

New born macrosomia in gestational diabetes mellitus

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Abstract. Gestational diabetes mellitus (GDM) is a metabolic complication of pregnancy. The pathogenesis of GDM is considered to involve β -cell dysfunction and insulin resistance (IR). GDM is associated with a significant risk of macrosomia in addition to a high probability of metabolic complications for the offspring. The precise mechanism underlying GDM remains unclear. The aim of the present study was to analyse the factors associated with insulin resistance and β -cell dysfunction involved in the pathophysiology of GDM complicated with macrosomia compared with GDM without macrosomia. In addition, another aim of the present study was to assess the relationship between GDM complicated with macrosomia and anthropometric, clinical and para-clinical parameters. The following group of patients were recruited as part of a case-control study: Patients with GDM without macrosomia, patients with GDM complicated with macrosomia and healthy gestational controls. Blood samples were collected at the third trimester of pregnancy and tested for adiponectin, leptin, insulin, proinsulin and C-peptide. Homeostatic model assessment-IR (HOMA-IR), steady state β -cell function (HOMA%B), insulin sensitivity (HOMA%S) and body mass index (BMI) were also calculated. All patients diagnosed with GDM showed an impairment in HOMA%B and a decrease in C-peptide maternal serum concentration. Additionally, diabetic status leading to the birth of offspring with macrosomia did not induce changes in the maternal serum levels of insulin, proinsulin, adiponectin or leptin, which was also the case in patients with GDM but not macrosomia. HOMA%B presented a stronger positive correlation with pre-pregnancy BMI and maternal weight gain, and a

stronger negative correlation with adiponectin. Furthermore, HOMA%S in this group exhibited strong positive correlations with maternal serum levels of high-density lipoprotein cholesterol (HDL) and aspartate aminotransferase, and a strong negative correlation with pre-pregnancy BMI. In the same patients, HOMA-IR was also found to have a high negative correlation with HDL levels, and highly positive correlations with gestational age and triglyceride levels. In conclusion, the present study suggests that the different correlations among the factors involved in the pathogenesis of GDM may explain the evolution of GDM pregnancy to macrosomia.

Introduction

Gestational diabetes mellitus (GDM) is a metabolic complication of pregnancy that is defined by the development of glucose intolerance, which is first recognized during pregnancy (1). The prevalence of GDM has been reported to be at ~20% of all pregnancies worldwide (2), but it is increasing due to the epidemic nature of obesity among women during reproductive age (2). Maternal genetic predisposition, fetoplacental and environmental factors have all been proposed to initiate vascular damage events that result in long term complications at the level of the heart, kidneys and nerves and are becoming a serious public health burden (2). Individuals with a personal history of GDM and her offspring both have an increased risk of developing type 2 diabetes, obesity, cardiovascular diseases and metabolic syndrome (3). Therefore, it is critical to understand the physiopathology of GDM, since it generates a transgenerational vicious circle of various metabolic diseases. Risk factors for GDM, such as advanced maternal age, overweight, obesity, high parity, previous delivery of a macrosomic infant, are associated with impaired β -cell function and insulin resistance (IR), which are essential components in the pathogenesis of GDM (3). Early prevention of adverse pregnancy outcomes, such as excessive fetal growth, jaundice, neonatal hypoglycaemia, stillbirth, polyhydramnios, hypertensive disorders of pregnancy and fetal growth restriction, may improve the quality of life and health care efficiency (3).

β -cell dysfunction occurs when β -cells lose their capacity to respond correctly to blood glucose changes, resulting in insufficient insulin secretion (4). IR aggravates β -cell dysfunction

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by excessively stimulating insulin production in response to chronic hyperglycaemia (4). This mechanism is defined as glucotoxicity which, over time β -cell dysfunction leads to a vicious cycle starting with hyperglycaemia and followed by insulin resistance, eventually β -cell apoptosis (5). Malfunction of β -cells can typically appear at different levels: Pro-insulin synthesis or post-translational modifications, secretory vesicle storage and exocytosis, or sensing of blood glucose concentrations (5). Proinsulin is the precursor molecule of insulin that is produced by pancreatic cells and incorporates the A and B chains of insulin connected between amino acid residues 31 and 65 by the C-peptide (5). In normal conditions, all proinsulin is cleaved to produce C-peptide and insulin, whilst a small amount of intact proinsulin may also be released into the circulation (5). In response to IR, pancreatic β -cell function is affected, which results in an increased release of both intact and split forms of proinsulin (5).

Physiological IR during pregnancy is necessary for fetal growth and when insulin secretion fails to compensate for IR, hyperglycaemia and GDM develops (6). Specifically, IR is a state in which normal levels of insulin cannot initiate a response in target cells to uptake glucose from the circulation (7). It is this lack of response that stimulates the pancreas to secrete more insulin (7). Other factors involved in the development of IR during pregnancy are adipocyte-derived hormones, such as adiponectin and leptin (8). Adiponectin regulates insulin action and glucose homeostasis, the serum levels of which decrease during pregnancy (8). During the third trimester of pregnancy, when the severity of maternal IR is at its highest, circulating adiponectin reaches its lowest level (9). Hypoadiponectinemia during pregnancy has several effects leading to GDM: It increases IR in skeletal muscles and reduces glucose uptake; increase pancreatic β -cell dysfunction; and induction of hyperglycaemia (9). Maternal adiponectin was previously found to be higher in healthy pregnant individuals compared with that in pregnant individuals complicated with GDM (10). Another hormone secreted by adipocytes is leptin. A previous study has reported that leptin levels are increased during GDM, which are associated with increased sizes of the fetus (11). In addition, higher plasma leptin concentrations have been reported in obese individuals, due to the associated inflammation (12).

A previous study has suggested that IR in GDM can result in differential pregnancy outcomes, such as macrosomia (7). Fetal macrosomia is defined as an infant birth weight of $\geq 4,000$ g, which affects 15–45% of all new-borns from women with GDM (13).

A previous study showed that non-alcoholic fatty liver disease (NAFLD) during pregnancy increases the risk of GDM whilst the presence of GDM also increases the risk of NAFLD (14). This association is bidirectional though the mechanism remains unclear. NAFLD is characterized by elevated levels of transaminases and leads to hepatic IR (14). Hepatic IR increases glycogen breakdown and free fatty acid secretion due to increased lipolysis, which contributes to macrosomia (15). To the best of our knowledge, there are insufficient data regarding the impact of maternal NAFLD on macrosomia in GDM.

Results of a 2005 clinical trial led to the recommendation of controlling maternal hyperglycaemia and gestational weight gain, in order to prevent macrosomia (16). However, there are

also data showing that insulin levels are elevated in the blood sampled from the umbilical cord of macrosomic infants from non-diabetic mothers (17). This suggests that there are other risk factors for macrosomia apart from pre-existing maternal diabetes and uncontrolled GDM. Therefore, further analyses on macrosomic GDM pregnancy are required to identify these other factors and their relationship with IR during the second half of pregnancy.

The aims of the present study were: i) To identify the factors involved in the pathophysiology of GDM complicated with or without macrosomia; and ii) to reveal the association between these factors and anthropometric, clinical and para-clinical parameters of the patient.

