Adipokine Levels of RBP4, Resistin and Nesfatin-1 in Women Diagnosed With Gestational Diabetes

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Summary

Gestational diabetes mellitus (GDM) is a common complication of pregnancy in which women without previously diagnosed diabetes develop chronic hyperglycemia during pregnancy. It is associated with a number of maternal and fetal/neonatal complications. The role of the adipokines retinol binding protein-4, resistin and nesfatin-1 in the development of GDM is relatively poorly understood, but their role in glucose metabolism is suspected and their use as early markers to predict the development of GDM is being sought. The aim of study was to determine the correlation between the levels of selected adipokines (retinol binding protein-4, resistin, nesfatin-1) in women with gestational diabetes mellitus (GDM) and healthy pregnant women and to compare their levels with other clinical and biochemical parameters. Patients with GDM had significantly higher BMI (28.4±4.5 vs. 24.6±4 kg/m^2), total cholesterol (6±1.3 vs. 5.3±1.4 mmol/l) and triacylglycerols (1.9±0.8 vs. 1.4±0.7 mmol/l) than women in the control group. RBP4 confirms the significant difference between the groups, it is higher in the control group of healthy pregnant women. The adipokines resistin and nesfatin-1 show no differences between the control and GDM groups, but their ratios with BMI, cholesterol and triacylglycerols, resistin shows elevated levels in the control group. In women with GDM, RBP4 was significantly positively correlated with C-peptide and negatively correlated with total, LDL, and non-HDL cholesterol. Resistin was also negatively correlated with total, LDL, HDL, and non-HDL cholesterol. Nesfatin-1 was only moderately positively correlated with glycated hemoglobin (HbA1C) and fasting glycemia.

There is ambiguity in the results of previous studies on the levels of the investigated adipokines in pregnant women with GDM and the interpretation depends on many factors.

Keywords

Gestational diabetes • Adipokines • Retinol-binding protein 4 • Resistin • Nesfatin-1

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Introduction

Gestational diabetes mellitus (GDM) is defined as a disorder of glucose tolerance that occurs during the second and third trimesters of pregnancy in women without a history of diabetes before pregnancy. It is associated with a number of maternal and fetal/neonatal complications. The pathophysiology of GDM involves a combination of relatively deficient insulin secretion with increased insulin resistance in peripheral tissues. While the normal pregnancy is accompanied by lower insulin sensitivity (IS) reduced hepatic insulin extraction, lower glucose effectiveness, higher levels of leptin at lower concentrations of adiponectin, increased lipolysis and higher levels of triglycerides in white adipose tissue (WAT) and by increased WAT expansion, the pregnancy

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complicated with GDM is additionally associated with increased levels of inflammatory cytokines, higher macrophage infiltration, and pronouncedly increased lipolysis at prominently lower IS [1].

Obesity is one of the major risk factors for GDM, and dysfunctional adipose tissue has been intensively studied in recent years as an important tool for the development of chronic inflammation and insulin resistance [2,3]. A large number of cytokines, adipokines, hepatokines and other regulatory molecules have been proposed as markers of dysfunctional adipose tissue or as their mediators. Adipokines are cell signaling proteins produced by adipose tissue (adipocytes). They include a variety of cytokines, peptide hormones, and enzymes that are involved in a wide range of biological functions. Adipokines are closely connected to insulin resistance and chronic inflammation [4]. For example, they are involved in the regulation of appetite, energy homeostasis, vascular hemostasis, blood pressure, inflammatory and immune processes, and play a role in carbohydrate and fat metabolism. Adipokine secretion is often abnormal in obese patients [5]. Several adipokines (adiponectin, leptin, adipocyte fatty acid binding protein, visfatin, omentin, resistin, vaspin, apelin, chemerin) secreted by adipose tissue may directly and/or indirectly contribute to the exacerbation of insulin resistance and promotion of GDM by enhancing chronic inflammation [6]. Most of these have been studied in recent years in association with GDM [3,6,7,8]. The role of retinol binding protein-4, resitin and nesfatin-1 in the development of GDM is relatively poorly understood.

