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Plasma Asprosin Levels Changes in Pregnant and Non-Pregnant Rats with and without Gestational Diabetes

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ABSTRACT

Objective: *We hypothesized that asprosin might be increased during pregnancy and gestational diabetes (GD) suggesting a potential role in food intake stimulation during pregnancy and suggesting a role in prediction of GD so, we analyzed changes in plasma asprosin levels in pregnant and non-pregnant rats with and without gestational diabetes.* ***Methods:*** *40 female rats divided into 4 groups; control non-pregnant, normal pregnant, untreated GD and insulin-treated GD groups. In all groups, body weights (BW), body length, body mass index (BMI) and food intake, levels of asprosin, estrogen, progesterone, serum levels of insulin, glucose and lipid profile were measured. HOMA-IR and HOMA-B were calculated.* ***Results:*** *Asprosin levels were found higher in pregnant, GDM and insulin-treated groups in comparison with control group ($p \leq 0.001$, $p \leq 0.0001$ and $p \leq 0.0001$ respectively). Asprosin levels were higher in GDM group during early and late pregnancy in comparison with the pregnant group ($p \leq 0.0001$). Asprosin levels decreased in insulin-treated group compared with GDM group ($p \leq 0.0001$). Asprosin levels correlated positively with body weight ($r=0.821$, $p<0.05$), body mass index ($p<0.05$), food intake, serum glucose ($r=0.9958$, $p<0.00001$), HOMA-IR, cholesterol, triglycerides, LDL-c and VLDL and negatively correlated to HOMA-B, HDL, estradiol and progesterone levels.* ***Conclusion:*** *asprosin levels were significantly elevated during normal pregnancy suggesting that asprosin may have a physiological role during pregnancy as it may participate in stimulation of appetite and food intake commonly occurring during pregnancy. GD rats were found to have significant higher asprosin compared to pregnant group. Asprosin may be a potential factor predicting diabetes mellitus during pregnancy.*

Keywords: Asprosin, Gestational, Diabetes, Glucose, Insulin, Estrogen

INTRODUCTION

Diabetes mellitus (DM) is a chronic multisystemic endocrinopathy, characterized by glucose intolerance, requiring multidisciplinary approach and strategies for glycemic control [1]. Gestational diabetes mellitus (GDM) is a harmful condition associated with abnormal glucose intolerance and insulin resistance primarily identified during pregnancy with many adverse effects to mother and her fetus. It may lead to fetal cardiac congenital malformations, macrosomia, neonatal hypoglycemia, premature birth, fetal mortality, and increased incidence of DM type 2 [2-4]. Furthermore, women with GDM have increased risk of developing DM type 2, hypertension, infection and operative delivery [5]. The elevated oxidative stress linked to maternal hyperglycemia may lead to altered cellular structures as DNA, protein and lipid disrupting the cell homeostasis and promoting maternal and fetal adverse effects development [6]. Asprosin is a glucogenic adipokine formed by white adipose tissue and cleaved from profibrillin C-terminal end. It stimulates

hepatic release of glucose in the circulation during fasting via G protein-cyclic adenosine monophosphate-protein kinase A pathway. However, food intake turns off its manufacture [7,8]. In addition, it promotes food intake and body weight propably by activating Agouti-related peptide (AgRP) neurons. Therefore, its genetic deficiency results in neonatal progeroid syndrome manifested by low appetite and severe leanness [9]. However, it is abnormally increased with insulin resistance, obesity, metabolic syndrome and type 2 DM [8,10]. Hyperinsulinemia linked to metabolic syndrome may be improved by reducing asprosin levels [7]. Therefore, asprosin might be a probable therapeutic target helping in DM. Herein, we investigated the changes of plasma asprosin levels in pregnant rats in comparison with non-pregnants to clarify its physiological role during normal pregnancy. Furthermore, we investigated asprosin role in the pathogenesis of GDM by measuring the plasma asprosin levels in the pregnant STZ-induced diabetic rats supposing a predictive role in its diagnosis.

MATERIALS AND METHODS

Experimental Animals

Forty female healthy albino rats weighing 200-250 gm, were obtained from animal house of Veterinary medicine Faculty-Zagazig University. Rats were kept for two weeks in laboratory for acclimatization and kept in steel wire cages, 5 rats per cage; they were kept under comfortable temperature (20˚C-24˚C) and were maintained on a normal light/dark cycle. All Rats were fed with the standard diet and provided with water ad libitum. Ten rats served as control non-pregnant group and 30 rats would be pregnant. For determination of estrous cycle, vaginal smears were daily collected and taken under the microscope [11]. Then, the estrous rats were paired with the normal male rats for conception. One male was housed up with four females for overnight periods. On the next morning, the presence of mucus plug or sperm observed by light microscopy was determined to be the 0th day of pregnancy. Pregnant rats were labeled and separated and non-pregnant rats were excluded from the study [12,13]. Pregnant rats were equally divided into three groups: control pregnant group, GDM group and insulin treated group. The study was conducted in accordance with the rules of the Animal Research Ethical Committee, Faculty of Medicine, Zagazig University, Egypt.

