Ginekol Pol. 2009, 80, 14-18

Serum visfatin concentration is elevated in pregnant women irrespectively of the presence of gestational diabetes

Stężenie wisfatyny w surowicy kobiet ciężarnych z zaburzeniami tolerancji glukozy

Szamatowicz Jacek[1], Kuźmicki Mariusz[2], Telejko Beata[3], Zonenberg Anna[3], Nikołajuk Agnieszka[3], Krętowski Adam[3], Górska Maria[3]

[1] Department of Gynaecology, Medical University of Bialystok, Poland
[2] Department of Pathophysiology of Pregnancy, Medical University of Bialystok, Poland
[3] Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Poland

Summary

Objectives: *The aim of the present study was to compare serum concentrations of a recently identified namely – visfatin between pregnant women with normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM), as well as non-pregnant healthy subjects.*

Materials and methods: *Serum visfatin concentration was measured in 61 patients with GDM, 63 pregnant subjects with NGT and 36 non-pregnant healthy women by means of an immunoassay.*

Results: *Median visfatin levels did not differ in the women with GDM (14.8 [10.8-17.3] μg/l) and NGT (15.3 [11.8-19.4] μg/l), but were significantly higher than those found in the non-pregnant women (11.4 [8.6-15.2] μg/l, p=0.0008 vs NGT and p=0.008 vs GDM group). Visfatin concentrations correlated significantly with fasting insulin (R=0.20, p=0.01), HOMA-IR (R=0.19, p=0.02) and HOMA-%B (R=0.23, p=0.004). Stepwise regression analysis revealed that serum visfatin levels were significantly predicted only by HbA1c values (b=0.21, p=0.04).*

Conclusions: *Serum visfatin concentrations are elevated in pregnant women, irrespectively of their glucose tolerance status. This elevation may be caused by an additional secretion of visfatin from the placenta, however other possible sources of visfatin should also be taken into account.*

Key words: **visfatin** / **pregnancy** / **gestational diabetes mellitus** /

Address for correspondence:
Jacek Szamatowicz
Department of Gynaecology, Medical University of Białystok,
M. Curie-Skłodowskiej 24A, 15-276 Białystok, Poland,
tel.: +48 85 746 8352
e-mail: szamatj@amb.edu.pl

Otrzymano: **09.07.2008**
Zaakceptowano do druku: **04.12.2008**

Streszczenie

Cel pracy: *Celem pracy było porównanie stężeń wisfatyny w osoczu kobiet ciężarnych z prawidłową tolerancją glukozy i cukrzycą ciążową oraz zdrowych kobiet nie będących w ciąży.*

Materiał i metody: *Stężenie wisfatyny oznaczono metodą immunoenzymatyczną w osoczu 61 pacjentek z cukrzycą ciążową, 63 ciężarnych z prawidłową tolerancją glukozy oraz 36 kobiet nie będących w ciąży.*

Wyniki: *Stężenia wisfatyny nie różniły się istotnie w grupie kobiet z cukrzycą ciążową (mediana 14,8 [10,8-17,3]µg/l) i prawidłową tolerancją glukozy (15,3 [11,8-19,4] µg/l), były jednak znamiennie wyższe niż w grupie kobiet nie będących w ciąży (11,4 [8,6-15,2] µg/l, p=0,008 vs cukrzyca ciążowa i p=0,0008 vs prawidłowa tolerancja glukozy). Stwierdzono istotną korelację pomiędzy stężeniem wisfatyny i insuliny (R=0,20, p=0,01), HOMA-IR (R=0,19, p=0,02) i HOMA-%B (R=0,23, p=0,004).*

Regresja wieloraka wykazała, że jedynym czynnikiem wpływającym znamiennie na stężenia wisfatyny w osoczu była wartość HbA1c (b=0,21, p=0,04).