Retinol binding protein-4 (RBP4) is a member of the lipocalin family and is the major transport protein for the hydrophobic retinol molecule, also known as vitamin A, in the circulation. Expression of RBP4 is highest in the liver, where most of the body's vitamin A is stored in the form of retinol esters. To mobilize vitamin A from the liver, retinyl esters are hydrolyzed to retinol, which is then bound to RBP4 in the hepatocyte. After binding to transthyretin (TTR), the retinol/RBP4/TTR complex is released into the circulation and delivers retinol to tissues via binding to specific membrane receptors [9]. Elevated serum levels of RBP4 have been associated with components of the metabolic syndrome, including increased body mass index, waist-to-hip ratio, serum triglyceride and systolic blood pressure levels, and decreased high-density lipoprotein cholesterol. Adipocyte GLUT4 protein and serum RBP4 levels were inversely correlated [10]. The glucose transporter GLUT4 mediates insulin-stimulated glucose uptake in adipocytes and muscle by rapidly translocating from intracellular storage sites to the plasma membrane. In insulin-resistant states such as obesity and type 2 diabetes, GLUT4 expression is reduced in adipose tissue but maintained in muscle [11]. Serum RBP4 levels have been found to correlate positively with fasting serum insulin levels, fasting triglyceride levels, and systolic blood pressure. Serum RBP4 concentrations were inversely correlated with glucose disposal rate independent of body weight and age [12].

Resistin is a proinflammatory adipokine that is predominantly expressed and secreted by mononuclear cells, adipose tissue, and placental trophoblastic cells during pregnancy [8]. Plasma resistin concentrations are elevated in obese subjects and correlate with the inflammatory state underlying the initiation and progression of atherosclerotic lesions. A correlation between resistin concentration and the extent of atherosclerotic plaques in coronary arteries has also been reported [13]. Many recent studies have reported a physiological role of resistin in glucose homeostasis, one of which is to increase glucose production from the liver by regulating gluconeogenic enzymes such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase [14]. Anti-resistin antibodies improved insulin sensitivity and glucose tolerance in mice that developed obesity and insulin resistance on a high-fat diet. In addition, treatment with rosiglitazone, an antidiabetic drug that improves insulin action, also reduced resistin levels. These findings suggest that resistin may explain the insulin resistance associated with obesity and type 2 diabetes in humans [15]. Some studies have reported that obese individuals have elevated serum resistin levels compared to lean individuals [16,17].

Nesfatin-1 is secreted by neurons (paraventricular nucleus of the hypothalamus, supraoptic nucleus, arcuate nucleus, lateral hypothalamic area, and spinal cord) and peripheral tissues (pancreas, liver, subcutaneous and visceral adipose tissue, brown adipose tissue, and skeletal muscle) [18]. NUCB2/nesfatin-1 expression in adipose tissue increases with obesity and is altered under conditions of feeding and starvation [19]. Plasma levels are altered under conditions of chronic altered body weight such as obesity. Nesfatin-1 has also been implicated in the modulation of emotions. Some studies suggest that nesfatin-1 is involved in the regulation of stress and, consequently, in the regulation of food intake and weight [20]. The results of a study by Yijing SU (2010) describe the anorexigenic and antihyperglycemic effects of

nesfatin-1 in mice and its influence on the metabolic control of the body through its effect on food intake and glucose metabolism [21].

Based on these findings, it is expected that circulating levels of the above adipokines will be different in women with GDM compared to healthy pregnant women and that they will correlate with possible manifestations of insulin resistance. Early detection of GDM allows timely intervention to normalize blood glucose levels and prevent adverse pregnancy outcomes [22].

Methods

Study design, inclusion and exclusion criteria

The study was designed as a case-control study in accordance with the tenets of the Declaration of Helsinki as revised in 2008. It was reviewed and approved by the Ethics Committee of the University Hospital in Olomouc (approval no. 120/17). All participants signed an informed consent form. The diagnosis of GDM was based on a "one-step" 75g OGTT derived from the IADPSG criteria, with fasting plasma glucose and 1-hour and 2-hour measurements at 24-28 weeks' gestation in subjects without previously diagnosed diabetes. A diagnosis of GDM was made if any of the following plasma glucose values were met or exceeded: fasting 5.1 mmol/l; 1 hour after a 75 g oral glucose challenge, 10.0 mmol/l; 2 hours after a 75 g oral glucose challenge, 8.5 mmol/l [23]. Exclusion criteria were as follows: type 1 or type 2 diabetes, secondary or genetic diabetes. Controls were healthy women with no personal history of glucose intolerance or diabetes (including GDM or history of delivery of a large gestational age baby, i.e. ≥ 4.5 kg). Body mass index (BMI), waist circumference, systolic and diastolic blood pressure (SBP and DBP), and laboratory tests were also performed.

Laboratory analyses

Venous blood samples were collected in the morning after a 12-hour fast. Routine serum analyses for biochemical parameters (glucose, HbA1C, cholesterol, triacylglycerols, and C-peptide) were performed on the day of blood collection. Adipokine concentrations were measured in aliquots of samples stored at -80 °C for a maximum of 6 months.

