Available online www.ijpras.com

International Journal of Pharmaceutical Research & Allied Sciences, 2017, 6(1):236 - 250

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Relationship between Levels of Retinol Binding Protein 4, Vaspin and Chemerin and Insulin Resistance in Gestational Diabetes Mellitus

Hana M. Gashlan

Biochemistry Department, Faculty of Sciences, King Abdulaziz University, KSA
**Corresponding authors*
Hana M. Gashlan
Biochemistry Department, Faculty of Sciences, King Abdulaziz University, KSA
E-mail: hgashlan@yahoo.com

ABSTRACT

Gestational diabetes mellitus (GDM) is a common medical complication associated with pregnancy. The incidence of GDM in Saudi Arabia ranged from 3.8% to 12.5 according to the American Diabetes Association and World Health Organization criteria respectively. The goal of the present study was to investigate the changes of some adipokines including: retinol binding protein 4 (RBP4), vaspin and chemerin at 2nd and 3rd trimesters and their associations with markers of insulin resistance in GDM compared to normal pregnancy. The study included 88 pregnant women classified into 4 groups: normal pregnancy (n=19) and pregnancy with GDM (n=25) at 2nd trimester and normal pregnancy (n=18) and pregnancy with GDM (n=26) at 3rd trimester. Both GDM and control groups were matched for maternal age (MA), gestational age (GA) and body mass index (BMI). Results indicated that GDM subjects either 2nd or 3rd trimesters had significantly elevated fasting blood glucose (FBG), glycated hemoglobin (HbA1c) and markers of IR compared to matched normoglycemic control. At 2nd trimester, RBP4 was significantly decreased in GDM compared to control, however vaspin and chemerin were comparable between the two groups. RBP4 was significantly correlated with IR in GDM group only. No associations were detected between either vaspin or chemerin with IR in GDM. On the other hand, at 3rd trimester, chemerin was significantly higher in GDM compared to control, while vaspin showed no variations between both groups. In addition, chemerin was correlated with FBG and HOMA1-% B. It could be concluded that RBP4 and chemerin might be involved in the increased of IR in associated with GDM.

Key words: *Gestational diabetes mellitus, vaspin, chemerin, glycated hemoglobin, insulin resistance*

INTRODUCTION

Human pregnancy is characterized by a series of endocrine, metabolic and vascular changes to provide sufficient energy and nutrients to the fetus [1]. It is characterized by insulin resistance (IR), traditionally attributed to the effects of placental hormones [2]. As pregnancy advances, IR becomes more intense and could lead to the development of gestational diabetes, a majority of which manifests at the 24–28th weeks of gestation [3]. In early pregnancy, insulin secretion increases, while insulin sensitivity is unchanged, decreased, or may even increase [4, 5]. In late pregnancy, maternal adipose tissue depots decrease, whereas postprandial free fatty acids (FFA) levels increase and insulin-mediated glucose disposal rates decline by approximately 50% as compared to pre pregnancy values. Moreover, insulin shows reduced ability to suppress lipolysis [1]. Dysregulation of these physiological changes during pregnancy contributes to the complications of pregnancy.

Adipokines are proteins that are secreted by adipose tissue and are involved in a wide range of physiological processes including haemostasis, lipid metabolism, atherosclerosis, blood pressure regulation, insulin sensitivity and

angiogenesis [6]. A large body of evidence has supported the role of adipose tissue in the regulation of insulin resistance in both non pregnant and pregnant participants. In this respect, adipocytokines, which are adipocyte-derived hormones, have been implicated in the regulation of maternal metabolism and gestational insulin resistance [2,6]. Retinol binding protein 4 (RBP4) is a 21-kDa protein synthesized in hepatocytes and adipocytes, and it acts as a carrier for retinol in the blood stream. Increased circulating RBP4 levels is indicated in several metabolic complications including obesity, insulin resistance, polycystic ovary syndrome and cardiovascular disease [7]. Vaspin, a visceral adipose tissue-derived serine protease inhibitor family, has been identified as an adipokine with potential insulin-sensitizing effects that might be implicated in endogenous glucose regulation [8,9]. Chemerin is a chemokine [10] that is highly expressed in liver and white adipose tissue [11]. Chemerin is essential for normal adipocyte differentiation and modulates the expression of adipocyte genes involved in glucose and lipid homeostasis, such as glucose transporter-4, fatty acid synthase, and adiponectin via its own receptor [11,12,13]. Alterations in adipokines (RBP4, vaspin and chemerin) were detectable in prediabetic states, and may reflect dysfunction as an early pathogenetic event in T2DM development [14].

The aim of this study was to investigate the changes in and association of some adipokine (RBP4, vaspin and chemerin) with markers of IR in GDM compared to normal pregnancy.

MATERIAL AND METHODS

I. Materials

a- Subjects and study Design:

This study was approved by Directorate of Health Affairs, Jeddah, Saudi Arabia. Blood samples were collected from Maternity and Children's Hospital, al-Mesadiah. All women were requested to perform oral glucose tolerance test (OGTT) using 100g / 250 ml glucose solution. Glycated haemoglobin (HbA1c) was performed for each pregnant woman with gestational diabetes mellitus (GDM) and with normal pregnancy to identify blood glucose status during the last 3 months of gestation. GDM was diagnosed according to world Health Organization criteria [15].

Eighty-eight pregnant women (37 normal pregnant women and 51 pregnant women with GDM) were enrolled in the study. All normal and GDM pregnant women had a regular follow up visits and were diagnosed by hospital physicians. The exclusion criteria were presence of hypertension, preeclampsia, urinary tract infection, fever (>37.5 °C), fetal / placental abnormalities, remarkable previous medical – surgical – and gynaecological maternal history, alcohol intake and no history of diabetes; type I or type II. Almost all GDM women included in 2nd (14-27 weeks of gestation) and 3rd (28-42 weeks of gestation) trimesters were treated with nutrition therapy except 3 and 4 cases in 2nd and 3rd trimesters, respectively, were treated with insulin therapy. Normal pregnant women and women with GDM were classified according to pregnancy status. Normal pregnant women at 2nd trimester: included 19 women with a range of age from 21 to 44 years. Pregnant women with GDM at 2nd trimester: included 25 women with a range of age from 21 to 42 years. Normal pregnant women at 3rd trimester: included 18 women with a range of age from 24 to 41 years. Pregnant women with GDM at 3rd trimester: included 2٦ women with a range of age from 21 to 45 years.

