Increased visfatin concentrations in women with gestational diabetes mellitus

Katarzyna KRZYZANOWSKA*, Walter KRUGLUGER†, Friedrich MITTERMAYER‡, Rupa RAHMAN*, Dominik HAIDER‡, Nadja SHNAWA* and Guntram SCHERNTHANER*

*Department of Internal Medicine I, Rudolfstiftung Hospital, Vienna, Austria, †Central Laboratory, Rudolfstiftung Hospital, Vienna, Austria, and ‡Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria

ABSTRACT

The recently discovered adipocytokine visfatin has insulin-like properties. It lowers blood glucose and improves insulin sensitivity; however, clinical data on visfatin are limited. To evaluate the role of visfatin in GDM (gestational diabetes mellitus), we determined visfatin levels in women with GDM and in healthy pregnant controls. Furthermore, visfatin concentrations were investigated longitudinally during pregnancy and after delivery in a subgroup of women with GDM. Blood for measurement of visfatin and metabolic parameters was obtained from 64 women with GDM [median week of gestation, 34 (interquartile range, 27–36) weeks] and 30 healthy pregnant controls [median week of gestation, 34 (interquartile range, 28–36) weeks]. In a subgroup of 24 women with GDM, visfatin, leptin and metabolic parameters were investigated twice during pregnancy (28–30 and 38–40 weeks of gestation) and 2 weeks after delivery. In the cross-sectional analysis, median visfatin levels were significantly elevated in women with GDM [64.0 (interquartile range, 50.9–74.8) ng/ml] compared with controls [46.0 (interquartile range, 36.9–54.6) ng/ml; P < 0.0001]. In women with GDM, visfatin correlated with week of gestation at the time of blood draw (R = 0.35, P = 0.005). No association with fasting glucose, insulin, homoeostasis model assessment-insulin resistance or body mass index was observed. According to the longitudinal analysis, visfatin increased during pregnancy (P = 0.002) and rose further after delivery (P = 0.014), whereas leptin and insulin levels decreased after parturition (both P < 0.001). In conclusion, visfatin is elevated in women with GDM and increases during the course of pregnancy as well as after delivery. Furthermore, visfatin shows no association with insulin and leptin in women with GDM.

INTRODUCTION

GDM (gestational diabetes mellitus), which is defined as glucose intolerance with onset or first recognition during pregnancy [1], is a state of temporary insulin resistance. Women with GDM have an increased risk for the development of Type II diabetes mellitus [2]. Insulin resistance is linked to obesity, inflammation, cardiovascular disease and secretion of adipocytokines [3]. A variety of polypeptides secreted from adipose tissue, such as TNF-α (tumour necrosis factor-α) [4], resistin [5] and leptin [6], might play an important role in metabolic homoeostasis and the development of Type II diabetes, dyslipidaemia and atherosclerosis [7].

Recently, the novel adipocytokine visfatin, which was previously known as PBEF (pre-B-cell colony-enhancing factor), was identified in visceral fat [8]. It is found further in skeletal muscle, liver, bone marrow and lymphocytes [9]. Acute administration of recombinant visfatin to mice leads to a reduction of plasma glucose independent of

Key words: adipocytokine, delivery, gestational diabetes mellitus, insulin resistance, leptin, pregnancy, visfatin.

Abbreviations: BMI, body mass index; GDM, gestational diabetes mellitus; HbA1c, glycated haemoglobin; HOMA-IR, homoeostasis model assessment-insulin resistance; PBEF, pre-B-cell colony-enhancing factor; TNF-α, tumour necrosis factor-α.

Correspondence: Dr Katarzyna Krzyzanowska (email katarzyna.krzyzanowska@wienkav.at).
changes in plasma levels of insulin. Thus it works synergistically with insulin to lower blood glucose concentrations [8]. Chronic elevation of visfatin in mice reduces insulin plasma concentrations [8], and it was suggested that visfatin improves insulin sensitivity [9]. Visfatin affects the insulin signal transduction pathway by inducing tyrosine phosphorylation of the insulin receptor and IRS1 and 2 (insulin receptor substrate 1 and 2) in the liver. Furthermore, an autocrine/paracrine function on visceral adipose tissue as well as an endocrine role modulating insulin sensitivity in peripheral organs might be modes of action [9]. According to Fukuhara et al. [8], plasma concentrations of visfatin are strongly correlated with the amount of visceral fat in humans, but a weak association with the amount of subcutaneous fat was found. In contrast, Berndt et al. [10] described a lack of association between visfatin and visceral fat in humans with a wide range of obesity, body fat distribution, insulin sensitivity and glucose tolerance. Visfatin correlated with BMI (body mass index) and percentage body fat in men, but not in women [10]. Increased visfatin concentrations have also been reported for patients with Type II diabetes [11]; however, data on visfatin in clinical settings are rare up until now.

The expression of the adipocytokine leptin, which decreases hepatic glucose production and increases glucose uptake in muscle and brown adipose tissue, is influenced by alterations in glucose metabolism [12]. As visfatin is considered to contribute to the regulation of glucose levels, it could be presumed that changes in leptin might be related to visfatin.