Materials and methods

Study population. A case-control study was conducted on 36 pregnant women who presented in the Second Department of Diabetes ‘N. Paulescu’ National Institute of Diabetes, Nutrition and Metabolic Diseases (Bucharest, Romania) between Jan 2018 and Nov 2021. The inclusion criteria were as follows: i) Women aged 18–40 years; ii) 24 to 32 weeks of gestation; and iii) if their fasting glucose levels exceed the cut-off levels [fasting (OGTT 0-h) ≥ 92 mg/dl; after 1 h of fasting (OGTT 1-h) ≥ 180 mg/dl; and after 2 h of fasting (OGTT 2-h) ≥ 153 mg/dl]. The present study was approved by the Ethics Committee of the ‘N. Paulescu’ National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania (approval no. 1680/01.11.2017). Each patient involved in the study signed the written informed consent form as specified in the Declaration of Helsinki and agreed to the use of their samples for scientific research.

The patients with GDM and non-diabetic individuals were divided into the following three groups: i) Gestational healthy control group (GC; $n=8$; age, 26 ± 2.33 years); ii) gestational diabetes mellitus group (GDM) with normal offspring (GDM-N; birth weight of child $< 4,000$ g; $n=23$; age, 31.52 ± 3.80 years); and iii) GDM with macrosomia (GDM-M; birth weight of child $\geq 4,000$ g; $n=5$; age, 32.00 ± 6.67 years).

The exclusion criteria were: Aged < 18 or > 40 years; hypertension; preeclampsia; retinopathy; nephropathy; and psychiatric treatment. A total of 28 patients with GDM maintained normoglycemia with medical nutrition therapy from the moment of GDM diagnosis (24–32 weeks of gestation). Specifically, an individualized nutrition plan was enacted to provide an adequate caloric intake, optimal glycemic levels and maternal weight gain according to the 2009 Institute of Medicine (US) and National Research Council (US) Committee recommendations (18). They received recommendations for ≥ 175 g carbohydrates, ≥ 71 g proteins and 28 g fibres. In addition, at GDM diagnosis, 10 ml blood samples were collected from all patients and sera were isolated for biochemical and immunological testing as detailed below (Fig. 1). A baby's Apgar score was calculated using heart rate, respiratory effort, muscle tone, skin color and reflex irritability (19).

Definition of glucose tolerance. The screening test for the diagnosis of GDM is based on 75-g oral glucose tolerance test (OGTT) at 2 h after administration (recommendations of National Institute for Health and Care Excellence, American

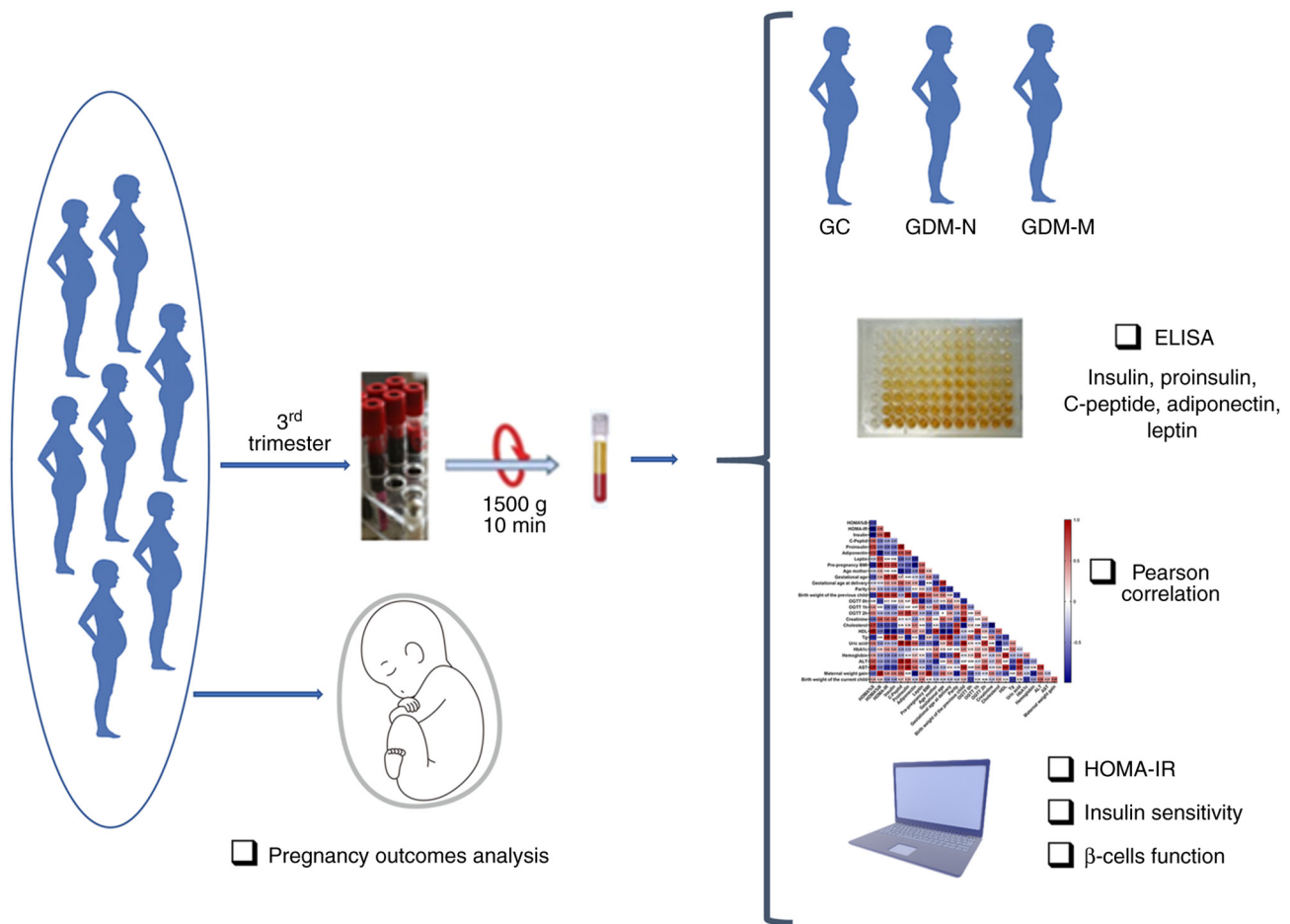


Figure 1. Experimental protocol of the present case-control study design and methodological approaches. The selected subjects were divided into three groups based on oral glucose tolerance test at 0, 1 and 2 h on the third trimester of pregnancy and the baby's weight at birth. Among the patients, 8 were with normal pregnancy, 23 had GDM with normal birth weight of the child, whilst 5 had GDM with macrosomia. The serum was adequately prepared for biochemical and immunological analysis. Homeostatic model assessment of insulin resistance, insulin sensitivity and β -cell function were calculated followed by Pearson correlation analysis were performed on the clinical and paraclinical variables. GC, gestational healthy control group; GDM, gestational diabetes mellitus; GDM-N, GDM group with normal offspring; GDM-M, GDM group with macrosomia; HOMA-IR, homeostatic model assessment of insulin resistance.

Diabetes Association) (20). Diagnosis of GDM would be confirmed if ≥ 1 values of glucose levels exceed the cut-off levels: Fasting (OGTT 0-h) ≥ 92 mg/dl; after 1 h of fasting (OGTT 1-h) ≥ 180 mg/dl; and after 2 h of fasting (OGTT 2-h) ≥ 153 mg/dl.