Induction of Gestational Diabetes Mellitus (GDM)

After fasting for 12 hours, the pregnant rats in GDM group and insulin treated group received a single intraperitoneal injection of fresh 2% streptozotocin (STZ) solution (60 mg/kg) dissolved in citrate solution at the 3rd day of pregnancy. Rats with a glucose level ≥ 200 mg/dL are considered as GDM model rats [14,15]. Rats in the control group recieved equal volume of citrate buffer. Rats died during the study are excluded.

Anthropometric Parameters Measurement

The animals were weighed in by an electronic balance; at day 7 and 20. The results were written in a record for each labeled rat. The nose to anus lengths of rats were used for calculation of body mass index (BMI) (gm/cm^2) by dividing body weight (gm)/$length^2$ (cm^2) [16,17]. Then data were plotted in records of each labeled rat.

Biochemical Analysis

Glycemia was measured in tail blood every 2 weeks (at day 7 and day 20) up to the end of pregnancy, at approximately 9 a.m. At days 7 and 20 of pregnancy, body weight and food intake were measured. At gestational day 20, rats were injected by sodium thiopental anaesthetic (50 mg/kg b. w., i.p.) then blood samples were collected from the tail vein for biochemical analysis. The blood samples were left at room temperature to clot before centrifugation at 3000 rpm for 15 minutes (Centrifuge Zentriguanhan, Engelsr-of DDR- 7123.Engels-drf/-eipzig/Banug/Fabr.NO.08/30 type T30, Max. 1 min: 6400 Max Fullgew Kgr. Frequency 50 Hz made in Germany). The serum was stored at -20° C.

Serum glucose levels (mg/dl) were measured according to Tietz, et al. [18], and serum insulin levels (μIU/ml) were measured by enzyme-linked immuno-sorbent assay (ELISA) according to Reaven, et al. [19]. Serum glucose and insulin kits were purchased from sigma chemicals Co. Homeostasis model assessment-

insulin resistance (HOMA-IR) was calculated using HOMA-IR index according to Bonora, et al. [20] as follows: [HOMA-IR]=fasting serum glucose (mg/dL) × fasting serum insulin (μIU/mL)/405. Serum total cholesterol (TC), triglycerides (TG) and serum HDL levels were estimated according to Tietz, et al., Fossati, et al., and Nauck, et al. [18,21,22] respectively. Serum LDL levels was calculated according to Friedewald, et al. [23] as follows: LDL=TC-HDL–TG/5.

Plasma asprosin was determined using ELISA kits (Abbexa, Cambridge, United Kingdom). Kit had a sensitivity of 0.938 ng/mL, with a range between 1.56 ng/mL and 100 ng/mL. The intra-assay and interassay variations were 8% and 10% respectively [24]. Serum estrogen and progesterone levels were estimated according to Tietz, et al. [18] using rat kits purchased from Sigma Co.

Statistical Analysis

The data obtained in the present study were expressed as mean ± SD. Unpaired T test was done for comparing means. The statistical analysis is done by using Graphpad Quickcalc internet site. p-value<0.05 was considered statistically significant.

RESULTS

Tables 1-5 represent the demographic characteristics; Values are presented as mean ± standard deviation.

Table 1 Anthropometric parameters

Variables		Body Weight (BW)	Body Length (cm)	Body Mass Index (BMI) (gm/cm^2)	Food Intake in gm
Group I (Control non-pregnant)		225 ± 4	24 ± 0.3	0.39 ± 0.02	31.7 ± 1.2
Group II (Normal Pregnant)	day 7	235 ± 5a***	24 ± 0.4	0.41 ± 0.03	32.5 ± 1.4
	day 20	270 ± 6a***	25 ± 0.5a***	0.43 ± 0.01a***	37.6 ± 1.3a***
Group III (Gestational Diabetic (GDM))	day 7	310 ± 3a***b***	25 ± 0.4a***b***	0.49 ± 0.02a***b*	38.7 ± 1.1a***b***
	day 20	340 ± 7a***c***	26 ± 0.6a***c***	0.50 ± 0.02a***	40.4 ± 1.2a***c***
Group VI (Insulin-treated GDM)	day 7	245 ± 6a***b***d***	25 ± 0.5a***b***	0.39 ± 0.03d***	32.1 ± 1.1a#B#d**
	day 20	310 ± 5a***c***e***	26 ± 0.4a***b***, e***	0.46 ± 0.03a***	37.7 ± 1.2a***C #e***

*a: Significant in comparison with control group; *b: Significant in comparison with preganant day 7 group; *c: Significant in comparison with preganant day 20 group; *d: Significant in comparison with Gestational diabetic day 7 group; *e: Significant in comparison with gestational diabetic day 20 group; #: Non significant

Table 2 Pearson correlation between Asprosin, BMI and food intake

Asprosin ng/ml	Body Mass Index (BMI) (gm/cm^2)	Food Intake in gm
12.05 ± 1.5	0.39 ± 0.02	31.7 ± 1.2
15.03 ± 2.5a**	0.41 ± 0.03	32.5 ± 1.4
17.07 ± 2.4a***	0.43 ± 0.01a***	37.6 ± 1.3a***
59.05 ± 5.7a***b***	0.49 ± 0.02a***b*	38.7 ± 1.1a***b***
73.03 ± 6.5a***c***	0.50 ± 0.02a***c*	40.4 ± 1.2a***c***
15.23 ± 2.6a**b#d***	0.36 ± 0.02a***c*	32.1 ± 1.1a#B#d**
19.03 ± 3.5a***c#e***	0.46 ± 0.03a***e**	37.7 ± 1.2a***C#e***
R (Pearson correlation)	Strong Positive correlation 0.8318 (p<0.05)*	Strong Positive correlation 0.7698 (p<0.05*)

