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Molecular communication between Apelin-13, Apelin-36, Elabela, and nitric oxide in gestational diabetes mellitus

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Abstract. – OBJECTIVE: Gestational diabetes mellitus (GDM) is a type of diabetes that affects from 3.8% to 6.9% of pregnancies worldwide, causing significant mortality and unfavorable obstetric outcomes, such as delivery trauma and macrosomia risk. The fundamental processes of this metabolic disorder that first appeared during pregnancy are still unknown. Tissue hormones, particularly adipokines, have aided in understanding the pathophysiology of numerous disorders in recent years. This study aims to determine if Apelin-13 (APLN-13), Apelin-36 (APLN-36), Elabela (ELA), and nitric oxide (NO) molecules have all a part in the pathophysiology of GDM.

PATIENTS AND METHODS: The study included 30 pregnant control women and 30 pregnant women who had been diagnosed with GDM in the second trimester and whose body mass index and age were compatible with each other. Blood samples were collected from 60 participants during the second trimester (30 control pregnant women and 30 GDM pregnant women) and postpartum (17 controls *vs.* 14 GDM). In these blood samples, the amounts of APLN-13, APLN-36, ELA, and NO were studied using the ELISA method. In addition, the participants' glucose, lipid profiles, and other parameters were obtained from the hospital record files. At postpartum, 29 pregnant women (13 control and 16 pregnant women with GDM) dropped out of the study without explanation.

RESULTS: In the second trimester and postpartum plasma of mothers with GDM, APLN-13, APLN-36, NO, and ELA molecules were found to be significantly higher (< 0.05), compared to those of the control mothers, while APLN-13, APLN-36, NO values were significantly lower (0.05). While APLN-13, APLN-36, NO amounts in mothers with GDM were positively correlated with glucose amounts, they were negatively correlated with ELA amounts. Similarly, the triglyceride amounts in mothers with GDM were positively correlated with APLN-13, APLN-36 and NO, while they were negatively correlated with the ELA amounts. Due to gestational diabetes, APLN-13, APLN-36, NO, glucose, and triglyceride increased, and ELA decreased.

CONCLUSIONS: It is predicted that the glucose increase in GDM is because Apelins reduce glucose transport to erythrocytes by inhibiting the sodium-dependent glucose transporter (SGLT) and that the increase in triglyceride and NO may be associated with high glucose levels in GDM. As a result, we believe that the above-mentioned chemicals may cause GDM Pathology by triggering one another.

Key Words:
Apelin-13, Apelin-36, Elabela, Gestational diabetes mellitus, Nitric oxide.

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Introduction

Gestational diabetes mellitus (GDM) is a glucose intolerance first described in pregnant women without previous diabetes, and its prevalence (which can vary from 3.8 to 6.9%, depending on the data source) is increasing day by day worldwide[1]. Today, the following is a current explanation of the potential mechanism of GDM formation. The pancreatic reserve is insufficient in women with GDM, and there is no compensatory increase in insulin release. Postprandial hyperglycemia develops due to beta-cell dysfunction and reduced early-phase insulin response caused by GDM, and fasting hyperglycemia develops due to the failure to inhibit hepatic glucose synthesis. Furthermore, because maternal insulin cannot pass through the placenta, even if more insulin is secreted from the pancreas to eliminate this condition, the fetus is exposed to hyperglycemia, and it is assumed that insulin causes many complications in infants, including macrosomia, as a result of its anabolic effects[2,3].

However, it is suggested that in highly organized living beings, whether pregnant or not, a vast number of tissue hormones (adipokines and myokines) other than insulin and glucagon control carbohydrate and energy metabolism, and that adipokines and myokines play a role in the etiopathology of GDM[4]. For example, it is reported that GDM resistin, visfatin, copeptin, adropin, obestatin, preptin, ghrelin, subfatin, spexin, hepcidin, and insulin increase in circulation while the amounts of apelin, ghrelin, salusin, nesfatin-1, and irisin decrease or not change[5-13].

Elabela (ELA), a hormone that increases glucose intake and insulin sensitivity by acting on the apelin receptor (APJ), has recently been revealed to function in glucose homeostasis[14,15]. Patients with Type 2 Diabetes Mellitus (T2DM) have lower levels of this hormone in their bloodstream due to diabetes[16,17]. In zebrafish embryos, this hormone was discovered as an APJ ligand[18]. It was later discovered[19] in the prostate, placenta, heart endothelium, blood vessels, hepatic cells, and hepatic, renal cells.

Although studies[20-22] have shown that circulating APLN levels rise with Type 1 Diabetes Mellitus (T1DM) and T2DM, there is no consensus on whether or not GDM raises APLN levels[10,23,24]. Some researchers say[23,24] apelins with GDM and diabetes mellitus decrease or remain unghanged, while others[10,25] claim they actually increase[26]. Apelins have different forms in many biological fluids[27]. The two most important biological and physiological forms are apelin-13 (APLN-13) and apelin-36 (APLN-36)[28]. However, diabetes research has not pinpointed which form is actively studied, and the literature is ambiguous. APLN is an endogenous ligand of the APJ, and it manifests its effects by binding to APJ[29]. Apelins also show their physiological effects using the nitric oxide (NO) molecule[21]. There is no thorough understanding of the association between NO levels and diabetes. According to some researchers[30,31], NO decreases because of diabetes while others[32,33] report it increases. NO reduction has also been reported to damage small and large blood vessels[34]. Using NO inhalation to treat neonatal pulmonary hypertension significantly improve oxygen supply by reducing pulmonary artery pressure[35].

ELA, apelins, and NO are synthesized in cardiac endothelium, blood vessels, hepatic cells, and renal cells[19]. The ability to produce NO has also been documented in macrophages and pancreatic islet cells, brain astrocytes, and platelets[36-39]. As mentioned above, ELA provides glucose homeostasis by increasing glucose intake and insulin sensitivity[40]. While performing this biological task, ELA activates NO molecules through apelins[41].

As we mentioned earlier, although GDM is a condition defined by carbohydrate metabolism disorders[42], we have yet to find a study in our exhaustive literature analysis that suggests that APLN, ELA and NO may play a role together in this disease. Furthermore, diabetic studies do not identify exatly which version of APLN is being studied, and there is no agreement on whether it is rising or decreasing, leading to uncertainty. There is also no consensus on whether NO levels rise or fall due to diabetes[30-33]. In a more controlled study, we aimed to determine the amounts of ELA, APLN-13, APLN-36, and NO in the blood of mothers with GDM in their second trimester (24-28 week) and at postpartum using the ELISA method for the first time and reveal whether these parameters interact with each other in this disease.

