Journal of Diabetes Research

Diabetes in Pregnancy: Diagnosis, Complications, and Outcomes

Lead Guest Editor: Pasquale De Franciscis
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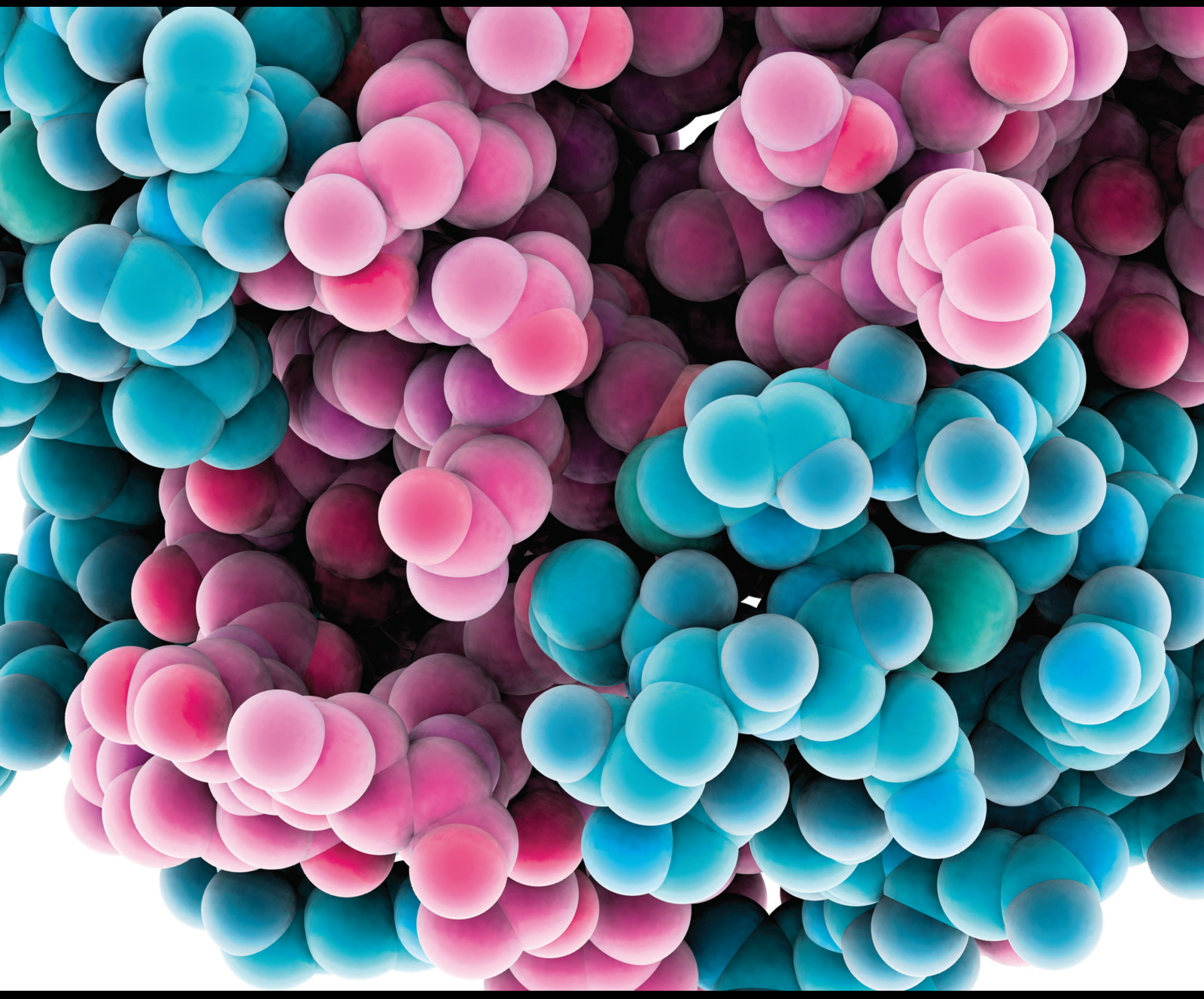

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Contents

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Research Article

Analysis of Risk Factors for the Development of Gestational Diabetes Mellitus in a Group of Romanian Patients

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Introduction. Gestational diabetes mellitus (GDM) is caused by numerous risk factors, the most common being old age, obesity, family history of diabetes mellitus, GDM, history of fetal macrosomia, history of polycystic ovary syndrome or treatment with particular drugs, multiple births, and certain races. The study proposed to analyze the risk factors causing GDM. *Method*. In the study, we included 97 pregnant women to whom there was an OGTT performed between weeks 24th and 28th of pregnancy, divided into two groups, with GDM and without GDM. The statistical analysis was performed with SPSS 26.0, the tests being statistically significant if p value < 0.05. *Results*. The favoring risk factors for the onset of GDM were analyzed, with statistically significant differences between the GDM group and the group without GDM related to the delivery age (32.39 ± 4.66 years old vs. 28.61 ± 4.71 years old), history of fetal macrosomia (13.7% vs. 0%), presence of GDM during previous pregnancies (7.8% vs. 0%), HBP before pregnancy (9.8% vs. 0%), gestational HBP (17.6% vs. 0%), glycemia value at first medical visit (79.37 ± 9.34 mg/dl vs. 71.39 ± 9.16 mg/dl), and weight gain during pregnancy (14.61 ± 4.47 kg vs. 12.48 ± 5.87 kg). *Conclusions*. Identifying the risk factors for the GDM onset has a special importance, implying an early implementation of interventional measures in order to avoid the onset of GDM and associated maternal and fetal complications.

1. Introduction

Gestational diabetes mellitus (GDM) is defined by the American Diabetes Association (ADA) as "not previously known diabetes, diagnosed during the second or third trimester of pregnancy" [1]. The most common risk factors involved in the onset of GDM are represented by age over 40 years old, obesity, family history of diabetes mellitus (DM) in 1st degree relatives, history of GDM or fetal macorsomia, personal history of polycystic ovary syndrome or treatment with drugs like corticosteroids or antipsychotic drugs, multiple births, and race (Asian, African-American, Middle East, and some islands in the Pacific) [2]. An important role in the pathogenesis of GDM is played by insulin resistance and endothelial dysfunction, aggravated by unhealthy diet and sedentary lifestyle, which induce oxidative stress and the appearance of chronic inflammation and increasing inflammatory markers such as C-reactive protein, tumor necrosis factor-alpha (TNF-α), and interleukin (IL) 6. The recommendation to the pregnant woman, as early as possible of a vegetarian diet, rich in dietary fiber seems to decrease inflammation, oxidative stress, endothelial dysfunction, and insulin resistance. Mediterranean diet might favorably impact the onset of GDM and its complications, having a favorable role in metabolic control of pregnant women, decreasing the risk of maternal-fetal complications [3]. During the COVID-19

time period, more risk factors for GDM were added, such as prolonged stress, weight gain, as a result of movement and/or access to healthy food limitation, or even SARS-Cov-2 infection, which may lead to direct pancreatic lesions and insulin resistance, or it may even cause type 1 DM in predisposed women, through an immune mechanism. Starting a Mediterranean diet could limit the onset of GDM, by preventing gestational weight gain, immune system improvement, and modulation of IL-6, C-reactive protein, and nuclear factor (NF)-Kb [4]; the role played by diet and physical exercise in preventing GDM is also supported by Mijatovic-Vukas et al. [5]. COVID-19 pandemic led to changes in the diagnosis, supervision of the progression, and births in women with GDM, both through the limitation of medical care access and due to the pregnant woman self-limitation of contacts [6].

The purpose of the study was to analyze the risk factors favoring the onset of GDM in a group of Romanian patients.

2. Material and Method

2.1. Participants. We performed an epidemiological, prospective, noninterventional study, over a period of 2 and a half years (December 2018–April 2021); the study was conducted in Romania, Craiova city, including women monitored at two medical units: Emergency Clinical County Hospital and Clinical Municipal Hospital "Philanthropy". We included in the study a group of 97 pregnant women monitored during pregnancy, in whom there was an oral glucose tolerance test (OGTT) performed with 75 g pulvis anhydrous glucose on 3 times, between weeks 24 and 28 of pregnancy. After the results of OGTT, the pregnant women were divided into 2 groups, namely, group 1: 51 pregnant women with GDM and group 2: 46 pregnant women without GDM.

The inclusion criteria were age over 18 years old, pregnant women who signed the informed consent for study inclusion and were monitored during pregnancy within the Emergency County Hospital of Craiova and the Clinical Municipal Hospital "Philanthropy."

The exclusion criteria were represented by women with type 1 and 2 DM diagnosed before pregnancy, women who later gave birth outside the Clinical County Emergency Hospital of Craiova and the Clinical Municipal Hospital "Philanthropy," of Craiova, women with severe comorbidities that may influence the maternal and perinatal outcome (kidney disease, neoplasia, anemia, thyroid disorders, etc.), and women who did not present to the follow-up visits after delivery.

All the pregnant women included in the study consciously signed an informed consent. The study was performed according to the ethical principles from the Helsinki Declaration—updated, according to the Good Clinical Practice (GCP), respecting the right to integrity, confidentiality, and giving the subject the option to withdraw from the study at any moment.

The data of every participant in the study included demographic characteristics, personal physiological history (number of pregnancies and previous deliveries, number of miscarriages, number of interrupted pregnancies, history of in utero fetal death or fetal macrosomia), and familial history. The pregnancies were considered interrupted if the fetus death occurred until the gestational age of 20 weeks. After this age, the fetus death was considered in utero fetal death.

The patients were physically examined, and there were anthropometric data recorded regarding weight and height; the body mass index (BMI) was calculated, previous to pregnancy, according to the following formula: BMI = weight (kg)/height^2 (in meters). The gestational age was determined according to the echographic data and by calculating the duration from the first day of the last period. BMI was classified according to the guidelines of the World Health Organization (WHO) [7]. Blood pressure (BP) was measured by using an automatic sphygmomanometer in the subjects on a sitting position, after 10 minutes of rest. We considered the pregnant women having high blood pressure (HBP)in the study who presented systolic BP values $\geq$ 140 mmHg and/or diastolic BP values $\geq$ 90 mmHg and/or following a high blood pressure treatment at home. Gestational HBP was considered HBP diagnosed after 20 weeks of amenorrhea.

2.2. Blood Tests. The blood tests were represented by fasting plasma glucose (FPG) during first prenatal visit, subsequently followed by 3 measurements of a jeun, one hour and 2 hours glycemia after uploading 75 g anhydrous glucose within OGTT, performed between weeks 24 and 28 of pregnancy. FPGs were obtained after a fasting period of 8-12 hours. In women with GDM, there was an OGTT performed with 75 g anhydrous glucose, and there were determined a jeun and 2 hours glycemia, 4-12 weeks after delivery.

2.3. Evaluation of Gestational Diabetes. GDM diagnosis was established according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) (Table 1) [8].

In order to exclude a prediabetes or prior to pregnancy diabetes, we performed an a jeun glycemia during first prenatal visit, using the standard diagnosis criteria. 4-12 weeks after delivery, we performed an OGTT with 75 g glucose in all women with GDM, using the standard diagnosis criteria, outside pregnancy, in order to exclude a possible diabetes before pregnancy.

2.4. Statistical Analysis. The data were recorded on a computer, in a database, EXCEL, then transferred to Statistical Package for the Social Sciences (SPSS) 26.0 (SPSS Inc., Chicago, IL, USA), codified and analyzed using this program. All the data were analyzed according to the presence or absence of GDM in women included in the study.

The distribution of continuous variables were tested for normal values using the Kolmogorov-Smirnov test. Normal distribution data were presented as average ± standard deviation (SD); the data that did not have a normal distribution were presented as a median and interquartile range (IQR). In order to determine the statistical significance of the differences between the two groups, we used Student's

Table 1: Diagnosis criteria for GDM (OGTT with 75 g glucose).

	A jeun glycemia	1 hour glycemia (OGTT)	2 hours glycemia (OGTT)	Observations
GDM	≥92 mg/dl (5.1 mmol/l)	≥180 mg/dl (10 mmol/l)	≥153 mg/dl (8.5 mmol/l)	A single pathological value may support the GDM diagnosis

Reproduced from Medicina 2021, 57(11), 1170; doi:10.3390/medicina57111170. Analysis of maternal and neonatal complications in a group of patients with gestational diabetes mellitus [under the Creative Commons Attribution License/public domain] [9].

t test for comparing the averages, respectively, and the Mann-Whitney *U* test for comparing the medians. The percentages between the two groups were compared by using the chi square test.

All the performed tests were considered statistically significant if they recorded a p value < 0.05.

3. Results

We analyzed the risk factors known in the literature as responsible for the GDM onset. For the studied groups, the characteristics related to heredocholateral and personal history are summarized in Tables 2 and 3.

Women with GDM had twice more frequently 1st degree relatives with type 2 DM than the ones without GDM, yet with no statistically significant differences ($p = 0.073$) (Table 2).

There was the analyzed physiological personal history of the pregnant women included in the study, a studied parameter being older age at delivery, when there were recorded high statistically significant differences between the groups, pregnant women with GDM being older than the ones who did not develop GDM ($p < 0.001$) (Table 2).

The statistical analysis of previous pregnancies did not identify statistically significant differences between the 2 groups, although women with GDM had a higher number of pregnancies ($p = 0.169$) (Table 2).

There were not recorded any statistically significant differences regarding the number of previous births ($p = 0.228$) (Table 2).

Regarding the number of previous miscarriages, there were more cases observed in the group with GDM, without any statistically significant differences between the two groups ($p = 0.412$) (Table 2).

The number of patients who presented interrupted pregnancies (until the age of 20 weeks of pregnancy) was higher in the group with GDM, still with no statistically significant differences between the 2 groups ($p = 0.754$) (Table 2).