*a: Significant in comparison with control group; *b: Significant in comparison with preganant day 7 group; *c: Significant in comparison with preganant day 20 group; *d: Significant in comparison with Gestational diabetic day 7 group; *e: Significant in comparison with gestational diabetic day 20 group; #: Non significant

Table 3 Glucosemic parameters

Variables		Asprosin (ng/ml)	Insulin (mIU/ml)	Glucose (mg/dl)	(HOMA-IR)	HOMA-β
Group I (Control non-pregnant)		12.05 ± 1.5	8.75 ± 0.07	95.79 ± 4.55	2.08 ± 0.07	79.06 ± 4.12
Group II (Normal Pregnant)	day 7	15.03 ± 2.5a**	8.67 ± 0.07a*	105.35 ± 3.50a***	2.14 ± 0.05a*	75.05 ± 3.11a*
	day 20	17.07 ± 2.4a***	7.65 ± 0.05a***	120.54 ± 4.33a***	2.97 ± 0.06a***	71.06 ± 3.12a***
Group III (Gestational Diabetic (GDM))	day 7	59.05 ± 5.7a***b***	4.45 ± 0.06a***b***	205.39 ± 3.45a***b***	4.64 ± 0.05a***b***	41.82±2.55a***b***
	day 20	73.03 ± 6.5a***c***	3.65 ± 0.05a*** c***	240.67 ± 4.98a***c***	5.45 ± 0.06a***c***	34.67±3.60a***c***
Group VI (Insulin-treated GDM)	day 7	15.23 ± 2.6a**b#d***	8.61 ± 0.09a**b# d***	107.34 ± 3.95a***b#d***	2.17 ± 0.04a**b#d***	74.05 ± 4.14a*b#d***
	day 20	19.03 ± 3.5a***c#e***	7.59 ± 0.08a***c#e***	124.58 ± 4.35a***c#e***	2.95 ± 0.05a***, c#, e***	72.06 ± 5.13a**c#e***
R (Pearson correlation)			-0.9865 (p=0.000044**)	0.9958 (p<0.00001***)	0.977 (p=0.000152**)	-0.9963 (p<0.00001***)

*a: Significant in comparison with control group; *b: Significant in comparison with preganant day 7 group; *c: Significant in comparison with preganant day 20 group; *d: Significant in comparison with Gestational diabetic day 7 group; *e: Significant in comparison with gestational diabetic day 20 group; #: Non significant

Table 4 Hormones

Variables		Asprosin (ng/ml)	Estradiol (pgm/ml)	Progesterone (ngm/ml)
Group I (Control non-pregnant)		12.05 ± 1.5	30 ± 1.6	20 ± 0.5
Group II (Normal Pregnant)	day 7	15.03 ± 2.5a**	40 ± 1.5a***	25 ± 0.3a***
	day 20	17.07 ± 2.4a***	65 ± 1.9a***	30 ± 0.5a***
Group III (Gestational Diabetic (GDM))	day 7	59.05 ± 5.7a***b***	32 ± 0.9a**b***	15 ± 0.6a***b***
	day 20	73.03 ± 6.5a***c***	33 ± 1.6a**c***	17 ± 0.9a***c***
Group VI (Insulin-treated GDM)	day 7	15.23 ± 2.6 a**b#d***	41 ± 1.5a***b#d***	13 ± 0.6a***b***d***
	day 20	19.03 ± 3.5a***c#e***	66 ± 1.7a***c#e***	14 ± 0.6a***c***e***
R (Pearson correlation)			-0.4259 (week negative correlation) # (p=0.341)	-0.3256 (week negative correlation) # (p=0.476)

*a: Significant in comparison with control group; *b: Significant in comparison with preganant day 7 group; *c: Significant in comparison with preganant day 20 group; *d: Significant in comparison with Gestational diabetic day 7 group; *e: Significant in comparison with gestational diabetic day 20 group; #: Non significant

Table 5 Serum Lipids (mM) (mean ± SD)

Variables		Total cholesterol (TC)	Triglycerides (TG)	HDL-c	LDL-c	VLDL
Group I (Control non-pregnant)		0.98 ± 0.09	1.08 ± 0.19	1.13 ± 0.31	48.65±1.81	15.21±0.45
Group II (Normal Pregnant)	day 7	1.03 ± 0.07a#	1.09 ± 0.23a#	1.12 ± 0.28a#	49.64±1.82a#	15.32±0.46a#
	day 20	1.05 ± 0.08a*	1.16 ± 0.17a#,	1.11 ± 0.25a#	50.56±2.31a#	15.65±0.35a*
Group III (Gestational Diabetic (GDM))	day 7	1.97 ± 0.09a***b***	1.91 ± 0.25a***, b***	0.77 ± 0.23a**b**	78.62± 1.74a***b***	19.11± 0.36a***b***
	day 20	2.06 ± 0.06a***c***	2.13 ± 0.33a***c***	0.71 ± 0.25a**, c**	98.63± 2.42a***c***	21.13± 0.36a***c***
Group VI (Insulin-treated GDM)	day 7	1.04 ± 0.09a#b#d***	1.08 ± 0.21a#b#d***	1.13 ± 0.35 a#b#	49.73± 1.75a#b#d***	15.53± 0.47a#b#d***
	day 20	1.08 ± 0.06a**c#e***	1.29 ± 0.31a**c#e***	1.12 ± 0.40a#c#e*	50.73± 1.82a*c#e***	15.72± 0.37a*c#e***
R (Pearson correlation)		0.9938 (p<0.00001)	0.9944 (p<0.00001)	-0.9952 (p<0.00001)	0.9898 (p<0.0001)	0.9956 (p<0.0001)