Biochemical tests. Blood samples were collected to establish the diagnosis of GDM during the third trimester of pregnancy. The samples were harvested after ≥ 8 h of fasting and blood samples were centrifuged at $1,000 \times g$ for 15 min at room temperature and stored at -80°C . According to the manufacturer's protocols (DRG Instruments GmbH), specific ELISA kits were used to measure the concentration of human serum adiponectin (cat. no. EIA-4177), C-peptide (cat. no. EIA-1293), insulin (cat. no. EIA-2935), leptin (cat. no. EIA-2395) and proinsulin (cat. no. EIA-1560).

Commercial kits from DIALAB GmbH were used to measure the serum cholesterol (cat. no. D95116), high-density lipoprotein cholesterol (HDL; cat. no. F03100), triglycerides (Tg; cat. no. DK0740), creatinine (cat. no. D06450), uric acid (cat. no. D00720), alanine aminotransferase (ALT; cat. no. D98625) and aspartate aminotransferase (AST; cat. no. D98617) levels, according to the manufacturer's

protocols. Fasting plasma glucose was determined using the glucose oxidase method using a glucose analyser (AU480 Clinical Chemistry System; Beckman Coulter, Inc.). Plasma HbA1c levels were determined using the Variant II Turbo HbA1c analyser (Bio-Rad Laboratories, Inc.) and the Variant II Turbo HbA1c kit (cat. no. 12000447; Bio-Rad Laboratories, Inc.), which uses cation exchange high-performance liquid chromatography, according to the manufacturer's protocols.

Assessment of IR and β -cell function. The homeostasis model assessment (HOMA-IR) is a validated method for quantifying IR and β -cell function (21). It is calculated based on the plasma levels of fasting glucose and insulin and it is a mathematical assessment that yields an estimate of an individual's degree of insulin sensitivity (HOMA%S) and the level of steady-state β -cell function (HOMA%B) (21). The following formulas were used: $\text{HOMA-IR} = [\text{fasting insulin (mU/l)} \times \text{fasting glucose (mmol/l)}] / 22.5$; $\text{HOMA}\%B = [20 \times \text{fasting insulin (mU/l)}] / [\text{fasting glucose (mmol/l)} - 3.5]$; and $\text{HOMA of insulin sensitivity (HOMA}\%S) = 1 / \text{HOMA-IR} \times 100$.

Sociodemographic data. Sociodemographic data of the subjects, including maternal age, parity, smoking status, family

Table I. Characteristics of the individuals included in the present study.

Characteristics	Gestational healthy control (n=8)	GDM-N (n=27)	GDM-M (n=5)
Age, years	26.00±2.33	31.52±3.80 ^c	32.00±6.67 ^a
Pre-pregnancy BMI, kg/m ²	24.21±5.13	26.00±5.26	32.50±0.14
BMI at gestational age, kg/m ²	27.69±5.29	29.41±5.56	32.45±2.00 ^a
Gestational age at diagnosis, weeks	25.88±1.55	29.29±2.77 ^b	30.00±3.81 ^b
Gestational age at delivery, weeks	38.50±0.71	37.93±8.22	38.40±0.55
Parity (number of gestations)	1.13±1.13	1.31±1.25	1.80±2.05
OGTT 0-h, mg/dl	75.25±7.21	92.87±13.49 ^b	99.70±13.30 ^b
OGTT 1-h, mg/dl	115.17±19.86	185.70±36.23 ^c	198.80±39.70 ^c
OGTT 2-h, mg/dl	81.81±23.32	150.34±29.43 ^c	168.98±36.84 ^c
Creatinine, mg/dl	0.45±0.14	0.47±0.08	0.47±0.08
Cholesterol, mg/dl	253.73±43.9	239.28±41.90	271.77±17.85
High-density lipoprotein cholesterol, mg/dl	75.83±23.67	72.08±18.27	77.90±17.95
Triglycerides, mg/dl	146.29±29.10	237.2±74.36 ^a	207.66±84.97
Uric acid, mg/dl	3.26±0.75	3.57±1.04	4.57±2.18
Glycated haemoglobin, %	5.2±0.17	5.49±0.41	5.42±0.37
Hemoglobin, g/dl	11.13±0.82	11.12±1.02	10.7±0.66
Alanine aminotransferase, Ui/l	28.85±34.23	16.88±13.03	12.84±11.89
Aspartate aminotransferase, Ui/l	19.49±9.63	16.07±6.80	13.13±3.07

Data are expressed as mean ± standard deviation. ^aP<0.05, ^bP<0.01 and ^cP<0.001 vs. GC group. GDM, gestational diabetes mellitus; GDM-N, GDM group with normal offspring; GDM-M, GDM group with macrosomia; BMI, body mass index; OGTT, oral glucose tolerance test.

history of diabetes, family medical history, socioeconomic status, were obtained by anamnesis. Anthropometric measurements, including height, present body weight, pre-pregnancy body weight, BMI and blood pressure, were also measured.

Statistical analysis. Data obtained are expressed as the mean ± standard deviation. Statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad Software, Inc.) using unpaired Student's t-test or one-way ANOVA followed by Tukey's post-hoc test. Pearson's correlation analysis was performed on serum level parameters and patient characteristics associated with GDM. P<0.05 was considered to indicate a statistically significant difference.

Results

Maternal characteristics. The serum samples collected from the patients used to establish the diagnosis of GDM were collected at the third trimester of pregnancy for which these subjects were treated only by dietary modulation. A summary of the study patient groups characteristics is shown in Table I.

It was observed that patient age, a well-known risk factor for GDM development (3), was significantly higher in the GDM-N (1.21-fold; P<0.001) and in the GDM-M (P<0.05; 1.23-fold) groups compared with that in the GC group. In addition, GDM-M women had higher BMI at gestational age at diagnosis (1.17-fold) compared with that in the GC group (P<0.05).

Diabetes was confirmed by OGTT, for which both the GDM-N and GDM-M groups were exhibiting significantly

higher levels compared with those in the GC group at 0, 1 and 2 h (P<0.01) (Table I). The serum Tg levels were also higher in the GDM-N group (1.62-fold; P≤0.05) compared with those in the GC group. However, GDM did not significantly alter the gestational age at delivery, parity or serum levels of creatinine, cholesterol, HDL, uric acid, HbA1c, haemoglobin, ALT or AST.

Adverse outcomes induced by GDM. The adverse outcomes induced by GDM, despite the diet introduced during the third trimester of pregnancy, were analysed to achieve optimal glucose levels (Table II).

There were five offspring weighing >4,000 g (macrosomia) born from women with a controlled diet regulating the serum blood glucose levels during pregnancy. There were no significant differences in terms of neonatal hypoglycaemia, jaundice, Apgar score, hypertension, edema or the type of delivery (natural or Caesarean section) between the GDM-N and GDM-M groups.

Altered β -cell homeostasis is an essential component in the pathogenesis of GDM. The primary function of β -cells is to produce and release insulin. Based on fasting insulin and glucose serum concentrations, HOMA%B, HOMA%S and HOMA-IR were calculated (Fig. 2A-D).

In the GDM-N group, a significant decrease in HOMA%B (1.67-fold; P<0.01), serum insulin (1.47-fold; P<0.05), proinsulin (1.34-fold; P<0.05) and C-peptide (1.43-fold; P<0.05) were observed compared with those in the GC group (Fig. 2A and D-F).

Pancreatic dysfunction was verified by the observed decrease in HOMA%B (2.2-fold; P<0.01) and in serum

Table II. Pregnancy outcomes of patients with GDM included in the present study.

