U: Visfatin is an adipokine, but it is not regulated by thiazolidinediones. *J Clin Endocrinol Metab* 91:1181–1184, 2006
- Manco M, Fernandez-Real JM, Equitani F, Vendrell J, Valera Mora ME, Nanni G, Tondolo V, Calvani M, Ricart W, Castagneto M, Mingrone G: Effect of massive weight loss on inflammatory adipocytokines and the innate immune system in morbidly obese women. *J Clin Endocrinol Metab* 92:483–490, 2007
- Ingelsson E, Larson MG, Fox CS, Yin X, Wang TJ, Lipinska I, Pou KM, Hoffmann U, Benjamin EJ, Keaney JF Jr, Vasan RS: Clinical correlates of circulating visfatin levels in a community-based sample. *Diabetes Care* 30:1278–1280, 2007
- Pagano C, Pilon C, Olivieri M, Mason P, Fabris R, Serra R, Milan G, Rossato M, Federspil G, Vettor R: Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. *J Clin Endocrinol Metab* 91:3165–3170, 2006
- Tanaka M, Nozaki M, Fukuhara A, Segawa K, Aoki N, Matsuda M, Komuro R, Shimomura I: Visfatin is released from 3T3-L1 adipocytes via a non-classical pathway. *Biochem Biophys Res Commun* 359:194–201, 2007
- Varma V, Yao-Borengasser A, Rasouli N, Bodles AM, Phanavanh B, Lee MJ, Starks T, Kern LM, Spencer HJ III, McGehee RE Jr, Fried SK, Kern PA: Human visfatin expression: relationship to insulin sensitivity, intramyocellular lipids, and inflammation. *J Clin Endocrinol Metab* 92:666–672, 2007
- Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM: Leptin enters the brain by a saturable system independent of insulin. *Peptides* 17:305–311, 1996
- Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr: Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 2:589–593, 1996
- McGlothlin JR, Gao L, Lavoie T, Simon BA, Easley RB, Ma SF, Rumala BB, Garcia JG, Ye SQ: Molecular cloning and characterization of canine pre-B-cell colony-enhancing factor. *Biochem Genet* 43:127–141, 2005
- Fasshauer M, Bluher M, Stumvoll M, Tonessen P, Faber R, Stepan H: Differential regulation of visfatin and adiponectin in pregnancies with normal and abnormal placental function. *Clin Endocrinol (Oxf)* 66:434–439, 2007
- Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, Considine RV: Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 348:159–161, 1996
- Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Schwartz MW: Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. *Diabetes* 49:1525–1533, 2000
- Cline MA, Nandar W, Prall BC, Bowden CN, Denbow DM: Central visfatin causes orexigenic effects in chicks. *Behav Brain Res* 186:293–297, 2008
- de Luis DA, Gonzalez SM, Conde R, Aller R, Izaola O, Romero E: Effect of a hypocaloric diet on serum visfatin in obese non-diabetic patients. *Nutrition* 24:517–521, 2008
- Haider DG, Pleiner J, Francesconi M, Wiesinger GF, Muller M, Wolzt M: Exercise training lowers plasma visfatin concentrations in patients with type 1 diabetes. *J Clin Endocrinol Metab* 91:4702–4704, 2006