Glucose levels were determined using the hexokinase method (GluH_3, Siemens Healthineers, Siemens Healthcare GmbH, Germany) on an Atellica automated analyzer (Siemens). Cholesterol was determined on an Atellica analyzer (Siemens) using the Cholesterol_2 kit (Siemens Healthineers, Siemens Healthcare GmbH, Germany) based on the enzymatic colorimetric assay principle. The concentration of triacylglycerols was determined using the Triglycerides_2 enzymatic assay (Siemens Healthineers, Siemens Healthcare GmbH, Germany). HbA1C levels were measured by ion exchange chromatography on an Arkray Adams HA-8180V analyzer (Arkray Corporation, Kyoto, Japan). All these assays meet the requirements of the EN ISO 15189:2013 accreditation standard. C-peptide levels were determined using a commercially available C-peptide immunochemiluminescence assay (Siemens Healthineers, Siemens Healthcare GmbH, Germany) on the immunochemistry module of an Atellica analyzer (Siemens). HDL cholesterol levels were determined on an Atellica analyzer (Siemens) using an enzymatic HDLC kit (Siemens Healthineers, Siemens Healthcare GmbH, Germany). RBP4 quantification was performed with a Human RBP4 (High Sensitivity) ELISA immunochemical kit (Biovendor Laboratory Medicine Inc., Brno, Czech Republic) according to the manufacturer's instructions. The antibodies used in this ELISA are specific for human RBP4. The sensitivity of the assay was 380 pg/mL, and the CV accuracy was 2.7 % (intra-assay) and 5.0 % (inter-assay). Resistin was determined using the Human Resistin ELISA immunochemical kit (Biovendor Laboratory Medicine Inc., Brno, Czech Republic) according to the manufacturer's instructions. The antibodies used in this ELISA are specific for human resistin. The sensitivity of the assay was 0.012 ng/mL, and the CV accuracy was 5.9 % (intra-assay) and 7.6 % (inter-assay). Nesfatin-1 was measured by ELISA (Human Nesfatin-1 ELISA Kit, Biovendor Laboratory Medicine Inc., Brno, Czech Republic) according to the manufacturer's instructions. The antibodies used in this ELISA are specific for human nesfatin-1. The sensitivity of the assay was 0.021 ng/mL and the CV accuracy was 4.25 % (intra-assay) and 5.9 % (inter-assay). The Evolis system (Bio-rad), a stand-alone microplate processor for automated EIA testing on microtiter plates, was used for all ELISA methods.

Statistical analysis

All data evaluation was performed in GraphPad Prism 10.0 (GraphPad Software, Boston, MA, USA). Normality of the data was tested by Saphiro-Wilk. Outliers were identified by the Grubbs test. Groups of patients and controls were compared by nonparametric Man-Whitney

U test and parametric t test. All parameters were correlated by nonparametric Spearman test and heatmap was performed. P-values < 0.05 were evaluated as statistically significant.

Results

Results were obtained from 20 pregnant women without a diagnosis of gestational diabetes mellitus (control group, median age 30 years) and 21 patients (pregnant women with a diagnosis of gestational diabetes mellitus) who met the study criteria. Patients with GDM had on average a higher BMI (28.4±4.5 vs. 24.6±4 kg/m^2), slightly higher fasting glucose, slightly higher HbA1C, and higher LDL and total cholesterol (6±1.3 vs. 5.3±1.4 mmol/l) than women in the control group. There was also a significant difference in triacylglycerol levels - GDM group (1.9±0.8 mmol/l) vs control group (1.4±0.7 mmol/l). The clinical and biochemical parameters found are summarized in Table 1.

Table 1. Descriptive clinical and biochemical parameters of the GDM/without GDM groups. (GDM - gestational diabetes mellitus, BMI - body mass index, BP – blood pressure, HbA1c – glycated hemoglobin, HDL – high-density lipoprotein cholesterol, LDL - low-density lipoprotein cholesterol, FPG – fasting plasma glucose.)

	non GDM (n=20)		with GDM (n=21)		t-test p-value	fold change
	mean	**SD**	**mean**	**SD**		
Age (years)	30.2	3.2	31.5	3.5	0.208	1.05
Height (cm)	172.5	4.4	166.4	6.2	0.00106	0.96
Weight (kg)	73.1	11.6	79	15.1	0.183	1.08
BMI (kg/m^2)	24.6	4	28.4	4.5	0.00736	1.16
Waist (cm)	88	11	95.9	15.1	0.0815	1.09
Systolic BP (mmHg)	122.2	9.1	124.5	14.5	0.573	1.02
Diastolic BP (mmHg)	75.4	8.2	77.4	9.4	0.487	1.03
Heart rate (BPM)	83.7	12.5	89	8.2	0.131	1.06
HbA1c (mmol/mol)	30.3	2.7	31.6	3.4	0.2	1.04
Cholesterol (mmol/l)	5.3	1.4	6	1.3	0.0619	1.13
Triacylglycerols (mmol/l)	1.4	0.7	1.9	0.8	0.0484	1.34
HDL (mmol/l)	2	0.3	1.9	0.4	0.64	0.97
LDL (mmol/l)	2.7	0.9	3	1	0.193	1.14
nonHDL (mmol/l)	3.3	1	3.9	1.2	0.0833	1.18
FPG (mmol/l)	4.5	1	5	0.5	0.107	1.09
C-peptide (pmol/l)	798.8	256.8	795.7	293.3	0.979	1