In utero fetal death (after the age of 20 weeks of pregnancy) was found in a single pregnant woman with GDM, unlike the group without GDM, where there was no case, with a nonstatistically significant difference ($p = 0.340$) (Table 2).

Fetal macrosomia was found exclusively in the pregnant women with GDM, with a statistically significant difference ($p = 0.009$) (Table 2).

The pathological personal history was the next studied objective, the obtained results being described in Table 3.

Regarding obesity, we analyzed the BMI previous to pregnancy in women who developed GDM, in comparison to those who did not develop GDM, still with no statistically significant differences between the 2 groups ($p = 0.734$) (Table 3).

Also, we recorded the data regarding the presence of GDM in previous pregnancies, and we identified some differences at the limit of statistical significance ($p = 0.05$) in the group with GDM (Table 3).

HBP previous to pregnancy with high values detected during the first 20 weeks of amenorrhea was found exclusively in the group with GDM, a statistically significant difference ($p = 0.029$) (Table 3).

Even from the beginning of pregnancy and during its progression, there were a series of parameters synthesized in Table 4.

One of these parameters was the value of glycemia during the first prenatal visit. Its value in the pregnant women who developed GDM was higher than the one in the group of those who did not develop GDM, with a high statistically significant difference ($p < 0.001$) (Table 4).

During pregnancy, there was an excessive weight gain analyzed, and we observed statistically significant differences between the two groups, pregnant women who have developed GDM presenting a higher weight gain ($p < 0.05$) (Table 4).

Gestational HBP (diagnosed after 20 weeks of amenorrhea) was observed more frequently in pregnant women who developed GDM than in the ones without GDM, a statistically significant difference ($p = 0.003$) (Table 4).

Preeclampsia, as a pregnancy associated complication, was found only in the group with GDM, namely, in 58.3% of the patients (Table 4).

4. Discussions

Obesity is a risk factor commonly associated with the development of GDM [10, 11]. In our study, obesity was strictly found only in pregnant women with GDM, even though there were not recorded any statistically significant differences between the two groups;

Age at delivery time was highly correlated with a statistically significant risk for GDM, data which are in accordance with those in the literature [11, 12].

Similar to numerous studies, the family history of DM increases the risk for GDM development [13]. In our study, even though the number of pregnant women who developed GDM had a family history of DM in a higher percentage, there were not recorded any statistically significant differences.

History of fetal macrosomia, also known as a risk factor for GDM [14], was found in a higher percentage in pregnant

Table 2: Physiological heredocholateral and personal history.

		Without GDM	With GDM	p
Heredocholateral history of type 2 DM		8 (17.4%)	17 (33.3%)	0.073
Age at delivery time (years old)—*average* ± *DS*		28.61 ± 4.71	32.39 ± 4.66	**<0.001**
Age at delivery time (years old)	20-25	8 (17.4%)	1 (2%)	**<0.001**
	25-30	24 (52.2%)	14 (27.5%)	
	30-35	10 (21.7%)	16 (31.4%)	
	≥35	4 (8.7%)	20 (39.2%)	
No. of previous pregnancies	0 pregnancy	26 (56.5%)	20 (39.2%)	0.169
	1 pregnancy	14 (30.5%)	21 (41.2%)	
	2 pregnancies	6 (13%)	5 (9.8%)	
	3 pregnancies	0 (0%)	4 (7.8%)	
	≥4 pregnancies	0 (0%)	1 (2%)	
No. of previous deliveries	0 delivery	32 (69.6%)	29 (56.9%)	0.228
	1 delivery	14 (30.4%)	20 (39.2%)	
	2 deliveries	0 (0%)	2 (3.9%)	
No. of miscarriages	0 avorturi	42 (91.3%)	43 (84.3%)	0.412
	1 miscarriage	4 (8.7%)	5 (9.8%)	
	2 miscarriages	0 (0%)	2 (3.9%)	
	3 miscarriage	0 (0%)	1 (2%)	
No. of stopped pregnancies	0 pregnancy	40 (87%)	42 (82.4%)	0.754
	1 pregnancies	4 (8.7%)	6 (11.8%)	
	2 pregnancies	2 (4.3%)	2 (3.9%)	
	3 pregnancies	0 (0%)	1 (2%)	
History of in utero fetal death	Yes	0 (0%)	1 (2%)	0.340
History of fetal macrosomia	Yes	0 (0%)	7 (13.7%)	**0.009**

Table 3: Pathological personal history in the 2 studied groups.

		Without GDM	With GDM	p	Total
BMI (kg/m^2)—*average* ± *SD*		22.75 ± 2.60	22.96 ± 3.44	0.734	22.86 ± 3.06
BMI (kg/m^2)—categories	<18.5	6 (13%)	3 (5.9%)	0.075	9 (9.3%)
	18.5-25	31 (67.4%)	37 (72.5%)		68 (70%)
	25-30	9 (19.6%)	6 (11.8%)		15 (15.5%)
	≥30	0 (0%)	5 (9.8%)		5 (5.2%)
GDM in previous pregnancies	Yes	0 (0%)	4 (7.8%)	**0.05**	4 (4.1%)
	No	46 (100%)	47 (92.2%)		93 (95.9%)
HBP previous to pregnancy	Yes	0 (0%)	5 (9.8%)	**0.029**	5 (5.2%)
	No	46 (100%)	46 (90.2%)		92 (94.8%)

women who developed GDM, similarly to the data in the literature.

Excessive weight gain during pregnancy is frequently quoted in the literature as a risk factor for the onset of GDM [11, 15]. In our study, there was a higher weight gain recorded in the case of pregnant women who developed GDM.

GDM was associated with the presence of gestational HBP, an important weight gain probably representing one of the connection factors, as there was no significant difference regarding the BMI prior to pregnancy.

Despite the fact that there were more pregnant women with GDM who presented a family history of type 2 DM, a higher number of previous pregnancies, births, and

Table 4: Parameters observed during pregnancy.

		Without GDM	With GDM	p
Glycemia value at first prenatal visit (mg/dl)—*average* ± *SD*		71.39 ± 9.16	79.37 ± 9.34	**<0.001**
Glycemia value at first prenatal visit (mg/dl)—categories	<100	46 (100%)	51 (100%)	—
	100-125	0 (0%)	0 (0%)	
	≥126	0 (0%)	0 (0%)	
Weight gain (kg)—*average* ± *SD*		12.48 ± 5.87	5	**0.046**
Weight gain (kg)—categories	<10	8 (17.4%)	8 (15.7%)	0.449
	10-15	20 (43.5%)	16 (31.4%)	
	15-20	12 (26.1%)	21 (41.1%)	
	≥20	6 (13%)	6 (11.8%)	
Gestational HBP	Yes	0 (0%)	9 (17.6%)	**0.003**
	No	46 (100%)	42 (82.4%)	
Preeclampsia	Yes	—	7 (58.3%)	—
	No	—	5 (41.7%)	

miscarriages, as well as a number of interrupted pregnancies, namely, in utero fetal death, in comparison to the pregnant women without GDM, still with no statistical significance, could explain the limitations of this study due to the low number of pregnant women included in the study. GDM represents a risk factor not only for a future development of type 2 DM, mainly, but also of early cardiovascular diseases; therefore, prevention measures are required [16]. More clinical studies showed the efficiency of inositols, mainly, myo-inositol, in the prevention and treatment of GDM. At present, inositols are considered candidates for classical insulin sensitizers, being useful in the prevention and treatment of GDM; they reduce insulin resistance, the need for insulin in GDM, also improving the lipidic profile [17–20].

5. Conclusions

In conclusion, we highlight the importance of identifying the risk factors for the GDM onset, early detection, and therapeutic intervention; the screening is required not only for pregnant women at risk but also of those out of risk, and the start of interventional measures as soon as possible, in order to prevent the onset of GDM and its associated complications.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

The authors contributed equally to the manuscript and share first authorship. All authors have read and agreed to the published version of the manuscript.

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Review Article

Microbiome and Gestational Diabetes: Interactions with Pregnancy Outcome and Long-Term Infant Health

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Microbiota composition is progressively being connected to different physiologic effects, such as glucose metabolism, and also to different pathologies, such as gestational diabetes mellitus (GDM). GDM is a public health concern that affects an important percentage of pregnancies and is correlated with many adverse maternal and neonatal outcomes. An increasing number of studies are showing some connections between specific microbial composition of the gut microbiota and development of GDM and adverse outcomes in mothers and neonates. The aim of this review is to analyze the available data on microbial changes that characterize healthy pregnancies and pregnancies complicated by GDM and to understand the correlation of these changes with adverse maternal outcomes; this review will also discuss the consequences of these maternal gut microbiome alterations on neonatal microbiota composition and neonatal long-term outcomes.

1. Introduction

The human microbiome is the wide community of microorganisms that live in and on the human body. It consists of more than 100 trillion cells [1, 2] and contains 27 times more genes than the human genome [3–5]. The microbiome plays an important role in regulating metabolism, immune function, and behavior in humans [6].

The microbiota is represented by the community of microorganisms present on a certain body site, in particular the gastrointestinal tract (also called gut microbiota), the oral cavity, the skin, the lungs, and the genitourinary tract [2].

Until recently, the intrauterine environment was considered to be sterile except in the case of chorioamnionitis related to bacterial infections and usually associated with adverse pregnancy outcomes including preterm birth [7]. However, it is now clear that the placenta has its own "healthy" microbiota which is not necessarily associated with infections. Therefore, a specific microbiota characterizes also the placenta and the amniotic fluid [8], and it is subject to modifications with the progression of the pregnancy [9].

Gestational diabetes mellitus (GDM) is an increasing public health concern that affects approximately 5-20% of pregnancies, and its prevalence is progressively rising [10, 11]. It has been defined as any glucose intolerance with the first onset or recognition during pregnancy [12] and is associated with many adverse maternal and neonatal outcomes, such as preeclampsia, cesarean delivery, macrosomia, shoulder dystocia, and neonatal hypoglycemia [13, 14].

The role of intestinal microbiota in modulating insulin resistance and the body inflammatory response is well known [15, 16]. Therefore, the potential impact of specific interventions on the gut bacteria composition and function is of considerable interest when seeking the optimal strategy to prevent and treat GDM.

The aim of the present work is to review the role of the microbiome during pregnancy, its physiological modifications among trimesters, and its pathological changes when

pregnancy is complicated by gestational diabetes. A brief excursus on the molecular approaches to study the gut microbiome will be presented as well. In addition to this, a review of the mechanisms implicated in the correlation between microbiota alterations, adverse pregnancy outcomes, and neonatal long-term outcome will be performed.

2. Gut Microbiota Modifications during Pregnancy

With the term "gut microbiota," we refer to the microorganisms that colonize the gastrointestinal tract [17]. By now, we know that all these microorganisms, which are more than 100 trillion [18] classified in over 35,000 bacterial species [19], have a symbiotic exchange with the human host through the performance of multiple functions [20]: they are involved in nutrients, xenobiotic and drug metabolisms, antimicrobial protection, immunomodulation, integrity of the gut barrier, and structure of the gastrointestinal tract.

The gut microbiota is composed by several types of microorganisms, including bacteria and viruses. Bacteria are classified in phyla, classes, orders, families, genera, and species [21]. The dominant phyla are *Firmicutes* and *Bacteroides*, which represent 90% of the microbiota, followed by *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia* [22]. For each phylum, there are predominant genera and species, for example, *Firmicutes* phyla are represented by Clostridium genera for 95% of its composition but includes also other important genera like *Lactobacillus*, *Bacillus*, and *Ruminococcus*. The composition of the gut microbiota changes between individuals and within the same individual in relation to different factors: gestational age at birth, mode of delivery, age, diet, antibiotics, use of probiotics, body mass index (BMI), and exercise are some of the more studied elements that can influence the human gut microbiota composition. Another factor that can influence the microbiota composition is represented by pregnancy, which is characterized by profound hormonal and metabolic changes [21].

2.1. Changes of Gut Microbiota throughout Different Trimesters of Gestation. During normal pregnancies, the composition of the gut microbiota changes through the course of gestation: during the first trimester, it resembles that of a healthy nonpregnant individual [9, 23, 24], then it changes gradually, and by the third trimester, it is like the microbiota of people affected by metabolic syndrome, with the capacity to induce it if transplanted in germ-free mice [9]. In particular, the main change is represented by a reduction in alfa-diversity (which is the complexity of species diversity in the sample) and an increase in beta-diversity (which is between-subject diversity) [25]. At the phylum level, there is an increase in *Actinobacteria* and *Proteobacteria* and a decline in butyrate-producing bacteria. Butyrate is an important short-chain fatty acid (SCFA) that can serve as a second messenger as well as a source of energy. These changes might be linked with the maternal metabolic profile, consisting of a decline in insulin sensitivity and an increase in nutrient absorption that are necessary to support a healthy pregnancy [26].

More recent studies have focalized on differences between nonpregnant and pregnant individuals at a genera and species levels, with results that are not always concordant. The genera *Blautia* and *Collinsella* have been shown to increase not only in normal pregnancy [27, 28] but also in pregnancy complicated by GDM [29]; its reduction has been associated with digestive diseases that are common in pregnancy, such as vomit and constipation, and also in disease that is more rare like acute fatty liver; these diseases have also been associated with an increase in the presence of *Paenibacillus*, *Acinetobacter*, and *Enterococci* [25], while in normal pregnancy, there is a reduction in *Acinetobacter* [27].

Recently, it has been shown that there is a change in the gut microbiota composition from first trimester to second trimester: in particular, there is an increase in *Firmicutes*/*Bacteroides* ratio, *Blautia*, *Rothia*, and *Bilophila* and a decrease in *Bacteroides* and *Parabacteroides* [27] (Figure 1).