A, Child			
Outcomes	GDM-N (n=23), N (%)	GDM-M (n=5), N (%)	P-value
Birth weight			
Infants <4,000 g	23 (100)	0 (0)	0.0001
Infants >4,000 g	0 (0)	5 (100)	
Neonatal hypoglycaemia	1 (4.34)	1 (20)	0.07
Jaundice	2 (8.69)	0 (0)	0.2560
Apgar score			
≤8	4 (17.39)	1 (20)	0.24
>8	19 (82.6)	4 (80)	
B, Mother			
Outcomes	GDM-N (n=23), N (%)	GDM-M (n=5), N (%)	P-value
Hypertension in pregnancy	3 (13.04)	0 (0)	0.33
Edema	2 (8.69)	1 (20)	0.24
Delivery			
Natural	7 (30.43)	0 (0)	0.08
Caesarean section	16 (69.56)	5 (100)	

GDM, gestational diabetes mellitus; GDM-N, GDM group with normal offspring; GDM-M, GDM group with macrosomia. Data are expressed both as percentages (%) and as means with corresponding statistical significance P-value.

C-peptide concentration (2.34-fold; $P<0.05$) in the GDM-M group compared with that in the GC group (Fig. 2A and F). The insulin and proinsulin levels did not show a significant difference between the patients with GDM-M and those in the GC group (Fig. 2D and E). In addition, no significant differences in HOMA%B and HOMA-IR were observed between the GDM-M and GDM-N groups (Fig. 2B and C). Serum concentrations of insulin, proinsulin, C-peptide, the HOMA%B, HOMA%S and HOMA-IR also did not show a significant difference between the GDM-M and GDM-N groups.

Insulin resistance is an important component of GDM. Insulin resistance in gestational diabetes can also be caused by the alterations in hormones produced during pregnancy, such as adiponectin and leptin, which can render the secreted insulin less effective (10).

Serum collected from patients in the GDM-N group presented a significantly decreased level of adiponectin (1.62-fold; $P<0.01$) and a significantly increased level of leptin (1.42-fold; $P<0.05$) compared with those in the GC group (Fig. 3A and B). This meant that the GDM-N group exhibited a significantly decreased ratio of adiponectin/leptin (3.34-fold; $P<0.05$) compared with that in GC (Fig. 3C).

However, in the GDM-M group the change in the calculated ratio is not statistically significant compared with the GC group. In addition, the GDM-M group showed a significantly increase in the adiponectin level (1.55-fold; $P<0.05$) and a significantly increased ratio of adiponectin/leptin (2.01-fold; $P<0.05$) compared with those in the GDM-N group (Fig. 3A and C).

The patients with GDM-M, specifically in their third trimester of pregnancy when GDM was diagnosed, had a mean BMI at gestational age of 32 ± 2 kg/m² (Fig. 3D), which was 1.17-fold higher compared with that of GC ($P<0.05$). By contrast, there was no difference in the pre-pregnancy BMI between the GDM-M and GDM-N groups (Fig. 3E).

Correlation between clinical and paraclinical characteristic of GDM patients. The Pearson R statistical test and Pearson correlation matrices were used to measure the strength of the correlation between the different parameters of women with GDM-N (Fig. 4A). In addition, correlation matrices between the clinical and paraclinical parameters in women with GDM who have given birth to children with macrosomia (birth weight of child >4,000 g) were shown, which were compared with GDM-N (Fig. 4A and B).

In the entire GDM-N and GDM-M groups, several significant Pearson's correlations were identified between the principal factors involved in the pathophysiology of GDM. The following factors are associated with β -cell dysfunction in GDM: HOMA%B; HOMA%S; HOMA-IR; insulin; proinsulin; and C-peptide serum levels.

In GDM-N, there was a significant correlation between HOMA%B and HOMA%S ($r=-0.73$; $P<0.001$), HOMA-IR ($r=0.78$; $P<0.001$), insulin ($r=0.73$; $P<0.001$), leptin ($r=-0.435$; $P<0.05$) and pre-pregnancy BMI ($r=0.371$; $P=0.04$). By contrast, HOMA%B in the GDM-M group presented a strong correlation with pre-pregnancy BMI ($r=0.95$; $P<0.05$) and specifically for this group, with maternal weight gain ($r=0.94$; $P<0.03$) and adiponectin ($r=-0.96$; $P<0.01$).

It was observed that HOMA%S presented a correlation with HOMA%B ($r=-0.73$; $P<0.001$), creatinine ($r=0.43$; $P<0.05$), pre-pregnancy BMI ($r=-0.40$; $P<0.05$) and C-peptide ($r=-0.36$; $P<0.05$) in the GDM-N group. Additionally, the correlations between HOMA%S and serum levels of HDL ($r=0.97$; $P<0.05$), AST ($r=0.87$; $P<0.05$) and pre-pregnancy BMI ($r=-0.86$; $P<0.05$) are specific to GDM-M.

The present study found that in the GDM-N group the calculated HOMA-IR was significantly correlated with HOMA%B ($r=0.78$; $P<0.001$), pre-pregnancy BMI ($r=0.57$; $P<0.01$) and serum creatinine levels ($r=-0.46$; $P<0.05$). By contrast, in the GDM-M group, HOMA-IR was not correlated with HOMA%B or creatinine concentration, but was strongly correlated ($P<0.05$) with serum levels of HDL ($r=-0.96$), Tg ($r=0.89$) and gestational age at which diabetes was diagnosed ($r=0.81$).

Maternal insulin level was positively correlated with HOMA%B ($r=0.73$; $P<0.001$) and C-peptide ($r=0.379$; $P<0.05$) in the GDM-N group. By contrast, in the GDM-M group a specific and strong correlation ($P<0.05$) was found between insulin level with HDL ($r=-0.97$) and gestational age at which GDM was diagnosed ($r=0.80$).