Wnioski: *Stężenie wisfatyny w osoczu wzrasta u kobiet w ciąży, niezależnie od współistniejących zaburzeń tolerancji glukozy, prawdopodobnie wskutek dodatkowej sekrecji tej adipokiny przez tkankę łożyska.*

Słowa kluczowe: **wisfatyna** / **ciąża** / **cukrzyca ciążowa** /

Wstęp

Visfatin was originally identified as pre-B cell colony-enhancing factor (PBEF) – a 52-kilodalton protein expressed in lymphocytes that synergized with interleukin-7 and stem cell factors to promote the growth of B cell precursors [1].

Visfatin mRNA and protein is expressed in subcutaneous fat tissue (SAT) [2, 3], visceral fat tissue (VAT) [2, 3], as well as in the liver [1], muscle [1], macrophages [4], placenta and foetal membranes [5, 6]. The same gene was also identified as a cytosolic enzyme micotinamide 5-phosphoribosyl-1-pyrophosphate transferase (Nampt) that catalyzes the rate-limiting step in nicotinamide adenine dinucleotide (NAD) biosynthesis [7].

Insulin-mimetic effects, initially ascribed to visfatin, have not been confirmed in recent studies but visfatin is involved in glucose metabolism at the beta-cell level by regulating insulin secretion via the NAD pathway [8]. However, clinical data linking circulating visfatin to the parameters of glucose metabolism and insulin resistance are contradictory, since both increased [9, 10, 11, 12, 13, 14] and decreased levels [15] have been observed in patients with type 1 and type 2 diabetes. Conflicting results have been also obtained in obese subjects [2, 16, 17, 18].

It has been demonstrated that circulating visfatin levels are elevated in pregnant women with intrauterine growth retardation (IUGR) [19] and preeclampsia [20], whereas significantly lower [21, 22] or higher [23, 24] visfatin concentrations have been shown in patients with gestational diabetes mellitus (GDM) when compared with healthy pregnant controls. Therefore, in the present study we investigated serum visfatin concentrations in pregnant women with various degree of glucose intolerance in order to establish its relation to a range of anthropometric and metabolic factors conferring glucose metabolism and insulin resistance.

Materials and methods

The group studied consisted of 61 patients with GDM and 63 pregnant women with NGT, between 24 and 31 week of gestation, attending the gynaecological out-patient clinic of the Medical University of Bialystok: GDM was diagnosed according to the WHO criteria [25].

Blood samples for visfatin assay were collected before the initiation of diet or insulin therapy. All subjects were non-smokers and had not taken any drugs known to affect carbohydrate metabolism in the previous 3 months. Patients with abnormal glucose readings before pregnancy, as well as with pregnancy induced hypertension (PIH), preeclampsia and other pregnancy complications (except GDM), were not included. All pregnancies were singletons. The estimation of pregnancy duration was based on routine ultrasonographic examination preformed between 10 and 12 weeks of gestation. The control group consisted of 36 non-pregnant healthy women, nine of whom had one or more pregnancies without a history of GDM and 27 had no previous pregnancy. Written informed consent was obtained from all participants, and the protocol was approved by the local ethics committee (Medical University of Bialystok).

For each patient body mass index (BMI) was calculated at the time of blood collection as weight in kilograms divided by height in meters squared. Medical records were also analyzed to collect information concerning subjects' weight shortly before conception, and prepregnancy BMI was calculated as described above.

Plasma glucose concentration was measured using oxidase method (CORMAY, Poland), fasting serum insulin level was assayed by immunoradiometric method (Biosource Europe SA, Belgium) and glycated haemoglobin (HbA1c) was evaluated by a high performance liquid chromatography technique (HPLC Variant™, BIO-RAD Laboratories, Germany).

Serum visfatin concentration was measured using commercial Visfatin-C Terminal Human Enzyme Immunoassay kit (Phoenix Pharmaceuticals, Inc., USA) with the detection

Table I. Clinical and biochemical characteristics of the population studied.