Patients and Methods

The study was carried out by obtaining the written consent of the non-invasive Kafkas University Local Ethics Committee following the information given to the participants verbally

about the study with the number 80576354-050-99/01 and the decision dated 12/13/2017, observing the Ethical Principles of the World Medical Association (WMA) Declaration of Helsinki. The study comprised pregnant women who applied to Gynecology and Obstetrics Polyclinics in their second trimester (weeks 24-28). Criteria for inclusion in the study are as follows: being 21 years of age or older; not having been diagnosed with T1DM or T2DM before pregnancy, having a singleton pregnancy. Anamnesis was used to record the existence of the chronic disease, body mass index (BMI), weight gain during pregnancy, having a GDM screening test, having GDM in a prior pregnancy, and GDM risk factors[42].

Oral glucose tolerance test (OGTT) was performed based on the diagnostic criteria of the pregnancy diabetes[43]. All pregnant women who agreed to participate were tested for fasting blood sugar (FBS) after at least eight hours of fasting, and then administered 75 grams of glucose, according to current GDM diagnostic criteria. Then, blood glucose levels were checked again at the 1st and 2nd hours. In normal cases, fasting blood glucose, 1st- and 2nd-hour blood glucose are < 92/180/153mg/dL, respectively. If one of these three values was high, the pregnant woman was GDM. Thirty pregnant women with GDM who met this criterion and whose glucose levels could be kept under control by diet (Dietary content: 40-50% complex high-fiber carbohydrate, 15-30% protein, 20-35% fat) were included in the study (pregnant women who needed metformin or insulin to eliminate the effect of the drug on the study parameters were not included in the GDM group). After 75 g of glucose loading, the 1st-hour plasma glucose (PG) threshold value was accepted as 140 mg/dL. Fasting blood glucose ≥ 140 mg/dL was found to be sufficient for the diagnosis of GDM, and in this case, OGTT was not performed.

The control group consisted of 30 volunteers who did not have a history of GDM in their previous pregnancy, who did not have a diagnosis of glucose intolerance before pregnancy, who did not have T2DM in their first-degree relatives, did not have a BMI > 25 kg/m² before pregnancy, who did not have a history of infants with large for gestational age (LGA) (> 4000 gr) in their previous pregnancy, who did not have a bad perinatal history (missed abortion, malformation, polyhydramnios, dead or preterm birth), who did not gain 20 kg or more in their previous pregnancy, who did not have a history of the polycystic ovarian syndrome, and who had a fasting blood sugar < 95 mg/dL and had a healthy medical pregnancy. In addition, these pregnant participants were followed up on until term. 5 mL of blood was drawn into biochemistry tubes containing aprotinin with EDTA when GDM was identified in GDM and control moms and at birth (only vaginal delivery). It was centrifuged at 4,000 rpm and the obtained samples were stored at -40°C until they were analyzed[44].

Biochemical Analyses

APLN-13 (Human APLN, Catalog no: EH2174 Fine Biotech Co., Ltd., Wuhan, China), APLN-36 (Human ELISA Kit, Catalog no: 201122038, Sun-Red, Shanghai, China), ELA (Human Elabela, Catalog no: S1508 Peninsula Laboratories International, Inc; San Carlos, CA, USA) and NO amounts (Human NO, catalog no: 201-12-1511 Sunred Biological Technology Co., Ltd., Shanghai, China) were studied following the working procedures specified in the kit catalogs by ELISA method. APLN-36, APLN-13, ELA and NO measurement ranges were 0.01-4 ng/mL, 62.5-4000 pg/mL, 0-100 ng/mL and 4-600 µmol/L, respectively. APLN-36, APLN-13 and NO sensitivities were 0.09 ng/mL, 37.5 pg/mL, 2.052 µmol/L, intra-Assay CV < 10%, < 10%, < 8% and inter-Assay CV values were 15%, 12% and 10%, respectively. Automatic washer Bio-Tek ELX50 (BioTek Instruments, Winooski, The USA) was used for plate washes, and ChroMate and Microplate Reader P4300 devices (Awareness Technology Instruments, Palm City, FL, USA) were used for absorbance readings. Glucose, HbA1c, hemoglobin, Triglyceride (TG), Total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), VLDL cholesterol (VLDL-C) values were obtained from the hospital registration files of the participants. The insulin resistance test (HOMA-IR) was taken after 8-10 hours of fasting and the level of fasting insulin was multiplied by each other and divided by 405, thus calculating the level of insulin resistance called homeostasis model assessment-estimated insulin resistance (HOMA-IR)[45].

Statistical Analysis

The analyzes were performed with the Statistics for Social Sciences (SPSS 22, IBM Corp., Armonk, NY, USA) package program. When analyzing the differences between the measured values of the two groups they seemed to not comply with the normal distribution, Mann-Whitney

Table I. Comparison of demographic characteristics and birth weights of babies with GDM and control mothers.

Parameters	Control	GDM	*p* value
Age (year)	32.8 ± 5.9	30.8 ± 6.48	0.652
BMI (kg/m^2)	[a]26.4 ± 2.35 / [b]29.14 ± 2.22	[a]26.9 ± 1.8 / [b]31.44 ± 2.2	0.354
Pregnancy (week)	37.7 ± 1.98	36.94 ± 1.23	0.776
Baby weight (g)	2987 ± 104	3418 ± 125	0.05

[a], Body mass index in the second trimester; [b], Body mass index at postpartum. Mean ± standard deviation. Statistical significance.

U-test was used and when they did, Student's *t*-test was used. The one-way ANOVA test was applied to determine whether there was a statistically significant difference between the mean values of the independent groups. Spearman's correlation test was used for correlation analysis. In the statistical analysis, *p*-values lower than 0.05 were considered statistically significant.

Results

Initially, this study was started with 60 pregnant women (30 controls and 30 GDM) in the second trimester, but only 31 pregnant women remained until delivery (17 controls, 14 GDM). There was only one cesarean section in the healthy group and thus she was excluded, while 12 of them dropped out of the study without giving any reason (13 people left the study in total at postpartum). In the group with GDM, 3 were excluded from the study due to cesarean section while 13 pregnant women dropped out of the study without explanation (14 people left the study in total at birth).

While the mean age of the participants was 32.8 ± 5.9 in the control groups, the mean age of the group with GDM was 30.8 ± 6.48.

In the postpartum, the mean BMI of the controls was calculated as 29.14 ± 2.22 and the mean BMI of the mothers with GDM was calculated as 31.44 ± 2.2. BMI values at the beginning of the pregnancy are unknown. Other details and term infant weights are also shown in Table I. Infant weights of mothers with GDM were significantly heavier than those in the control group.