II. Methods:

a. Blood sample collection:

Whole blood was collected from each participant. After overnight fasting, each blood sample was divided into three parts. First part was used for blood glucose measurement (fasting and postprandial plasma glucose levels). Second part was for HbA1c measurement. Third part was for hormonal assay including: Retinol Binding Protein 4, Vaspin and Chemerin.

b. Biochemical Assays:

i. Oral Glucose Tolerance Test:

Determination of plasma glucose was carried out, using kit provided by Siemens Healthcare Diagnostic Limited, UK based on glucose hexokinase method.

ii. Glycated Hemoglobin (HbA1c):

ELISA kit provided by Human (cat. No. OKEH00660) was used for determination of both HbA1C and total hemoglobin. The HbA1C measurement is based on a turbid metric inhibition immunoassay principle, and the measurement of total hemoglobin is based on a modification of the alkaline hematin reaction. Using the values obtained for each of these two analyses, the relative proportion of the total hemoglobin that is glycated is calculated and reported.

iii. Markers of Insulin Resistance:

Serum Insulin was determined using a kit purchased from Cobas (cat. No. 12017547 122) based on electro chemiluminescence immunoassay technique. Glucose/Insulin ratio (G/I), Homeostasis Model Assessment (HOMA), HOMA-IR, HOMA1-%B, HOMA β cell, Log (HOMA-IR) and fasting Insulin Resistance Index (FIRI) were calculated according to Singh and Saxena [16].

iv. Hormonal assays:

All kits used for hormonal assays were purchased from Elabscience Company (Wuhan, China). Hormonal parameters included: Serum retinol binding protein 4 (RBP4) (cat. No. E0929h), serum vaspin (cat. No. E0706h) and serum chemerin (cat. No. E-EL-H0698).

III. Statistical Analysis:

Statistical analysis was performed using SPSS 22.0 for windows (SPSS Inc, USA). The Kolmogorov-Smirnov statistic, with a Lilliefors significance level, the Shapiro-Wilk statistic with a significance level of 5% &Skewness were applied in the statistical analysis to verify the normality of samples. Levene's robust tests for homogeneity of variance were displayed. The parametric tests, was chosen for the statistical analysis due to the normal distribution of the data. Descriptive statistics are shown as means ± standard error of means. Pearson correlations were used to measure how RBP4, vaspin and chemerin were related with each other and with other variables in GDM and normal pregnancy at 2nd and 3rd trimesters groups. Independent-Samples T Test (t) was performed for comparing means for GDM and normal pregnancy at 2nd and 3rd trimester groups. P value smaller than 0. 05 was considered statistically significant.

RESULTS

I- Subjects' Characteristics:

Pregnant women with GDM, either at 2nd or 3rd trimesters, had maternal age (MA) and body mass index (BMI) comparable to their matched control. However, GDM at 3rd trimester had significantly higher mean for GA than normoglycemic control (Table 1).

II- Fasting blood glucose (FBG), glycated hemoglobin (HbA1c) and markers of IR:

Results revealed highly significant change in FBG, HbA1c and most markers of IR in GDM relative to normal control, both at 2nd and 3rd trimesters (Table 2).

III-Retinol binding protein 4 (RBP4), vaspin and chemerin:

At 2nd trimester, only significant reduction in the mean value of RBP4 was noted in GDM relative to control mean value, however, other hormonal parameters showed no significant variations. At 3rd trimester, pregnant subjects

with GDM had significantly elevated chemerin mean value compared to matched normoglycemic mean values. Other hormones showed comparable values to control (Table 3)

Table 1: Subject characteristics in normal pregnancy and pregnancies complicated with GDM groups at 2nd and 3rd trimesters ($\overline{X}$ ± SE).

Groups / Parameters	2nd trimester		*P* value	3rd trimester		*P* value
	Normal n= 19	GDM n= 25		Normal n=18	GDM n= 26	
MA (year)	34.0 ± 1.58	32.4 ± 0.98	NS	31.9 ± 1.33	33.4 ± 1.36	NS
GA (week)	21.3 ± 1.10	21.6 ± 0.80	NS	31.3 ± 0.78	34.4 ± 0.61	0.001
BMI (Kg/m^2)	33.8 ± 1.01	33.4 ± 0.81	NS	34.3 ± 1.02	34.1 ± 0.82	NS

- MA: maternal age, GA: gestational age, BMI: body mass index.
- *P* value is significant at < 0.05 NS: non-significant, *P* value > 0.05.

Table 2: FBG, HbA1c and markers of IR in normal pregnancy and in GDM groups at 2nd and 3rd trimesters ($\overline{X}$ ± SE).

Groups / Parameters	2nd trimester		*P* value	3rd trimester		*P* value
	Normal n=19	GDM n=25		Normal n=18	GDM n=26	
FBG (mg/d)	82 ± 2.31	114.42 ± 3.66	0.00	83.33 ± 1.85	120.92 ± 4.62	٠,٠٠
HbA_{1c} (%)	5.48 ± 0.05	6.95 ± 0.14	0.00	5.20 ± 0.09	7.13 ± 0.17	٠,٠٠
FI (µu/ml)	13.29 ± 1.68	17.58 ± 2.57	NS	10.94 ± 1.12	17.5 ± 3.69	NS
G/I	0.46 ± 0.05	0.56 ± 0.06	NS	0.48 ± 0.04	0.71 ± 0.05	٠,٠٠
FIRI	2.45 ± 0.33	4.56 ± 0.75	0.02	2.04 ± 0.23	5.13 ± 1.18	٠,٠٤
HOMA–IR	2.72 ± 0.37	5.17 ± 0.82	0.02	2.26 ± 0.26	5.71 ± 1.31	0.04
Log (HOMA-IR)	0.36 ± 0.05	0.6 ± 0.05	0.00	0.31 ± 0.04	0.54 ± 0.05	0.00
HOMA1- B%	312.7 ± 58.57	138.7 ± 24.87	0.00	213.3 ± 18.09	108.5 ± 17.25	0.00
HOMA β cell	185.9 ± 28.32	103.٢ ± 13.41	0.01	139.6 ± 11.76	9٠.4 ± 15.02	0.02

- FBG: fasting blood glucose, HbA_{1c}: glycated hemoglobin, FI: fasting insulin, G/I: glucose / insulin ratio, FIRI: fasting insulin resistance index, HOMA-IR: homeostasis model assessment of insulin resistance.
- *P* value is significant at < 0.05. NS: non-significant, *P* value > 0.05

Table 3: RBP4, vaspin and chemerin in normal and in GDM groups at 2nd and 3rd trimesters ($\overline{X}$ ± SE).