Parameter	Control group	NGT	GDM	Significance
Number	36	63	61	NS
Age (years)	28 (25-30)	28 (26-31)	30 (27-33)	NS
Parity	1 (1-2)	1 (1-2)	1 (1-2)	NS
Gestational age (week)	-	30 (28-32)	28 (26-32)	NS
Prepregnancy BMI (kg/m^2)	21.8 (20.3-23.8)	21.5 (19.4-23.4)	22.8 (20.2-24.0)	p=0.01[a]
Current BMI (kg/m^2)	21.8 (20.3-23.8)	25.7 (24.2-28.7)	26.6 (24.0-28.9)	NS
Fasting glucose (mmol/l)	4.8 (4.3-5.2)	4.1 (3.9-4.4)	4.4 (4.2-4.8)	p=0.01[a] p<0.0001[b] p<0.0001[c]
120 min post-load glucose (mmol/l)	5.6 (4.9-6.3)	5.8 (5.0-6.5)	8.4 (7/9-9.0)	p<0.0001 [a,b]
Fasting insulin (pmol/l)	73.9 (60.3-103.0)	80.4 (64.6-117.7)	99.7 (72.5-133.4)	p=0.02[a] p=0.035[c]
HOMA-IR	1.6 (1.2-2.1)	1.6 (1.3-2.3)	2.0 (1.5-2.7)	p=0.007[a]
HOMA%-B	142.8 (121.1-175.2)	214.6 (172.3-249.2)	204.5 (161.9-231.9)	p=0.0002[a] p<0.0001[c]
HbA1c (%)	4.9 (4.7-5.2)	4.8 (4.6-5.1)	4.95 (4.65-5.2)	NS
Visfatin (µg/l)	11.4 (9.6-15.2)	15.3 (11.8-19.4)	14.8 (10.8-17.3)	p=0.008[a] p=0.0008[c]

Data are shown as medians (interquartile range), [a]GDM vs control, [b]GDM vs NGT group,
[c]NGT vs control, NGT-normal glucose tolerance, GDM-gestational diabetes mellitus, NS-not significant

limit of 0.1µg/l. The intraassay and interassay coefficients of variation (CVs) were less than 5.0% and 14.0%, respectively.

HOMA2 (the Homeostasis Model Assessment 2) Calculator was used to estimate steady state beta cell function (%B) and insulin resistance (HOMA-IR) according to the updated HOMA2 model (www.OCDEM.ox.ac.uk).

Statistical analysis was performed using the STATISTICA 7.0 for Windows software package (StatSoft.Inc, Tulsa, USA). Non-normally distributed variables were expressed as medians and interquartile ranges (IR). The differences between the groups were compared by Mann - Whitney U test. Multivariate least–square regression procedures were used to estimate mean case–control differences in plasma visfatin concentrations after allowing for potential confounders. Relationships between variables were tested by Spearman's rank correlations. Multivariate linear regression analysis was performed to establish which of the metabolic and anthropometric factors (age, gestational age, BMI, glucose, insulin, HbA1c) were significantly and independently associated with the variance in plasma visfatin concentration. P value less than 0.05 was regarded as statistically significant.

Results

Clinical and biochemical characteristics of the groups studied are summarized in Table I. There were not significant differences in the mean age, parity, gestational age, current BMI (except non-pregnant subjects) and HbA1c values between the groups. The patients with GDM had markedly higher fasting and post-load glucose than the women with NGT (p<0.0001), as well as significantly higher pre-pregnancy BMI (p=0.01), fasting insulin (p=0.02), HOMA-IR (p=0.007) and HOMA-%B (p=0.007) in comparison with the control group. The pregnant women with NGT had significantly lower fasting glucose levels (p<0.0001), but higher fasting insulin (p=0.035) and HOMA-%B values (p<0.0001) in comparison with the non-pregnant women.

Serum visfatin concentrations were comparable in the NGT and GDM groups but significantly higher than those found in the non-pregnant subjects (p=0.0008 *vs* NGT group and p=0.008 *vs* GDM group, Table I).