While FBS, glycated hemoglobin A1c (HbA1c), HOMA-IR, TG, TC, HDL-CLDL-C, VLDL-C values in blood samples taken from mothers with GDM during the second trimester and after delivery were found to be higher when compared to blood values of healthy mothers (Table II), HDL-C values were found to be lower (Table II).

The correlation values of the biochemical variables of the groups are summarized in full detail in Table III, Table IV, Table V, and Table VI. The negative sign (-) indicates a negative correlation, which means that as one increases, the other

Table II. Comparison of the biochemical values of GDM and control mothers in the second trimester and postpartum.

Biochemical parameters	Second trimester			Postpartum		
	Control (n: 30)	GDM (n: 30)	*p*	Control (n: 17)	GDM (n: 14)	*p*
FBS (mg/dL)	86.42 ± 11.22	137.8 ± 12.9	0.05	91.16 ± 9.12	129.12 ± 12.0	0.06
Hemoglobin (g/dL)	14. 41 ± 1.1	16.22 ± 1.8	0.03	13.11 ± 0.91	15.89 ± 1.3	0.226
HbA1c (%)	4.91 ± 0.01	6.3 ± 0.02	0.05	5.1 ± 0.01	5.9 ± 0.03	0.05
HOMA-IR	1.9 ± 1.1	4.3 ± 2.3	0.05	2.2 ± 1.4	3.9 ± 1.8	0.05
TG (mg/dL)	178.16 ± 29.1	210.35 ± 75.2	0.06	189.41 ± 62.4	260.23 ± 84.41	0.05
TC (mg/dL)	197.11 ± 17.8	221.3 ± 16.8	0.06	199.8 ± 27.9	236.3 ± 67.8	0.05
LDL-C (mg/dL	166.2 ± 19.8	171.1 ± 42.8	0.07	172.4 ± 26.9	202.9 ± 47.8	0.05
HDL-C (mg/dL)	61.92 ± 11.44	57.8 ± 9.13	0.08	59.76 ± 11.12	47.96 ± 9.23	0.05
VLDL-C (mg/dL)	32.68 ± 3.9	44.56 ± 4.5	0.06	36.42 ± 3.9	52.63 ± 5.6	0.05

FBS = Fasting blood sugar; GDM = gestational diabetes mellitus; HbA1c = glycated hemoglobin A1c; HDL-C = High-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment-estimated insulin resistance; LDL-C = Low-density lipoprotein cholesterol; TC = Total cholesterol; TG = Triglyceride; VLDL-C = Very low-density lipoprotein cholesterol. Values are means ± SD.

Table III. Comparison of correlation analyzes of APLN-13 values of gestational diabetes and control mothers with other biochemical parameters.

	Second trimester				Postpartum			
	Control (n: 30)		GDM (n: 30)		Control (n: 17)		GDM (n: 14)	
Variable	Correlation	p	Correlation	p	Correlation	p	Correlation	p
APLN-13	0.522*	0.000	0.603**	0.00	0.556**	0.00	0.644**	0.00
NO	0.704**	0.000	0.694**	0.00	0.598**	0.00	0.766*	0.00
ELA	-0.498*	0.001	-0.708**	0.00	-0.498*	0.01	-0.698**	0.00
FBS	0.088	0.666	0.572**	0.00	0.240	0.25	0.497*	0.02
Hemoglobin	-0.077	0.612	-0.102	0.69	0.109	0.58	0.096	0.77
HbA1c	0.032	0.882	0.594*	0.00	0.044	0.91	0.493*	0.03
HOMA-IR	0.059	0.78	0.498**	0.02	0.082	0.83	0.498*	0.02
TG	0.048	0.08	0.548	0.05	0.498*	0.05	0.711**	0.00
TC	0.094	0.21	0.212	0.24	0.241	0.33	0.312	0.42
LDL-C	0.374	0.36	-0.251	0.17	0.204	0.32	0.284	0.36
HDL-C	0.096	0.22	0.042	0.46	0.088	0.23	0.055	0.38
VLDL-C	0.064	0.11	0.104	0.37	0.102	0.17	0.162	0.09

APLN-13 = apelin-13; APLN-36 = apelin-36; ELA = elabela; FBS = fasting blood sugar; GDM = gestational diabetes mellitus; HbA1c = glycated hemoglobin A1c; HDL-C = High-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment-estimated insulin resistance; LDL-C = Low-density lipoprotein cholesterol; NO = nitric oxide; TC = total cholesterol; TG = triglyceride; VLDL-C = Very low-density lipoprotein cholesterol. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

decreases. If there is no sign in the data in the tables, then there is a positive correlation, meaning that both values increase.

Furthermore, APLN-13, APLN-36, and NO levels in blood samples taken from GDM mothers during the second trimester and after delivery were found to be statistically significantly higher than blood values from healthy mothers (Figure 1), while ELA levels were found to be statistically significantly lower (Figure 1).

Table IV. Comparison of correlation analyzes of APLN-36 values of gestational diabetes and control mothers with other biochemical parameters.

	Second trimester				Postpartum			
	Control (n: 30)		GDM (n: 30)		Control (n: 17)		GDM (n: 14)	
Variable	Correlation	p	Correlation	p	Correlation	p	Correlation	p
APLN-36	0.497*	0.010	0.583*	0.01	0.504*	0.01	0.628**	0.00
NO	0.698**	0.000	0.716**	0.00	0.596**	0.01	0.712*	0.00
ELA	-0.516*	0.001	-0.623**	0.00	-0.488*	0.01	-0.693**	0.00
FBS	0.092	0.625	0.476*	0.02	0.220	0.25	0.493*	0.02
Hemoglobin	-0.084	0.622	-0.092	0.67	0.102	0.57	0.092	0.76
HbA1c	0.027	0.779	0.571*	0.02	0.038	0.88	0.493*	0.03
HOMA-IR	0.055	0.74	0.507*	0.01	0.078	0.82	0.492*	0.02
TG	0.043	0.07	0.522	0.05	0.482	0.06	0.623**	0.02
TC	0.086	0.19	0.181	0.22	0.232	0.32	0.312	0.42
LDL-C	0.248	0.23	-0.274	0.18	0.196	0.31	0.284	0.36
HDL-C	0.082	0.16	0.039	0.44	0.076	0.22	0.055	0.38
VLDL-C	0.072	0.12	0.098	0.31	0.094	0.16	0.162	0.09

APLN-13 = apelin-13; APLN-36 = apelin-36; ELA = elabela; FBS = fasting blood sugar; GDM = gestational diabetes mellitus; HbA1c = glycated hemoglobin A1c; HDL-C = High-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment-estimated insulin resistance; LDL-C = Low-density lipoprotein cholesterol; NO = nitric oxide; TC = total cholesterol; TG = triglyceride; VLDL-C = Very low-density lipoprotein cholesterol. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Table V. Comparison of correlation analyzes of NO values of gestational diabetes and control mothers with other biochemical parameters.