Groups / Parameters	2nd trimester		*P* value	3rd trimester		*P* value
	Normal n=19	GDM n=25		Normal n=18	GDM n=26	
RBP4 (ng/ml)	3.86 ± 0.71	2.22 ± 0.29	0.02	3.20 ± 0.46	3.16 ± 0.36	NS
Vaspin (ng/ml)	0.07 ± 0.03	0.27 ± 0.10	NS	0.2 ± 0.10	0.19 ± 0.04	NS
Chemerin(ng/ml)	7.68 ± 1.36	7.21 ± 1.19	NS	5.56 ± 0.92	6.57 ± 0.95	0.03

- RBP4: Retinol Binding Protein 4
- *P* value is significant at < 0.05.
- NS: non-significant, *P* value > 0.05.

IV-Pearson's correlations:

Between BMI and each of FBG, HbA1c and markers of IR in normal pregnancy and in GDM groups:

Results revealed no associations between BMI and all markers in normal pregnancy at either 2nd or 3rd trimesters. In pregnancy complicated with GDM, at 2nd trimester, BMI was only correlated with HbA1c, while at 3rd trimester, it showed significant correlation with G/I ratio (Table 4).

Between each hormone and MA, GA and BMI in normal pregnancy and in GDM:

In normal pregnancy at 2nd and 3rd trimesters, no correlations were obtained between any of the studied hormonal parameters and patient's characteristics (Table 5). In GDM at 2nd trimester, RBP4 was associated with MA and GA. On the other hand, chemerin was negatively associated with GA, however, vaspin showed no association with any of the tested characteristics. In GDM at 3rd trimester, vaspin showed significant association with MA and BMI while RBP4 and chemerin showed no associations with MA, GA & BMI (Table 5).

Between each hormone (RBP4, Vaspin and Chemerin) and each of FBG, HbA1c, and markers of IR in normal pregnancy and in GDM groups:

Results in Table 6 revealed no association between RBP4 and each of FBG, HbA1c, FI, G/I and all markers of IR in normal pregnancy either at 2nd or 3rd trimesters. However, in GDM at 2nd trimester, RBP4 was associated with FI, G/I and almost all markers of IR. In GDM at 3rd trimester, RBP4 was only correlated with G/I and HOMA1-% B.

No association between vaspin and each of FBG, HbA1c and markers of IR in normal pregnancy either at 2nd or 3rd trimesters. However, in GDM at 2nd trimester, vaspin was only associated with G/I, while in GDM at 3rd trimester, vaspin was correlated with HOMA1- % B (Table 7).

Results in table 8 reveal no association between chemerin and all tested parameter in normal or in GDM groups at 2nd trimester. In 3rd trimester, same trend was obtained in normal pregnant control, however in GDM, chemerin showed significant correlations with each of FBG and HOMA1-%B.

Table 4: Pearson's correlations between BMI and each of FBG, HbA_{1c} and markers of IR in normal and in GDM groups at 2nd and 3rd trimesters:

Parameters \ Groups	2nd trimester		3rd trimester	
	Normal n=19	GDM n=25	Normal n=18	GDM n=26
FBG	-0.02 (NS)	-0.28 (NS)	-0.02 (NS)	-0.23 (NS)
HbA_{1c}	0.36 (NS)	0.47 (0.02)	-0.14 (NS)	-0.01 (NS)
FI	-0.11 (NS)	-0.01 (NS)	0.01 (NS)	-0.06 (NS)
G/I	-0.13 (NS)	0.11 (NS)	0.01 (NS)	-0.45 (0.02)
FIRI	-0.13 (NS)	-0.04 (NS)	0.01 (NS)	-0.08 (NS)
HOMA-IR	-0.13 (NS)	-0.05 (NS)	0.01 (NS)	-0.08 (NS)
Log(HOMA-IR)	-0.03 (NS)	-0.02 (NS)	-0.01 (NS)	0.16 (NS)
HOMA1-%B	-0.06(NS)	0.02(NS)	0.06(NS)	0.00(NS)
HOMA β cell	-0.07 (NS)	0.08 (NS)	0.04 (NS)	-0.01 (NS)

- FBG: fasting blood glucose, HbA_{1c}: glycated hemoglobin, FI: fasting insulin, G/I: glucose / insulin ratio, FIRI: fasting insulin resistance index HOMA-IR: homeostasis model assessment of insulin resistance.
- Numbers outside parenthesis indicate r value, while inside parenthesis indicate p- value.
- *P* value is significant at < 0.05, NS: non-significant, *P* value > 0.05.

Table 5: Pearson's correlations between each hormones and MA, GA and BMI in normal and in GDM groups at 2nd and 3rd trimesters:

Hormones	Vs	2nd trimester		3rd trimester	
		Normal n=19	GDM n=25	Normal n=18	GDM n=26
RBP4	**MA**	0.32 NS	-0.52 (0.01)	-0.02 (NS)	0.28 (NS)
	GA	-0.20(NS)	0.66 (0.00)	-0.25 (NS)	-0.29 (NS)
	BMI	0.09(NS)	-0.09(NS)	0.13(NS)	-0.32(NS)
Vaspin	**MA**	0.07 (NS)	0.13 (NS)	0.17 (NS)	-0.39 (0.05)
	GA	0.10 (NS)	0.25 (NS)	-0.6 (NS)	-0.26 (NS)
	BMI	-0.01 (NS)	-0.32 (NS)	-0.17 (NS)	-0.53 (0.01)
Chemerin	**MA**	0.02 (NS)	-0.16 (NS)	-0.35 (NS)	-0.04 (NS)
	GA	-0.12 (NS)	-0.48 (0.02)	0.24 (NS)	0.26 (NS)
	BMI	-0.12 (NS)	-0.05 (NS)	0.20 (NS)	0.18 (NS)

- MA: maternal age, GA: gestational age, BMI: body mass index.
- Numbers outside parenthesis indicate r value, while inside parenthesis indicate p- value.
- *P* value is significant at < 0.05. NS: non-significant, *P* value > 0.05.

Table 6: Pearson's correlations between RBP4 and each of FBG, HbA_{1c} and markers of IR in normal and in GDM groups at 2nd and 3rd trimesters:

Parameters \ Groups	2nd trimester		3rd trimester	
	Normal n=19	GDM n=25	Normal n=18	GDM n=26
FBG	-0.16 (NS)	0.27 (NS)	-0.11 (NS)	0.20 (NS)
HbA_{1c}	0.26 (NS)	0.23 (NS)	-0.16 (NS)	0.34 (NS)
FI	0.17 (NS)	0.54 (0.01)	-0.16 (NS)	-0.18 (NS)
G/I	-0.15 (NS)	-0.54 (0.01)	0.08 (NS)	0.40 (0.05)
FIRI	0.13 (NS)	0.51 (0.01)	-0.18 (NS)	-0.15 (NS)
HOMA-IR	0.14 (NS)	0.53 (0.01)	-0.18 (NS)	-0.15((NS)
Log(HOMA-IR)	0.16 (NS)	0.60 (0.00)	-0.23 (NS)	-0.15 (NS)
HOMA1-%B	0.36 (NS)	0.49 (0.01)	0.04 (NS)	-0.42 (0.03)
HOMA β cell	0.23 (NS)	0.54 (0.01)	-0.04 (NS)	-0.36 (NS)

- FBG: fasting blood glucose, HbA_{1c}: glycated hemoglobin, FI: fasting insulin, G/I: glucose / insulin ratio, FIRI: fasting insulin resistance index, HOMA-IR: homeostasis model assessment insulin of resistance.
- Numbers outside parenthesis indicate r value, while inside parenthesis indicate p- value.
- *P* value is significant at < 0.05. NS: non-significant, *P* value > 0.05.