Another genera that increases during pregnancy is represented by *Bifidobacterium* with a demonstrated causal role of progesterone in this variation as shown by using murine models with progesterone implanted subcutaneously [28]. *Bifidobacterium* abundance has been shown to be directly correlated with high-fat diet before and during pregnancy, just like *Akkermansia* [30], suggesting a possible role of prepregnancy diet on the type of microbial changes that occur during pregnancy, even if a previous study had shown an inverse correlation between gestational weight gain and reduced abundance of *Bifidobacterium* and *Akkermansia* [31].

Further studies are needed to elucidate the differences between the gut composition of nonpregnant individuals and healthy pregnancies. In consideration of the role played by the gut microbiota in different metabolic processes, research in this field of interest could help understanding the physiology of pregnancy microbiome modifications, and consequently, it could allow developing a strategy of interventions and prevention in high-risk pregnancies.

3. Gut Microbiota in Pregnancy: Molecular Approaches

Until recently, information about the microbes inhabiting the human body was obtained via conventional culture-based microbiology techniques, where fluid or epithelial swabs from a given body site were placed in culture media, and the organisms that grow were phenotypically and genetically characterized [32]. Nowadays, Real-Time-q Polymerase Chain Reaction (RT-qPCR), shotgun sequencing of 16S rRNA/rDNA gene sequence, and fluorescent in situ hybridization coupled with flow cytometry are most widely used to characterize the gut microbiome in human and animal models [15].

Animal models have advanced our understanding of the gut microbiome and its relationship to fetal programming. A murine model of parental high-fat diet consumption found that offspring of Western diet breeders had a significantly

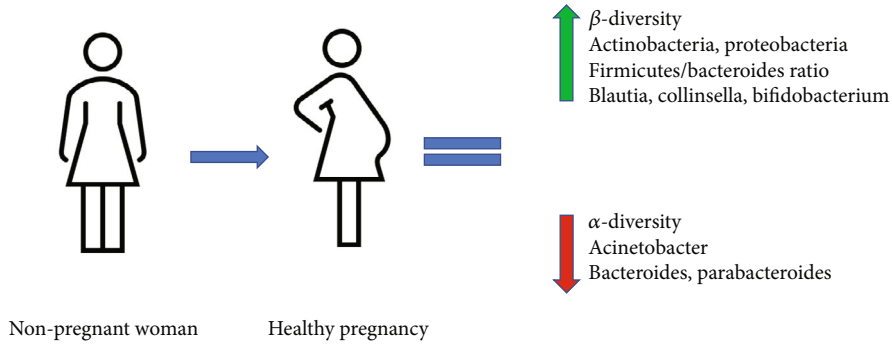

Figure 1: Differences in the gut microbiota between a nonpregnant woman and healthy pregnancy.

increased *Firmicutes*-to-*Bacteroides* ratio compared with control offspring and showed heightened colonic inflammatory responses and dysregulated autoimmunity and allergic sensitization [33]. Similar deleterious changes in the maternal and infant microbiome have also been noted in the setting of human studies, advocating the potential protective role of oral administration of probiotic bacteria to pregnant women resulting in colonization of the infant gut lasting from six to 24 months postpartum [34].

Further mechanistic studies, especially in humans, are needed to better understand how gut microbiota interact with the host immune response, especially in the setting of maternal metabolic syndromes, in order to develop targeted interventions during pregnancy and prevent chronic disease in future generations.

4. Gut Microbiota in Pregnancies Complicated by GDM

Several studies have shown some differences in microbial composition between healthy pregnancy and pregnancy complicated by GDM, even if not all studies are concordant.

It has been shown that GDM patients have a higher *Firmicutes*/*Bacteroides* ratio when compared with healthy pregnancy patients [23]. The same study found an abundance of *Akkermansia* in the control patients and increased levels of *Lachnospiraceae*, *Phascolarctobacterium*, and *Christensenellaceae* in women with GDM, but no differences at a genera level between the two groups of patients [23].

Some differences were found in the composition of gut microbiota during the third trimester of pregnancy, with the identification of phylum *Actinobacteria* as biomarkers of GDM; in the same study, the genera *Collinsella*, *Rothia*, *Actinomyces*, *Desulfovibrio*, *Leuconostoc*, *Granulicatella*, and *Mogibacterium* were biomarkers of GDM, while the genera *Marvinbryantia*, *Acetivibrio*, and *Anaerosporobacter* were markers of normal glucose regulation [35]. Another small study found that in the third trimester of women with GDM, there is a higher relative abundance of *Bacteroides caccae*, *Bacteroides massiliensis*, and *Bacteroides thetaiotaomicron* and a reduction of *Bacteroides vulgatus*, *Eubacterium eligens*, *Lactobacillus rogosae*, and *Prevotella copri* [36].

Differences between gut microbiota in healthy pregnancy compared to pregnancy complicated with GDM are shown in Figure 2.

More recently, several studies have focused on the identification of differences in abundance and composition of the gut microbiota in the first half of pregnancy that correlate to GDM, diagnosed with the standard oral glucose tolerance test at 24-28 weeks of gestation, aimed at discovering an early biomarker for the diagnosis and treatment of gestational diabetes [27, 37–40].

During the first and second trimesters, a decreased relative abundance of *Coprococcus* and *Streptococcus* has been found, which are, respectively, a butyrate-producing bacterium and a lactate-producing bacterium. The same study also showed a positive association between GDM and *Megasphaera* and *Eggertella* [27].

Another study showed, other than a reduced alfa-diversity in patients that will develop GDM, that the genera *Bacteroides*, *Dialister*, and *Campylobacter* were taxonomic biomarkers of GDM, while the genera *Gemminer* and *Bifidobacterium* were markers of normal glucose levels during pregnancy [37]. In contrast with the result of this study, a change in GDM patients from the second to the third trimester has been reported, represented by a higher alfa-diversity, an increment in the colonization of *Firmicutes*, and a reduction in the presence of *Bacteroidetes* and *Actinobacteria* [38].

The increase of relative abundance of *Ruminococcaceae* in the early pregnancy has also been associated with the subsequent development of GDM [39]. In a recent metagenomics study, an association between *Parabacteroides distasonis* and *Klebsiella variicola* in GDM in comparison to healthy pregnancies has been shown [40].

Further studies are needed to understand if interventions on gut microbiota composition in the first half of pregnancy in women with an abundance of microorganisms connected to development of GDM may help prevent the onset of the disease or reduce its severity, with consequent reduction of maternal and neonatal adverse outcomes.

Another group of studies have focused on the identification of specific microorganisms as markers of carbohydrate metabolism. The genera *Blautia* and *Eubacterium hallii* group was positively correlated to fasting blood glucose while the relative abundance of *Faecalibacterium* was negatively correlated to it [38, 41]: the authors suggested the possibility of using these as markers of GDM that is not controlled by diet.

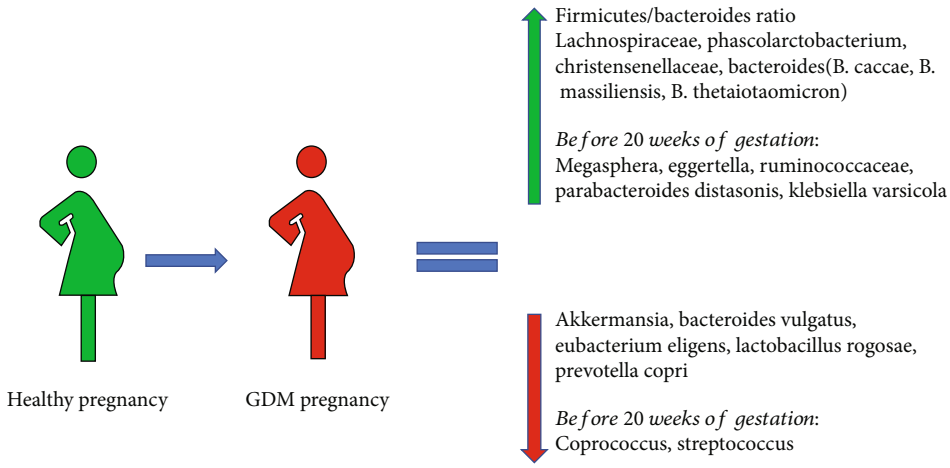

Figure 2: Differences in the gut microbiota between healthy pregnancy and GDM pregnancy.

High blood glucose values corresponded to low intestinal *Faecalibacterium*/*Fusobacterium* ratios, with the correlation highly significant between the bacterial rations and two-hour blood glucose levels, representing the regulatory and recovery capability after sugar intake [42].

Ketonuria, which is an indirect marker of glucose metabolism, has been shown to be associated with a relative abundance of *Roseburia* and also with *Faecalibacterium* and *Dialister* in overweight and obese women at 16 weeks of gestation [43], even if previous studies showed a decrease in *Roseburia intestinalis* and *Faecalibacterium prausnitzii* in patients with type 2 diabetes [44, 45].

Insulin, c-peptide, and HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) have been positively associated with the genus Collinsella in early pregnancy of obese and overweight women [26]; the same study has noted a positive correlation between the genus Coprococcus and the levels of GIP (Gastric Inhibitory Peptide), an incretin that acts by stimulating insulin secretion.

HbA_{1c} levels have been found to be correlated with *Bacteroides* and *Prevotella* [38].

It would be interesting to discover if the use of these biomarkers in the clinical practice may help improve the management of patients with GDM by recognizing patients that are not well controlled with therapy and may need further treatments.

There are some contrasts in the results of some studies, like a recent one [46] that showed no correlation between specific microbial species and GDM in obese and overweight women. The authors linked this result to the use of a more accurate approach, even if the same authors admit that other studies, using the same technique, have found a correlation between GDM and gut microbiota composition [41, 47].

A study that compared the gut microbiome composition between women with a history of GDM and nondiabetic women found no differences after five years from delivery, suggesting that there is no causal role of microbiome composition in GDM appearance [48]; however, another study evidenced a different composition in GDM after eight months from delivery compared to healthy patients, with the genera *Collinsella* and *Olsenella* found to be biomarkers of previous GDM [35].

Further studies are needed to better understand if the differences in gut microbiota composition continue after the term of pregnancies, playing a role in the development of GDM in subsequent pregnancies and if interventions on its composition in the interpregnancies interval may help prevent the onset of GDM.

5. Microbiota Alterations in GDM and Adverse Pregnancy Outcome

Over the course of a normal pregnancy, women undergo several physiological changes, including an increase in insulin resistance (IR). In order to compensate for this physiological resistance, insulin secretion increases gradually during gestation [49]. However, some pregnant women have a limited capacity to increase insulin production and, consequently, develop GDM [50]. Dysbiosis, an altered microbiota composition, has been hypothesized to play a key role in the pathogenesis of many acute and chronic conditions, including metabolic diseases, such as obesity, insulin resistance, and both type 1 and type 2 diabetes mellitus (T2DM) [51, 52].

The composition of the microbiome changes during pregnancy. It has recently been proposed that intestinal microflora and their metabolic activities (intestinal dysbiosis) may play a critical role in body weight control, energy homeostasis, fermentation, and absorption of nondigestible carbohydrate and also in the development of IR; therefore, it may also participate in the pathogenesis of several metabolic disorders, such as obesity, diabetes mellitus, and GDM [50, 53].

In addition to the gut microbiome, the composition of the microbial community in other body sites seems to also be involved in systemic health [54–56]. The oral microbiome seems to play an important role in obesity and diabetes, through the release of inflammatory mediators that may increase the IR, suggesting a link between pathogenic periodontal bacteria (such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) with glycemic control and risk of diabetes [54].

During pregnancy, there is a change in the structure of the vaginal bacterial community, leading to the production of metabolites such as lactic acid that helps to maintain low pH, which contribute to increasing the presence and stabilization of Lactobacillus in the vaginal microbiome. New data concerning the relationship between the vaginal microbiome and metabolic diseases, such as GDM, have been reported [57]. An increase of inflammatory cytokine expression has been shown in GDM, as well as an increase in the abundance of potential pathogenic bacteria, characterizing a dysbiotic profile of the vaginal microbiome [23, 57].

Currently, the etiology is unknown for some of the most important obstetric conditions, such as preeclampsia, premature preterm rupture of membranes, premature labor, preterm delivery, intrauterine growth restriction, gestational diabetes, abruptio placentae, late abortions, stillbirth, hyperemesis gravidarum, and gestational trophoblastic disease, although a microbial role has been implicated in all these conditions. In a recent publication, Romero coined the term "The great obstetrical syndromes" [58] referring to syndromes characterized by multiple etiologies, long preclinical stage, frequent fetal involvement, often adaptive clinical manifestations, and predisposing genetic interactions. Diagnosis and treatment for any of these conditions is challenging, although changes in the microbiota were suggested to play a role [59].

5.1. Cardiometabolic Adverse Outcome. There is mounting evidence supporting the role of the gut microbiome in cardiometabolic diseases in pregnancies [60, 61], and the imbalance in the gut microbiome is nowadays considered an important contribution to the development of GDM [61, 62] being already demonstrated that differences in gut microbiome composition, and its related metabolic activities, distinguish lean versus obese individuals and those with type 2 diabetes mellitus versus those without. Moreover, the finding that a different microbial pattern precedes the onset of GDM leads to the hypothesis that microbiota alterations might have a role in the pathogenesis of GDM [9, 39].

More difficult is the topic over the relationship between the composition of the microbiome in pregnancies complicated by GDM and adverse obstetrical outcomes.

5.2. Preterm Birth. Actually, the proof of a link between alteration of the microbiome in pregnancy and adverse obstetrical outcomes is various: a review of the literature [63] and a meta-analysis of 22 studies including 12,047 pregnant women showed that women with periodontitis had an increased risk of preterm delivery (PTD) and of delivering a low-birth-weight infant [64].