	Second trimester				Postpartum			
	Control (n: 30)		GDM (n: 30)		Control (n: 17)		GDM (n: 14)	
Variable	Correlation	p	Correlation	p	Correlation	p	Correlation	p
APLN-13	0.618**	0.000	0.626**	0.00	0.544**	0.00	0.606**	0.00
APLN-36	0.692**	0.000	0.608**	0.00	0.602**	0.00	0.678**	0.00
ELA	-0.598**	0.000	-0.684**	0.00	-0.502*	0.01	-0.698**	0.00
FBS	0.094	0.688	0.598**	0.00	0.250	0.26	0.498*	0.02
Hemoglobin	-0.084	0.664	-0.122	0.71	0.112	0.59	0.098	0.78
HbA1c	0.034	0.883	0.594**	0.00	0.048	0.92	0.516*	0.01
HOMA-IR	0.062	0.782	0.512**	0.01	0.086	0.84	0.499*	0.02
TG	0.054	0.081	0.528*	0.05	0.502*	0.05	0.733**	0.00
TC	0.102	0.255	0.216	0.25	0.255	0.34	0.341	0.43
LDL-C	0.302	0.290	-0.256	0.17	0.232	0.33	0.288	0.37
HDL-C	0.105	0.262	0.048	0.47	0.089	0.23	0.062	0.39
VLDL-C	0.068	0.122	0.098	0.36	0.111	0.18	0.169	0.11

APLN-13 = apelin-13; APLN-36 = apelin-36; ELA = elabela; FBS = fasting blood sugar; GDM = gestational diabetes mellitus; HbA1c = glycated hemoglobin A1c; HDL-C = High-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment-estimated insulin resistance; LDL-C = Low-density lipoprotein cholesterol; NO = nitric oxide; TC = total cholesterol; TG = triglyceride; VLDL-C = Very low-density lipoprotein cholesterol. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

Discussion

Although glucose intolerance is an endocrinological condition that fades after birth, the physiopathology and endocrinology behind how this disease arises during pregnancy have yet to be thoroughly understood. Although pregnant women with borderline pancreatic insulin reserve

Table VI. Comparison of correlation analyzes of ELA values of gestational diabetes and control mothers with other biochemical parameters.

	Second trimester				Postpartum			
	Control (n: 30)		GDM (n: 30)		Control (n: 17)		GDM (n: 14)	
Variable	Correlation	p	Correlation	p	Correlation	p	Correlation	p
APLN-13	-0.544**	0.000	-0.588**	0.00	-0.566**	0.00	-0.558**	0.00
APLN-36	-0.706**	0.000	-0.576**	0.00	-0.618**	0.00	-0.608**	0.00
NO	-0.608**	0.000	-0.664**	0.00	-0.552**	0.00	-0.582**	0.00
FBS	-0.098	0.694	-0.602**	0.00	-0.256	0.26	-0.543*	0.01
Hemoglobin	0.088	0.668	0.128	0.73	-0.118	0.60	-0.099	0.78
HbA1c	-0.036	0.991	-0.604**	0.00	-0.044	0.90	-0.504*	0.01
HOMA-IR	0.058	0.780	-0.502**	0.01	-0.082	0.83	-0.488*	0.02
TG	-0.052	0.079	-0.542*	0.05	-0.518*	0.05	-0.682**	0.00
TC	-0.097	0.244	-0.222	0.26	-0.256	0.34	-0.366	0.21
LDL-C	0.298	0.284	0.256	0.17	0.234	0.34	0.284	0.36
HDL-C	-0.133	0.302	-0.044	0.48	-0.078	0.22	-0.060	0.39
VLDL-C	-0.056	0.118	-0.104	0.37	-0.104	0.17	-0.153	0.12

APLN-13 = apelin-13; APLN-36 = apelin-36; ELA = elabela; FBS = fasting blood sugar; GDM = gestational diabetes mellitus; HbA1c = glycated hemoglobin A1c; HDL-C = High-density lipoprotein cholesterol; HOMA-IR = homeostasis model assessment-estimated insulin resistance; LDL-C = Low-density lipoprotein cholesterol; NO = nitric oxide; TC = total cholesterol; TG = triglyceride; VLDL-C = Very low-density lipoprotein cholesterol. *Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

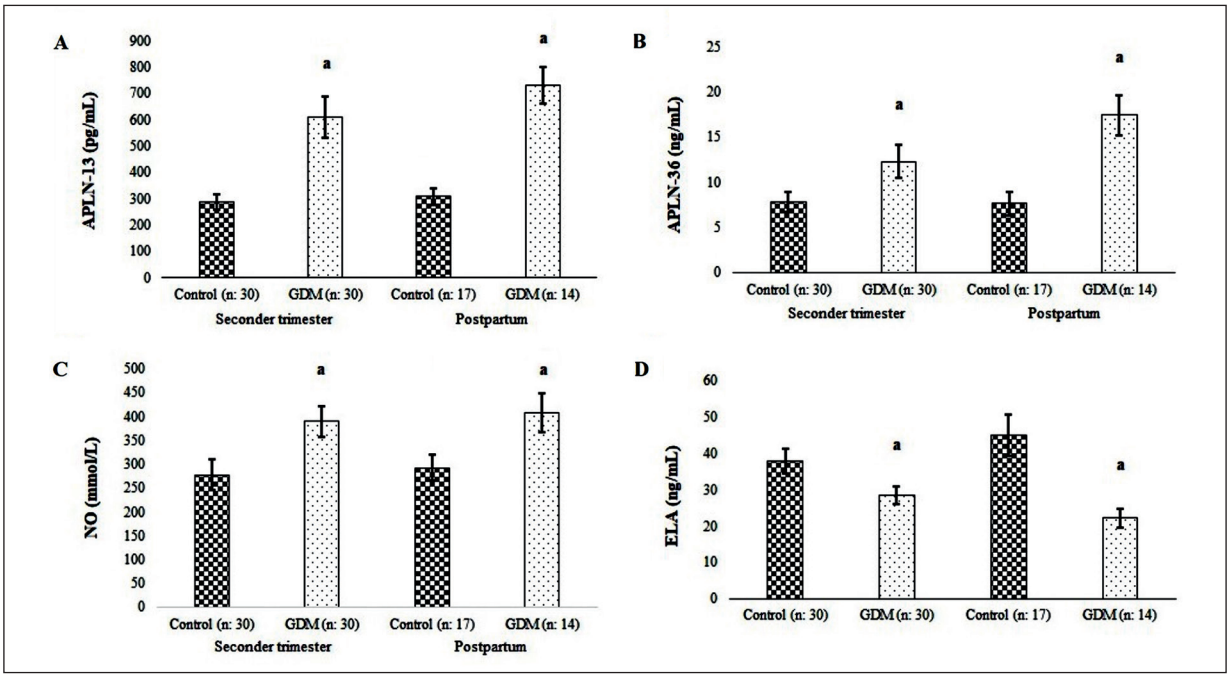

Figure 1. Comparison of Apelin-13 (APLN-13), Apelin-36 (APLN-36), Elabela (ELA) and nitrix oxide (NO) values in the second trimester and postpartum blood samples of gestational diabetes and control mothers. **A,** APLN-13 amounts in the second trimester and postpartum; **B,** APLN-36 amounts in the second trimester and postpartum; **C,** NO amounts in the second trimester and postpartum; **D,** ELA amounts in the second trimester and postpartum. a = Control group *vs*. GDM group ($p < 0.05$).