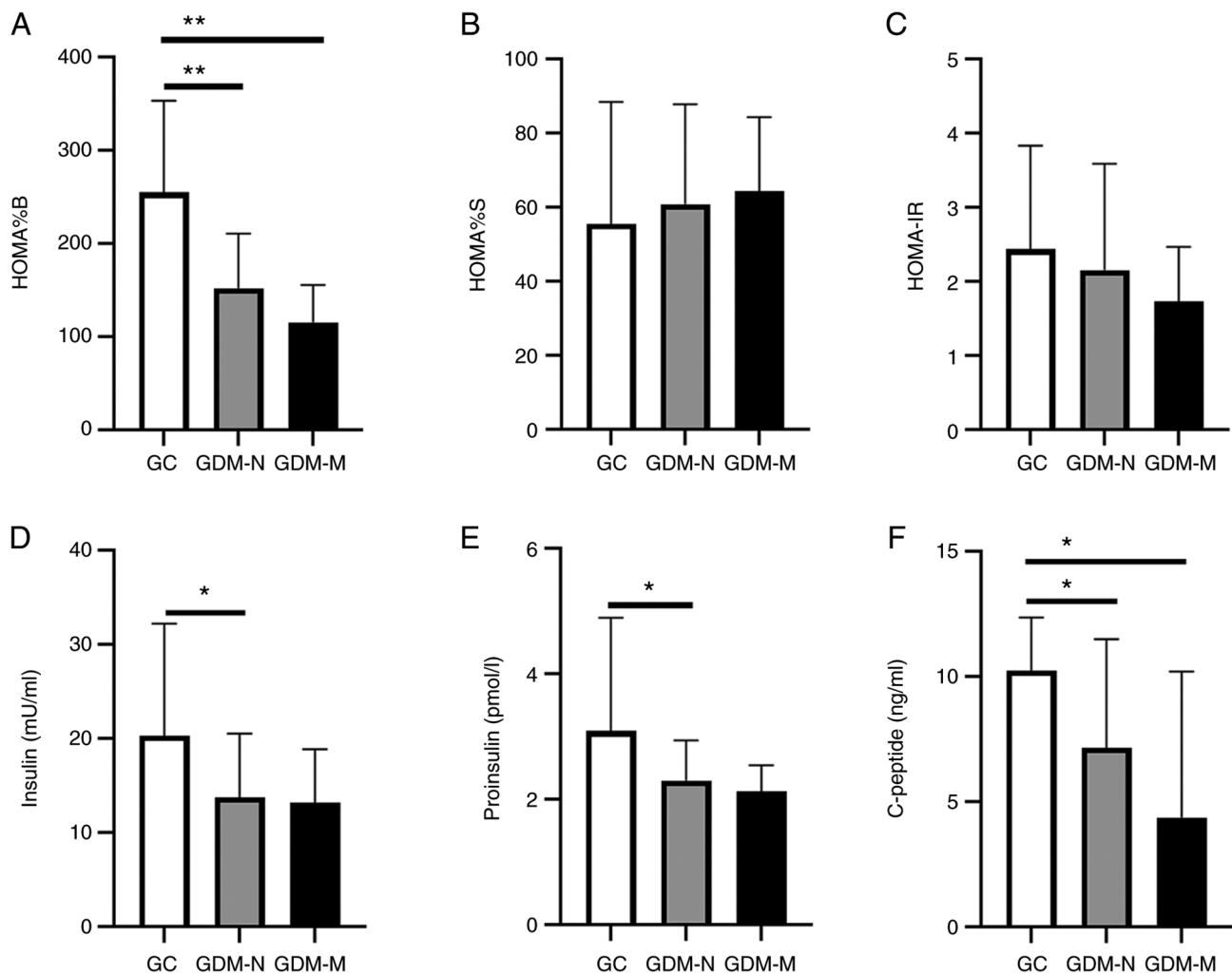


Figure 2. Altered β -cell homeostasis during the third pregnancy trimester. Histograms showing (A) steady state β -cell function, (B) insulin sensitivity, (C) homeostatic model assessment of insulin resistance calculated from serum level of fasting glucose, (D) Insulin, (E) proinsulin and (F) C-peptide levels were measured in serum collected at the third trimester of pregnancy from the GC, GDM-N and GDM-M groups. Data represent the means \pm standard deviation; * $P < 0.05$ and ** $P < 0.01$. HOMA%B, steady-state β -cell function; HOMA%S, insulin sensitivity; HOMA-IR, homeostatic model assessment of insulin resistance; GC, gestational healthy control group; GDM, gestational diabetes mellitus; GDM-N, GDM group with normal offspring; GDM-M, GDM group with macrosomia.

Additionally, the Pearson analysis showed a positive correlation ($P < 0.05$) in proinsulin with HbA1c ($r = 0.76$) and OGTT1h ($r = 0.42$) in GDM-N. Compared with these findings, in GDM-M, proinsulin levels presented a strong positive correlation with C-peptide ($r = 0.95$; $P < 0.05$), with uric acid ($r = 0.96$; $P < 0.05$), AST ($r = 0.93$; $P = 0.01$), ALT ($r = 0.91$; $P < 0.05$), birth weight of previous child ($r = 0.99$; $P < 0.05$) and OGTT 2-h ($r = 0.8$; $P < 0.05$).

The C-peptide serum level in the GDM-N individuals showed a significant positive correlation with proinsulin ($r = 0.41$; $P = 0.01$) and haemoglobin ($r = 0.5$; $P < 0.05$). However, in the GDM-M group, C-peptide serum levels were more strongly correlated with proinsulin ($r = 0.95$; $P = 0.01$), uric acid ($r = 0.97$; $P < 0.05$), ALT ($r = 0.93$; $P = 0.01$) and age mother ($r = -0.89$; $P < 0.05$).

In pregnancies with GDM, insulin resistance was associated with HOMA%S, serum levels of adiponectin, leptin and maternal weight gain (8). In GDM-N, maternal adiponectin levels were correlated with OGTT 0-h ($r = -0.57$; $P < 0.01$), OGTT 1-h ($r = -0.42$; $P < 0.05$) and AST ($r = 0.43$; $P < 0.05$). A defining characteristic of GDM-M is the negative correlation between maternal adiponectin serum level and HOMA%B ($r = -0.96$;

$P < 0.001$), pre-pregnancy BMI ($r = -0.95$; $P = 0.01$), maternal weight gain ($r = -0.96$; $P < 0.05$) and leptin ($r = -0.82$; $P < 0.05$).

Furthermore, in the GDM-N group, there was a correlation ($P < 0.05$) between serum leptin concentration and HOMA%B ($r = -0.435$), OGTT 0-h ($r = 0.431$) and HbA1c ($r = 0.52$). In the GDM-M group, leptin levels presented both strong negative correlations ($P < 0.05$) with OGTT 0-h ($r = -0.87$) and adiponectin ($r = -0.82$). In GDM-N, maternal weight gain was correlated ($P < 0.05$) with the gestational age at which GDM was diagnosed ($r = 0.57$), AST ($r = 0.52$), birth weight of current child ($r = 0.46$) and parity ($r = -0.45$). Unlike the GDM-N group, maternal weight gain in the GDM-M was correlated specifically and strongly with pre-pregnancy BMI ($r = 0.98$; $P = 0.01$), maternal adiponectin serum level ($r = -0.96$; $P < 0.05$) and HOMA%B ($r = 0.94$; $P < 0.05$).

Discussion

During pregnancy, maternal metabolism undergoes various changes facilitate fetal growth (3). The present study compared