HDL-c: High-density lipoprotein-cholesterol; LDL: Low-density lipoprotein; VLDL: Very-Low-density lipoprotein-cholesterol; *a: Significant in comparison with control group; *b: Significant in comparison with preganant day 7 group; *c: Significant in comparison with preganant day 20 group; *d: Significant in comparison with Gestational diabetic day 7 group; *e: Significant in comparison with gestational diabetic day 20 group; #: Non significant

DISCUSSION

Asprosin is a newly discovered glucogenic adipokine [25] that increases with insulin resistance [15]. Gestational diabetes mellitus (GDM) is a harmful condition associated with an abnormal insulin resistance with many adverse effects to mother and fetus [4]. Asprosin might be a predicting factor in diagnosis of DM and could be a therapeutic target for prediabetes and diabetes mellitus type 2 [26]. The role of asprosin in gestational diabetes is in controversy so we investigated the changes of asprosin levels in pregnant rats in comparison with non-pregnants to clarify its physiological role during normal pregnancy. Furthermore, we investigated asprosin role in the pathogenesis of gestational diabetes by measuring the plasma asprosin levels in the pregnant STZ-induced diabetic rats supposing a predictive role in its diagnosis.

In the present study, we found significant increase in in body weight in all pregnant groups compared with the control group ($p \leq 0.0001$). Also, a significant increase in body weight was found in gestational diabetic group day 7 in comparison with nondiabetic pregnant day 7 and in gestational diabetic day 20 in comparison with nondiabetic pregnant day 20 ($p \leq 0.0001$). A significant reduction in body wight is found in insulin treated group day 7 and day 20 compared with gestational diabetic group (day 7 and day 20) ($p \leq 0.0001$). Significant differences were found between group 4 at day 7 and day 20 compared with control group. We found a positive correlation between plasma asprosin and body weight ($r=0.821$, $p<0.05$). These findings are in agreement with Li et al. [15], who found that overweight participants have a significant higher asprosin than lean subjects. Our findings are also supported by Duerrschmid, et al. [27], who reported high concentrations of circulating asprosin in obese humans and mice.

In the present study, body length increased significantly in pregnant group at day 20 and groups 3 and 4 compared with control group. no significant change was found between preganant group at day 7 and control group. Highly significant increases in body length were found in group 3 at day 7 and day 20 compared with group 2 day 7 and day 20 respectively ($p \leq 0.0001$). Insulin could not decrease body length in group 4 at day 7 and day 20 compared with group 3 (gestational diabetic group) at day 7 and day 20 respectively.

Body mass index (BMI) increased significantly in pregnant group 2 day 20 ($p \leq 0.0001$) howeer no significant differences in group 4 day 7 compared with control group. We also found a significant increase in BMI in group 3 day 7 and day 20 compared with group 2 at days 7 and 20 respectively ($p \leq 0.0001$) and significant differences in group 4 compared with group 2 ($p \leq 0.0001$). We also found that asprosin is positively correlated with body mass index ($p<0.05$). The results of our study are in agreement with Duerrschmid, et al. [27], who found a significant higher asprosin levels in obese humans and mice. However, our findings are in controversy with Long, et al. [28], who found significant lower asprosin concentrations in obese children than controls. The controversy with the latter study might be due to species and age differences. Our findings are also supported by Wang, et al., [29], who found that circulating asprosins are increased in obese children associated with insulin resistance.

In the current study, food intake increased significantly in group 2 day 20 and in group 3 at days 7 and 20 compared with control group ($p \leq 0.0001$). In group 4 food intake increased significantly at day 20 compared with control group ($p \leq 0.0001$) however no significant differences at day 7. Significant increases in food intake were found in group 3 at day 7 and day 20 compared with group 2 at day 7 and 20 respectively ($p \leq 0.0001$). We found a significant positive correlation between asprosin level and food intake. These results are in agreement with Duerrschmid, et al. [27], who demonstrated that asprosin crosses the blood-brain barrier and directly activates orexigenic AgRP+ neurons via a cAMP-dependent pathway. This signaling results in inhibition of anorexigenic neurons in a GABA-dependent manner stimulating appetite and resulting in increased adiposity and body weight.

In the present study, a significant reduction in food intake was found in insulin treated group 4 at day 7 and day 20 compared with group 3 at day 7 and day 20 respectively ($p \leq 0.0001$). We suggested that lower levels of asprosin represent a major cause of reduction in food intake in this group. Our suggestion could be confirmed by Duerrschmid, et al. [27], who reported that a deficiency in asprosin in humans causes Marfanoid–progeroid-lipodystrophy syndrome characterized by low food intake and extreme leanness which can be fully rescued by asprosin.