To evaluate the role of visfatin in GDM we determined this novel adipocytokine in women with GDM and healthy pregnant controls. Furthermore, we investigated in a longitudinal manner the levels of visfatin and leptin during the third trimester of pregnancy and after delivery in a subgroup of women with GDM.

**METHODS**

All clinical investigations were conducted in accordance with the Guidelines in The Declaration of Helsinki and approved by the Institutional Review Committee. All subjects were carefully instructed about the aims of the study and written informed consent was given. Sixty-four women with GDM (mean age, 32 years) and 30 healthy pregnant controls (mean age, 31 years) were included in the study. All subjects were non-smokers. GDM was diagnosed according to the criteria of the 4th Workshop Conference of Gestational Diabetes [13]. Twenty GDM women were treated with insulin and 44 with diet alone. Serum samples were obtained at a median week of gestation of 34 (interquartile range, 27–36) weeks in women with GDM and at a median week of gestation of 34 (interquartile range, 28–36) weeks in healthy pregnant control women. Insulin resistance was estimated by HOMA-IR (homeostasis model assessment-insulin resistance) [14]. BMI was obtained during the early pregnancy.

A subgroup of 24 women with GDM was included in a longitudinal analysis. Outcome parameters were determined between 28–30 weeks of gestation (examination 1) as well as 38–40 weeks of gestation (examination 2). In addition, subjects were examined 2 weeks after delivery.

**Laboratory analysis**

Glucose, insulin and HbA1c (glycated haemoglobin) were determined with standard laboratory methods. Visfatin and leptin were measured by enzyme immunometric assay (Visfatin EIA and Leptin EIA; Phoenix Pharmaceuticals). The inter- and intra-assay variability for visfatin were < 6 %.

**Statistics**

Differences between subjects with and without GDM and with and without insulin therapy were analysed with the Mann–Whitney U test. A bivariate linear model was applied to adjust the difference in visfatin between women with GDM and controls for BMI. For assessment of changes of outcome parameters during pregnancy and after delivery, a Wilcoxon matched pairs test was applied. Correlations were calculated using Spearman rank correlations. Multiple regression analysis was performed to detect possible independent predictors for visfatin. Skewed variables (insulin and week of gestation) were log-transformed for the multiple regression analysis. Statistica software version 6.0 (StatSoft) was used for all analyses. A P value < 0.05 was considered the level of significance. Data are presented as medians (interquartile range).

**RESULTS**

**Cross-sectional study**

Women with GDM had significantly higher (P < 0.0001) visfatin concentrations than healthy pregnant controls (Table 1). This difference persisted after adjustment for BMI. Glucose, insulin, HOMA-IR and HbA1c, were also significantly increased in women with GDM compared with healthy pregnant women (Table 1); however, BMI did not differ between the two groups. Visfatin concentrations did not differ significantly between women with GDM on insulin therapy and those treated with diet [59.9 (interquartile range, 44.1–68.1) ng/ml compared with 65 (interquartile range, 51.3–76.8) ng/ml; P = 0.138] and were elevated in both groups compared with controls (P = 0.017 and P < 0.0001 respectively).

Visfatin concentrations were correlated with the week of gestation in women with GDM (R = 0.35, P = 0.005). No associations between visfatin and parameters of the
Comparison of outcome parameters between women with GDM and healthy pregnant controls

Data are medians (interquartile range). Mann–Whitney U test was applied to test for significant differences between groups.

<table>
<thead>
<tr>
<th></th>
<th>Women with GDM</th>
<th>Healthy pregnant controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>64</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>64.0 (50.9–74.8)</td>
<td>46.0 (36.9–54.6)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.6 (4.1–5.4)</td>
<td>4.1 (3.8–4.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>20.9 (10.6–36.3)</td>
<td>7.6 (4.9–10.5)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.3 (2.3–7.9)</td>
<td>1.3 (0.9–2.0)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.3 (5.1–5.5)</td>
<td>5.1 (4.9–5.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.4 (26.5–32)</td>
<td>28.1 (24.7–30.5)</td>
<td>0.190</td>
</tr>
</tbody>
</table>

Visfatin, leptin and insulin concentrations according to week of gestation and at 2 weeks after delivery in women with GDM

Data are medians (interquartile range); n = 24. *P < 0.05 compared with 28–30 weeks of gestation; †P < 0.01 compared with 38–40 weeks of gestation (Wilcoxon matched pairs test).