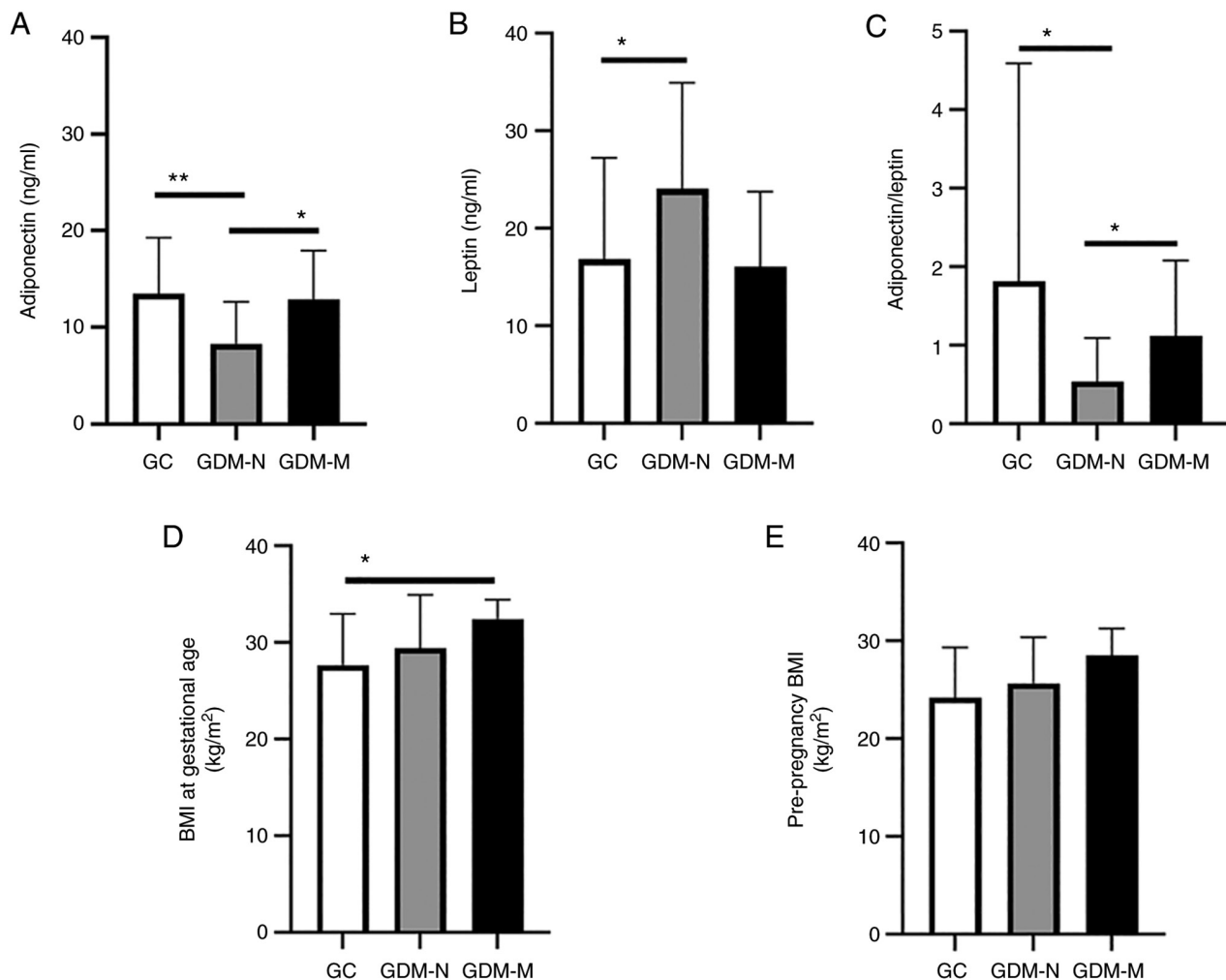


Figure 3. Changes in serum adipocyte-derived hormones and BMI during pregnancy. Histogram showing the serum levels of (A) adiponectin, (B) leptin, (C) calculated ratio of adiponectin/leptin serum concentrations, (D) BMI at gestational age and (E) pre-pregnancy BMI of individuals in the GC, GDM-N and GDM-M groups. Data represent the means \pm standard deviation; * $P < 0.05$ and ** $P < 0.01$. BMI, body mass index; GC, gestational healthy control group; GDM, gestational diabetes mellitus; GDM-N, GDM group with normal offspring; GDM-M, GDM group with macrosomia.

the principal factors involved in the pathophysiology of GDM, namely β -cell dysfunction and insulin resistance, whilst also examining their potential impact on the onset of macrosomia in new-borns. The present study included 36 patients that were followed up from the third trimester of pregnancy until birth. Risk factors for GDM, such as advanced maternal age, overweight, obesity, high parity, previous delivery of a macrosomic infant, are associated with impaired β -cell function and insulin resistance (3). However, there remains a great deal of complexity involved in the interaction between β -cell dysfunction and insulin resistance during GDM.

β -cell function was analysed in patients with GDM by measuring fasting serum insulin, C-peptide and proinsulin levels, where their correlations with other anthropometric, clinical and paraclinical parameters were also analysed.

The data showed that insulin levels decreased in GDM compare with those in the GC group, but significant difference was only observed in the GDM-N group. Several studies previously reported contradictory results regarding the regulation trend of insulin secretion in GDM when defects in β -cell function are present (22,23). In comparison with the GC group, the present study analysed the insulin levels in GDM-M and found

no significant difference but, the GDM-M group revealed a negative correlation between maternal serum insulin level and HDL level. In the case of insulin deficiency or peripheral IR, the HDL level decreases (24). The present study could not find significant differences in HDL levels in the GC group compared with those in the GDM-M or GDM-N groups. Other previous studies have also failed to find any associations between HDL and macrosomia in GDM pregnancies (24,25). In addition, information regarding the association of insulin level, HDL and macrosomia with GDM remain inconsistent. Compared with the present results, another previous study showed that GDM group (300 women with gestational diabetes mellitus) resulted in lower HDL concentrations throughout pregnancy compared with GC group (1,283 healthy pregnant women) (26). This inconsistency may be due to the different study populations and sample size. Therefore, there is a demand for improving the knowledge regarding changes in the maternal lipid profile in GDM and their association with macrosomia.

In the present study, C-peptide levels were decreased in both GDM groups, with significant differences compared with GC. A previous study showed that in the third trimester of GDM pregnancy, C-peptide levels were increased until