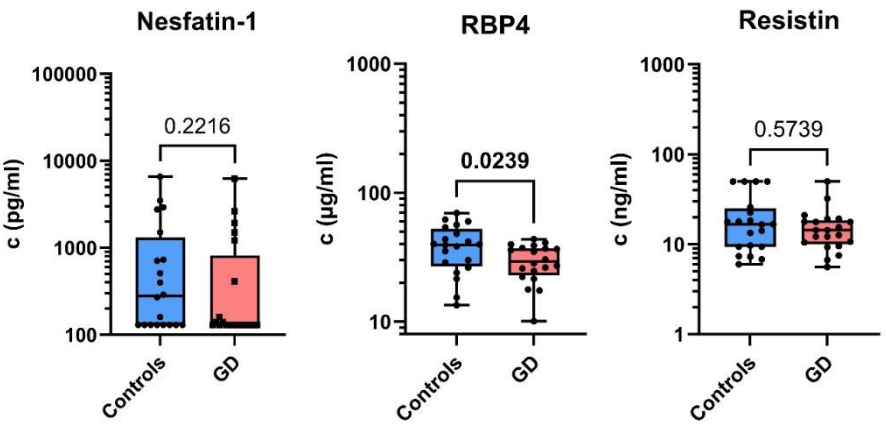

Fig. 1. Univariate statistical analysis of adipokines - non-parametric Man-Whitney U test (NESF, RES,) and parametric t-test (RBP4) RBP4 listed first. (GD – gestational diabetes.)