5.3. Gestational Hypertension, Preeclampsia, and Instrumental Delivery. A dysbiotic microbiome is implicated in the diffusion of gut bacterial endotoxin into systemic circulation, inducing a low-grade inflammatory response, which is a common feature of cardiometabolic diseases and that in turn raises the risk of maternal complications of pregnancies. Combined with insulin resistance, chronic subclinical inflammation characterizes the hallmark pathway to the development of both gestational diabetes and gestational hypertension [65]. The maternal oral, vaginal, and gut microbiome influence the risk of pregnancy outcomes and have profound impacts upon the health of the neonate and infant, potentially affecting the possibility that patients affected by GDM—given the microbiome imbalance—can be super exposed to preterm birth, preeclampsia, and excessive gestational weight gain.

The alteration of the microbiome associated with GDM may contribute to the elevated risk of pregnancy complications, including preeclampsia and instrumental or operative delivery for the mother. Fetal complications include macrosomia (birthweight greater than 4500 g), polyhydramnios, preterm birth, shoulder dystocia, and neonatal complications of admission to high-level care, respiratory distress, hypoglycemia, and jaundice. Both women with GDM and their infants are also at increased risk of diabetes mellitus and metabolic dysfunction later in life [66, 67], and this risk can be connected to the favorable outcome of subsequent pregnancies.

Although incompletely elucidated, there are a number of modifiable factors that shape the composition of the maternal microbiome, including maternal diet, prepregnancy weight and gestational weight gain, and hygiene practices. The maternal microbiome and perinatal factors establish the fetal and infant microbiome.

Indeed, treatment of GDM improves pregnancy outcomes with significant reductions in the rate of serious perinatal outcomes including macrosomia, shoulder dystocia, and caesarean delivery [68, 69]. Primary prevention of GDM rather than treatment would however be ideal in preventing both the economic and health costs associated with GDM.

As a strategy for reducing the risk of adverse pregnancy outcomes that negatively impact neonatal and infant health, practitioners should evaluate women's attainment of a healthy maternal microbiome before and during pregnancy (via preconception and prenatal care) through the promotion of a healthy diet, achievement of a healthy weight status and weight gain during pregnancy, and oral hygiene (such as regular brushing, flossing, and dental care). In the perinatal period, the key target for promoting a healthy infant microbiome includes the promotion of breastfeeding and kangaroo care along with the judicious use and appropriate selection of antibiotics.

There is a need for research to further elucidate maternal microbiome patterns that protect against and elevate the risk for adverse pregnancy outcomes that impact neonatal and infant health and, thereafter, to identify modifiable factors that influence the composition of the maternal and infant microbiome to support the targeting of health strategies to improve pregnancy outcomes and infant health.

6. Neonatal Microbiota in Pregnancies Complicated by GDM and Neonatal Long-Term Outcome

It is well known that the maternal environment affects the offspring health. The newborn gut microbiota is strongly influenced by maternal health and pregnancy conditions

and participates in the development programming of the newborns [70–72]. Early disruption of the infant microbiota has been associated with many inflammatory, immune-mediated, allergic, and dysmetabolic diseases in later life [70–73].

GDM was found to be associated with specific changes in the gut microbiota composition [23, 35, 38, 40, 42]. The altered microbiome may have a crucial role in the underlying metabolic dysregulation that underpins the pathogenesis of gestational hyperglycemia, as well as the consequence of the increased adiposity frequently coexisting in GDM patients [74, 75].

6.1. Neonatal Microbiota. A possible vertical mother-to-child transmission of maternal gut bacteria has already been reported, even if, to date, certainty about the way of intrauterine microbial acquisition is lacking [76–78]. Besides breastfeeding and vaginal microbiota, placenta and amniotic fluid have also been reported to be a vehicle for this transmission [9].

Human and animal studies investigating possible causal linkage of disease programming suggest that gut microbiota dysbiosis negatively affects metabolic health triggering cardiometabolic disease onset later in life [79]. In alignment with the "developmental origin of health and disease" hypothesis, increasing evidence supports that exposure to prenatal metabolic disorders during fetal growth may contribute to health outcomes in the offspring [80].

Among full-term infants, gut microbiota consists primarily of anaerobic organisms. The "normal" infant gut microbiota develops by the colonization of facultative anaerobic organisms, later developing obligate anaerobes, including *Bifidobacterium*, *Bacteroides*, and *Clostridium* [81]. These anaerobes are associated with producing polysaccharides that mediate microbiota colonization, immune modulation, and host-gut cross-talk [70]. For example, *Clostridium* in the infant's gut, at high levels, is pathogenic and considered unhealthy.

6.2. Childhood Microbiota. After the age of 3 years, the microbial environment changes rapidly; compositional stability occurs to resemble an adult becoming dominated by *Firmicutes* and *Bacteroidetes* [82].

Gut microbiota is associated with metabolic and immune-inflammatory axes in the liver, muscle, and brain through host pathways. Dysbiosis, or imbalance of the infant gut microbiome, may be facilitated by early exposure to environmental factors such as bacteria and viruses, which can also alter host microbiota. This dysbiosis of microbiota has long-term effects on host metabolism, leading to metabolic changes, in particular, type 1 diabetes, autoimmune disease, and obesity [70]. In humans, it is suggested that early microbial patterns may predict excessive weight gain in offspring during childhood and later in life [70, 83] and that microbiota-related epigenetic changes during early development can affect phenotypic characteristics such as obesity later in life [83]. All these data support the hypothesis that the infant's early exposure to maternal microbiomes through a transfer of maternal gut microbiota may alter the composition of the infant's gut microbiome.

6.3. Long-Term Health Status. Recent research reported that GDM alters the microbiota of newborns, contributing to the current understanding of intergenerational obesity and diabetes prevalence [41]. In particular, one study observed a significant reduction in the diversity of various bacterial types in GDM newborns indicating that there might be serious dysbiosis in the gut of GDM newborns [84]. Compared with those of healthy newborns, GDM newborns could be more predisposed to develop gastrointestinal diseases and metabolic syndrome at later stages in their lives [84]. These findings are consistent with previous data showing that the gut microbiota in the GDM group was associated with a lower alpha-diversity level compared with that in the healthy groups [46] which, in turn, is associated with a higher BMI [85]. Research supports that the future health of infants may be affected as the offspring of GDM mothers is more likely to develop obesity during childhood and later in life [79], and this is information that deserves to be included in the prenatal counselling of patients affected by GDM.

Future studies are needed to improve our current knowledge in terms of infant gut microbiome and weight management interventions, important for decreasing risks for obesity and cardiometabolic disorders. Studies that connect diet, microbiota, and metabolism in mothers with GDM and their offspring remain a critical key point in obstetrics research. Further work is needed to determine specific mechanisms of compositional changes in newborns and infants over time.

Finally, efforts to identify biomarkers that detect neonatal dysbiosis are required to define appropriate diagnostic approaches and design effective early intervention strategies to optimize infancy, childhood, and adult health outcomes.

7. Clinical Implications

It is clear from the literature published in this field the crucial role of proper maternal nutrition throughout pregnancy in order to maintain a balanced microbiota colonization, which is demonstrated to positively influence intrauterine and vaginal environment, thus leading to reduced risk of both maternal and neonatal metabolic dysfunction and preterm birth.

According to this, probiotics administered during pregnancy are supposed to be helpful in preventing complications such as gestational diabetes. Several studies, investigating the possible role of probiotic use versus placebo in overweight and obese pregnant women, suggest that this can be a valid proposal of prevention strategy in order to reduce maternal and neonatal complications related to dysbiosis [86, 87].

In this scenario, the use of antibiotics during pregnancy should be extremely well weighted, considering risks and benefits of treating mothers with drugs potentially harmful for the microbiota composition.

It is demonstrated that antibiotics may alter the gut microbiota, in terms of the total number of bacteria and also its composition [88, 89]. Whether this change may improve or worsen the risk of developing GDM is yet to be demonstrated. While in nonpregnant individuals it has been shown

that antibiotic exposure may increase the risk of type 2 diabetes [90], a retrospective study on 12,551 patients found no differences in the risk of GDM between pregnant women that used antibiotics during pregnancy versus women who did not [91]. Another study found that antibiotic treatment in adolescent mice reduced *Bacteroidetes* [92]. In contrast to this, as it has already been discussed before, *Bacteroidetes*, along with *Dialister* and *Campylobacter*, is considered taxonomic biomarkers of GDM [37]. So, the real impact of antibiotics on the risk of developing GDM is far to be demonstrated.

Future research should focus on demonstrating the usefulness of "mapping" the maternal microbiome early during pregnancy as a preventive strategy to detect and treat unbalanced microbiota colonization that can be later related to adverse maternal and neonatal outcomes.

8. Conclusions

It is clear that the maternal microbiome widely influences neonatal and infant microbiome, and it has been shown that microbiome pathological alterations occurring during pregnancy can lead to adverse pregnancy outcomes that negatively affect neonatal and infant long-term health status, with a consistent socioeconomic impact as well.

Further characterization of the maternal microbiome and identification of various factors that facilitate changes in microbial profiles during preconception and in the course of pregnancy may elucidate preconception and prenatal strategies for improving pregnancy outcomes and, thereby, neonatal and infant health.

Conflicts of Interest

The authors declare no conflict of interest.

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Review Article

MiRNAs in Gestational Diabetes Mellitus: Potential Mechanisms and Clinical Applications

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Gestational diabetes mellitus (GDM) is a common pregnancy complication which is normally diagnosed in the second trimester of gestation. With an increasing incidence, GDM poses a significant threat to maternal and offspring health. Therefore, we need a deeper understanding of GDM pathophysiology and novel investigation on the diagnosis and treatment for GDM. MicroRNAs (miRNAs), a class of endogenic small noncoding RNAs with a length of approximately 19-24 nucleotides, have been reported to exert their function in gene expression by binding to proteins or being enclosed in membranous vesicles, such as exosomes. Studies have investigated the roles of miRNAs in the pathophysiological mechanism of GDM and their potential as noninvasive biological candidates for the management of GDM, including diagnosis and treatment. This review is aimed at summarizing the pathophysiological significance of miRNAs in GDM development and their potential function in GDM clinical diagnosis and therapeutic approach. In this review, we summarized an integrated expressional profile and the pathophysiological significance of placental exosomes and associated miRNAs, as well as other plasma miRNAs such as exo-AT. Furthermore, we also discussed the practical application of exosomes in GDM postpartum outcomes and the potential function of several miRNAs as therapeutic target in the GDM pathological pathway, thus providing a novel clinical insight of these biological signatures into GDM therapeutic approach.

1. Overview of Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is a common maternal complication that occurs or is recognized during pregnancy. Since the pathophysiology of GDM is characterized by chronic insulin resistance in the second half of pregnancy, it is not diagnosed until the late second or early third trimester of gestation. A globally estimated prevalence of 1.8-31% has been reported due to the lack of consistency in GDM diagnostic criteria between countries [1]. GDM exerts various adverse implications for mothers and their offspring. Mothers complicated by GDM have higher rates of preeclampsia and adverse pregnancy outcomes, such as cesarean deliveries and shoulder dystocia [2]. They are more susceptible to developing postpartum type 2 diabetes mellitus (T2DM) compared with normal women [3]. Additionally, their offspring may suffer from long-term metabolic disorders and related health conditions such as obesity, T2DM, and cardiovascular disease (CVD) [4, 5]. According to a new set of diagnostic criteria published by IADPSG, pregnant women should perform an oral glucose tolerance test (OGTT) during 24-28 weeks of gestation [6]. However, compliance with the test may decline because it requires fasting and multiple blood types and may cause discomfort such as vomiting. However, OGTT is not recommended as a routine screening for GDM at an earlier trimester of gestation [6], and hence, treatment cannot be applied promptly for the prevention of GDM. Therefore, finding early predictors of GDM is significant to improve the prognosis of mothers and fetuses.

Moreover, since coronavirus disease 2019 (COVID-19) pneumonia pandemic-induced local lockdown measures have been carried out worldwide, their negative effects on psychosocial states of pregnant women and on the glycemic

balance in GDM patients have been observed. A review indicated negative implications of lockdowns and unhealthy lifestyle for pregnancy [7]. Solitude and mental burden such as anxiety and depression may lead to unhealthy dietary habits and reduced exercise. On the other hand, increased snack consumption and carbohydrate intake were revealed with a high glycemic index; increased total diet intake was found to be associated with a rise in HbA1c levels [8, 9] during the COVID-19 pandemic lockdown. A retrospective study conducted in France reported a lower postprandial glycemic control and a higher use of insulin therapy during quarantine (18 March–7 May 2020). These observations were explained by anxiety, reduced physical activity, and changes in diet [10]. These risk factors, coordinating with self-reported boredom/solitude and enhanced consumption of snacks, unhealthy foods, and sweets, have caused increased weight gain in some obese individuals [11]. A higher rate of GDM was observed in pregnant women during March–April 2020 compared with the same period in 2019 [12].

miRNAs, first discovered from C. elegans by Ambros and Ruvkun, represent small, short noncoding, and single-stranded RNA sequences consisting of approximately 22 nucleotides (nt) in length and act as negative regulators by inhibiting mRNA translation or leading to its degradation [13]. In most cases, miRNAs can also mediate posttranscriptional gene silencing by complementary binding to the target mRNA $3'$-untranslated region ($3'$-UTR) or $5'$-UTR or open reading frame (ORF) regions via their seed sequence region [14, 15]. Many animal model systems have been established to detect miRNAs, and their number is primarily associated with the organism's complexity [16]. miRNAs present potential roles in the regulation of β-cell function and mass, as well as in metabolic processes [17]. The genome-wide analysis has demonstrated over 600 miRNAs expressed in placenta and their essential role in pregnancy and GDM [17–19]. Given the high stability of placental miRNAs in maternal circulation and their accessibility from maternal blood, they may become an early diagnostic biomarker of GDM [19]. Meanwhile, the role of a low glycemic or Mediterranean diet and particularly the favorable impact of plant-derived foods (e.g., vegetables, fibers, and fruits) on oxidative stress by enhancing antioxidant compounds has represented a new aspect in the pathogenesis of GDM [20]. Moreover, the correlation with miRNAs was not fully understood. This review is aimed at reporting updated literature in miRNA regarding to pathogenesis of GDM and the associated potential application.