In the present study, we found that insulin significantly decreased in all pregnant groups (2, 3 and 4) compared with control group 1 ($p \leq 0.0001$). Highly significant reductions in insulin levels were found in group 3 at day 7 and day 20 compared with group 2 at day 7 and 20 respectively ($p \leq 0.0001$). Insulin increased significantly in group 4 at day 7 and day 20 compared with group 3 at day 7 and day 20 respectively ($p \leq 0.0001$). We did not find any significant differences ($p>0.05$) in insulin levels in group 4 at day 7 and day 20 in comparison with group 2 at day 7 and 20 respectively. We found a negative correlation between asprosin and insulin levels ($p \leq 0.0001$). Our findings are in agreement with Long, et al. [28], who found that asprosin was correlated with insulin.

In the currect study, glucose increased significantly in pregnant group 2 compared with control group. In addition, highly significant increases in glucose levels were found in group 3 and 4 compared with control group. We also found significant reductions in glucose levels in group 4 at day 7 and day 20 compared with group 3 at day 7 and day 20 respectively ($p \leq 0.0001$). No significant differences in glucose levels in group 4 at day 7 and day 20 in comparison with group 2 at day 7 and day 20 respectively which confirmed insulin potent effect in restoration of nondiabetic pregnant levels of glucose. We also found a strong positive correlation between asprosin and glucose levels ($r=0.9958$, $p<0.00001$). These findings are supported by Alan, et al. [30], who reported that asprosin promotes hepatic glucose production. The findings of our study is also supported by Wiecek, et al. [25], who found a positive correlation between asprosin concentration and glucose concentration. Our results are also supported by the findings of Romere, et al. [7], who reported that asprosin activates the G protein-cAMP-PKA pathway stimulating hepatic glucose release. Our findings are also in agreement with Wang, et al. [26], who found that plasma asprosin levels were higher in impaired glucose regulation ($p<0.001$) and in newly diagnosed T2DM ($p<0.001$) groups in comparison with those in the normoglycemic group.

In the present study, we found a significant and highly significant increase in HOMA-IR in group 2 at day 7 and day 20 compared with the control group ($p<0.05$ and $p \leq 0.0001$) respectively. We also found highly significant and significant increases in HOMA-IR in gestational diabetic group and insulin treated group compared with control group ($p \leq 0.0001$ and $p \leq 0.001$ respectively). HOMA-IR increased significantly in gestational diabetic group at day 7 and 20 in comparison with nondiabetic pregnant group at day 7 and 20 respectively ($p \leq 0.0001$). In the current study, asprosin was positively correlated with HOMA-IR. Our results are supported by the findings of Romere, et al. [7], who elevated plasma asprosin in humans and mice with insulin resistance and loss of asprosin function decreased significantly glucose and insulin. They concluded that asprosin represents a glucogenic protein hormone and a therapeutic target could be beneficial in type II diabetes and metabolic syndrome. Our findings are also in agreement with Wang, et al. [26], who showed that plasma asprosin levels were positively correlated with homeostasis model assessment for insulin resistance (HOMA-IR). They revealed that plasma asprosin concentrations were significantly correlated with impaired glucose regulation (IGR) and newly diagnosed T2DM. We also found highly significant reductions in HOMA-IR in group 4 at day 7 and day 20 compared with group 3 at day 7 and day 20 respectively ($p \leq 0.0001$). No significant differences between group 4 at day 7 and 20 and group 2 at day 7 and 20 respectively which indicates that HOMA-IR in gestational diabetes treated by insulin nearby nondiabetic pregnant values. Our findings are also supported by Alan, et al. [31], who found that asprosin levels correlated positively with insulin resistance and BMI. They also reported that increased asprosin levels resulted in high probability of having polycystic ovary syndrome risk associated with insulin resistance. Our findings are also supported by Romere, et al. [7], who found that overexpression of fibrillin-1 (asprosin encoding gene) up regulating plasma asprosin might be characteristic to insulin resistance pathogenesis.

In the current study, highly significant reductions in HOMA-β were found in group 2 day 20 and group 3 day 7 and 20 in comparison with control group and significant reductions in group 2 day 7 and group 4 in comparison with the control group. We found that HOMA-β significantly decreased in group 3 at day 7 and day 20 compared with group 2 day 7 and 20 respectively ($p \leq 0.0001$). HOMA-β significantly increased in group 4 at day 7 and 20 in comparison with group 3 day 7 and 20 respectively ($p \leq 0.0001$). We found that HOMA-β values in group 4 at day 7 and day 20 are nearby values in group 2 at day 7 and 20 as no significant differences between both groups in comparable days were found ($p>0.05$). We found a significant negative correlation between asprosin and HOMA-B. Our findings are in agreement with Wiecek, et al. [25], who found a postitive correlation between asprosin concentration and metabolic disorder risk factors. Our findings are also in agreement with Wang, et al. [26], who showed that plasma asprosin levels were negatively correlated with HOMA-β (all $p<0.05$).