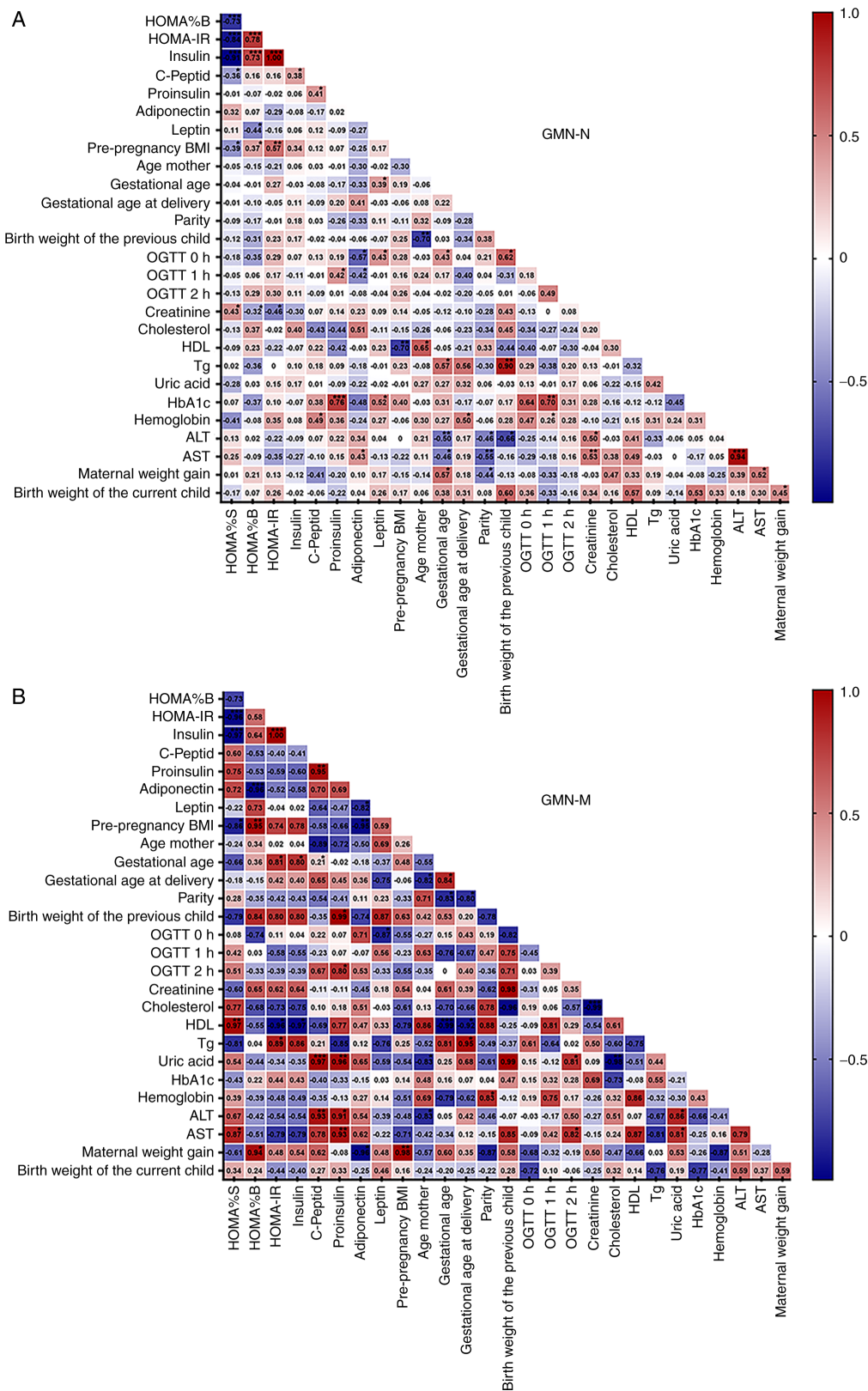


Figure 4. Graphical representation of the Pearson correlation analyses in GDM groups. Correlation matrix plots were presented with Pearson correlation coefficients and significance levels between all clinical and paraclinical parameters measured in women from (A) GDM group with normal offspring (GDM-N) and (B) GDM group with macrosomia (GDM-M). The intensity of red and blue colours represent the strength of positive ($r > 0.4$) and negative ($r < -0.4$) correlations between any two parameters tested. Characteristics included in this Pearson correlation analysis were: Insulin sensitivity; β -cell function; serum levels of insulin, C-Peptide, proinsulin, adiponectin and leptin; pre-pregnancy BMI; mother age; gestational age; gestational age at delivery; parity (number of gestations); birth weight of the previous child; oral glucose tolerance test at 0, 1 and 2 h; serum levels of creatinine, cholesterol, high-density lipoprotein, triglycerides, glycated haemoglobin, haemoglobin, alanine aminotransferase and aspartate aminotransferase in the third trimester; maternal weight gain and birth weight of the current child. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. HOMA%B, steady state β -cell function; HOMA-IR, homeostatic model assessment of insulin resistance; OGTT, oral glucose tolerance test; BMI, body mass index; OGTT, oral glucose tolerance test; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein cholesterol; Tg, triglycerides; HbA1c, glycated haemoglobin; GDM, gestational diabetes mellitus.

delivery, suggesting a β cell dysfunction as possible cause of IR (27). However, these authors analysed a slightly different experimental setting by evaluating changes in plasma C-peptide levels in patients with GDM, gestational impaired glucose tolerance and in normal pregnant women (27).

In GDM-N individuals, the C-peptide serum level correlated positively with proinsulin level. However, in GDM-M individuals, a strong and positive correlation of C-peptide serum level with proinsulin and ALT was registered. Over the past decade, the prevalence of NAFLD during pregnancy has nearly tripled and has been found to be independently associated with pregnancy and postpartum complications, such as preeclampsia, disorders, preterm birth and postpartum haemorrhage (28). This previous study (28) also showed that patients with NAFLD during pregnancy experienced GDM more frequently. In the general population, C-peptide was found to be an independent risk factor for NAFLD and a surrogate marker for monitoring IR during NAFLD (29). In the present study, patients with GDM-M had a strong positive correlation between C-peptide levels and ALT levels, which was not observed in the GDM-N group. However, a conclusion cannot be drawn because the present study enrolled patients that were not screened for NAFLD. Therefore, one of the possible future directions could be evaluating the relationship between NAFLD, C-peptide levels and ALT in GDM and the risk of macrosomia.

Proinsulin levels in GDM have been previously documented, although with contradictory results (5,30). The present study registered a decrease in maternal serum proinsulin levels in the GDM-N group and not in the GDM-M group compared with those in the GC group. However, no significant difference in the proinsulin levels between GDM and healthy controls could be found in previous studies, although they did not divide the GDM group by baby birth weight (30).

Although the bidirectional association between NAFLD and GDM has been extensively documented (31), the subjects were not screened for NAFLD or hepatic steatosis in the present study. In GDM-M, proinsulin levels presented a strong and positive correlation with ALT and AST levels. Several studies previously reported a significant correlation between NAFLD and proinsulin concentrations, where high proinsulin levels can increase the risk of developing hepatic steatosis (32,33). These studies concluded with the significant association among hepatic steatosis, proinsulin and ALT concentration. Existing data regarding the correlation between birth weight of the child and liver enzymes are also controversial. A previous study revealed that elevated ALT levels during the first trimester conferred a 4-fold increase in the risk of giving birth to a child with high birth weight, but without an explanation (15). However, to the best of our knowledge, scant data regarding the impact of maternal NAFLD on macrosomia in GDM exist, such that the association among ALT levels during third trimester, proinsulin and macrosomia remain unexplored.

During pregnancy, alterations in glucose metabolism induce IR, which progressively accentuates in parallel with gestation (5). This form of IR is influenced by several factors, such as adipokines (leptin and adiponectin), maternal weight gain and pre-pregnancy BMI (5). Adipokines are secreted by the adipose tissue and are one of the most important regulators of neurohormonal metabolism (34,35). In the GDM-N group,

adiponectin levels were decreased compared with those in the GC group. In GDM-N, maternal adiponectin levels in the third trimester of pregnancy were positively correlated with AST levels whilst being negatively correlated with OGTT 0-h and 1-h. By contrast, in the GDM-M group adiponectin level increased compared with that in the GDM-N group, to that comparable to the GC group. In addition, adiponectin levels presented strong negative correlations with HOMA%B, leptin, pre-pregnancy BMI and maternal weight gain. Consistent with the present study, previous studies (36,37) showed that maternal adiponectin levels between 24 and 31 weeks of gestation in women with GDM were significantly lower compared with those in the control group.

The liver is a major site of insulin action and clearance, serving an important role in maintaining glucose and insulin homeostasis (38). Therefore, it is predisposed to IR-induced injury and other metabolic diseases (38). Liver enzymes ALT and AST are associated with different cardiometabolic diseases, including type 2 diabetes (39-41). Therefore, their levels are useful and cost-effective tools for the routine diagnosis of liver diseases. However, data on the effects of pregnancy on serum ALT and AST levels are contradictory. The present study found that ALT and AST levels decreased non-significantly in both of the GDM groups examined. In several previous studies, serum ALT and AST values did not change during pregnancy or remain within the normal range compared with women who are not pregnant (38,42). A previous report analysing the association between GDM and the ratio of ALT/AST showed that ALT/AST was higher in the GDM group compared with that in the control group (43). The relationship between transaminases and macrosomia has also been analysed previously, where a positive correlation between infant birth weight and ALT levels in macrosomic babies has been observed (44). This study showed that asymptotically elevated ALT values measured during the first trimester can be used to predict a macrosomia foetus. To the best of our knowledge, the present study is the first to analyse the differences in transaminase levels during the third trimester of pregnancy in GDM.