2. The Biogenesis Pathway for miRNAs

During the process of miRNA biogenesis (shown in Figure 1), miRNAs located in intergenic regions and introns are transcribed by RNA polymerases II and III, from their promoter or cotranscribed with their own host gene or other miRNAs in the initial stage. The primary miRNA (pri-miRNA), an ~1000 nt capped and polyadenylated transcript, is known to contain a stem-loop structure in the nucleus [21]. The microprocessor complex subsequently crops this pri-miRNA to produce a precursor miRNA (pre-miRNA) with a length of 60 nt. The Exportin5-RanGTP system then exports this pre-miRNA to the cytoplasm for further processing. Eventually, the Dicer/TRBP complex cleaves the terminal loop of the pre-miRNA to create a miRNA duplex [21].

The remaining double-stranded RNA is loaded into a multiprotein complex called an RNA-induced silencing complex (RISC) and further unwinds in the center of RISC (an Argonaute protein) [21, 22]. During this process, the guide RNA strand from the miRNA duplex is selected as the mature miRNA, while the other passenger RNA strand is degraded. This guide strand remains in the RISC to form the miRNA-RISC complex as an essential component and serves to regulate gene expression epigenetically [23].

The miRNA-RISC has the capacity to regulate gene expression through base-pairing to the $3'$-untranslated region (UTR), $5'$-UTR, and protein-coding region of the messenger RNA (mRNA) target [13, 24]. The specific interaction between miRNAs and the target mRNA is primarily directed by the miRNA binding. This binding requires a certain number of nucleotides to match the sequence flanking the seed region [25]. The processes of the regulation in gene translation by miRNA-RISC are divided into two steps [26]: (i) the miRNA-RISC complex obstructs the binding between ribosomes and the mRNA target [27]; (ii) this consequently leads to mRNA degradation characterized by mRNA deadenylation and decapping, leading to accelerated destabilization and decay, thus suppressing translation of the target mRNA ultimately [28].

A single miRNA can target hundreds of mRNAs, and a specific target mRNA is often under the control of several distinct miRNAs. It has been established that miRNAs have potential function in many essential biological activities, such as cell proliferation, differentiation, apoptosis, disease initiation, and development [29–33]. Their dysregulation or dysfunction was revealed in many metabolic researches regarding obesity, T2DM, and cardiovascular disease. In addition, extracellular miRNAs are present in biological fluids such as plasma and are being packed into various carriers such as microvesicles (e.g. exosomes) or lipoproteins, rendering them a potential role as biomarkers or therapeutic targets [34].

3. miRNA Identification and Quantification Techniques

Several specific and sensitive approaches were applied to detect, validate, and quantify miRNAs, including quantitative reverse transcriptase PCR (RT-qPCR) [35, 36], in situ hybridization [37], Northern blot analysis [38, 39], miRNA microarray [40, 41], and next-generation sequencing(NGS) [42]. Deciding on the optimal miRNA profiling and quantification technology depends on the experimental designs, specific types of sample, research objective, and intended therapeutic use.

However, the expressions of several miRNAs in some findings we will review in detail further are not shared across each other. Different source materials such as serum or

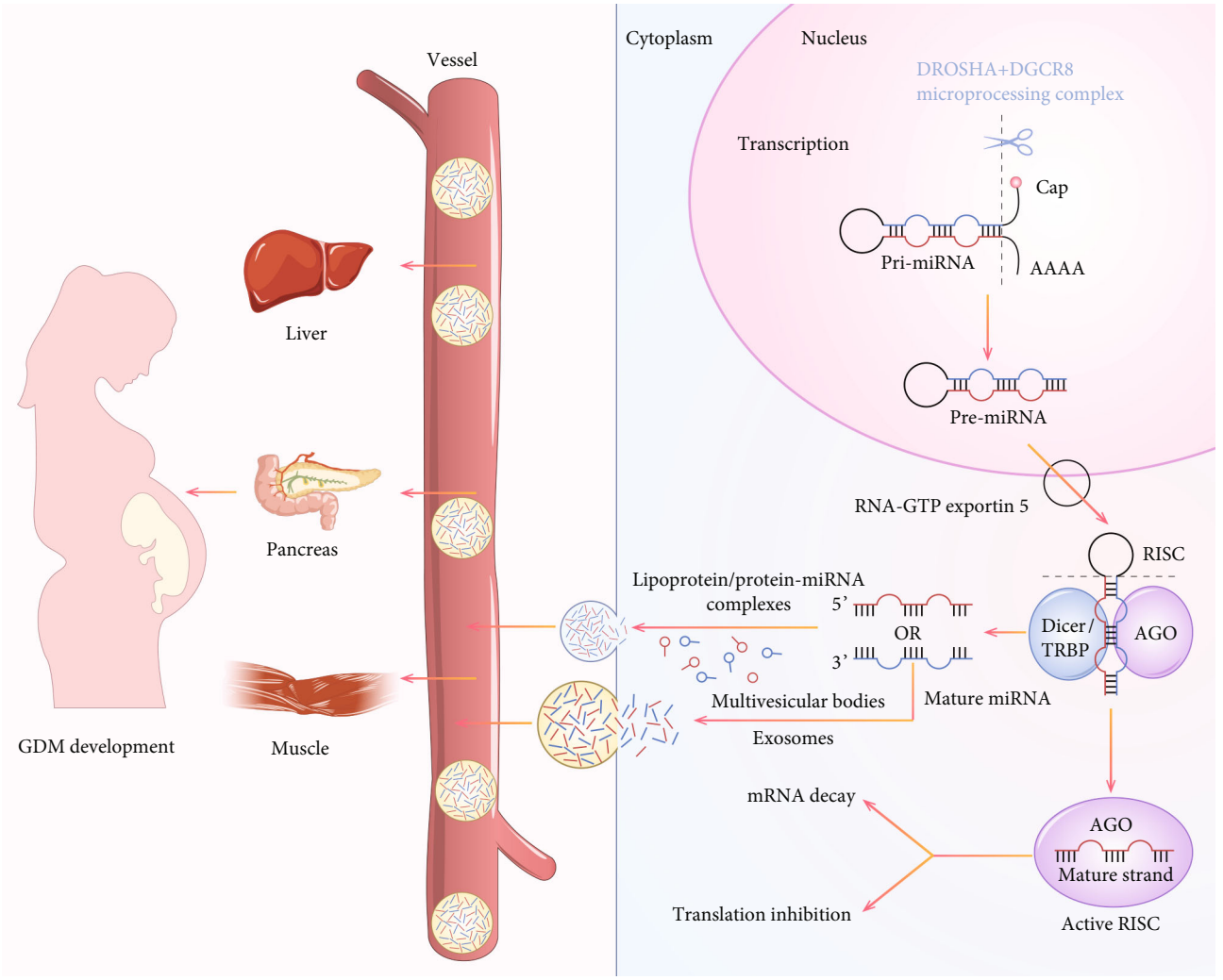

Figure 1: Overview of the biogenesis and potential functions of miRNAs in GDM. The biogenesis pathway for miRNAs is shown on the right side: pri-miRNA is cropped by the microprocessor complex to produce a pre-miRNA, which is then exported to the cytoplasm by the Exportin5-RanGTP system. The Dicer/TRBP complex cleaves the terminal loop of the pre-miRNA to create a miRNA duplex [21]. The latter is loaded into the RISC and further unwinds in the Argonaute (AGO) protein, the center of RISC [21, 22]. During this process, the guide RNA strand from the miRNA duplex is selected as the mature miRNA, while the other passenger RNA strand is degraded. This guide strand remains in the RISC to form the miRNA-RISC complex as an essential component [23], serving to play a role in mRNA decay or translation inhibition [13, 24–28]. Furthermore, miRNA is exported to the extracellular space through various carriers such as lipoproteins, proteins, and exosomes [34]. The participation of miRNA in the pathogenesis of GDM is shown on the left: these miRNA complexes are transported in the vessel and exert their potential functions on the pathogenesis of GDM through tissues/organs that linked to glucose metabolism (e.g., liver, pancreas, and muscle), serving as diagnostic biomarkers and therapeutic target in GDM.

plasma used during the detecting process or discrepancy in the analysis platform's application might contribute to such differences [43]. Therefore, minimizing experimental variations through experimental normalization, data processing, and optimization is also significant for the precise evaluation of the level of miRNA from a specific sample [44].

Currently, quantitative reverse transcriptase PCR (RT-qPCR) is known as the gold-standard approach for miRNA quantification, which serves as the most reproducible and sensitive method [45–47]. Stem-loop RT-based TaqMan miRNA assay is widely used as the main PCR technique in research due to the advantage of high sensitivity and specificity [48]. Besides, direct RT-based and poly(A) tailing-based SYBR miRNA assays are considered as practical alternatives for miRNA detection and quantification [48]. A high-throughput qRT-PCR platform has been established as a more available approach for rapid miRNA profiling of a great quantity of biological samples. Some advances have been achieved in quantification using low amounts of miRNA [49]. TaqMan low density array (TLDA) possesses the advantage of cost-effectivity and serves as the most widely used qRT-PCR miRNA expression profiling method [50].

Efforts have been made for the possibility of shortening the technique execution time as well as lowering amounts of miRNA used in quantification [51–53]. Microarrays represent a practicable discovery tool used for miRNA

identification on the basis of the principle in hybridization of cDNA to the DNA probe [54]. However, this technique is not quite promising for miRNA profiling, since they are not capable of detecting highly expressed miRNAs or distinguishing between mature and immature miRNAs [55, 56]. Moreover, several limitations related to this technique including low sensitivity, high requirement for RNA input amount (100 ng to 1 μg), background, and cross-hybridization still remain to be solved.

Another two alternatives for miRNA identification are also applied in research. In situ hybridization serves to contrast the level of miRNAs in different cells through utilizing radioactive, fluorescent, or dioxygen in probes [57]. It is noted that ISH also presents several disadvantages, including long processes, strenuous steps, and a higher rate of errors [57]. Additionally, next-generation sequencing technology (NGS) is a highly accurate technique with an advantage over other technologies, as it has the capability to identify novel miRNAs. Nevertheless, NGS is a more laborsome technique compared with qRT-PCR and microarrays and presents a higher requirement for RNA input amount (500 ng to 5 μg). Of note, high costs of this technique may contribute to a limitation of its wider availability [58].

Moreover, the most frequent normalization technique involves strategies using exogenous spike-in miRNA, such as C. elegans miR-39, which is validated to be more reliable compared with endogenous reference genes like miR-16 [59]. However, researchers prefer an application of combining both exogenous and endogenous miRNA reference genes, due to no ideal normalization strategy exists and the application of a single type of reference gene is insufficient for accurate miRNA results [60].

4. miRNA: The Role in Pathophysiology of GDM

4.1. miRNA-Related Maternal Metabolic Adaptation. In the past decade, people have been interested in the link of novel placenta-derived factors such as placenta-derived miRNA to pregnancy. More and more studies have explored the biological functions of placental-derived miRNA and their applicability as biomarkers in some pregnancy complications, such as GDM. Moreover, it is well established that an improper maternal metabolic adaptation to these placental-derived miRNAs has been observed [61, 62]. Therefore, variations in the expression of placental-related miRNA may indicate changes to maternal metabolic adaptation mechanism, thus providing insight into the pathogenesis of GDM pregnancy. Besides, variations in the expression of miRNAs in circulating samples may also indicate their involvement in maternal metabolic adaptation. Several studies have investigated the regulation of placenta-associated miRNA and circulating miRNAs as well as their related metabolic adaptation in GDM (Table 1).

Kokkinopoulou et al. first described a T2DM-specific expression profile of miRNAs that target disease-susceptibility genes, such as CDKN2A, CDK5, IGF2BP2, KCNQ1, and TSPAN8. miR-98-5p, one of miRNAs expressing decreased levels in T2DM patients compared with controls, was reported [63]. Moreover, miR-98 is also known to be implicated in embryo implantation during the initial stage of pregnancy. In 2016, Cao et al. showed a significant upregulation of miR-98 derived from placenta at gestation of 37–40 weeks in GDM patients ($n = 193$) compared to normal pregnant subjects ($n = 202$). Additionally, experimental validation in JEG-3 (human choriocarcinoma cell line) provided supportive evidence for its role in the regulation of glucose uptake. Specifically, by regulating Mecp2 and in turn targeting Trpc3, it has subsequent regulative effects on insulin-mediated glucose uptake in GDM [64]. This experimental evidence further confirmed the role of miR-98 in the development of GDM.

Zhao et al. reported a significantly upregulated concentration of miR-518d in the placenta of women affected by GDM compared with the normal subjects at 37-40 weeks of gestation. It is further proven that concentration of miR-518d in term placenta was negatively correlated with the expression of peroxisome proliferator-activated receptor-α (PPARα) [65]. PPAR plays a role in regulating the pathway related to inflammation, accidental formation, oxidative stress, and insulin signaling metabolism [66, 67]. The downregulation of the PPARγ expression in GDM may accelerate glucose intolerance [68]. Reduced expression of PPARα and RXRα were also found in the placenta of women with GDM [69].