After an adjustment for glucose and insulin values, the differences in visfatin levels between the control group and the pregnant women remained statistically significant (p=0.003 *vs* NGT group and p=0.009 *vs* GDM group).

Table II. Serum visfatin concentrations with respect to prepregnancy BMI values.

Group / Parameter	Control group		NGT		GDM		Total	
BMI (kg/m^2)	< 25	≥ 25	< 25	≥ 25	< 25	≥ 25	< 25	≥ 25
n	29	7	55	8	52	9	136	24
Visfatin (μg/l)	11,5 (9.8-16.1)	9.5 (7.6-11.6)*	15.3 (11.8-19.5)	16.3 (12.8-18.5)	14.9 (10.8-17.7)	13.9 (11.9-15.8)	14.8 (10.8-17.8)	12.8 (9.7-16.3)

NGT-normal glucose tolerance, GDM-gestational diabetes mellitus, * p=0.047

When all the women studied were divided into two groups according to their prepregnancy BMI values (Table II), there was no significant difference in serum visfatin levels between the subjects with BMI < and ≥25kg/m^2 both in the whole population studied, as well as in the subgroups with NGT and GDM, whereas in the control group serum visfatin concentrations were apparently lower in obese than in slim subjects (p=0.047, Table II).

In a pooled analysis serum visfatin levels correlated significantly with fasting insulin (R=0.20, p=0.01), HOMA-IR (R=0.19, p=0.02) and HOMA-%B (R=0.23, p=0.004). However, when the three subgroups were analysed separately, visfatin levels were not associated with any of the clinical or metabolic parameters studied. Stepwise multiple regression analysis with visfatin as a dependent value (independent values: age, gestational age, BMI, glucose, insulin, HbA1c) revealed that in the whole group of pregnant women HbA1c was the only significant predictor (b=0.26, p=0.005), explaining about 7% of the variance in serum visfatin concentration. In the subgroup with GDM visfatin levels were also significantly predicted by HbA1c values (b=0.29, p=0.03), whereas in the subjects with NGT visfatin concentrations were not associated with any of the parameters analyzed (age, gestational age, BMI, glucose, insulin, HbA1c). In the control group stepwise multiple regression analysis showed that visfatin concentrations were significantly related to BMI values (b=0.47, p=0.01).

Discussion

In the present study we showed that patients with GDM had markedly higher fasting glucose than women with NGT, as well as significantly higher fasting insulin and the index of insulin resistance as compared with the control group, whereas pregnant women with NGT demonstrated markedly lower fasting glucose and higher insulin concentrations in comparison with the non-pregnant group. These findings correspond to the well-known metabolic changes that occur in normal and diabetic pregnancy, since non-diabetic pregnancy produces an "accelerated starvation" in the fasting state, with an earlier and more profound hypoglycaemia and an increased fasting insulin level, whereas women with GDM demonstrate elevated fasting insulin concentrations i the first place [26].

Serum visfatin levels in the NGT and GDM groups were comparable, but markedly higher than those found in the non-pregnant women. Because of the differences observed in glucose and insulin values, an adjustment for these covariates was made, but the differences in visfatin concentrations between the pregnant and the non-pregnant subjects remained statistically significant. Our findings are in part consistent with the results obtained by Mastorakos et al. [27] who demonstrated that serum visfatin concentrations increased during healthy pregnancy, especially between the 1st and the 2nd trimester, in parallel with b-cell secretion indices, probably compensating for a gradual increase in insulin resistance. Moreover, serum visfatin concentration in the 1st trimester was a significant predictor of insulin sensitivity during the 2nd trimester, however later this close association disappeared, possibly because of an increase in visfatin secretion by an additional source other that adipose tissue, namely the placenta [27].