In the present study, asprosin levels increased significantly in pregnant group 2, gestational group 3 and insulin treated group 4 in comparison with control group ($p \leq 0.001$, $p \leq 0.0001$ and $p \leq 0.0001$ respectively). We found significant elevations in asprosin levels in gestational diabetic group day 7 and day 20 in comparison with pregnant group day 7 and day 20 respectively ($p \leq 0.0001$). These findings are in agreement with Baykus, et al. [24], who found a significant increase in asprosin levels in gestational diabetic and preeclamptic women in comparison with the control group.

In the present study, insignificant differences in asprosin levels were found in insulin treated group day 7 and day 20 compared with pregnant group day 7 and 20 respectively ($p \leq 0.05$). Asprosin levels were decreased significantly in insulin treated group day 7 and day 20 in comparison with gestational diabetic group day 7 and 20 respectively ($p \leq 0.0001$). Our findings are in agreement with Zhang, et al. [10], who found a significant increase in asprosin concentrations in diabetes mellitus type 2 in cmparison with the control ($p<0.001$). They suggested that asprosin might be a risk factor associated with the pathogenesis of diabetes mellitus type 2. Our findings are also supported by Long, et al. [28], who found that asprosin was correlated with insulin.

In the current study, we found highly significant elevations in estradiol level in pregnant, gestational diabetic and insulin treated groups compared with control group ($p \leq 0.0001$). Estradiol significantly decreased in gestational diabetic group day 7 and day 20 compared with pregnant group day 7 and day 20 respectively ($p \leq 0.0001$) however, no significant differences in insulin treated group day 7 and day 20 compared with pregnant group day 7 and day 20 respectively ($p \leq 0.05$). Highly significant increases in estradiol levels were found in insulin treated group day 7 and day 20 compared with gestational diabetic group day 7 and day 20 respectively ($p \leq 0.0001$). We found a negative correlation between plasma asprosin and estradiol and progesterone levels. These findings are in agreement with Li, et al. [15], who reported the correlation of asprosin level with sex-related hormones in females. They also reported a negative correlation between asprosin and sex hormone binding globulin (SHBG) because insulin resistance could increase androgen levels by stimulating ovarian steroidogenesis.

In the current study, progesterone increased significantly in pregnant group 2 in comparison with control group ($p \leq 0.0001$). Highly significant reductions in progesterone levels in gestational diabetic group and insulin treated group in comparison with the control group ($p \leq 0.0001$). In addition, highly significant reductions in progesterone levels in gestational diabetic group day 7 and 20 and insulin treated group day 7 and 20 in comparison with the pregnant group day 7 and 20 respectively ($p \leq 0.0001$). Progesterone was also decreased significantly in insulin treated group compared with gestational diabetic group. These findings are supported by Li, et al. [15], who found that sex-related hormones were significantly correlated with asprosin. Our findings are also in agreement with Wei, et al. [31], who demonstrated that the Asprosin-OLFR734 (Olfactory receptor) signaling axis promotes sperm progressive motility and enhances fertility.

In the present study, no significant differences were found in cholesterol level between pregnant day 7 and control groups however, significant and highly significant differences were found in pregnant day 20 and gestational diabetic groups respectively ($p \leq 0.05$ and $p \leq 0.0001$). These findings are in agreement with Bequer, et al. [32], who reported that human pregnancy increases serum triglycerides, cholesterol, glycerol,

and fatty acids. In our study, Insignificant differences were also found in group 4 (gestational diabetic treated with insulin) day 7 compared with control group. Total cholesterol increased significantly in gestational diabetic group at day 7 and day 20 in comparison with pregnant group 2 day 7 and day 20 respectively ($p \leq 0.0001$). These findings is also in agreement with Bequer, et al. [32], who found that total cholesterol was significantly higher at the end of the pregnancy in the diabetic group. No significant differences were found between group 4 (insulin treated) day 7 and day 20 and group 2 (pregnant) day 7 and 20 respectively. Highly significant reductions in total cholesterol were found in group 4 day 7 and day 20 compared with group 3 day 7 and 20 respectively ($p \leq 0.0001$). In present study, A significant positive correlation between asprosin level and cholesterol level ($p \leq 0.0001$). Our findings are supported by Li, et al. [15], who found that plasma asprosin concentration was positively correlated with total cholesterol (TC) triglycerides (TG) and LDL.

We did not find any significant differences in triglyceride level in pregnant group day 7 and day 20 and group 4 day 7 and day 20 in comparison with control group ($p<0.05$) however highly significant increases were found in gestational diabetic group 3 ($p \leq 0.0001$) compared with the control group. In our study, triglycerides increased significantly in gestational diabetic group at day 7 and day 20 compared with pregnant group day 7 and day 20 respectively ($p \leq 0.0001$). These findings are in agreement with Bequer, et al. [32], who found that triglycerides were significantly higher in gestational diabetes day 20. Insignificant differences were found in group 4 day 7 and 20 compared with control group day 7 and 20 respectively ($p<0.05$) however, highly significant reductions in triglycerides were found in group 4 day 7 and 20 compared with group 3 day 7 and 20 respectively ($p \leq 0.0001$). We also found a strong positive correlation between asprosin level and triglycerides ($r=0.9944$, $p \leq 0.0001$). Our findings are in agreement with Wang, et al. [26], who showed that plasma asprosin levels were positively correlated with triglyceride (TG). Our findings are also supported by Li, et al. [15], who reported that increased asprosin has been correlated with triglycerides in diabetes mellitus type 2.