are recognized to be major candidates for the development of GDM, several recent researches[4-13] have found a link between tissue hormones and GDM development.

The link between APLN-13, APLN-36, ELA, and NO together in GDM was explored in this study for the first time, and their association with GDM was revealed. Our research discovered that the quantities of APLN-13, and APLN-36 in the mothers' circulation were considerably higher than those in the control group at the time of GDM diagnosis and the end of pregnancy. While these findings were consistent with the results of Guo et al[24] APLN samples taken during the diagnosis of GDM, they were not compatible with the results of Akinci et al[46], who reported low apelin levels in GDM. There are many different forms of apelins in circulation. These two groups of researchers did not specify in their studies which form of APLN they were studying in GMD. Therefore, it is useful to interpret the above comparison carefully.

It is also unclear whether these researchers utilized protease inhibitors (such as aprotinin) before transferring the material to biochemistry tubes. Because apelins have low molecular weights[28] and are rapidly broken down by proteases (there are more than 700 proteases in circulation), when protease inhibitors are not used[47], their levels in serum or plasma are assessed at very low levels and fluctuate from disease to disease. The biological samples used for this study were taken into biochemistry tubes containing a protease inhibitor (aprotinin). APLN levels in GDM may have been assessed *in sera* without protease inhibitors, and apelins may have been degraded by proteases, and therefore, in Akinci et al[46] it was low. In this present work we added a protease inhibitor to the biochemistry tubes for protection apelins before collection samples. This may be one possible reason for the high amount of APLN in our study compared to Akinci et al[46]. Furthermore, the increased levels of APLN-13 and APLN-36 registered in our study in the blood of mothers could be attributable to an increase in fat mass originating from the placenta or related to pregnancy, since placenta produces APLN[48]. Elsehmawy et al[49] reported that apelins are also produced and secreted by mature adipocytes of humans. Therefore, in our study, it is thought that the increased fat mass due to placenta and pregnancy is found to be significantly higher because

it contributes to the APLN pool in the mother's circulation. However, when comparing whether apelins will be elevated in GDM in the future, one should consider whether the researchers used protease inhibitors before collecting biological samples.

In our study, glucose levels in blood samples taken from mothers with GDM in the second trimester were considerably higher than glucose levels in blood samples taken from control mothers. Apelins also inhibit the sodium-dependent glucose transporter (SGLT), reducing glucose transport to erythrocytes[50]. Since APLN blocks glucose transport to erythrocytes, it is expected that glucose levels will rise indirectly due to the high APLN levels in the blood of GDM mothers[50].

Furthermore, in a study[32] conducted on patients having T2DM and coronary diseases, it was reported that high glucose amounts increased the amount of NO. In this GDM study, a significant increase was found when the NO amounts were compared to the control maternal NO amounts. The increase in NO amounts in our study may be related to the increased glucose levels due to GDM. Because high glucose levels increase the number of eNOS and iNOS genes and proteins, NO production has been reported to increase[51]. Plasma NO levels increase in T2DM patients with insulin resistance[52]. Also, NO biosynthesis is increased during pregnancy, which is associated with pregnancy-associated maternal vasodilation[53,54]. NO is the most critical vascular signaling molecule that relaxes and dilates blood vessels and reduces platelet sensitivity against anticoagulants[55]. Therefore, to eliminate the endothelial dysfunction in GDM, NO may be increased by a comparator mechanism. Furthermore, a rise in NO levels in GDM may be linked to higher glucose levels in the circulation due to GDM, with increased apelins limiting glucose transport to erythrocytes[50]. The increased glucose levels in diabates causes to increase NO levels[51]. While the NO results of this study are compatible with Yang et al's findings[51], they are inconsistent with the results of Vanizor et al[31]. So, the clarification of this situation has remained an important research topic.

Again, in this study, as in previous ones[56-58], it was detected that babies' birth weights increased due to GDM. However, the mechanisms and ways of increasing babies' birth weights due to GDM have not been fully explained. With this study, we hypothesize that an increase in apelins due to GDM inhibits red blood cells and increases the amount of glucose in circulation[50]. The increase in glucose quantity may be related to the increase of NO in the circulation[51], therefore to the increased birth weight of newborns. In other words, high glucose in the maternal circulation may have increased the amount of NO and thus increased maternal vasodilation, allowing the fetus to take more nutrients, leading to the formation of macrosomic babies[52]. NO biosynthesis rises during pregnancy[53,54], and increased NO may be associated with increased vasodilation, which allows the fetus to get more nutrients (good nutrition means more weight)[59]. There is no complete understanding of how NO levels change in diabetes. According to some researchers[31,60], NO levels increase due to diabetes[52,53], while others suggest that actually the amount of NO decreases. Increased NO amounts due to diabetes have both advantages and disadvantages. Namely, while NO causes blood vessels to relax and hypertension to decrease[34], on the other hand it can interact with the superoxide radical (O_2^-) and cause the inactivation of NO. The interaction of O_2^- with NO is rapid and leads to the strong oxidant radical peroxynitrite. This may contribute to endothelial dysfunction by stimulating arachidonic acid metabolism, lipid peroxidation, and prostanoid production[61,62]. Therefore, for a healthy life, the balance of NO levels should be kept within physiological limits.

Another possible reason for the increase in babies' birth weight is the increased transplacental glucose transport from mother to fetus, usually due to improved apelins caused by GDM, which could lead to the formation of macrosomic babies. A previous study[63] reported that intravenous injection of apelin to pregnant rat mothers boosted transplacental glucose transfer from mother to fetus. Based on this observation, it has also been suggested that the APLN derived from the placenta can contribute to fetal glycemia[50]. Increased glucose (in the case of an excess of substrate) may lead to increased fat synthesis and deposition in fetuses, resulting in macrosomic babies. Aside from glucose, aminoacids and free fatty acids have been shown to help with fetal-maternal metabolic regulation and baby weight gain[64]. Kitajima et al[65] showed that plasma triglyceride concentrations play a role in developing macrosomia in obese diabetic mothers' infants. In our current study, triglyceride and total cholesterol levels resulted to be higher in mothers with GDM. Therefore, high triglyceride amounts may have contributed to fetal weight gain.