Table 7: Pearson's correlations between vaspin and each of FBG, HbA_{1c} and markers of IR in normal pregnancy and in GDM groups at 2nd and 3rd trimesters:

Parameters \ Groups	2nd trimester		3rd trimester	
	Normal n=19	GDM n=25	Normal n=18	GDM n=26
FBG	0.16 (NS)	0.20 (NS)	0.05 (NS)	-0.08 (NS)
HbA_{1c}	0.29(NS)	-0.25 (NS)	-0.02 (NS)	-0.12 (NS)
FI	-0.18 (NS)	-0.19 (NS)	0.12 (NS)	0.22 (NS)
G/I	0.20 (NS)	0.47 (0.02)	-0.20 (NS)	-0.16 (NS)
FIRI	-0.16 (NS)	-0.13 (NS)	0.14 (NS)	0.21 (NS)
HOMA-IR	-0.16 (NS)	-0.14 (NS)	0.14 (NS)	0.21 (NS)
Log(HOMA-IR)	-0.15 (NS)	-0.17 (NS)	0.16 (NS)	0.08 (NS)
HOMA1-%B	-0.19 (NS)	-0.20 (NS)	0.03 (NS)	0.40 (0.04)
HOMA β cell	-0.19 (NS)	-0.25 (NS)	0.05 (NS)	0.34 (NS)

- FBG: fasting blood glucose, HbA_{1c}: glycated hemoglobin, FI: fasting insulin, G/I: glucose / insulin ratio, FIRI: fasting insulin resistance index, HOMA-IR: homeostasis model assessment of insulin resistance.
- Numbers outside parenthesis indicate r value, while inside parenthesis indicate p- value.
- *P* value is significant at < 0.05. NS: non-significant, *P* value > 0.05.

Table 8: Pearson's correlations between Chemerin and FBG, HbA_{1c} and markers of IR in normal and in GDM groups at 2nd and 3rd trimesters:

Groups / Parameters	2nd trimester		3rd trimester	
	Normal n=19	GDM n=25	Normal n=18	GDM n=26
FBG	0.48 (NS)	-0.03 (NS)	0.23 (NS)	-0.45 (0.02)
HbA_{1c}	-0.43 (NS)	0.09 (NS)	0.11 (NS)	-0.23 (NS)
FI	0.21 (NS)	-0.11 (NS)	-0.09 (NS)	0.08 (NS)
G/I	-0.16 (NS)	0.04 (NS)	0.32 (NS)	-0.29 (NS)
FIRI	0.34 (NS)	-0.11 (NS)	-0.06 (NS)	0.03 (NS)
HOMA-IR	0.34 (NS)	-0.13 (NS)	-0.06 (NS)	0.03 (NS)
Log(HOMA-IR)	0.28 (NS)	-0.10 (NS)	-0.13 (NS)	-0.14 (NS)
HOMA1-%B	-0.21 (NS)	-0.09 (NS)	-0.20 (NS)	0.43 (0.03)
HOMA β cell	-0.12 (NS)	-0.08 (NS)	-0.18 (NS)	0.33 (NS)

- FBG: fasting blood glucose, HbA_{1c}: glycated hemoglobin, FI: fasting insulin, G/I: glucose / insulin ratio, FIRI: fasting insulin resistance index, HOMA-IR: homeostasis model assessment of insulin resistance.
- Numbers outside parenthesis indicate r value, while inside parenthesis indicate p- value.

P value is significant at < 0.05. NS: non-significant, *P* value > 0.05.

DISCUSSION

Insulin resistance plays a major role in the development of GDM. In the present study, in relation to control, pregnant women with GDM had significantly higher FBG, HbA1c and markers of IR in terms of FIRI, HOMA-IR, log (HOMA-IR), HOMA1 -% B and HOMA ß cell at 2nd and 3rd trimesters, indicating increased IR in GDM. These results were supported by other study [1] indicating higher IR in 2nd and 3rd trimesters of normal pregnancy, which was worsened in GDM.

Several trials were carried out by many researchers to explore the causes of increased IR in GDM. Among factors contributed to the increased IR during pregnancy was obesity [17]. In this study, it is worth mentioning to indicate that both normoglycemic and GDM groups were matched for BMI. In addition, no significant correlations were obtained between BMI and markers of IR, indicating that BMI was not a confounder in the elevated IR among the enrolled GDM subjects.

Numerous studies had implicated adipose tissue in the regulation of insulin resistance in both non-pregnant and pregnant individuals, through the excretion of adipocyte-derived hormones, the adipokines. In pregnancy in particular, adipokines seem to be implicated in maternal glucose metabolism and gestational insulin resistance [6]. The current investigation revealed significantly decreased maternal RBP4 in GDM group compared to normoglycemic at 2nd, but not at 3rd trimesters. Inconclusive results by other researchers on the relationship between circulating RBP4 in GDM in relation to normal pregnancy are existing. Different researches have reported increased RBP4 in pregnancies complicated with GDM compared to uncomplicated ones [18,19,20,21]. However, other report observed no significant difference between GDM and control for serum RBP4 [22]. Results concerning RBP4 obtained from the present study are in line with Kryzanowska et al. [23]. The authors attributed the reduced maternal RBP4 in GDM subjects to be due to decreased retinol. They suggested that RBP4/ retinol ratio may be appropriate for asserting metabolic syndrome during pregnancy. In the same study, retinol was found to be lower in GDM than control subjects. Furthermore; other researches indicated that RBP4 must bound to retinol to be secreted

from hepatocytes [24,25]. Therefore, decreased RBP4 in GDM may be linked to reduction in retinol levels. Other factors that might explain the inconsistency between available researches and might explain the decreased RBP4 in GDM could be differences in renal clearance, where increased renal clearance of RBP4 was reported to be a cause for reduced maternal circulating levels [26].