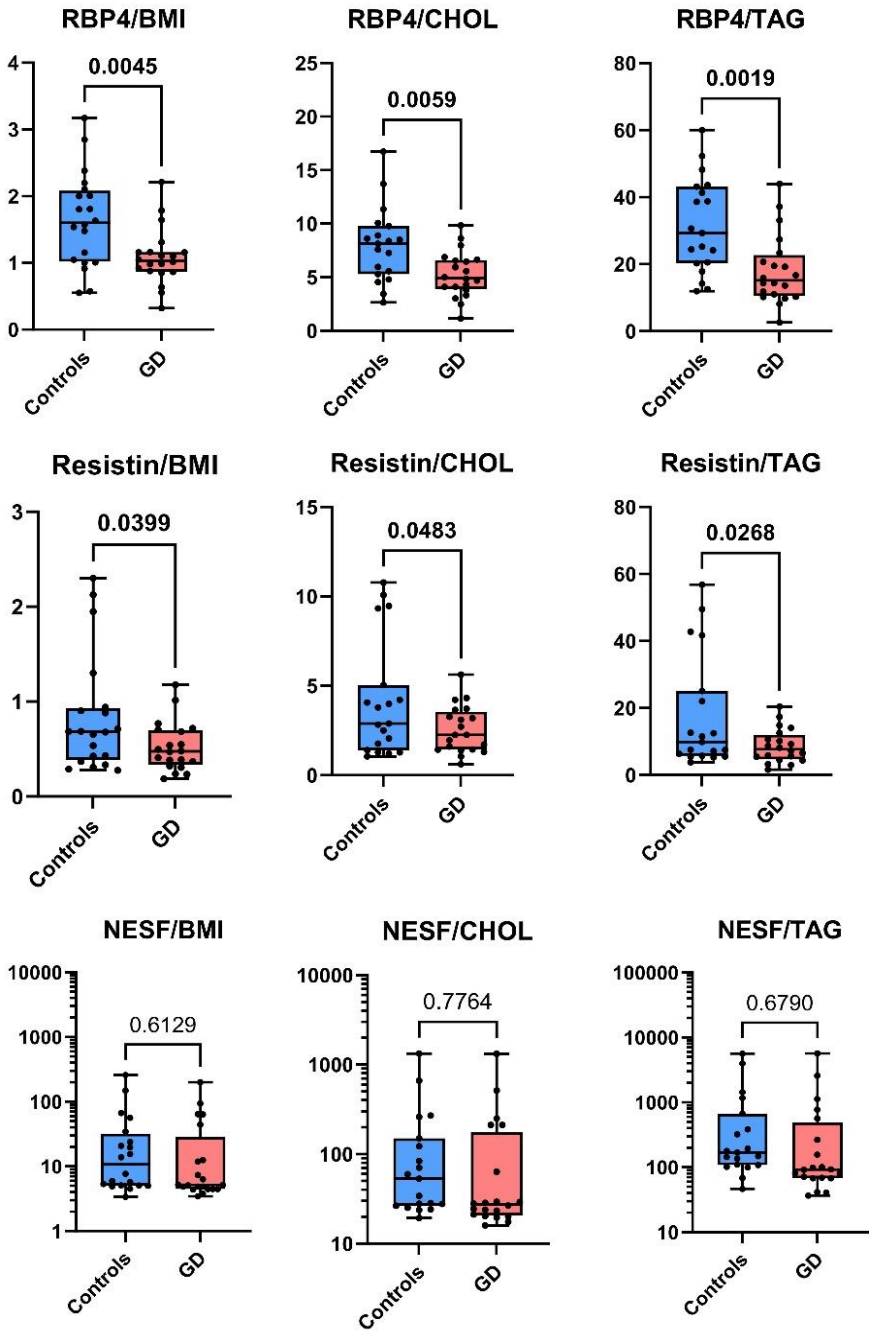

Fig. 2. Univariate statistical analysis (p-value from t-test) of RBP4/resistin/nesfatin-1 ratio versus BMI, cholesterol, TAG. (BMI - body mass index, CHOL – cholesterol, TAG – triacylglycerols.)

RBP4 levels were significantly lower (at p-value ≤ 0.05 level of significance) in the group of pregnant women with DM compared to the control group. In contrast, no significant differences were found for the adipokines nesfatin-1 and resistin between the control group and the DM patients. The levels of the adipokine groups are shown in Figure 1.

The adipokines were further compared with selected anthropometric and biochemical parameters, showing a difference between the selected groups (BMI, cholesterol and triacylglycerols) - Figure 2. RBP4 confirms a significant difference (at a significant level of p-value ≤ 0.05) even as ratio with the selected lipid parameters, with the control group showing higher RBP4 results compared to the GDM patients, and the difference was even more pronounced as ratio. Resistin shows a difference between groups after as ratio with BMI, cholesterol and triacylglycerols, with elevated levels in the control group (p-value ≤ 0.05).

Correlation analysis of adipokines with biochemical markers and clinical-anamnestic data is divided into patient and control groups - Figure 3. The