Our study found that ELA levels were low in blood samples taken during the second trimester of pregnancy and postpartum. Comparing the amounts of ELA between the GDM and control groups shows that ELA may be directly related to the pathogenesis of GDM. A previous study[24] reported decreased ELA levels in GDM patients in the second trimester, showing that this was related to the pathogenesis of GDM and glucose metabolism. In addition, another study group[17] reported that ELA was decreased in diabetic nephropathy. ELA amounts also decrease in other pathological conditions, such as pre-eclampsia[66]. However, in the above-mentioned GDM studies, pregnant women were not followed up until the end of delivery. Our current study was the first to detect a decrease in the amount of ELA in blood taken from pregnant women after delivery and to investigate about the relationship with the baby's weight. One study[67] on mice showed that ELA was maximally produced by the placenta in midle of pregnancy. We think that the low ELA levels in the GDM group in our study were related to the deterioration of the vascular system caused by GDM, since endothelial dysfunction is commonly due to GDM[68].

Like in other studies, also this one shows some limitations. First, the number of participants was limited, thus a study with a higher number of participants should be conducted. Especially in follow-up studies, many participants should be included in the study from the beginning, since participants can then drop out of the study without giving a reason, as it happened in the current study. Thus, the number of participants in the postpartum period in our research was extremely low, and this should be considered in the future. In addition, it is difficult today to explain the pathophysiology of a disease by studying a single peptide or protein. As in this study, more than one interrelated molecule should be studied together, as well as possible peptidomics and protonomic studies. We do not have peptidomic and protonomic study facilities at our university, therefore this created another limitation for our study. Despite all these limitations, we foresee that this study may shed light on new research by providing a different perspective on the pathophysiology of GDM.

Conclusions

The development of GDM is an extremely complex and complicated process. It is hard and difficult to come to the right conclusion without studying related molecules together. Thus, this study was the first to examine the metabolically interdependent hormones APLN-13, APLN-36, and ELA, both ligand molecules for APJ, and NO (apelin-mediated vasodilation), which are metabolically interdependent and reveal their associations with GDM. While the amounts of APLN-13, APLN-36 and NO increased because of GDM, the amounts of ELA decreased significantly. Together, these working molecules (APLN-13, APLN-36 and NO) are thought to play a direct role in the pathophysiology of GDM. Because apelins exceeding the physiological threshold inhibit the SGLT, causing glucose accumulation in the blood and increasing the high glucose NO levels in the blood[50], and since ELA and APLN are ligands of APJ[69], they were all affected. The molecules studied herein were laboratory evidence, suggesting that these molecules might have a role in the pathogenesis of GDM. However, it should be remembered that GDM is an extremely complex metabolic event (it is not possible to reach the target by studying a single adipokine or myokine), thus it needs advanced peptidomics and proteomic studies to fully reveal the underlying mechanisms.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Lawrence RL, Wall CR, Bloomfield FH. Prevalence of gestational diabetes according to commonly used data sources: an observational study. BMC Pregnancy Childbirth 2019; 19: 349.
2) Buchanan TA. Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. J Clin Endocrinol Metab 2001; 86: 989-993.
3) Lende M, Rijhsinghani A. Gestational Diabetes: Overview with Emphasis on Medical Management. Int J Environ Res Public Health 2020; 17: 9573.
4) Gutaj P, Sibiak R, Jankowski M, Awdi K, Bryl R, Mozdziak P, Kempisty B, Wender-Ozegowska E. The Role of the Adipokines in the Most Common Gestational Complications. Int J Mol Sci 2020; 21: 9408.

5) Karatas A, Tunçay Işikkent N, Ozlü T, Demirin H. Relationship of maternal serum resistin and visfatin levels with gestational diabetes mellitus. Gynecol Endocrinol 2014; 30: 355-358.

6) Aydin S, Kuloglu T, Aydin S. Copeptin, adropin and irisin concentrations in breast milk and plasma of healthy women and those with gestational diabetes mellitus. Peptides 2013; 47: 66-70.

7) Baykus Y, Gurates B, Aydin S, Celik H, Kavak B, Aksoy A, Sahin I, Deniz R, Gungor S, Guzel SP, Minareci Y. Changes in serum obestatin, preptin and ghrelins in patients with Gestational Diabetes Mellitus. Clin Biochem 2012; 45: 198-202.

8) Derbent AU, Simavli SA, Kaygusuz I, Gumus II, Yılmaz S, Yildirim M, Uysal S. Serum hepcidin is associated with parameters of glucose metabolism in women with gestational diabetes mellitus. J Matern Fetal Neonatal Med 2013; 26: 1112-1115.

9) Yavuzkir S, Ugur K, Deniz R, Ustebay DU, Mirzaoglu M, Yardim M, Sahin İ, Baykus Y, Karagoz ZK, Aydin S. Maternal and umbilical cord blood subfatin and spexin levels in patients with gestational diabetes mellitus. Peptides 2020; 126: 170277.

10) Aslan M, Celik O, Celik N, Turkcuoglu I, Yilmaz E, Karaer A, Simsek Y, Celik E, Aydin S. Cord blood nesfatin-1 and apelin-36 levels in gestational diabetes mellitus. Endocrine 2012; 41: 424-429.

11) Aydin S, Celik O, Gurates B, Sahin I, Ulas M, Yilmaz M, Kalayci M, Kuloglu T, Catak Z, Aksoy A, Ozercan IH, Kumru S. Concentrations of preptin, salusins and hepcidins in plasma and milk of lactating women with or without gestational diabetes mellitus. Peptides 2013; 49: 123-130.

12) Aydin S. The presence of the peptides apelin, ghrelin and nesfatin-1 in the human breast milk, and the lowering of their levels in patients with gestational diabetes mellitus. Peptides 2010; 31: 2236-2240.

13) Ersahin SS, Yurci A. Cord blood and maternal serum preptin and irisin concentrations are regulated independently in GDM. Eur Rev Med Pharmacol Sci 2021; 25: 1954-1958.

14) Palmer ES, Irwin N, O'Harte FP. Potential Therapeutic Role for Apelin and Related Peptides in Diabetes: An Update. Clin Med Insights Endocrinol Diabetes 2022; 15: 11795514221074679.