Most of the RBP4 in serum is bound to retinol. It is not known whether unbound apolipoprotein-RBP4 has different biological effects from bound RBP4. However, the proportion of RBP4 bound to retinol is decreased in pregnancy [27]. This altered RBP4/ retinol ratio in pregnancy may reflect the possibility that a ligand other than retinol can bind to RBP4 and that this 'alternative ligand' may be associated with features of the metabolic syndrome. Other retinoids, such as retinaldehyde, have metabolic effects and have been shown to bind to RBP4 [28]. Most of serum RBP4 is also bound to transthyretin, which prevents glomerular filtration of RBP4. C-terminal truncated forms of RBP4 are thought to have decreased binding affinity for transthyretin, leading to excretion in the urine. These forms, which are abundant in urine, have also been detected in serum and levels can vary in different metabolic states [29]. It is not known whether these forms of RBP4 have the same biological activity as full-length RBP4. Thus, total RBP4 could be an inaccurate measure of biologically active RBP4.

The employed methods for assessing RBP4 could not be ruled out as a factor that might affect the results obtained from different researches. RBP4 levels could be measured by different techniques such as ELISA, competitive enzyme immunoassay, western blot. The most reliable method is western blot technique [30]. However, it has been shown that ELISA is the most frequently preferred [31].

The current study demonstrated significant correlations between RBP4 and each of FI, G/I, FIRI and HOMA-IR in second trimester, but not in normoglycemic control group. This result may point out to the role of RBP4 in the pathogenesis of GDM. Yang et al. [25] demonstrated that the transgenic over expression of human RBP4 or injection of recombinant RBP4 in normal mice caused increased IR. RBP4 was suggested to be a factor that might increase the activity of gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, in hepatocytes. In addition, RBP4 also inhibits insulin receptor activity by blocking the insulin-stimulated phosphorylation of insulin receptor substrate-1 at serine in position 307. These actions of RBP-4 have been proposed to increased IR [25,32,33].

Although vaspin is an insulin sensitizing adipokine, which might be implicated in the regulation of maternal glucose, this study did not obtain significant difference between maternal vaspin in GDM compared to matched normal pregnancy, both, at 2nd and 3rd trimesters. On the other hand, no significant associations between vaspin and each of FBG, FI and markers of IR were obtained in either GDM or normal pregnancy, at 2nd or at 3rd trimesters. Other contradictory findings are existing, where maternal vaspin levels in GDM were reported to be significantly elevated in some studies [34,35], but not all [36,37].

Circulating vaspin is known to reflect its expression in adipose tissue [38]. In T2DM, the available literature reported either higher [39,40] or similar vaspin levels [41,38,42] compared to normoglycemic controls. Similarly, inconsistent results indicated that vaspin levels were positively correlated with FI and HOMA-IR in T2DM [38], in induced weight loss [43] and in obese children [44]. Others reported no significant association between vaspin and IR in T2DM [42] and in GDM [36].

Variations in characteristics of the investigated subjects in the different studies might contribute to the diverse findings. Differences in GA might represent an explanation for inconsistency between literatures including ours. Since during pregnancy, vaspin expression in the placenta has been reported to increase gradually, reaching the highest levels at the end of gestation [45]. Glycemic control in GDM, either dietary or using anti- diabetic treatment, might also affect vaspin levels. A study carried out by Tan et al. [46] indicated that diabetic women under metformin treatment or PCOS had significantly lower vaspin than control.

Differences in race [47] and in BMI [44,47] as well as physical training [38,47], few days of life style modifications [47] or even type of exercise as exercise – induced oxidative stress [48], all might contribute to inconstant result in literature.

In the present investigation, vaspin was not correlated with BMI or FI. In previous studies conducted on normal or obese subjects, positive correlations between vaspin and BMI, FI and IR as estimated by HOMA-IR were noted [44,47]. Other reports [42,47] detected negative or even no correlations [49,50]. Differences in sample size, population characteristics or presence of concomitant disease that might affect glucose metabolism or methodology differences were attributed to be responsible for discrepancies in literature.

Giomisi et al. [51] investigated vaspin levels at 24 – 30 weeks and found no relation with BMI, maternal blood glucose, FI and IR at later GA. The authors suggested to extend their study to include advanced GA > 30 weeks to explore the differences in vaspin levels between GDM and control. The present investigation was carried out both at early (14 - 26) and at late (29 - 39) gestations, and still no significant differences in vaspin levels between normoglycemic control and GDM at both examined trimesters. However, at late gestation, vaspin was correlated with MA, BMI and HOMA1-% B (as a marker of IR). In line with our observation, Gkiomisi et al. [37] pointed out to the existence of correlations between vaspin and IR at 3rd trimesters in women with GDM but not with controls. The authors were not able to obtain such correlations at 2nd trimesters.

In the current study, maternal serum chemerin in GDM was comparable to GA and BMI matched normal controls at 2nd trimesters. However, at 3rd trimester chemerin was significantly elevated in pregnancy complicated with GDM relative to normoglycemic pregnancy. In line with the present data Nazarian et al. [52] and Li [53] reported higher serum chemerin in GDM during 3rd trimester compared to normal glucose tolerance. Same results were obtained between normal weight GDM and normal weight pregnancy groups [54]. The authors found no significant differences in CMKIRI mRNA expression between the two groups, suggesting that GDM may be a factor resulting in elevated serum chemerin. However, other researches [55] reported decreased serum chemerin in GDM compared to control, finding that was further supported by Li et al. [54] indicating decreased chemerin in GDM at 3rd trimester. Other contradictory results indicated unaltered maternal chemerin in GDM at 3rd trimester relative to normal glucose tolerance group [56,57,58]. Although the precise reason for such discrepancy is unclear, however, various reasons have been postulated by many researchers.

Finding by Barker et al. [57] indicated that GDM group managed with dietary modification alone had comparable maternal chemerin mean value to control group at term. Pfau et al. [56] study suggested that circulating chemerin may increase in a more severe form of GDM. In animal model induction of IR followed by treatment with metformin decreases chemerin expression [59]. The last studies indicated that control and severity of GDM might be a factor affecting maternal chemerin levels in GDM. Li et al. [54] found that obese normoglycemic pregnant women had higher chemerin relative to normal weight pregnant controls. This finding let the authors to suggest that obesity may contribute to elevated chemerin. Additionally, in the same study, normal and overweight GDM had higher chemerin levels than normal and overweight controls, although no difference was found between obese GDM and obese- control for chemerin. In the last study, chemerin was determined 24- 48 hours before delivery. The authors suggested that GDM might be responsible for elevated chemerin in GDM before delivery. However, this was not the same in other studies [57,59].