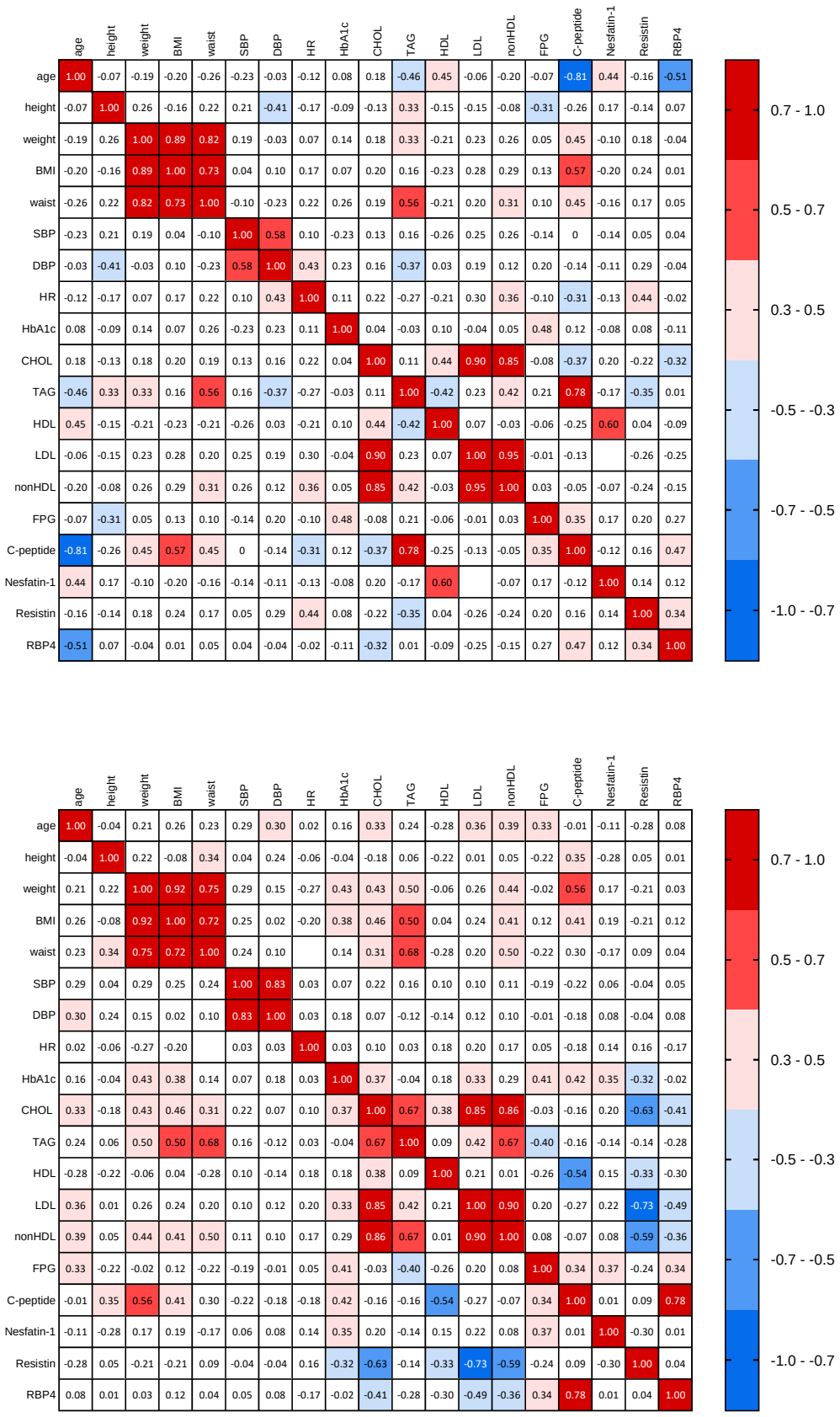

Fig. 3. Correlation analysis (Spearman) of the control group of pregnant women without gestational diabetes (top part) and the group of pregnant women with gestational diabetes (bottom part). Values in boxes represents correlation coefficients. (BMI - body mass index, BP – blood pressure, HR – heart rate, HbA1c – glycated hemoglobin, CHOL – cholesterol, HDL – high-density lipoprotein cholesterol, LDL - low-density lipoprotein cholesterol, TAG – triacylglycerols, FPG – fasting plasma glucose.)

adipokine RPB4 shows a negative correlation with total cholesterol in both selected groups, while patients with GDM also show a negative correlation with LDL (or non-HDL) cholesterol. Also interesting is the finding of a positive association of RPBP4 with c-peptide in both groups, with a strong correlation in GDM patients. The results are complemented by the weak positive correlation of RBP4 with fasting glucose levels in the GDM group. Resistin shows a strong negative correlation with total and LDL (or non-HDL and HDL) cholesterol in the GDM group. In contrast, these parameters show no association or only a weak negative association with triacylglycerols in the non-DM group. In the pregnant control group, nesfatin-1 showed a positive correlation with HDL cholesterol, whereas this was not confirmed in the GDM group. In GDM patients, nesfatin-1 showed a weak correlation with HbA1C and fasting glucose.

Discussion

Women with GDM had significantly lower levels of RBP4, whereas they did not differ in resistin and nesfatin-1 levels. As ratio with selected clinical and biochemical parameters that showed a difference between the selected groups (BMI, cholesterol, and triacylglycerols), women with GDM showed a difference compared with the control group not only in RBP4 but also in resistin, complementing the finding of a strong negative correlation of GDM patients with cholesterol. Nesfatin-1 showed no difference after correction for these selected clinical and biochemical parameters, but there is a trend towards higher levels in controls.

In women with GDM, RBP4 correlated significantly positively with C-peptide and negatively with total, LDL, and non-HDL cholesterol. Resistin was also negatively correlated with total, LDL, HDL, and non-HDL cholesterol. Nesfatin-1 was only moderately positively associated with glycated hemoglobin (HbA1C) and fasting glucose.