In 2011, the same group demonstrated a significant downregulation of miR-132, miR-29a, and miR-222 in serum derived from GDM patients ($n = 24$) at gestation during the 16th and 19th weeks in comparison with healthy pregnant women ($n = 24$) [50]. Contrarily, a significant upregulation of miR-222, 1 of 17 differentially expressed miRNAs identified by Shi et al., was found in omental adipose tissues from GDM patients. By conducting a validation study in 10 GDM pregnant women compared with 10 healthy subjects of normal glucose tolerance, they further confirmed that the level of miR-222 was negatively correlated with the protein concentration of transporter glucose transporter 4 (GLUT4) in omental adipose tissue, as well as estrogen receptor- (ER-) α; the implication of the latter was validated in glucose homeostasis and insulin regulation [70–72]. Furthermore, they also validated the involvement of miR-222 in insulin resistance induced by estrogen in GDM through experiments performed on 3T3-L1 adipocytes by using antisense oligonucleotides [55].

Later on, Stirm et al. demonstrated a significant upregulation of miR-340 in whole blood cells (WBC) and lymphocytes from GDM women ($n = 8$) at 24–32 weeks of gestation, compared to healthy subjects ($n = 8$) [73]. A significant downregulation of polyadenylate- (poly(A)-) binding protein- (PABP-) interacting protein 1 (PAIP1), known as a key promoter of translation that was never described in GDM before [74], was observed only in WBCs in GDM women, in comparison with normal glucose tolerant (NGT) subjects. An inverse correlation between miR-340 and PAIP1 expression in lymphocytes was observed, indicating that miR-340 might negatively regulate PAIP1. They further conducted experiments and observed reduced expression of miR-340 in human lymphocytes cultured in high-glucose medium. After adding insulin to the high-

Table 1: Studies investigating the regulation of miRNA and related maternal metabolic adaptation in GDM.

miRNA	Regulation	Stage of pregnancy	Source	Cell studied	Putative target	Related metabolic adaptation
miR-222 [55]	↑	38-39 wk	Omental adipose tissue	3T3-L1 cells	ER-α	↑estrogen induced insulin resistance
miR-98 [64]	↑	37-40 wk	Placenta	JEG-3 cells	Mecp2, Trpc3	↓insulin-mediated glucose uptake
miR-518d [65]	↑	37-40 wk	Placenta	HEK-293 cells	PPARα	↓glucose intolerance
miR-340 [73]	↑	24-32 wk	Whole blood cells	Lymphocytes	PAIP1	↑maternal fasting insulin
miR-130b, miR-148a [75]	↑	Newborns	HUVECs	HUVECs & BeWo cells	AMPKα1	↓glucose metabolism

glucose medium, the miR-340 level presented an inversely significant increase, indicating that miR-340 expression was regulated in a context of insulin resistance. Accordingly, the expression of miR-340 in leukocytes is positively correlated with the level of maternal fasting insulin in vivo.

Finally, Tryggestad et al. identified differentially expressed miRNAs by using a miRNA microarray in HUVECs from GDM-exposed newborns ($n = 7$) with respect to normal newborns ($n = 12$) [75]. Seven upregulated miRNAs were found in HUVECs from GDM-exposed newborns and selected for validation by RT-qPCR, including miR-130b-3p and miR-148a-3p. They also observed the reduced expression of AMP-activated protein kinase α1 subunit (AMPKα1) in GDM-exposed placenta. Notably, miR-130b and miR-148a were validated to posttranscriptionally regulate AMPKα1 [75]. AMPKα1 is known to be involved in regulation of genes related to energy homeostasis, fatty acid synthesis, protein synthesis, and glucose metabolism by functioning as a central enzyme [76]. Recent data have also demonstrated that AMPK, whose activity is significantly reduced in adipose tissue and skeletal muscle of GDM women, was downregulated in placenta of pharmacologically treated GDM patients [77, 78]. Furthermore, pAMPK was confirmed to activate the mTOR pathway and contribute to the conversion toward aerobic glycolysis in GDM.

4.2. miRNA-Related Maternal Pancreatic β-Cell Dysfunction. The development of GDM may be attributed to the dysfunction of maternal pancreatic β-cell during the compensatory mechanism for insulin resistance. Recent studies have established a conceivable link between circulating miRNAs, placental miRNAs, and maternal pancreatic β-cell dysfunction in GDM (Table 2).

Feng et al. assessed the level of miRNAs in peripheral blood samples derived from 12 GDM pregnancies and 12 healthy pregnancies. miR-33a-5p was demonstrated to be significantly upregulated in GDM group with respect to the NGT group. Furthermore, the authors found a positive correlation between miR-33a-5p expression and blood glucose. Notably, overexpression or inhibition of miR-33a-5p performed on INS-1 cells was revealed to significantly inhibit or promote cell growth and insulin production under high glucose condition, respectively. miR-33a-5p was found to directly target its downstream gene ABCA1, and lnc-DANCR exerts as a sponge in the regulation of antagonizing the function of miR-33a-5p [79]. These results confirmed that the lnc-DANCR-miR-33a-5p-ABCA1 signaling pathway exerts a significant role in regulating the biological function of INS-1 cells.

Similarly, Sebastiani et al. evaluated the level of miR-330-3p and found its hyperexpression in the blood sample of 21 GDM pregnancies versus 10 normal pregnancies at 24–33 weeks of pregnancy using a highly standardized approach. Interestingly, circulating miR-330-3p expression was negatively associated with fasting insulin only in GDM patients. Furthermore, two age- and BMI-matched populations were distinguished by differential level of miR-330-3p that divided into high and low groups, respectively [80]. Moreover, overexpression of miR-330-3p was validated to target and downregulate key genes, such as E2F1, known as essential modulators in glucose-stimulated insulin secretion and β-cell maintenance, such as β-cell growth and proliferation [81, 82]. The authors thus postulated that the hyperexpression of miR-330-3p in the blood sample may be harmful for β-cell function and/or proliferation.

Oppositely, He et al. analyzed the expression of miR-494 in the blood sample from 20 pregnancies affected by GDM and 20 normal women [83]. A significant downregulation of miR-494 was found in GDM pregnancies compared to CTRLs and was negatively associated with blood glucose. Furthermore, overexpression of miR-494 enhanced insulin secretion, induced cell proliferation, and inhibited cell apoptosis, whereas miR-494 knockdown achieved the opposite results. miR-494 was revealed to directly target phosphatase and tensin homolog (PTEN), known to exert a crucial role in apoptosis, in pancreatic β-cells. Notably, downregulation of PTEN induced by siRNA rescued the impact brought by miR-494 knockdown on insulin secretion, cell proliferation, and apoptosis of pancreatic β-cells. In conclusion, the results underline implication of miR-494 in β-cell dysfunction of GDM.

Li et al. also reported a significant downregulation of miR-96 in placental tissue from 3 GDM pregnancies compared to 3 healthy pregnancies. In addition, miR-96 expression was also found inversely correlated with blood glucose. It is noted that the knockdown of miR-96 reduced insulin level, lowered cell viability, and increased apoptosis in INS-1 cells under high glucose condition. Interestingly, similar correlation between miRNA and blood glucose was also observed in GDM rats. Zhao et al. analyzed the miRNA-

Table 2: Studies investigating the regulation of miRNA and related maternal pancreatic β-cell dysfunction in GDM.

miRNA	Regulation	Stage of pregnancy	Source	Cell studied	Putative target	Related pancreatic β-cell dysfunction
miR-33a-5p [79]	↑	24-28 wk	Blood samples	INS-1, HEK293T cells	ABCA1	↓cell growth, ↓insulin production
miR-330-3p [80]	↑	24-33 wk	Plasma samples	—	E2F1, CDC42	↓cell proliferation, ↓insulin secretion
miR-494 [83]	↓	—	Peripheral blood	INS-1 cells	PTEN	↓insulin secretion, ↓cell proliferation, ↑cell apoptosis
miR-96 [85]	↓	—	Placental tissue	INS-1, HEK293T cells	PAK1	↓insulin secretion, ↓cell viability
miR-221 [84]	↓	—	Placental tissue of GDM rats	INS-1 cells	PAK1	↓insulin secretion, ↓cell proliferation, ↑cell apoptosis

221 expression in placental tissues of GDM rats by the microarray. A downregulation of miRNA-221 was reported in GDM rats, and a negative correlation between the miRNA-221 level and the blood glucose level was demonstrated. Notably, knockdown of miRNA-221 lowered insulin production and increased apoptosis in INS-1 cells, while opposite results were observed in miRNA-221-overexpressed INS-1 cells. Of note, miRNA-221 and miR-96 were proven to directly target PAK1 in two researches, and these results suggested that the dysfunction of β-cell might be attributed to dysregulation of miRNA-221 and miR-96 with a subsequent effect through targeting PAK1 [84, 85].

5. miRNAs in Placental Function and Fetal Complication

We have reviewed the role of several placenta-associated and circulating miRNAs in maternal metabolic adaptation and pancreatic β-cell dysfunction. In addition, several studies have investigated the role of miRNAs in placental function, as well as GDM-related fetal complication of the next generation.

By using RNA sequence and qRT-PCR validation, Ding et al. confirmed several dysregulated miRNAs in the placenta derived from 8 GDM pregnancies versus 8 healthy subjects. These differentially expressed miRNAs were predicted to be involved in placenta morphology and development. Notably, miR-138-5p was selected for biological functional assay due to its significant overexpression in GDM. Its overexpression inhibited the proliferative and migration ability of HTR-8/SVneo trophoblast cells. A specific target of miR-138-5p was TBL1X, an oncogene in the activation of the WNT/β-catenin signaling pathway. This pathway crucially participates in placental biological processes, such as proliferation, differentiation, and invasion [86–88]. Moreover, miR-138-5p was validated to target sirtuin 1 (SIRT1) [89]; although limited studies reported the association between SIRT1 and GDM, reliable data confirmed its implication in the inflammation and glucose metabolic pathway in human placenta. Mac-Marcjanek et al. conducted experiments to investigate SIRT1-dependent specific gene alteration in GDM pregnancies and identified four diabetes-relevant genes linked to metabolism, inflammation, and transporting functions in SIRT1-overexpressed leukocytes [90]. SIRT1 was also found increasingly expressed in GDM women exposed to hyperglycemia at one day postpartum [91]. However, other authors observed a reduced level of SIRT1 in fetal endothelial colony-forming cells (ECFCs) and HUVECs in GDM pregnancies [92, 93], suggesting that dysregulation of SIRTs may be related to fetal complication. These evidences suggested that miR-138-5p serves as a potential biomarker in GDM management.

Li et al. identified 29 differentially expressed placenta-derived miRNAs from 15 GDM pregnancies in respect to 15 normoglycemia subjects to investigate the alteration of miRNAs. By conducting a miRNA microarray and RT-qPCR analysis approach, they validated 9 dysregulated miRNAs (miR-508-3p, miR-27a, miR-9, miR-137, miR-92a, miR-33a, miR-30d, miR-362-5p, and miR-502-5p). Furthermore, these miRNAs were predicted to target key genes implicated in the EGFR/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway [56]. Of note, it is well known that the insulin tyrosine kinase receptor could activate the PI3K/AKT pathway and promote glucose transporting by enhancing the delivery of intracellular GLUT4 to the cell surface. Specifically, miR-508-3p, one of the overexpressed miRNAs, was revealed to directly regulate PIKfyve, a reverse modulator of the epidermal growth factor receptor (EGFR). PIKfyve exerts an essential role in adequate placental development and fetal growth [94]. The upregulation of miR-508-3p was validated to repress the expression of PIKfyve and aberrantly activate the EGFR/PI3K/AKT signaling [56]. Thus, the dysregulation of miR-508-3p may potentially promote the development of macrosomia, a specific fetal complication related to GDM.

Floris et al. reported an upregulated expression of miR-101 in HUVEC cells from GDM ($n = 22$) compared to healthy subjects ($n = 24$) and confirmed its crucial role in endothelial function and angiogenesis [95]. Moreover, miR-101 was found to target enhancer of zester homolog 2 (EZH2) [95–100], which exhibited reduced concentration in its isoform and histone H3K27 trimethylation in cultured human umbilical vein endothelial cells (HUVECs) from a GDM-exposed fetus [101]. A negative correlation between miR-101 and EZH2 was reported in a feedback loop of epigenetic regulation, suggesting a decreased functionality in GDM placenta. The dysfunctionality of the GDM placenta

may contribute to miR-101 upregulation and functional alterations observed in HUVECs, including cell apoptotic activities and angiogenic and migratory capacities [101]. However, the maintenance of the alteration in this pathway and associated adverse impact on generation's health still remain unclear. Some metabolic disorders such as cardiovascular disease might emerge in their adulthood life.

Notably, miRNAs could also function as a protective mechanism. Diaz-Perez et al. discovered another two differentially expressed miRNAs in GDM placental tissue and revealed their potential role in placental pathophysiology. Specifically, upregulation of miR-221 and miR-222 was reported in human fetoplacental endothelial cells (fpEC) isolated from four GDM placentas during the third trimester compared to four CTRLs [102]. What is more, miR-221 and miR-222 were validated to negatively regulate ICAM1 protein, whose reduced concentration was observed in the fetoplacental endothelium derived from GDM [103, 104]. These miRNAs may lead to the downregulation of ICAM-1 and function as a protective mechanism against inflammation characterized by leucocyte transmigration from blood to placenta due to hyperglycemia during GDM [102].

6. Exosomes and miRNAs in GDM

Exosomes are known specifically as extracellular vesicles (EVs), with the characteristic of a bilayered lipid and ~50-150 nm in diameter, originating from the endosomal compartment and actively secreted by multiple cell types [105]. Recently, exosomal miRNAs and their involvement in gene expression are gaining increasing scientific attention, suggesting their potential role for regenerating new therapies [106]. Exosomal miRs can be derived from different biological fluids, such as saliva, serum, amniotic fluid, urine, and breast milk, and can be released from various cells into the extracellular space [107, 108]; such a characteristic renders them to be potential clinical biomarkers and even novel targets for therapeutic intervention.

Three modes of mechanisms have been reported in the protection of miRNAs from degradation [34, 109–113]. These mechanisms could guarantee intercellular communication of miRNAs and their stability as cargos when delivered to recipient cells, subsequently inducing expressional and functional response. Therefore, similar to the cell-to-cell contact-dependent signaling pattern, the capacity of circulating EVs in conveying information is also considered an essential way for intercellular communication [114].