Sampling time may be another reason for discrepancy in literature concerning maternal chemerin in GDM. Blood samples were collected once in each study at different duration during pregnancy. One study was carried out 24 – 48 hours before delivery [54]. Other study [58] was carried in the 3rd trimester and 48 hours after delivery. The present study measured chemerin levels in 2nd and 3rd trimesters. Additionally, in this study we had a wide range of BMI, and we did not classify the included groups into overweight (BMI: $\geq 25 < 30$ Kg / m^2) and obese (BMI ≥ 30 Kg / m^2) which might also mask the significance between GDM and control at 2nd trimester.

As was described by Li et al. [54], women with normal weight GDM had 3 folds higher chemerin than normal weight control, and overweight GDM had only 50 % higher levels than overweight control. An interesting finding proved that circulating chemerin was down - regulated by dietary restriction or exercise, while elevated by refeeding [60,61,62]. Therefore, it might be reasonable that chemerin was reduced to values comparable to control at 2nd trimester during dietary restriction as soon as GDM developed, and sampling time was during dietary intervention.

In the present investigation no significant correlations were detected between maternal chemerin and each of FBG, HbA1c, FI, HOMA-IR or log (HOMA-IR), only significant associations with FBG and HOMA1-% B were obtained

at 3rd trimester. Absence of significant correlations with IR might prove the suggestion made by Li et al. [54] that GDM might be a cause for elevated chemerin.

Different studies have shown that pregnancy is a pro- anti-inflammatory state depending on GA [63], and IR is associated with high levels of TNF-α [64,65]. The expression and release of chemerin by adipocytes was up regulated by TNF-α [66]. It was also postulated that GDM subjects have pronounced elevation in TNF-α both at 2nd and 3rd trimesters [67,68,69]. Therefore, obese GDM women are expected to have higher chemerin levels compared to normal obese pregnant women; as was obtained in the present study at 3rd trimester. Chemerin blood level is mainly elevated due to chemerin producing organ such as adipose tissue, liver [70]. Although it is well known that chemerin has an inhibitory effect on uptake of glucose by adipose tissue [70] however, others reported increasing insulin stimulated glucose uptake by 3T3 adipocytes by chemerin [71]. Therefore, chemerin may have different actions. The role of chemerin in GDM is not established yet and the mechanism of action and its relationship with IR and FBG is not well known.

The present study revealed significantly higher FBG, HbA1c and markers of IR in GDM, at 2nd and 3rd trimesters, compared to matched normoglycemic control. At 2nd trimester, pregnancies complicated with GDM had decreased RBP4 compared to normal pregnant control group. No significant differences were noted in maternal vaspin and chemerin levels between GDM and control. At 3rd trimester, chemerin showed higher circulating levels in GDM relative to matched control, while other studied hormones showed no variations. It is worth mentioning to indicate that most enrolled GDM cases were managed with dietary control, while only 7 cases were under insulin treatment, indicating the inclusion of mild GDM cases which is evident from mean values of FBG (falls within normal value). In addition, all studied groups, either control or GDM, included wide range of BMI ranging from overweight (BMI: ≥ 25 to < 30 Kg / m²) and obese (BMI: ≥ 30 Kg/ m²). Since both severity of GDM and BMI are known to affect levels of the studied hormones, therefore the non- significant variations appeared between GDM and control at 2nd and at 3rd trimesters for some hormones might be due to inclusion of controlled GDM and a wide range of BMI. An important note is that, in GDM subjects, sampling time was carried out once during management of blood glucose, which might mask the change in the studied maternal circulating hormones.

REFERENCES

1- Barbour, L.A., McCurdy, C.E., Hernandez, T.L., et al. (2007): Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diab Care. 30(Suppl. 2), S112–S119.

2- Briana, D.D.and Malamitsi-Puchner, A. (2009): Reviews: adipocytokines in normal and complicated pregnancies, ReprodScien. 16: 921- 937.

3- Butte, N.F. (2000): Carbohydrate and lipid metabolism in pregnancy:normal compared with gestational diabetes mellitus. Am J ClinNut. 71: 1256S- 1261S.

4- Catalano, P.M., Tyzbir, E.D., Wolfe, R.R., Calles, J., Roman, N.M., Amini, S.B. and Sims, E.A. (1993): Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. Am J Physiol. 264: 60–67.

5- Catalano, P.M., Huston, L., Amini, S.B. and Kalhan, S.C. (1999): Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. Am J Physiol. 180: 903–916.

6- Miehle, K., Stepan, H. and Fasshauer, M. (2012): Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. ClinEndocrinol. 76: 2–11.

7- Esteve, E., Ricart, W. and Fernandez-Real, J.M. (2009): Adipocytokines and insulin resistance: the possible role of lipocalin-2, retinol binding protein-4, and adiponectin. Diab Care. 32: S362–S367.

8- Hida, K.,Wada, J., Eguchi, J., Zhang, H., Baba, M., Seida, A., Hashimoto, I., Okada, T., Yasuhara, A., Nakatsuka, A., Shikata, K., Hourai, S., Futami, J., Watanabe, E., Matsuki, Y., Hiramatsu, R., Akagi, S., Makino, H., and

Kanwar, Y.S. (2005): Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. PNAS. 102: 10610–10615.

9- Aust, G.; Richter, O.; Rohm, S., Kerner, C., Hauss, J., Klo¨ting, N., Ruschke, K., Kovacs, P., Youn, B.S. and Blu¨ her, M. (2009): Vaspin serum concentrations in patients with carotid stenosis. Atherosclero. 204 262–266.

10- Wittamer, V. et al. (2003): Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. J Exp Med. 198: 977– 985.

11- Bozaoglu, K., et al. (2007): Chemerin is a novel adipokine associated with obesity and metabolic syndrome. Endocrinol. 148:4687–94.

12- Goralski, K.B., et al. (2007): Chemerin: A novel adipokine that regulates adipogenesis and adipocyte metabolism. J Biol Chem. 282: 28175–88.

13- Roh, S.G., et al. (2007): Chemerin—A new adipokine that modulates adipogenesis via its own receptor. BiochemBiophys Res Commun. 362:1013–8.

14- Tönjes, A., Fasshauer, M., Kratzsch, J., Stumvoll, M., Blüher, M. (2011): Adipokine Pattern in Subjects with Impaired Fasting Glucose and Impaired Glucose Tolerance in Comparison to Normal Glucose Tolerance and Diabetes. Eur J clin Invest.41: 703- 09.

15- World Health Organization WHO. (1999): Consultation: Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications: Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus, Geneva, WHO/NCD/ NCS/99.2.

16- Singh, B. and Saxena, A. (2010): Surrogate markers of insulin resistance: A review. World J Diabetes. 15; 1(2): 36- 47

17- Warnick, G. R., Benderson, J. and Albers, J. J. (1982): Dextran sulfate-Mg21 precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem. 28:1379–88.