Regarding the association of RBP4 levels with insulin resistance and the development of DM, previous studies are inconsistent. Thus, it is suggested that ethnicity may be a factor modulating the effect of obesity on insulin resistance in pregnancy. Both Asian and South Asian ethnicity are independently associated with increased insulin resistance in late pregnancy. Pre-pregnancy BMI has a much greater effect on insulin resistance in pregnancy in Asian women than in Caucasian women [24]. This claim is supported by subsequent studies by authors from Asia and elsewhere. Wang (2012) links elevated RBP4 levels, DM and metabolic syndrome parameters [25]. Du (2019) publishes that RBP4 levels were significantly higher in the GDM group compared to the control group [25,26]. In contrast, some studies from Thailand and USA report that RBP4 is not associated with insulin resistance factors in pregnancy [27,28]. On the other hand, results of measurements performed in a Caucasian population reported higher RBP4 levels in a control group without GDM [29,30], or no significant differences in RBP4 levels were found in pregnant women with or without GDM [8]. The analysis of our study showed a significantly higher RBP4 result in the control group of pregnant patients without GDM compared to those with GDM, and this difference was further exacerbated after as ratio with BMI. Our results support the theory of different RBP4 levels in pregnant women with and without GDM, where higher RBP4 levels are not associated with insulin resistance in pregnancy. At the same time, as in the study by Krzyzanowska (2008) or Görkem (2016), our control group had lower RBP4 as well as lower BMI than the group with GDM [29,8]. Wolf (2007) described that in mice, elevated serum RBP4 levels led to impaired glucose uptake in skeletal muscle and increased glucose production by the liver, whereas reduced serum RBP4 levels significantly increased insulin sensitivity [12]. Our finding of a positive correlation between RBP4 and c-peptide levels in both groups confirms previous results of some studies where RBP4 correlated with insulin levels. The results of animal experiments, prospective studies, meta-analyses, genetic studies and intervention studies support a significant association between RBP4 levels and insulin resistance, but new attention is also being paid to its possible influence on pancreatic β-cell function [31]. It is possible that RBP4 directly affects insulin secretion via the JAK2/STAT1 pathway [32]. Thus, it could influence both pathophysiological mechanisms leading to GDM, insulin resistance and pancreatic β-cell dysfunction. This, in addition to other influences (differences in study populations, gender, metabolic status, renal function and differences in methodological assessment), could explain the discrepancies and different findings described in the above clinical studies [31]. Furthermore, RBP4 was negatively correlated with total cholesterol levels in both study groups. At the same time, the finding of a negative correlation supports the findings of higher RBP4 levels in the control group, which also had lower total cholesterol levels. Existing studies have reported a correlation

between RBP4 and indicators of cardiovascular risk. Feng (2015) suggested that RBP4 positively correlates with carotid atherosclerosis in T2DM patients and can be used as an early predictor of cardiovascular disease [33]. Chan Liu (2021) found that plasma RBP4 levels were significantly higher in hyperuricemic rats and positively correlated with plasma uric acid, creatinine, fasting insulin, total cholesterol, and triglycerides [34]. Of note, elevated serum RBP4 levels correlated specifically with cardiovascular risk factors [12]. Shen-Nien Wang (2010) published that serum RBP4 levels of all subjects were positively correlated with total cholesterol, triglycerides, creatinine, insulin resistance, and albumin, and inversely correlated with aspartate aminotransferase and alanine aminotransferase, and significantly lower serum RBP4 levels were found in patients with cholesterol gallstones compared to controls [35]. However, studies have focused on general comparisons of measured parameters or their comparison in patients with diabetes mellitus. The question remains whether these parameters behave differently in relation to gestational diabetes mellitus (i.e. the fact of pregnancy) and also in the case of RBP4 depending on the aforementioned fact of ethnicity. In general, cholesterol is an essential nutrient for fetal growth. It is also a precursor for the synthesis of steroid hormones and is essential for the development and maturation of fetal organs [36]. Therefore, specific changes in cholesterol levels would be expected in pregnant women. In general it can be said the development of gestational diabetes mellitus affects lipid metabolism during pregnancy. However, the magnitude of changes in lipid parameters is unclear [37].

The non-significant difference found in resistin levels between the selected groups may also be related to the site of its production, where in pregnant women placental trophoblast cells are added to the standard adipocytes and macrophages. Furthermore, resistin is classified as a pro-inflammatory adipokine and pregnancy is considered an inflammatory state due to the physiological adaptations of the mother to ensure appropriate conditions for fetal growth [7]. Schäffler (2004) reports higher levels of the adipokine resistin in a healthy control group (without DM) compared to individuals with DM1 or DM2 [16]. This source confirms the finding of higher resistin levels in the control group compared to pregnant women with GDM after as ratio with BMI, TAG and cholesterol levels. Chen (2005) reports significant differences in resistin levels only in the third trimester compared to the first and second trimesters. He also finds an association between pre-eclampsia and lower resistin levels in the third trimester of pregnancy [38]. This study was conducted at 24-28 weeks of gestation (after oGTT), i.e., at the start of the second and third trimesters, or patients were diagnosed with GDM in the first trimester based on high fasting glucose levels. Possible preeclampsia was not considered in the study. The question is whether sampling in these trimesters had an effect on the determination of this adipokine and whether it should not always be performed later in pregnancy. However, there are also authors, e.g. Yura 2003, who claim that the expression of the resistin gene in adipose tissue is relatively low and remains unchanged by pregnancy [39].