It is widely acknowledged that placenta is tightly linked to alteration of metabolic status in pregnancy. It is considered that adverse placental condition might be mirrored by the miRNA expression profile in placenta-derived exosomes (PdEs). In this part, we will emphasize PdE's contribution to the development of GDM and give our viewpoints for their application in GDM management.

6.1. Tissue-Derived Exosome and Exosomal miRNAs in GDM. Rice et al. performed the pilot study to demonstrate an altered exosomal concentration in GDM pregnancy. They observed a significantly higher exosomal level in the plasma sample of GDM women compared to normal subjects. The results also revealed that a high D-glucose level promotes exosomes released from trophoblast cells during the first-trimester pregnancy, suggesting a correlation between high glucose and exosomal bioactivity, which is of clinical relevance in GDM pathophysiology [115]. Furthermore, these exosomes released from trophoblast cells were confirmed to induce the expression of cytokine mediators such as interleukin-8 (IL-8) and TNF-a by in vitro experiments conducted on human umbilical vein endothelial cells (HUVECs), suggesting that exosomes could regulate immune responses to maternal metabolic adaptation during pregnancy.

Another study conducted by Salomon et al. also investigated the profile of PdEs in plasma during pregnancy. A progressive increase in the amount of these PdEs was observed, and the profile of these PdEs released into peripheral circulation at the 6-week gestation was characterized by gestational age. Furthermore, Salomon et al. confirmed these results in a prospective cohort through comparing the gestational-age PdE profile in GDM maternal plasma to normal subjects [116]. Similarly, they also observed an altered release of proinflammatory cytokines from HUVECs when treated with these PdEs derived from GDM pregnant women [117]. A more recent study conducted by Nardi et al. also reported similar results, indicating such pregnancy-related alterations of circulating EVs might provide a first hint for their role in the regulation of immune response during pregnancy [118].

Nakahara et al. also reported total PdE exosomal alterations in a cohort study and revealed their association with gestational age and pregnancy outcome. They also found a significantly higher PdE level in GDM pregnancies and PE versus normal pregnancies. In addition, several significant risk factors for GDM, including glucose concentration, maternal body mass index (BMI), and fetal body weight, were strongly associated with the PdE concentration during pregnancy, indicating that PdEs may reflect maternal metabolic adaptation and diagnostic utility to predict adverse pregnancy outcomes at an early stage [119]. Similarly, Elfeky et al. revealed a significant correlation between exosome concentration in maternal circulation and maternal BMI. Specifically, maternal BMI was inversely correlated with the contribution of PdEs to the total exosomes across gestation. A stronger effect was observed in exosomes derived from women of higher BMI in respect to lean, suggesting a potential influence of exosomes on the maternal systemic inflammation during gestation [120]. This study established the exosomal variation could be attributed to maternal BMI.

The role of exosomes derived from adipose tissue (exo-AT) is less investigated in the pathogenesis of GDM. Another study indicates that these exo-AT can function as regulators in the placental glucose metabolism through communication with placenta tissues in GDM, making it a potential to become an effective target for therapeutic intervention to prevent consequences complicated by GDM such as fetal overgrowth [121, 122]. Recently, it has been established that exo-AT might promote insulin resistance (IR) and other obesity-related metabolic statuses in obesity. Novel findings provided the evidence for the pivotal role of

the dysregulated release of exo-AT in the onset and development of GDM in obese mothers [122].

Interestingly, PdEs can also function as regulators in the communication with other organs/tissues. Recent studies have identified several exosomal miRs and suggested their potential roles as biomarkers for myogenesis, nutrient metabolism, and muscle mass variation in pathophysiological conditions [123–126]. There may exist a potential link between placenta-specific exosomal miRNAs and skeletal muscle. Nair et al. assessed the concentration of exosomal miRNA in chorionic villi explants derived from 12 pregnancies complicated by GDM compared to 12 normal subjects using next-generation sequencing (NGS) [123]. They further revealed a dysregulated set of 27 placenta-specific exosomal miRNAs and further explored the concentration of several exosomal miRs, including miR-22-3p, miR-125a-3p, miR-197-3p, miR-99b-5p, and miR-224-5p. These specific miRNAs were selected for their differentially expressed patterns between GDM and CTRLs, as well as variation in a consistent pattern in skeletal muscle samples and in GDM maternal circulation. Of note, several differentially expressed miRNAs were predicted to target glucose metabolism-associated genes such as the PI3K/AKT signaling pathway, suggesting their involvement in skeletal muscle insulin sensitivity of GDM. Therefore, placenta-specific exosomal miRNAs might exert a crucial role in the interrelation between gestational tissues and skeletal muscle with subsequent possible effects on peripheral insulin resistance in GDM.

Therefore, such research for the role of exosomes as paracrine vectors might help discover useful research hypotheses and novel knowledge for deciphering GDM pathophysiology and generating valuable and accessible biomarkers for the diagnostics and prediction in GDM. In addition, as regards the dysregulation of miRNA expression which has been linked to the complication of pregnancy, exosomal content including miRNA could be profiled and discovered as biomarkers for GDM. However, their involvement in the pathophysiology of GDM still needs to be further investigated for diagnostic purposes and therapeutic intervention.

6.2. Exosomes and miRNAs in GDM Treatment. Exosomes can be potential candidates for effective and regenerative therapies, thus establishing a new therapeutic area in regard to postpartum outcomes of GDM mothers, such as stress urinary incontinence (SUI), a common pathological state observed in nearly 30% of postpartum women [127]. Likewise, therapies based on MSC-exosomes have been also explored and represent as a promising approach in the improvement of GDM-caused myopathy.

Notably, Ni et al. demonstrated that some functional and histological improvements were achieved in a SUI rodent model when treated with hADSCs-exosomes. Additionally, several proteins contained in hADSCs-exosomes were linked to some crucial pathways such as Wnt, PI3K-Akt, and Jak-STAT signaling pathways, which were potentially implicated in skeletal muscle and nerve regeneration [127].

Similarly, Liu et al. reported the capacity of hADSCs-exosomes in increasing type I collagen content through stimulating collagen synthesis and inhibiting collagen degradation in vaginal fibroblasts from SUI women and established promising evidence in the field of therapeutic strategy for treating SUI [128]. Experimental evidence further confirmed the role of exosomes released from fibroblasts of SUI women in regulating endothelial cell angiogenesis [129].

Importantly, it has been established that miRNAs could be a potential candidate for effective and personalized therapy of GDM due to the discovery that exosomes possess diverse functions, including therapeutic function in the GDM avenues.

Moreover, several studies have reported promising approaches in treating GDM. By using microarray analysis, Chen et al. identified differentially expressed genes and miRNAs involved in the regulation of flotillin2 (FLOT2). The results indicated a negative correlation and a target relationship between miR-351 and FLOT2. Specifically, they treated GDM mice with a series of mimic, inhibitor, and small interfering RNA to investigate the bioactivity of miR-351 in insulin resistance (IR), cell apoptosis in pancreatic tissues, and liver gluconeogenesis [130]. The results showed that an upregulation of miR-351 suppressed the expression of FLOT2 with subsequent effects on liver gluconeogenesis by downregulating the PI3K/AKT pathway in GDM mice. These results indicated that miR-351 serve to prevent GDM development, and miR-351 was identified as a therapeutic target in the intervention of GDM.

Another study conducted by Tang et al. explored the role of miR-335-5p on insulin resistance and pancreatic islet β-cell secretion via activation of the TGFβ signaling pathway by downregulating VASH1 expression in GDM mice. They observed that overexpression of miR-335-5p and inhibition of VASH1 might contribute to the downregulation of insulin and insulin release levels [131]. These findings provided evidence for the role of miR-335-5p in the development of insulin resistance and the inhibition of pancreatic islet β-cell through downregulating VASH1 and subsequently activating the TGF-β pathway in GDM mice, thus providing more clinical insight into the GDM treatment.

7. Discussion

Gestational diabetes mellitus is regarded as one of adverse pregnancy complications, presenting an increasing prevalence throughout the world. It may lead to maternal postpartum metabolic disorders, such as obesity and diabetes, and bring about adverse influence on later development of the offspring. Although GDM is well known as a common pregnancy complication, it could not be diagnosed until the late second trimester [6]. Hence, novel biological signatures for timely diagnosis and therapeutic intervention are of significance. Nowadays, early recognition, diagnostic criteria, and therapeutic targets related to GDM are of great interest and with controversies, for diversity exists in race, region, genetics, environmental factors, and diagnostic criteria for GDM [132–135].

It is demonstrated that lifestyle strategy initiated in the first trimester of pregnancy has been proven effective

[136–141], reinforcing the importance of exploring biomarkers in early pregnancy. More importantly, identifying novel and available biomarkers in an early pregnancy provides clinical value not only for GDM early diagnosis but also for the prevention of obstetric and maternal-fetal complications.

Our team has investigated thyroid hormone in early pregnancy and revealed a negative correlation between its level and GDM. A low FT4 level in early pregnancy was found to increase the risk for developing GDM [142]. More recently, our team has established an advanced ML model for the early prediction of GDM [143]. Through employing machine learning(ML) models of high accuracy, a clinically cost-effective 7-variable logistic regression (LR) model that achieved effective discriminate power (AUC = 0.77) was ultimately investigated. The results demonstrated that low body mass index (BMI) (≤17) was revealed as a risk factor for GDM. Meanwhile, total 3,3,5′-triiodothyronine (T3) and total thyroxin (T4) showed superiority over free T3 and free T4 in predicting GDM, respectively. Besides, a promising predictive value of lipoprotein was also validated (AUC = 0.66).

As a class of short noncoding RNAs, miRNAs have achieved rising attention in GDM pathophysiology and development. Moreover, miRNAs have also induced interest as mediators of tissue cross-talk, such as adipose tissue and skeletal tissues, in the development of GDM. Notably, apart from previous findings related to miRNA in adipose tissue, adipocyte-derived markers also include adiponectin and leptin [144–146]. Likewise, some other placenta-derived markers, such as follistatin-like-3 [147–149] and placental growth factor [150, 151], could also function as biochemical predictors. These evidences may indicate a potential link of miRNAs to these serum biological signatures, suggesting their capacity as regulators of gene expression at the epigenetic level. Theoretically, the capacity of miRNAs in epigenetic modifications from an early pregnant stage holds evidence for their specific use in predicting GDM. Therefore, further investigation on miRNAs' changes in concentration and corresponding epigenetic alterations in various biological tissues should be carried out.

8. Conclusion

In conclusion, we reviewed miRNAs revealed in placental tissues and investigated their roles in metabolic adaptations (e.g., insulin resistance, pancreatic, and β-cell function), placental function, and fetal complication. We also reviewed plasma exosomes and molecular content involved in GDM etiology; these evidences help in elucidating GDM pathophysiological pathways. However, their clinically diagnostic and predictive value still needs further investigation. Although several miRs were detected in the first trimester of pregnancy, it is noted that sample collection for miRNA analysis in most studies reviewed were restricted to the late second trimester of gestation. There still exists a lack in the evidence for miRNAs. Therefore, further research is needed in the validation of miRNA profiles for the earlier prediction of GDM. We will conduct more research to establish the potentiality of miRNAs for their predicting value in the diagnosis of GDM later on.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Zhao-Nan Liu was responsible for conceptualization, PubMed search, and manuscript preparation. Ying Jiang was responsible for PubMed search and manuscript preparation. Xuan-Qi Liu was responsible for PubMed search and manuscript preparation. Meng-Meng Yang was responsible for review and editing and supervision and revision of the manuscript. Cheng Chen was responsible for manuscript review and editing. Bai-Hui Zhao was responsible for manuscript supervision and revision. He-Feng Huang was responsible for conceptualization and manuscript review, supervision, and revision. Qiong Luo was responsible for conceptualization and manuscript review, supervision, and revision. Zhao-Nan Liu, Ying Jiang, and Xuan-Qi Liu contributed equally to this work.

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Review Article

An Update of Medical Nutrition Therapy in Gestational Diabetes Mellitus

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Gestational diabetes mellitus (GDM) is a serious and frequent pregnancy complication that can lead to short and long-term risks for both mother and fetus. Different health organizations proposed different algorithms for the screening, diagnosis, and management of GDM. Medical Nutrition Therapy (MNT), together with physical exercise and frequent self-monitoring, represents the milestone for GDM treatment in order to reduce maternal and fetal complications. The pregnant woman should benefit from her family support and make changes in their lifestyles, changes that, in the end, will be beneficial for the whole family. The aim of this manuscript is to review the literature about the Medical Nutrition Therapy in GDM and its crucial role in GDM management.

1. Introduction

The American Diabetes Association (ADA) defines GDM as previously unknown diabetes, diagnosed during the second or third pregnancy trimester [1]. Although GDM is one of the most frequent perinatal complications, its definition, diagnosis criteria, or screening methods did not benefit from a uniform approach from different international organizations. Thus, although the effects of perinatal hyperglycemia were first described by Dr. J.P. Hoet in 1954 [2], the diagnosis criteria and medical care standards for GDM were established only in the last two decades. An important study regarding this matter was HAPO (Hyperglycemia and Adverse Pregnancy Outcomes Study) [3], a study performed on 23 316 pregnant women, whose results led to the establishment of the new criteria for diagnosis of GDM by IADPSG (International Association of Diabetes and Pregnancy Study Groups) in 2010 (Table 1) [4]. Still, an international consensus in this matter does not currently exist [5].