18- Su, Y.X., Hong, J., Yan, Q., et al. (2010): Increased serum retinol-binding protein-4 levels in pregnant women with and without gestational diabetes mellitus. Diabetes Metab. 36: 470–475

19- Abetew, D.F., Qiu, C., Fida, N.G., et al. (2013): Association of retinol binding protein 4 with risk of gestational diabetes. Diab Res ClinPract. 99: 48–53.

20- Du, M., Wang, B., Liang, Z., Dong, M. and Chen, D. (2015): The Relationship between Retinol-Binding Protein 4 Concentrations and Gestational Diabetes Mellitus in Chinese Women. GynecolObstet Invest. ISSN: 0378-7346.

21- Huang, Q. T., Huang, Q., Luo, W., Li, F., Hang, L., Yu, Y.H. and Zhong, M. (2015): Circulating retinol-binding protein 4 levels in gestational diabetes mellitus: a meta-analysis of observational studies. Obstet Gynecol. 31: 337-344.

22- Tepper, B.J., Kim, Y.K., Shete, V., Shabrova, E. and Quadro, L. (2010): Serum retinol-binding protein 4 (RBP4) and retinol in a cohort of borderline obese women with and without gestational diabetes. ClinBiochem. 43: 320–323.

23- Krzyzanowska, K., Zemany, L., Krugluger, W., et al. (2008): Serum concentrations of retinol-binding protein 4 in women with and without gestational diabetes. Diabetol. 51: 1115–1122.

24- Bellovino, D., Apreda, M., Gragnoli, S., Massimi, M., and Gaetani, S. (2003): Vitamin A transport: in vitro models for the study of RBP secretion. Mol Aspects Med. 24:411–420.

25- Yang, Q., Graham, T.E., Mody, N., et al. (2005): Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nat. 436: 356 – 362

26- Cheung, C.K., Lao, T. and Swaminathan, R. (1989): Urinary excretion of some proteins and enzymes during normal pregnancy. Clin Chem. 35:1978–1980.

27- Sapin, V., Alexandre, M.C., Chaib, S., et al. (2000): Effect of vitamin A status at the end of term pregnancy on the saturation of retinol binding protein with retinol. Am J ClinNutr 71:537 – 543.

28- Ziouzenkova, O., Orasanu, G., Sharlach, M., et al. (2007): Retinaldehyde represses adipogenesis and diet-induced obesity. Nat Med. 13:695 –702.

29- Kiernan, U.A., Tubbs, K.A., Nedelkov, D., Niederkofler, E.E. and Nelson, R.W. (2002): Comparative phenotypic analyses of human plasma and urinary retinol binding protein using mass spectrometric immunoassay. BiochemBiophys Res Commun. 297: 401–405.

30- Graham, T.E., Wason, C.J., Blüher, M. and Kahn, B.B. (2007): Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetol. 50: 814–823.

31- Christou, G.A., Tselepis, A.D. and Kiortsis, D.N. (2012): The metabolic role of retinol binding protein 4: An update. HormMetab Res. 44: 6–14.

32- Polonsky, K.S. (2006): Retinol-binding protein 4, insulin resistance, and type 2 diabetes. N Engl J Med. 354: 2596 – 2598.

33- Ost, A., Danielsson, A., Liden, M., Eriksson, U., Nystrom, F.H. and Stralfors, P. (2007): Retinol-binding protein-4 attenuates insulin-induced phosphorylation of IRS1 and ERK1/2 in primary human adipocytes. FASEB J. 21: 3696–3704.

34- Mm, W.Q., Fan, J., Khor, s., Song, M., Hong, W. and Dai, X. (2014): Serum vaspin levels and vaspin mRNA expression in subcutaneous adipose tissue in women with gestational diabetes mellitus. Eur J ObstetGynecolReprod Biol. 182: 98 – 101.

35- Jia, X., Wang, S., Ma, N., Li, X., Guo, L., Liu, X., Dong, T., Liu, Y. and Lu, Q. (2015): Comparative analysis of vaspin in pregnant women with and without gestational diabetes mellitus and healthy non-pregnant women. Endo. 48: 533 – 540.

36- Stepan, H., Kralisch, S., Klostermann, K., Schrey, S., Reisenbüchler, C., Verlohren, M., et al. (2010): Preliminary report: circulating levels of the adipokinevaspin in gestational diabetes mellitus and preeclampsia. Met.59:1054 – 6.

37- Gkiomisi, A., Kali, G., Makedou,,Athanasios, D., Anastasilakis, Stergios, A., Polyzos, Kourtis, A., Gerou, S., Gavana, E., Dagklis, T. and Rousso, D. (2013): Serum vaspin levels in women with and without gestational diabetes mellitus during pregnancy and postpartum. Cyto. 61: 127–132

38- Youn, B., Kloting, N., Kratzsch, J., Lee, N., Park, J.W., Song, E., et al. (2008): Serum vaspin concentrations in human obesity and type 2 diabetes. Diab.57: 372 – 7.

39- El-Mesallamy, H.O., Kassem, D.H., El-Demerdash, E. and Amin, A.I. (2011): Vaspin and visfatin/ Nampt are interesting interrelated adipokines playing a role in the pathogenesis of type 2 diabetes mellitus. Met. 60: 63–70.

40- Teshigawara, S., Wada, J., Hida, K., Nakatsuka, A., Eguchi, J., Murakami, K., et al. (2012): Serum vaspin concentrations are closely related to insulin resistance, and rs77060950 at SERPINA12 genetically defines distinct group with higher serum levels in Japanese population. J ClinEndocrinolMetab. 97: E1202-7.

41- Seeger, J., Ziegelmeier, M., Bachmann, A., Lossner, U., Kratzsch, J., Bluher, M., et al. (2008): Serum levels of the adipokinevaspin in relation to metabolic and renal parameters. J ClinEndocrinolMetab. 93: 247–51.

42- Gulcelik, N.E., Karakaya, J., Gedik, A., Usman, A. and Gurlek, A. (2009): Serum vaspin levels in type 2 diabetic women in relation to microvascular complications. Eur J Endocrinol. 160:65–70.

43- Handisurya, A., Riedl, M., Vila, G., Maier, C., Clodi, M., Prikoszovich, T. et al. (2010): Serum vaspin concentrations in relation to insulin sensitivity following RYGBinduced weight loss. Obes Surg. 20:198–203.

44- Suleymanoglu, S., Tascilar, E., Pirgon, O., Tapan, S., Meral, C. and Abaci, A. (2009): Vaspin and its correlation with insulin sensitivity indices in obese children. Diab Res Clin Pract.84: 325- 8.