It remains to be seen whether resistin can be a suitable early marker of insulin resistance in pregnant women. Previous studies have shown that serum resistin concentrations do not correlate with BMI, HOMA, fasting plasma glucose or fasting plasma insulin levels [16,40]. The results of our study are consistent with the aforementioned study and also showed no correlation between these parameters. However, resistin has also been shown to decrease cell surface glucose transporters and thus glucose uptake into cells, thereby increasing blood glucose levels and decreasing insulin sensitivity. The correlation did not confirm the binding of resistin to glucose and c-peptide in any of the groups studied. Resistin is also thought to act as a modulator of cholesterol transport in the body, increasing LDL cholesterol levels and enhancing hepatic LDL receptor degradation, thereby contributing to the pathogenesis of atherosclerosis [41], but this claim needs to be adjusted for pregnancy. In conclusion, Nava-Salazar (2022) described that resistin induces PCSK9 expression in placental explants and JEG-3 cells, which may be related to negative regulation of LDLR via lysosomal degradation. These findings suggest that resistin may significantly regulate the uptake and transport of LDL-C from the maternal circulation to the fetus, affecting its growth and lipid profile [7]. This theory is supported by our results showing a negative correlation of total cholesterol and LDL cholesterol with resistin in pregnant women with GDM. The lower resistin levels found in the GDM group and the negative correlation with cholesterol (and LDL cholesterol) provide an explanation for the association between the development of GDM and dyslipidemia. Furthermore, the demonstrated lower maternal LDL cholesterol levels in pregnancies with intrauterine growth restriction [42] should be considered. Fetal growth restriction was not evaluated in this study.

Most investigators found that nesfatin-1 levels were significantly lower in patients with GDM compared with healthy controls [43,44]. However, there are exceptions that claim higher nesfatin-1 levels in GDM patients [45] or have not shown differences between levels [46]. When comparing the healthy and GDM groups, our results did not show a significant difference, but after as ratio with for BMI and TAG, there was a noticeable trend toward slightly lower levels in the GDM group. In contrast, some studies investigating nesfatin-1 levels have shown lower nesfatin-1 levels in patients with high BMI, while others have shown lower nesfatin-1 levels in patients with DM2 compared to the healthy population [47]. As mentioned above, our GDM group had higher BMI values (mean 28.4 kg/m^2), i.e. in the overweight range, but of course pregnancy status has to be taken into account. Therefore, we might expect to see lower nesfatin-1 levels in women with GDM. Furthermore, our results showed a weak positive association between nesfatin-1 levels and glycated hemoglobin and glucose levels in GDM patients. However, glycated hemoglobin and glucose levels did not show a significant difference between the groups studied. Zhang (2022) also showed a positive association of glycated hemoglobin and glucose with nesfatin-1 levels in his study, but he claims that women with GDM have higher blood levels of nesfatin-1 than healthy pregnant women [48]. The positive association of HDL cholesterol in the healthy group is consistent with the concept of lower nesfatin-1 levels in patients with DM and the expected associated metabolic syndrome. Physiological changes in the dynamics of nesfatin-1 levels during pregnancy may be a possible explanation for the different results between studies. A decrease in serum nesfatin-1 levels until the end of pregnancy has been demonstrated in ad libitum fed rats [49]. Thus, it is questionable whether nesfatin-1 levels will behave in the same way during pregnancy in humans, and thus consideration of the critical timing of the appropriate week of pregnancy for blood sampling is warranted.

Although a number of papers have been published on the association of adipokines with GDM (see discussion above), none of the wide range of adipokines has been incorporated into routine diagnostic practice. The use of Adiponectin, or alternatively the use of the full set of adipokines in combination with advanced multivariate statistical methods, seems promising [50,51].

Study limitations

The author of the paper is aware of the small number of individuals in the selected groups with which these results must be considered. They can be considered as preliminary data before considering the design of a long-term study.

Conclusion

In general, there are inconsistencies in the results of previous studies of adipokine levels in pregnant women with GDM and require further in-depth investigation and unification of study design criteria (such as gestational age, gestational week of sampling or BMI of patients before and during pregnancy, or even possible consideration of racial origin of patients). RBP4 may be a kind of possible protective factor against the development of insulin resistance and the development of GDM in the pregnant ethnic Caucasian population. The use of resistin and nesfatin-1 as markers for early diagnosis of gestational diabetes is complicated by the interpretation of the results.

Conflict of Interest

There is no conflict of interest.

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