15) Wang Z, Yu D, Wang M, Wang Q, Kouznetsova J, Yang R, Qian K, Wu W, Shuldiner A, Sztalryd C, Zou M, Zheng W, Gong DW. Elabela-apelin receptor signaling pathway is functional in mammalian systems. Sci Rep 2015; 5: 8170.

16) Zhang H, Gong D, Ni L, Shi L, Xu W, Shi M, Chen J, Ai Y, Zhang X. Serum Elabela/Toddler Levels Are Associated with Albuminuria in Patients with Type 2 Diabetes. Cell Physiol Biochem 2018; 48: 1347-1354.

17) Onalan E, Doğan Y, Onalan E, Gozel N, Buran I, Donder E. Elabela levels in patients with type 2 diabetes: can it be a marker for diabetic nephropathy? Afr Health Sci 2020; 20: 833-840.

18) Yang P, Read C, Kuc RE, Buonincontri G, Southwood M, Torella R, Upton PD, Crosby A, Sawiak SJ, Carpenter TA, Glen RC, Morrell NW, Maguire JJ, Davenport AP. Elabela/Toddler Is an Endogenous Agonist of the Apelin APJ Receptor in the Adult Cardiovascular System, and Exogenous Administration of the Peptide Compensates for the Downregulation of Its Expression in Pulmonary Arterial Hypertension. Circulation 2017; 135: 1160-1173.

19) Liu Y, Wang L, Shi H. The biological function of ELABELA and APJ signaling in the cardiovascular system and pre-eclampsia. Hypertens Res 2019; 42: 928-934.

20) Soriguer F, Garrido-Sanchez L, Garcia-Serrano S, Garcia-Almeida JM, Garcia-Arnes J, Tinahones FJ, Garcia-Fuentes E. Apelin levels are increased in morbidly obese subjects with type 2 diabetes mellitus. Obes Surg 2009; 19: 1574-1580.

21) Dayem SMAE, Battah AA, Bohy AEME, Yousef RN, Ahmed AM, Talaat AA. Apelin, Nitric Oxide and Vascular Affection in Adolescent Type 1 Diabetic Patients. Open Access Maced J Med Sci 2017; 5: 934-939.

22) Du JH, Li X, Li R, Xu L, Ma RR, Liu SF, Zhang Z, Sun HZ. Elevation of serum apelin-13 associated with proliferative diabetic retinopathy in type 2 diabetic patients. Int J Ophthalmol 2014; 7: 968-973.

23) Oncul M, Tuten A, Erman H, Gelisgen R, Benian A, Uzun H. Maternal and cord blood apelin, resistin and visfatin levels in gestational diabetes mellitus. Minerva Med 2013; 104: 527-535.

24) Guo YY, Li T, Liu H, Tang L, Li YC, Hu HT, Su YF, Lin Y, Wang YY, Li C, Huang HF, Jin L, Liu XM. Circulating levels of Elabela and Apelin in the second and third trimesters of pregnancies with gestational diabetes mellitus. Gynecol Endocrinol 2020; 36: 890-894.

25) Bilir B, Ekiz Bilir B, Yilmaz I, Soysal Atile N, Yildirim T, Kara SP, Gumustas SA, Orhan AE, Aydin M. Association of apelin, endoglin and endocan with diabetic peripheral neuropathy in type 2 diabetic patients. Eur Rev Med Pharmacol Sci 2016; 20: 892-898.

26) Sun J, Ren J, Zuo C, Deng D, Pan F, Chen R, Zhu J, Chen C, Ye S. Circulating apelin, chemerin and omentin levels in patients with gestational diabetes mellitus: a systematic review and meta-analysis. Lipids Health Dis 2020; 19: 26.

27) Shin K, Pandey A, Liu XQ, Anini Y, Rainey JK. Preferential apelin-13 production by the proprotein convertase PCSK3 is implicated in obesity. FEBS Open Bio 2013; 3: 328-333.

28) Aydin S, Eren MN, Sahin I, Aydin S. The role of apelins in the physiology of the heart. Protein Pept Lett 2014; 21: 2-9.

29) Lee DK, Cheng R, Nguyen T, Fan T, Kariyawasam AP, Liu Y, Osmond DH, George SR, O'Dowd BF. Characterization of apelin, the ligand for the APJ receptor. J Neurochem 2000; 74: 34-41.

30) Tessari P, Cecchet D, Cosma A, Vettore M, Coracina A, Millioni R, Iori E, Puricelli L, Avogaro A, Vedovato M. Nitric oxide synthesis is reduced in subjects with type 2 diabetes and nephropathy. Diabetes 2010; 59: 2152-2159.

31) Vanizor B, Orem A, Karahan SC, Kiran E, Erem C, Aliyazicioğlu R, Uydu HA. Decreased nitric oxide end-products and its relationship with high density lipoprotein and oxidative stress in people with type 2 diabetes without complications. Diabetes Res Clin Pract 2001; 54: 33-39.

32) Adela R, Nethi SK, Bagul PK, Barui AK, Mattapally S, Kuncha M, Patra CR, Reddy PN, Banerjee SK. Hyperglycaemia enhances nitric oxide production in diabetes: a study from South Indian patients. PLoS One 2015; 10: e0125270.

33) Assmann TS, Brondani LA, Bouças AP, Rheinheimer J, de Souza BM, Canani LH, Bauer AC, Crispim D. Nitric oxide levels in patients with diabetes mellitus: A systematic review and meta-analysis. Nitric Oxide 2016; 61: 1-9.

34) Chen K, Pittman RN, Popel AS. Nitric oxide in the vasculature: where does it come from and where does it go? A quantitative perspective. Antioxid Redox Signal 2008; 10: 1185-1198.

35) Wu HW, Li ZG, Liu G, Lu GZ, Liang HY. Effect of nitric oxide inhalation for the treatment of neonatal pulmonary hypertension. Eur Rev Med Pharmacol Sci 2016; 20: 4607-4611.

36) Regunathan S, Piletz JE. Regulation of inducible nitric oxide synthase and agmatine synthesis in macrophages and astrocytes. Ann N Y Acad Sci 2003; 1009: 20-29.

37) Rabinovitch A, Suarez-Pinzon WL, Sorensen O, Bleackley RC. Inducible nitric oxide synthase (iNOS) in pancreatic islets of nonobese diabetic mice: identification of iNOS- expressing cells and relationships to cytokines expressed in the islets. Endocrinology 1996; 137: 2093-2099.

38) Fang FC, Vazquez-Torres A. Nitric oxide production by human macrophages: there's NO doubt about it. Am J Physiol Lung Cell Mol Physiol 2002; 282: L941-L943.

39) Gkaliagkousi E, Ritter J, Ferro A. Platelet-derived nitric oxide signaling and regulation. Circ Res 2007; 101: 654-662.