The results concerning circulating visfatin obtained by other authors in different populations with GDM are contradictory since Chan et al. [21] and Haider et al. [22] demonstrated significantly lower serum visfatin levels in patients with GDM, whereas Krzyzanowska et al. [23] and Lewandowski et al. [24] reported that median visfatin concentrations were significantly elevated in women with GDM when compared with healthy pregnant controls. The reasons for this discrepancy may be related to differences regarding sampling time during pregnancy, various diagnostic criteria or even racial differences, nevertheless they remain unclear. The mechanisms controlling visfatin secretion in humans are also under discussion. Haider et al. [28] showed that glucose induces an increase in visfatin release, whereas insulin can suppress this effect *in vitro* and *in vivo,* however it has no influence on basal visfatin concentrations. In clinical studies concerning pregnant women various factors, potentially influencing circulating visfatin levels, have been noted. Haider et al. [22] demonstrated a significant correlation between fasting glucose and visfatin concentrations in subjects with both NGT and GDM, whereas Krzyzanowska et al. [23] found no association between plasma visfatin and fasting glucose, insulin, HOMA-IR, HbA1c or BMI. In women with GDM visfatin correlated significantly only with the week of gestation at the time of sampling [23]. On the contrary, Chan et al. [21] observed no relationship between circulating visfatin and gestational age.

Finally, Lewandowski et al. [24] found positive correlations between serum visfatin concentrations and fasting insulin and HOMA-IR, which is consistent with our findings. In our population serum visfatin levels correlated significantly with fasting insulin, HOMA-IR and HOMA-%B – an indirect index of beta cell function. However, stepwise multiple regression analysis revealed that in the whole population studied, as well as in the subgroup with GDM, HbA1c was the only significant predictor, explaining less than 7% of the variance in serum visfatin concentration. In the non-pregnant group visfatin concentrations were significantly related to BMI values. The association between circulating visfatin and BMI was also demonstrated by Berndt et al. in a large population – based study, but in a subgroup of women with a wide range of obesity and insulin sensitivity the correlation did not reach statistical significance [2].

Conclusion

In conclusion, our results suggest that serum visfatin concentrations are elevated in pregnant women when compared with non-pregnant healthy subjects. This elevation was not related to the coexisting disturbances of glucose tolerance, however multiple regression analysis revealed that HbA1c – a metabolic parameter reflecting retrospectively mean plasma glucose levels - was the only significant predictor of serum visfatin values both in the whole population studied, as well as in the subgroup with GDM. The elevation of circulating visfatin during pregnancy may be caused by its additional secretion from the placenta, however other possible sources should be also taken into account. In fact, more research work is needed to determine the mechanism(s) regulating visfatin secretion and action during pregnancy – both physiological and complicated by GDM.