<table>
<thead>
<tr>
<th></th>
<th>Examination 1 (28–30 weeks)</th>
<th>Examination 2 (38–40 weeks)</th>
<th>After delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.2 (4.9–5.5)</td>
<td>4.0 (3.6–4.5)</td>
<td>4.1 (3.8–4.6)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.0 (1.9–5.7)</td>
<td>3.7 (1.7–10.7)</td>
<td>0.7 (0.4–3.0)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.2 (5.0–5.5)</td>
<td>5.3 (5.2–5.5)</td>
<td>5.4 (5.2–5.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.8 (26.4–30.4)</td>
<td>28.7 (27.3–31.2)</td>
<td>25.9 (24.5–29.6)</td>
</tr>
</tbody>
</table>

Table 2 Clinical parameters in women with GDM during pregnancy and 2 weeks after delivery

Data are medians (interquartile range); n = 24. Wilcoxon matched pairs test has been applied to test for significant differences between time points.* P < 0.05 compared with examination 1; †P < 0.001 compared with examination 2.

Longitudinal study

In women with GDM, visfatin levels increased significantly (P = 0.002) from examination 1 to examination 2. After delivery, visfatin increased further (P = 0.014) compared with examination 2. Leptin concentrations were comparable at the time of examinations 1 and 2 (P = 0.370). After delivery, leptin concentrations decreased significantly (P < 0.0005). Insulin increased from examination 1 to examination 2 (P = 0.011) and decreased after delivery (P = 0.001; Figure 1). Clinical parameters, including fasting glucose, HOMA-IR, HbA1c and BMI, are shown in Table 2.
DISCUSSION

This is the first study describing the recently identified adipokine visfatin in women with GDM and healthy pregnant controls. Women with GDM had significantly elevated visfatin serum concentrations. Furthermore, visfatin levels rose during pregnancy and 2 weeks after delivery in women with GDM.

Visfatin has physiological glucose-lowering effects similar to those of insulin and induces the expression of PPAR-γ (peroxisome-proliferator-activated receptor-γ), which might improve insulin resistance [8]. GDM is a state of temporary insulin resistance and elevated visfatin concentrations in GDM might counteract high glucose levels jointly with increased insulin. In the cohort of the present study, we could not find any relationship between insulin resistance and visfatin. In addition, no association between visfatin and BMI was observed. This is in accordance with recently published data showing that BMI is only related to visfatin in men, but not in women [10]. Moreover, Berndt et al. [10] demonstrated that visfatin is not correlated with insulin resistance measured by a euglycaemic–hyperinsulinaemic clamp in a study cohort with a wide range of obesity, body fat distribution and insulin sensitivity. A clear effect of obesity on visfatin levels has been described by Fukuhara et al. [8]. In the present study, women with GDM and healthy pregnant controls had comparable BMIs. Thus obesity is unlikely to contribute to high visfatin serum concentrations in women with GDM.

Visfatin correlated with the time of investigation during pregnancy in women with GDM in the cross-sectional analysis. One of the reasons might be the pronounced progression of disturbances of glucose homoeostasis during the course of pregnancy in GDM women. This implies that abnormalities in glucose metabolism contribute at least partly to visfatin concentrations in our cohort. Elevated visfatin concentrations in women with GDM may reflect an impairment of visfatin action in target tissues, dysregulation of biosynthesis or a response to hyperglycaemia. The increase of visfatin during pregnancy might be due to an aggravation of these factors. As a limitation to the present study, the cross-sectional design does not allow conclusions to be drawn as to whether elevated visfatin is a consequence or related to the development of GDM.

Hyperleptinaemia is present during pregnancies complicated by GDM [15]. Insulin influences leptin expression by its effects on glucose metabolism [12], and leptin mimics some of insulin’s actions in the liver, adipose tissue and muscle [16,17]. Visfatin imitates the effects of insulin in many features [8]; however, an influence of visfatin on leptin in GDM is unlikely given the fact that leptin decreases rapidly after delivery and is associated strongly with BMI, which is in accordance with previous findings [15]. Furthermore, leptin did not correlate with visfatin before or after adjustment for other covariates.

Visfatin is constitutively expressed in human placental tissue, including amniotic epithelium, mesenchymal cells, the chorionic cytotrophoblast and parietal decidua [18]. The inflammatory cytokine TNF-α enhances the expression of visfatin in human placenta cells [19]. Chronic inflammation, including elevated concentrations of TNF-α, is present in women with GDM [20]. Thus the placenta could be a source of increased visfatin in GDM; however, this has to be investigated in experimental studies.

Interestingly, visfatin levels were increased further after delivery in the longitudinal study, whereas insulin was clearly reduced and glucose metabolism was already normalized. This suggests that visfatin is regulated in a different manner than insulin. It has been reported that inflammatory stimuli, such as interleukin-1β or TNF-α, can induce PBEF (visfatin) expression in neutrophils [21]. Parturition is an inflammatory condition with elevated levels of interleukins [22,23]. As indicated from recent studies, women with GDM have increased inflammatory markers compared with women without GDM after delivery [24,25]. This might contribute partly to increased visfatin concentrations shortly after delivery in our present cohort. Further studies will be necessary to investigate the development of visfatin concentrations during a longer observation period after delivery in women with GDM.

In conclusion, visfatin is elevated substantially in women with GDM during pregnancy. It increases significantly during the course of pregnancy and after delivery. Furthermore, visfatin shows no association with insulin and leptin.

REFERENCES

Visfatin and gestational diabetes mellitus


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