40) Hu G, Wang Z, Zhang R, Sun W, Chen X. The Role of Apelin/Apelin Receptor in Energy Metabolism and Water Homeostasis: A Comprehensive Narrative Review. Front Physiol 2021; 12: 632886.

41) Catak Z, Kaya H, Kocdemir E, Ugur K, Pilten GS, Yardim M, Sahin I, Piril AE, Aydin S. Interaction of apelin, elabela and nitric oxide in schizophrenia patients. J Med Biochem 2020; 39: 184-190.

42) American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2009; 32 Suppl 1: S62-S67.

43) Rani PR, Begum J. Screening and Diagnosis of Gestational Diabetes Mellitus, Where Do We Stand. J Clin Diagn Res 2016; 10: QE01-QE04.

44) Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides 2015; 72: 4-15.

45) Majid H, Masood Q, Khan AH. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR): A Better Marker for Evaluating Insulin Resistance Than Fasting Insulin in Women with Polycystic Ovarian Syndrome. J Coll Physicians Surg Pak 2017; 27: 123-126.

46) Akinci B, Celtik A, Tunali S, Genc S, Yuksel F, Secil M, Ozcan MA, Bayraktar F. Circulating apelin levels are associated with cardiometabolic risk factors in women with previous gestational diabetes. Arch Gynecol Obstet 2014; 289: 787-793.

47) Rawlings ND, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD. The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. Nucleic Acids Res 2018; 46: D624-D632.

48) Mlyczyńska E, Kurowska P, Drwal E, Opydo-Chanek M, Tworzydło W, Kotula-Balak M, Rak A. Apelin and apelin receptor in human placenta: Expression, signalling pathway and regulation of trophoblast JEG 3 and BeWo cells proliferation and cell cycle. Int J Mol Med 2020; 45: 691-702.

49) Elsehmawy AAEW, El-Toukhy SE, Seliem NMA, Moustafa RS, Mohammed DS. Apelin and chemerin as promising adipokines in children with type 1 diabetes mellitus. Diabetes Metab Syndr Obes 2019; 12: 383-389.

50) Bertrand C, Valet P, Castan-Laurell I. Apelin and energy metabolism. Front Physiol 2015; 6: 115.

51) Yang P, Cao Y, Li H. Hyperglycemia induces inducible nitric oxide synthase gene expression and consequent nitrosative stress via c-Jun N-terminal kinase activation. Am J Obstet Gynecol 2010; 203: 185.e5-185.e11.

52) Chien WY, Yang KD, Eng HL, Hu YH, Lee PY, Wang ST, Wang PW. Increased plasma concentration of nitric oxide in type 2 diabetes but not in nondiabetic individuals with insulin resistance. Diabetes Metab 2005; 31: 63-68.

53) Maejima K, Nakano S, Himeno M, Tsuda S, Makiishi H, Ito T, Nakagawa A, Kigoshi T, Ishibashi T, Nishio M, Uchida K. Increased basal levels of plasma nitric oxide in Type 2 diabetic subjects. Relationship to microvascular complications. J Diabetes Complications 2001; 15: 135-143.

54) Ghosh A, Sherpa ML, Bhutia Y, Pal R, Dahal S. Serum nitric oxide status in patients with type 2 diabetes mellitus in Sikkim. Int J Appl Basic Med Res 2011; 1: 31-35.

55) Suchyta DJ, Handa H, Meyerhoff ME. A nitric oxide-releasing heparin conjugate for delivery of a combined antiplatelet/anticoagulant agent. Mol Pharm 2014; 11: 645-650.

56) Kc K, Shakya S, Zhang H. Gestational diabetes mellitus and macrosomia: a literature review. Ann Nutr Metab 2015; 66 Suppl 2: 14-20.

57) Tieu J, McPhee AJ, Crowther CA, Middleton P. Screening and subsequent management for gestational diabetes for improving maternal and infant health. Cochrane Database Syst Rev 2014; (2): CD007222.

58) Alfadhli EM. Maternal obesity influences Birth Weight more than gestational Diabetes author. BMC Pregnancy Childbirth 2021; 21: 111.

59) Simmonds MJ, Detterich JA, Connes P. Nitric oxide, vasodilation and the red blood cell. Biorheology 2014; 51: 121-134.

60) Honing ML, Morrison PJ, Banga JD, Stroes ES, Rabelink TJ. Nitric oxide availability in diabetes mellitus. Diabetes Metab Rev 1998; 14: 241-249.

61) Ishii N, Patel KP, Lane PH, Taylor T, Bian KA, Murad F, Pollock JS, Carmines PK. Nitric oxide synthesis and oxidative stress in the renal cortex of rats with diabetes mellitus. J Am Soc Nephrol 2001; 12: 1630-1639.

62) Francesco C, Keiichi H, Zvonimir SK. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997; 96: 25-28.

63) Ericsson A, Säljö K, Sjöstrand E, Jansson N, Prasad PD, Powell TL, Jansson T. Brief hyperglycaemia in the early pregnant rat increases fetal weight at term by stimulating placental growth and affecting placental nutrient transport. J Physiol 2007; 581: 1323-1332.

64) Mestyán J, Soltész G, Dobák E, Fekete M, Schultz K. The post-natal changes in the circulating free amino acid pool in the newborn infant. 4. The relationship between the changes of the plasma amino acid ratio, -amino-n, free fatty acid and blood glucose concentration after birth. Biol Neonate 1972; 21: 229-238.

65) Kitajima M, Oka S, Yasuhi I, Fukuda M, Rii Y, Ishimaru T. Maternal serum triglyceride at 24--32 weeks' gestation and newborn weight in nondiabetic women with positive diabetic screens. Obstet Gynecol 2001; 97: 776-780.

66) Deniz R, Baykus Y, Ustebay S, Ugur K, Yavuzkir Ş, Aydin S. Evaluation of elabela, apelin and nitric oxide findings in maternal blood of normal pregnant women, pregnant women with pre-eclampsia, severe pre-eclampsia and umbilical arteries and venules of newborns. J Obstet Gynaecol 2019; 39: 907-912.

67) Ma J, Hu H, Lin M, Chen L, Liu M, Li H, Quan S. ELABELA alleviates syncytiotrophoblast hypoxia/reoxygenation injury and preeclampsia-like symptoms in mice by reducing apoptosis. Placenta 2021; 106: 30-39.

68) He Y, Wu N. Research Progress on Gestational Diabetes Mellitus and Endothelial Dysfunction Markers. Diabetes Metab Syndr Obes 2021; 14: 983-990.

69) Kuba K, Sato T, Imai Y, Yamaguchi T. Apelin and Elabela/Toddler; double ligands for APJ/Apelin receptor in heart development, physiology, and pathology. Peptides 2019; 111: 62-70.