Adiponectin serum level is also known to predict type 2 diabetes (9). The association between liver markers and adiponectin has been reported and investigated in previous studies. To the best of our knowledge, there are no published data regarding the correlation between adiponectin and AST levels in GDM. Studies have been performed either on non-pregnant subjects or on male subjects, which showed a negative correlation between ALT and adiponectin levels (45,46). In another previous study, which analysed serum adiponectin levels and enzyme markers of liver dysfunction in both type 2 diabetic and non-diabetic Caribbean non-pregnant women, it did not identify any significant correlation between adiponectin and ALT in either group of patients (22). In the present analysis, only in the GDM-N group did the adiponectin levels in the third trimester correlate positively with AST, whilst maternal serum levels of AST and ALT were not modified by the GDM status with or without macrosomia. Future studies are necessary to elucidate this relationship.

Leptin is a satiety signal protein and regulates energy balance (11). If target organs are resistant to leptin's effects, which occurs during diabetes, leptin would be secreted in excess. Increased leptin levels are also associated with high

BMI and IR (11). The present results are generally consistent with other previous studies that also assessed maternal plasma leptin concentrations in pregnancies complicated with GDM in the GDM-N (47,48). Previously, maternal serum leptin levels in women with GDM have been shown to be significantly higher compared with those in women with uncomplicated pregnancies (47). The present study observed that the gestational diabetes status in the GDM-N group resulted in a decreased ratio of adiponectin/leptin by 2.82-fold compared with that in the GC group, consistent with results from another similar study (48).

The association between leptin levels and fasting plasma glucose has been analysed in several studies that enrolled either diabetic non-pregnant female subjects or pregnant women with GDM (11,44,47,49). In the present study, GDM-N showed a positive correlation between leptin levels and OGTT 0-h, whilst in the GDM-M group this correlation was negative. Vitoratos *et al* (47) previously investigated the changes in leptin levels and their relationship with plasma glucose in pregnant women with gestational-onset diabetes at 29 and 33 weeks of gestation. They identified a positive correlation between maternal leptin concentrations and OGTT 1-h in women with GDM (47). By contrast, another previous study found a positive correlation between leptin levels and OGTT 0-h in patients with GDM (49). Future studies are necessary to explain these differences. The relationship between serum leptin level and risk of macrosomia was also previously analysed, where a statistically significant correlation between plasma leptin levels and birth child weight was found (44). In addition, this previous study suggested that leptin levels are strongly associated with the level of body fat tissue in the macrosomic offspring (44). To the best of our knowledge, no correlation analysis among maternal leptin levels, OGTT 0-h, OGTT 1-h and macrosomia have been performed, which is warranted.

The present study found that in the GDM-N group maternal weight gain was negatively correlated with parity whilst being positively correlated with AST and child birth weight. By contrast, in the GDM-M group, maternal weight gain presented strong positive correlations with HOMA%B, pre-pregnancy BMI but negative correlation with adiponectin. Existing data are contradictory regarding the influence of maternal weight gain on birth child weight in GDM, where no correlation was found regardless of the pre-pregnancy BMI of the mother with GDM (50,51). It was suggested that the increased IR underlying GDM deviates the substances from maternal to fetal circulations, where the excessive supply of nutrients in women with diabetes decreases the influence of maternal weight gain on birth weight (50). In the present study, correlation between maternal weight gain and child birth weight could not be found in the GDM-N group, whilst a strong positive correlation was found between these two parameters in the GDM-M group. The present result concurs with a previous systematic review and meta-analysis of the association between pre-pregnancy body mass index and gestational weight gain on the perinatal outcomes in GDM subjects, which showed that excessive maternal weight gain increases the incidence of infant macrosomia (51). Compared with the present study, which enrolled only nulliparous women complicated with GDM, it also enrolled multiparous women.

Data regarding the role of parity in gestational weight gain is less clear, because both positive and negative relationships have been reported in previous studies (52,53). The present analysis found that only in the GDM-N group did the maternal weight gain correlate negatively with parity. By contrast, Harris *et al* (53) reported that parity is independently associated with maternal BMI and gestational weight gain.

In the present study, a positive correlation between maternal weight gain and AST was observed in the GDM-N group. During normal pregnancy, compared with their non-pregnant counterparts, ALT levels decrease whereas AST levels remain unchanged (54). In both GDM groups, AST and ALT levels remained unchanged when compared with GC. Understanding the correlation between AST and ALT levels during GDM pregnancy and modifiable factors, such as maternal weight gain, may facilitate the early recognition, diagnosis and prevention of impaired liver function in GDM. In addition, pregnancy complications that affect liver transaminases, such as preeclampsia, can also be recognised more easily. Further studies are necessary to elucidate the mechanism underlying maternal weight gain, GDM, liver enzymes and macrosomia. To the best of our knowledge, no studies have previously analysed the correlation between AST during the third trimester of GDM pregnancy and maternal weight gain, or differences between pregnancies with and without macrosomia.

HOMA-IR is a simplified measure of IR and is strongly associated with BMI (55,56). In the present study, in the GDM-N group, calculated HOMA-IR correlated positively with pre-pregnancy BMI. The results of the present analysis agree with the results from Lin *et al* (55), which showed in a retrospective study on 710 women diagnosed with GDM, that greater pre-pregnancy BMI values were associated with a higher risk of IR during the second trimester (56). In another prospective study, VanWiden *et al* (57) previously analysed the use of HOMA-IR measurements in pregnancies with and without GDM as an indicator of the degree of IR and as a potential tool for evaluating the improvement of insulin sensitivity after therapeutic interventions. They showed that HOMA-IR was significantly greater in the GDM group compared with that in non-GDM healthy controls. In comparison with this previous study, the present study divided the GDM group into subgroups with or without macrosomia, but did not find any significant differences in HOMA-IR levels between GC and GDM groups. It is known that HOMA-IR is an indicator of liver IR that can also be used to reflect the relationship between the liver and pancreas (58). In addition, it is also an indicator of insulin sensitivity that occurs in pregnancy (55). The present analysis found that HOMA-IR positively correlated with Tg levels but negatively with HDL only in the GDM-M group. Over the last decade, the relationship between lipid metabolism and IR has been the objective of various studies (24-26). Although IR and the levels of lipid parameters change during GDM, this correlation hasn't been elucidated. The present results are consistent with another study, where Tg was found to be associated with IR in GDM during the second trimester of pregnancy (56), since the present analysis found this correlation, but only in the GDM-M group. Another previous study analysed the relationship between IR and various risk factors for atherosclerosis

in a large group of male and women subjects, which found similar results (59). However, the mechanisms beyond these correlations remains unclear.

In conclusion, the present study adds to the current knowledge of the associations among factors involved in the physiology of GDM and macrosomia, which highlights novel correlations that could aid future studies to elucidate the mechanism underlying this pathology. In addition, it found different correlations of transaminases and lipid profiles with markers of IR or β -cell dysfunction in both of the GDM groups tested. However, further studies are warranted to establish their involvement in the evolution to macrosomia in GDM. The present analysis has drawn future directions to evaluate the relationship between NAFLD, C-peptide, proinsulin levels and transaminases in GDM and the risk of macrosomia.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

EGB and EU have equal contribution in performing the analysis of the data. FA designed the experiments, data acquisition and interpretation. EGB, DAM and IC confirm the authenticity of all the raw data. DAM and IC contributed to the enrolment of patients, collection of the biological samples and data acquisition. EGB, EU, VIS, LI and CIT performed analysis and interpretation of data. VIS, LI and CIT helped in the revision of the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the 'N. Paulescu' National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania (approval no. 1680/01.11.2017). Each patient involved in the study signed the written informed consent form as specified in the Declaration of Helsinki and agreed to the use of their samples for scientific research.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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