45- Caminos, J.E., Bravo, S.B., Garcés, M.F., González, C.R., Cepeda, L.A., González, A.C. et al. (2009): Vaspin and amylin are expressed in human and rat placenta and regulated by nutritional status. Histol Histopathol.24: 979–90.

46- Tan, B.K., Heutling, D., Chen. J., Farhatullah. S., Adya, R., Keay, S.D., et al. (2008): Metformin decreases the adipokinevaspin in overweight women with polycystic ovary syndrome concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. Diab.57:1501–7.

47- Lee, M.K., Jekal, Y., Im, J.A., Kim, E., Lee, S.H., Park, J.H., et al. (2010): Reduced serum vaspin concentrations in obese children following short-term intensive lifestyle modification. ClinChimActa. 411: 381–5.

48- Ghahramani, M., Rohani, H. and Ghiasi, A. (2012): Post-Resistance Exercise Response of VaspinAdipocytokin and its Relation to Insulin and Glucose Levels in Overweight Women. Middle-East J Scien Res. 11: 1328-1334.

49- Ye, Y., Hou, X.H., Pan, X.P., Lu, J.X. and Jia, W.P. (2009): Serum vaspin level in relation to postprandial plasma glucose concentration in subjects with diabetes.Chin Med J. 122: 2530 - 33.

50- Von Loeffelholz, C., Möhlig, M., Arafat, A.M., Isken, F., Spranger, J., Mai, K., et al. (2010): Circulating vaspin is unrelated to insulin sensitivity in a cohort of nondiabetic humans. Eur J Endocrinol.162:507–13.

51- Giomisi, A., Kourtis, A., Toulis, K.A., Anastasilakis, A.D., Makedou, K.G., Mouzaki, M., et al. (2011): Serum vaspin levels in normal pregnancy in comparison with nonpregnant women. Eur J Endocrinol. 164: 579–83.

52- Nazarian, A., Niknam, F., Mazloumzadeh, S., Kashanian, N. and Mazloumi, S. (2013): The Relation of Serum Chemerin Levels with Parameters of Metabolic Syndrome between Pregnant Women with Gestationl Diabetes and Normal Pregnant Women. Iran J Endocrinol Meta. 14: 423 – 429.

53- Li, X. (2013): Serum Chemerin Levels and Relationship between Chemerin and Insulin Resistance in Women with Gestational Diabetes Mellitus. ObstetGynaecol.

54- Li, X.M., Ji, H., Li, C., Wang, P.H., Yu, P. and Yu, D. (2015): Chemerin expression in Chinese pregnant women with and withoutgestational diabetes mellitus. Ann d'Endocrinol. 76: 19–24.

55- Hare, K. J., Bonde, L., Svare, J. A., Randeva,H. S., Asmar, M., Larsen, S., Vilsbøll T. and Knop, F. K.(2014): Decreased plasma chemerin levels in women with gestational diabetes mellitus. Diabet Med. 31: 936–940.

56- Pfau, D., Stepan, Kratzsch, Verlohren, Verlohren, Drynda, Lossner, Bluher, Stumvoll, Fasshauer. (2010): Circulating Levels of the AdipokineChemerin in Gestational Diabetes Mellitus. Horm Res Paediatr.74: 56 – 61.

57- Barker, G., Lim, R., Rice, G.E. and Lappas, M. (2012): Increased chemerin concentrations in fetuses of obese mothers and correlation with maternal insulin sensitivity. J MaternFetalal Neonatal Med. 25: 2274–2280.

58- Van Poppel MN, Zeck W, Ulrich D, Schest EC, Hirschmugl B, Lang U, et al. (2014): Cord blood chemerin: differential effects of gestational diabetes mellitusand maternal obesity. ClinEndocrinol (Oxf). 80:65–72.

59- Pie, L., Yang, J., Du, J., Liu, H., Ao, N. and Zhang, Y. (2012): Downregulation of chemerin and alleviation of endoplasmic reticulum stress by metformin in adipose tissue of rats. Diabetes Res ClinPract. 97: 267 – 275.

60- Ress, C., Tschoner, A., Engl, J., Klaus, A., Tilg, H., Ebenbichler, C.F., et al. (2010): Effect of bariatric surgery on circulating chemerin levels. Eur J Clin Invest. 40: 277 – 80.

61- Chakaroun, R., Raschpichler, M., Klöting, N., Oberbach, A., Flehmig, G., Kern, M., et al. (2012): Effects of weight loss and exercise on chemerin serum con-centrations and adipose tissue expression in human obesity. Metab. 61:706–14.

62- Stelmanska, E., Sledzinski, T., Turyn, J., Presler, M., Korczynska, J. and Swier-czynski, J. (2013): Chemerin gene expression is regulated by food restriction andfood restriction-refeeding in rat adipose tissue but not in liver. Regul Pep.181:22–9.

63- Hart R, and Greaves D.R. (2010): Chemerin contributes to inflammation by promoting macrophage adhesion to VCAM-1 and fibronectin through clustering of VLA-4 and VLA-5. J Immunol. 185: 3728–3739.

64- Sell, H., Eckel, J. and Dietze-Schroeder, D. (2006): Pathways leading to muscle insulin resistance—the muscle-fat connection. Arch PhysiolBiochem. 112: 105–113.

65- Sell, H. and Eckel, J. (2007): Monocyte chemotactic protein-1 and its role in insulin resistance. CurrOpinLipidol. 18: 258 – 262.

66- Arner P. (2003): The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. Trends EndocrinolMetab. 14:137–145.

67- Ategbo, J.M., Grissa, O., Yessoufou, A., Hichami, A., Dramane, K.L., Moutairou, K., Miled, A., Grissa, A., Jerbi, M., Tabka, Z. andKhan, N.A. (2006): Modulation of adipokines and cytokines in gestational diabetes and macrosomia. J ClinEndocrinol Met. 91: 4137-4143.

68- Gao, X.L., Yang, H.X. and Zhao, Y. (2008): Variations of tumor necrosis factor-alpha, leptin and adiponectin in mid-trimester of gestational diabetes mellitus. Chin Meical J Eng.121: 701–705.

69- Noureldeen, A.F.H., Qusti, S. Y., AL-Seeni, M.N. and Bagais, M. H. (2014): Maternal leptin, adiponectin, resistin, visvatin and tumor necrosis factor- alpha in normal and gestational diabetes. Ind J ClinBioch. 29: 462 - 470.

70- Sell, H., Laurencikiene, Taube, Eckardt, Cramer, Horrighs, Arner, Eckel. (2009): Chemerin is anovel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. Diab.58: 2731– 40.

71- Takahashia, M., et al. (2008): Chemerin enhances insulin signaling and potentiates insulin-stimulated glucose uptake in 3T3-L1 adipocytes. FEBS Letters.582: 573– 8.