References

1. Samal B, Sun Y, Stearns G, [et al.]. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol.* 1994, 14, 1431-1437.
2. Berndt J, Klöting N, Kralisch S, [et al.]. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes.* 2005, 54, 2911-2916.
3. Varma V, Yao-Borengasser A, Rasouli N, [et al.]. Human visfatin expression: relationship to insulin sensitivity, intramyocellular lipids, and inflammation. *J Clin Endocrinol Metab.* 2007, 92, 666-672.
4. Curat C, Wegner V, Sengenes C, [et al.]. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia.* 2006, 49, 744-747.
5. Ognjanovic S, Bao S, Yamamoto S, [et al.]. Genomic organization of the gene coding for human pre-B-cell colony enhancing factor and expression in human fetal membranes. *J Mol Endocrinol.* 2001, 26, 107-117.
6. Ognjanovic S, Bryant-Greenwood G. Pre-B-cell colony-enhancing factor, a novel cytokine of human fetal membranes *Am J Obstet Gynecol.* 2002, 187, 1051-1058.
7. Rongvaux A, Shea R, Mulks M, [et al.]. Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. *Eur J Immunol.* 2002, 32, 3225-3234.
8. Revollo J, Korner A, Mills K, [et al.]. Nampt/PBEF/visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. *Cell Metab.* 2007, 6, 363-375.
9. Haider D, Pleiner J, Francesconi M, [et al.]. Exercise training lowers plasma visfatin concentrations in patients with type 1 diabetes. *J Clin Endocrinol Metab.* 2006, 91, 4702-4704.
10. López-Bermejo A, Chico-Julia B, Fern_ndez-Balsells M, [et al.]. Serum visfatin increases with progressive beta-cell deterioration. *Diabetes.* 2006, 55, 2871-2875.
11. Chen M, Chung F, Chang D, [et al.]. Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2006, 91, 295-299.
12. Dogru T, Sonmez A, Tasci I, [et al.]. Plasma visfatin levels in patients with newly diagnosed and untreated type 2 diabetes mellitus and impaired glucose tolerance. *Diabetes Res Clin Pract.* 2007, 76, 24-29.
13. Fernández-Real J, Moreno J, Chico B, [et al.]. Circulating visfatin is associated with parameters of iron metabolism in subjects with altered glucose tolerance. *Diabetes Care.* 2007, 30, 616-621.
14. Hammarstedt A, Pihlajamäki J, Rotter Sopasakis V, [et al.]. Visfatin is an adipokine, but it is not regulated by thiazolidinediones. *J Clin Endocrinol Metab.* 2006, 91, 1181-1184.
15. Li L, Yang G, Li Q, [et al.]. Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. *Exp Clin Endocrinol Diabetes.* 2006, 114, 544-548.
16. Haider D, Schindler K, Schaller G, [et al.]. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. *J Clin Endocrinol Metab.* 2006, 91, 1578-1581.
17. Zahorska-Markiewicz B, Olszanecka-Glinianowicz M, Janowska J, [et al.]. Serum concentration of visfatin in obese women. *Metabolism.* 2007, 56, 1131-1134.
18. Pagano C, Pilon C, Olivieri M, [et al.]. Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. *J Clin Endocrinol Metab.* 2006, 91, 3165-3170.
19. Fasshauer M, Blüher M, Stumvoll M, [et al.]. Differential regulation of visfatin and adiponectin in pregnancies with normal and abnormal placental function. *Clin Endocrinol (Oxf).* 2007, 66, 434-443.
20. Fasshauer M, Waldeyer T, Seeger J, [et al.]. Serum levels of the adipokine visfatin are increased in pre-eclampsia. *Clin Endocrinol (Oxf).* 2008, 69, 69-73.
21. Chan T, Chen Y, Lee C, [et al.] Decreased plasma visfatin concentrations in women with gestational diabetes mellitus. *J Soc Gynecol Invest.* 2006, 13, 364-367.
22. Haider D, Handisurya A, Storka A, [et al.]. Visfatin response to glucose is reduced in women with gestational diabetes mellitus. *Diabetes Care.* 2007, 30, 1889-1891.
23. Krzyzanowska K, Krugluger W, Mittermayer F, [et al.]. Increased visfatin concentrations in women with gestational diabetes mellitus. *Clin Science (Lond).* 2006, 110, 605-609.
24. Lewandowski K, Stojanovic N, Press M, [et al.] Elevated serum levels of visfatin in gestational diabetes: a comparative study across various degree of glucose tolerance. *Diabetologia.* 2007, 50, 1033-1037.
25. Alberti K, Zimmet P. Definition, diagnosis, and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* 1998, 15, 539-553.
26. Carpenter M. Metabolic changes in gestational diabetes. *Clin Perinatol.* 1993, 20, 583-591.
27. Mastorakos G, Valsamakis G, Papatheodorou D, [et al.]. The role of adipocytokines in insulin resistance in normal pregnancy: visfatin concentrations in early pregnancy predict insulin sensitivity. *Clin Chem.* 2007, 53, 1477-1483.
28. Haider D, Schaller G, Kapiotis S, [et al.]. The release of the adipocytokine visfatin is regulated by glucose and insulin. *Diabetologia.* 2006, 49, 1909-1914.