In the present study, no significant change in HDL levels was found in group 2 and group 4 compared with group 1 ($p<0.05$). Highly significant reductions in HDL were found in group 3 compared with group 1 ($p \leq 0.0001$). We also found highly significant reductions in HDL in group 3 day 7 and day 20 compared with group 2 day 7 and day 20 respectively ($p \leq 0.0001$). HDL increased significantly in insulin treated group day 7 and 20 in comparison with gestational diabetic group day 7 and 20 respectively ($p \leq 0.0001$). We found a strong negative correlation between asprosin and HDL ($p \leq 0.0001$). Our findings are in agreement with Long, et al. [28], who found that asprosin was correlated with HDL after adjusting for age and they suggested a complex role for asprosin in energy metabolism.

Insignificant differences in LDL and VLDL were found in pregnant group day 7 and control group ($p<0.05$) and in LDL in pregnant group day 20 compared with control group however significant difference in VLDL was found in pregnant group day 20 compared with control group ($p \leq 0.05$). Highly significant elevations in LDL and VLDL in gestational diabetic group compared with control group ($p \leq 0.0001$). Insignificant diiferences were found in group 4 day 7 and control group however significant differences were found in group 4 day 20 compared with control group. Extremely significant increases were found in LDL and VLDL in gestational diabetic group day 7 and day 20 compared with pregnant group day 7 and day 20 respectively ($p \leq 0.0001$) however, no significant differences were found in insulin treated group day 7 and day 20 compared with preganant group day 7 and day 20 respectively. We also found highly significant reductions in LDL and VLDL in insulin treated group day 7 and 20 compared with gestational diabetic group ($p \leq 0.0001$). Our findings are in agreement with Li, et al. [15], who found that plasma asprosin was significantly higher in diabetic females than controls ($p<0.001$) and correlated positively with fasting blood glucose, HOMA-IR and LDL-c, ($p<0.05$). However, our findings are also supported by Zhang, et al. [10], who reported that fasting glucose and triglyceride were associated with asprosin levels in T2DM.

CONCLUSION

In the present study, asprosin levels increased significantly in pregnant group 2, gestational group 3 and insulin treated group 4 in comparison with control group ($p \leq 0.001$, $p \leq 0.0001$ and $p \leq 0.0001$

respectively). We found significant elevations in asprosin levels in gestational diabetic group day 7 and day 20 in comparison with pregnant group day 7 and day 20 respectively ($p \leq 0.0001$). Insignificant differences in asprosin levels were found in insulin treated group day 7 and day 20 compared with pregnant group day 7 and 20 respectively ($p \leq 0.05$). Asprosin levels were decreased significantly in insulin treated group day 7 and day 20 in comparison with gestational diabetic group day 7 and 20 resppectively ($p \leq 0.0001$).

We found a significant increase in body weight, food intake and body mass index in gestational diabetic group compared with the control and pregnant nondiabetic groups. We also found a strong positive correlation between plasma asprosin and body weight ($r=0.821$, $p<0.05$), body mass index ($p<0.05$) and food intake. We also found a strong positive correlation between asprosin and glucose levels ($r= 0.9958$, $p<0.00001$), HOMA-IR, total cholesterol, triglycerides, LDL and VLDL and a strong negative correlation with HOMA-B and HDL. We also found a negative correlation between plasma asprosin and estradiol and progesterone levels.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

[1] Marathe, Payal H., Helen X. Gao, and Kelly L. Close. "American Diabetes Association Standards of Medical Care in Diabetes 2017." *Journal of Diabetes,* Vol. 9, No. 4, 2017, pp. 320-24.

[2] Wendland, Eliana M., et al. "Gestational diabetes and pregnancy outcomes-a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria." *BMC Pregnancy and Childbirth,* Vol. 12, No. 1, 2012, p. 23.

[3] Roglic, Gojka, ed. "Global report on diabetes." World Health Organization, 2016.

[4] Coustan, Donald R. "Gestational diabetes mellitus." *Clinical Chemistry,* Vol. 59, No. 9, 2013, pp. 1310-21.

[5] Rodrigues, Fabiane, et al. "Influence of gestational diabetes on the activity of δ-aminolevulinate dehydratase and oxidative stress biomarkers." *Redox Report,* Vol. 23, No. 1, 2018, pp. 63-67.

[6] Baz, Baz, Jean-Pierre Riveline, and Jean-François Gautier. "Gestational diabetes mellitus: Definition, aetiological and clinical aspects." *European Journal of Endocrinology,* Vol. 174, No. 2, 2016, pp. R43-51.

[7] Romere, Chase, et al. "Asprosin, a fasting-induced glucogenic protein hormone." *Cell,* Vol. 165, No. 3, 2016, pp. 566-79.

[8] Greenhill, Claire. "Liver: Asprosin-New hormone involved in hepatic glucose release." *Nature Reviews Endocrinology,* Vol. 12, No. 6, 2016, p. 312.

[9] Duerrschmid, Clemens, et al. "Asprosin is a centrally acting orexigenic hormone." *Nature Medicine,* Vol. 23, No. 12, 2017, p. 1444.

[10] Zhang, Lei, et al. "Circulating asprosin concentrations are increased in type 2 diabetes mellitus and independently associated with fasting glucose and triglyceride." *Clinica Chimica Acta,* Vol. 489, 2019, pp. 183-88.

[11] Gerenutti, M., F. S. Del Fiol, and F. C. Groppo. "Reproductive performance of pregnant rats and embryotoxic effects of ciprofloxacin." *Die Pharmazie-An International Journal of Pharmaceutical Sciences,* Vol. 61, No. 1, 2006, pp. 79-80.

[12] Laurino, Leticia F., et al. "Lentinus edodes exposure before and after fetus implantation: Materno-fetal development in rats with gestational diabetes mellitus." *Nutrients,* Vol. 11, No. 11, 2019, p. 2720.

[13] Thornberry, Nancy A., et al. "A combinatorial approach defines specificities of members of the caspase family and granzyme B Functional relationships established for key mediators of apoptosis." *Journal of Biological Chemistry,* Vol. 272, No. 29, 1997, pp. 17907-11.

[14] Turhan, Ugur, et al. "Investigation of the effect of gestational diabetes on fetal cardiac tissue in streptozotocin ınduced in rats." *Acta Cirurgica Brasileira,* Vol. 33, No. 4, 2018, pp. 306-13.

[15] Li, Xing, et al. "Plasma asprosin levels are associated with glucose metabolism, lipid, and sex hormone profiles in females with metabolic-related diseases." *Mediators of Inflammation,* 2018, pp. 1-12.

[16] Nascimento, Andre F., et al. "Misclassification probability as obese or lean in hypercaloric and normocaloric diet." *Biological Research,* Vol. 41, No. 3, 2008, pp. 253-59.

[17] Novelli, E. L. B., et al. "Anthropometrical parameters and markers of obesity in rats." *Laboratory Animals,* Vol. 41, No. 1, 2007, pp. 111-19.

[18] Finley, Paul R., and N. W. Tietz, eds. "Clinical guide to laboratory tests." WB Saunders company, 1996.

[19] Reaven, Gerald M. "Insulin resistance, hyperinsulinemia, hypertriglyceridemia, and hypertension: Parallels between human disease and rodent models." *Diabetes Care,* Vol. 14, No. 3, 1991, pp. 195-202.

[20] Bonora, Enzo, et al. "Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: Studies in subjects with various degrees of glucose tolerance and insulin sensitivity." *Diabetes Care,* Vol. 23, No. 1, 2000, pp. 57-63.

[21] Fossati, Plero, and Lorenzo Prencipe. "Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide." *Clinical Chemistry,* Vol. 28, No. 10, 1982, pp. 2077-80.

[22] Nauck, M. A., J. J. Holst, and B. Willms. "Glucagon-like peptide 1 and its potential in the treatment of non-insulin-dependent diabetes mellitus." *Hormone and Metabolic Research,* Vol. 29, No. 9, 1997, pp. 411-16.

[23] Friedewald, William T., Robert I. Levy, and Donald S. Fredrickson. "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge." *Clinical Chemistry,* Vol. 18, No. 6, 1972, pp. 499-502.

[24] Baykus, Yakup, et al. "Asprosin in umbilical cord of newborns and maternal blood of gestational diabetes, preeclampsia, severe preeclampsia, intrauterine growth retardation and macrosemic fetus." *Peptides,* Vol. 120, 2019, p. 170132.

[25] Wiecek, Magdalena, et al. "Decreased blood asprosin in hyperglycemic menopausal women as a result of whole-body cryotherapy regardless of metabolic syndrome." *Journal of Clinical Medicine,* Vol. 8, No. 9, 2019, p. 1428.

[26] Wang, Yuren, et al. "Plasma asprosin concentrations are increased in individuals with glucose dysregulation and correlated with insulin resistance and first-phase insulin secretion." *Mediators of Inflammation,* 2018, pp. 1-7.

[27] Duerrschmid, Clemens, et al. "Asprosin is a centrally acting orexigenic hormone." *Nature Medicine,* Vol. 23, No. 12, 2017, p. 1444.

[28] Long, Wenjun, et al. "Decreased circulating levels of asprosin in obese children." *Hormone Research in Paediatrics,* Vol. 91, No. 4, 2019, pp. 271-77.

[29] Wang, Min, et al. "Serum asprosin concentrations are increased and associated with insulin resistance in children with obesity." *Annals of Nutrition and Metabolism,* Vol. 75, No. 4, 2019, pp. 205-12.

[30] Alan, Murat, et al. "Asprosin: a novel peptide hormone related to insulin resistance in women with polycystic ovary syndrome." *Gynecological Endocrinology,* Vol. 35, No. 3, 2019, pp. 220-23.

[31] Wei, Fangchao, Aijun Long, and Yiguo Wang. "The Asprosin-OLFR734 hormonal signaling axis modulates male fertility." *Cell Discovery,* Vol. 5, No. 1, 2019, pp. 1-3.

[32] Bequer, Leticia, et al. "Experimental diabetes impairs maternal reproductive performance in pregnant Wistar rats and their offspring." *Systems Biology in Reproductive Medicine,* Vol. 64, No. 1, 2018, pp. 60-70.