Regarding the screening for GDM, there is no doubt that all pregnant women should be tested for GDM between the 24th and 28th pregnancy weeks, but there is controversy regarding the testing in early pregnancy, during the first prenatal visit. ADA and NICE (National Institute for Health and Care Excellence) recommend screening for early GDM in women with risk factors during their first prenatal visit, using the classical criteria for diagnosing diabetes mellitus (DM) and thus identifying the pregnant women with early GDM and overt diabetes [6]. Other associations, such as FIGO (International Federation of Gynecology and Obstetrics), recommend universal screening for diabetes in early pregnancy, regardless of the presence or absence of the risk factors [7].

Table 1: Criteria of diagnosis for GDM- (OGTT with 75 g glucose)—adapted after [4].

	Fasting plasma glucose (FPG)	1 h plasma glucose (OGTT)	2 h plasma glucose (OGTT)	Observations
Gestational diabetes	≥92 mg/dl (5.1 mmol/l)	≥180 mg/dl (10 mmol/l)	≥153 mg/dl (8.5 mmol/l)	A pathological value may support the diagnosis for GDM

Between the 24th and 28th pregnancy weeks, it is recommended to test all pregnant women for GDM.

The main risk factors for GDM are as follows: age > 40 years old, obesity, personal history of GDM or delivery of a macrosomic baby, 1st degree relatives with DM, personal history of polycystic ovary syndrome, some medications (corticosteroids, antipsychotic drugs), multiple births, and race (Asian, Middle East, African-American, and Pacific Islanders) [8].

The last data published by IDF (International Diabetes Federation) in 2019 reported a number of 20 million women (16% of the live births) who presented a form of glucose intolerance during pregnancy, 84% of which being caused by GDM (1 of 6 pregnancies being affected by GDM) [9]. Most cases of hyperglycemia during pregnancy were present in women from low or average developed countries, where the access to medical care is quite limited. These data, however, should be carefully observed, taking into consideration the epidemics of type 2 DM in women of reproductive age and the fact that there is a high number of women with undiagnosed type 2 DM. Taking into consideration the multiple maternal and fetal complications of GDM (Table 2) [9], an early diagnosis and a rapid implementation of medical care standards are crucial. Lifestyle changes must be implemented both during pregnancy and postpartum, through a very close patient-diabetologist-obstetrician relationship. Doctors should make all the efforts to prevent the onset of GDM by controlling the changeable factors (for example obesity), but also, after diagnosis, they should promptly intervene in order to reduce the negative, sometimes catastrophic, effects that this disease may have on the mother and on the offspring.

A special remark should be made on the high risk of pregnant women with GDM to develop type 2 DM in the future [13]. This high risk imposes the indispensability of an appropriate and correct postpartum monitoring, as well as the reduction of modifiable risk factors for an early detection and treatment of possible changes in the glucose metabolism.

The actual pandemic context of SARS-CoV2 infection imposed new guidelines regarding the screening and the postpartum management of GDM. Canadian guidelines proposed HbA1c > 5.7% (39 mmol/mol) or random plasma glucose (RPG) > 200 mg/dl (11.1 mmol/l) as diagnostic criteria for GDM during the COVID-19 pandemic [14]. Australian guidelines diagnose GDM at a FPG > 92 mg/dl (5.1 mmol/mol) and recommend OGTT for levels between 85 and 90 mg/dl (4.7-5 mmol/mol) [15]. In May 2020, RCOG (Royal College of Obstetricians and Gynaecologists) established that women considered being at high risk for GDM should be tested at 28 weeks using HbA1c (GDM: HbA1c > 5.7%) or FPG (GDM: FPG > 95 mg/dl) or RPG (GDM: RPG > 162 mg/dl) [16]. All these measurements reduced the risk of contamination in pregnant women but also failed to detect 57% of cases [17].

Another downside to the pandemic is that pregnant women experience a low well-being state, and this fact has a negative impact on their physical and psychological health. Also, during the COVID-19 outbreak, visits to obstetric triage, gynecologic triage, and ultrasound units decreased by 36.4%, 34.7%, and 18.1%, respectively, according to a cross-sectional study that compared changes in outpatient clinic visits between March-April 2020 and March-April 2019 [18].

The postpartum screening of type 2 diabetes in women with GDM was postponed to 3-6 months after delivery using HbA1c (UK guidelines) [13] or 6-12 months using OGTT (Australian guidelines) [16].

The aim of this paper is to review recent studies and various methods of screening and management of GDM, focusing on the current recommendations concerning the Medical Nutrition Therapy.

2. Medical Nutrition Therapy in GDM

Medical Nutrition Therapy (MNT), together with physical exercise and frequent self-monitoring, represents the milestone for the GDM treatment in order to reduce the maternal and fetal complications, on both short and long times. All these interventions involve a strong collaboration between the pregnant woman and the medical care team, based on mutual trust and correct information; therefore, a sustained psychosocial support represents an important part of the therapy. It is a well-known fact that a good emotional state increases the compliance of the pregnant woman to the medical recommendations; stress, anxiety, depression, and nutritional disorders represent some limits that are difficult to overcome during an efficient therapy [19]. MNT, although follows some clear, generally accepted directions, needs to be individualized according to the cultural characteristics, the learning and decisional capacity, and the familial support of every pregnant woman.

Physical activity represents a very important aid for the MNT in GDM, both aerobic exercises (walking, swimming, biking, and prenatal exercises) and mild or moderate resistance exercises, both types being beneficial through increasing the insulin sensitivity. A duration of 30 minutes of physical activity/day is recommended [20]; this duration can be fractioned in 10-minute rounds. Exercises involving lying flat on the back, contact sports, tennis, horse riding, and nautical skiing are not recommended due to the risk of falling or injury. Also, the ones that involve intra-abdominal pressure increase (jumping) are forbidden. In addition, pregnant women should be advised to hydrate accordingly during exercise and to avoid performing

Table 2: Effects of maternal hyperglycemia on the mother and offspring—adapted after [8].

Maternal risks*	Short term	(i) Preeclampsia (ii) High blood pressure (iii) Premature birth (iv) Caesarean section (v) Polyhydramnios (vi) Postpartum bleeding (vii) Infection
	Long term	(i) GDM in the next pregnancies (ii) Diabetes Mellitus (5-6.5%, 6 months after birth) [10] (iii) Metabolic syndrome (iv) Cardiovascular/renal disease
Fetal/newborn baby risks	Short term	(i) Prematurity (especially in the case of important maternal hyperglycemia) [11] (ii) Macrosomia (especially in the case of important maternal hyperglycemia) [12] (iii) Fetal injury at birth (iv) Hypoglycemia (v) Polycythemia (vi) Cardiac malformations (hypertrophic cardiopathy) (vii) Stillbirth
	Long term	High risk of DM, obesity/overweight

*There is a clear relation of causality between the levels of hyperglycemia and the complications occurring in the mother and the offspring.

physical effort under conditions of high temperature or humidity, when they are hungry or do not feel well [21].

MNT, together with physical exercise, weight control, and implementing a self-control strategy, should begin as soon as possible after diagnosis, namely, in the first week. Pregnant women should be taught to self-monitor fasting and postprandial glucose and to keep a diary where to note down the values of self-measured blood glucose, data on the food, and physical exercise, a diary that should be presented to the medical team. They should also do it in order to identify the individual variations of glycemic values and the factors determining them, thus having the necessary data for taking appropriate decisions regarding their lifestyle changes.

Self-monitoring of blood glucose (SMBG) is an important part of standard diabetes care. Frequent SMBG helps patients understand better the influence that food and exercise have on their blood glucose values, thus increasing their adherence to the treatment plan. SMBG should be performed using capillary blood, and the number of tests required to adequately monitor blood glucose levels depends on several factors: diet, physical activity, type of treatment (diet/insulin), and the risk of hypoglycemia [22].

The German Diabetes Association (DDG) recommends SMBG 4 times/day (fasting, 1 h and 2 h postprandial) in the first two weeks after the diagnosis. If over 50% of the measurements are elevated during these two weeks, we should consider insulin therapy, in which case the patient will need at least 3 tests/day and nocturnal evaluations of the glucose levels. If the patient does not need insulin therapy, SMBG should be performed once/day on a rotation schedule along with two 4-point profiles/week [23].

The glycemic targets recommended for pregnant women with GDM are as follows: fasting plasma glucose < 95 mg/dl (5.3 mmol/l), 1-hour postprandial glucose < 140 mg/dl (7.8 mmol/l), and 2-hour postprandial glucose < 120 mg/dl (6.7 mmol/l). It was shown that reaching and maintaining fasting glucose < 95 mg/dl in the first 2 weeks from implementing MNT are correlated with a reduced possibility of introducing pharmacological treatment [24].

MNT in GDM has the following main objectives: providing the appropriate caloric intake for both mother and fetus, avoiding ketosis, promoting optimal fetal growth, and avoiding the mother's excessive weight gain. The nutrition plan is individualized, taking into consideration the mother's particularities (health state, weight, ethnic, cultural particularities, compliance, etc.), the medical team being the one informing the mother about the risks that this condition may have upon her and the fetus, in order to obtain maximum compliance and adherence. Studies showed that 70-85% of pregnant women diagnosed with GDM obtained and maintained glycemic targets only with MNT [25], its part being of utmost importance for the management of this condition. Although unanimously recognized as the milestone in the treatment of GDM, MNT remains a controversial subject. Even from the first official admission of GDM, various types of diets were proposed, starting from severe restrictions of the carbohydrate intake to more loose diets from this point of view. The data are limited, as the studies were not being conducted on a very large number of women, most studies being also deficient from various points of view (collecting and interpreting the results, data on compliance, etc.).

2.1. Caloric Intake. There are limited data that clearly establish the necessary caloric intake and the optimal weight gain in pregnant women with GDM. The Institute of Medicine (IOM) does not recommend weight loss during pregnancy [26], and if the caloric restriction is required, this should be performed in a controlled manner, taking into consideration the fact that severe food restriction may lead to a rapid turn of the body into using fatty acids (FA) and glucose saving [23]. Also, it is well known the negative effect of

maternal ketonemia, this being associated with neurological disorders and future cognitive deficits in the baby [27].

Still, we should take into consideration the high percentage of overweight and obese women at their reproductive age (25-40%) [28], most pregnant women with GDM being in this category (40% prevalence of GDM in European obese women) [29]. In this case, maternal hyperglycemia induces an excess of nutrients in the fetal blood stream which leads, through multiple mechanisms, to fetal macrosomia and its multiple complications: mechanical complications during delivery, obesity, and diabetes during the teenage period or adulthood. The results of a recent follow-up study of a cohort from the HAPO Study, performed 11 years after the pregnancy complicated with GDM, identified a high incidence of overweight/obesity in children correlated with the mothers' body mass index (BMI) before pregnancy [10]. Thus, it is very important to intervene on women's lifestyle, conducting information campaigns, and aggressively fight obesity before conception, in order to provide a healthy start in life for future generations.

Therefore, most international organizations (ADA, AND (Academy of Nutrition and Dietetics), and CDA (Canadian Diabetes Association)) recommend that normal and overweight pregnant women should be encouraged in having an adequate weight gain, according to the IOM recommendations. Regarding overweight and obese women, moderate caloric restriction is indicated (a reduction by approx. 30% of the caloric intake prior to pregnancy, taking into consideration that the diet should not have under 1600 kcal/day) (Table 3). No guide recommends weight loss during pregnancy, only to slow down weight gain, thus avoiding maternal ketosis and other side effects on the mother and fetus [30].

2.2. Carbohydrate Intake. The idea of carbohydrate (CH) dietary restriction in GDM has its origin even before the insulin era, when it was noted that a severe restriction of CH (8-10% of the total caloric intake) prolonged life in women with type 1 diabetes and reduced the incidence of fetal macrosomia and stillbirth. After the war, starting with the official admission of GDM, this trend was preserved, because of the evidence given by numerous studies that correlated maternal hyperglycemia with fetal macrosomia. In 1990, Jovanovic-Peterson and Peterson [31] proposed that the CH restriction should be considered the first line of treatment in GDM. In the following decades, there was an emphasis on identifying the most appropriate type of diet that provided optimal results, both for the mother and for the fetus. In 2018, Yamamoto et al. [32] published the results of a meta-analysis of 18 studies performed on a total of 1151 women with GDM that showed that a nutritional intervention (change in eating habits including, but not limited to CH restriction) led to the decrease of fasting glucose (by 4 mg/dl), postprandial glucose (by 8 mg/dl), and birth weight (by 171 g).

There are numerous controversies regarding the optimal intake of CH, in terms of quantity and type of CH (Table 4). It raises the question whether the best approach is represented by the CH restriction or by a more "liberal" diet. There are randomized controlled studies [33] showing that a more "liberal" intake of complex CH provided better control of maternal blood glucose in comparison to the more restrictive CH diets. Although there are numerous studies that have tried to determine which is the optimal quantity of CH that should be consumed by the pregnant women with GDM, a consensus has not been reached, so that, just like in the case of DM, a standard diet cannot be imposed, due to the numerous individual particularities (mother's age, anthropometric parameters, compliance, a correct report in the eating diary, and necessity for insulin), which makes these studies heterogeneous.

At present, the ADA recommendations are that pregnant women with GDM should consume a minimum quantity of 175 g CH/day, representing 35-50% of the total caloric intake. Regarding the CH distribution per meals, there is no evidence from studies highlighting a certain distribution that can be correlated with better results in controlling maternal blood glucose and the effects on the fetus, as well. The quantity and distribution of CH should be made according to the particularities of every pregnant woman: BMI, weight gain during pregnancy, fasting and postprandial glucose values, and presence or absence of ketonemia. Most guides recommend the distribution of CH into 3 main meals (breakfast: 10-15%, lunch: 20-30%, and dinner: 30-40%) and 3 small snacks (5-10% of the total CH intake). The CH intake during breakfast should be reduced to 15-30 g, taking into consideration the morning peak of cortisol secretion, which explains why most pregnant women with GDM present high blood glucose values after breakfast. In the last decades, the emphasis went more and more on the use of low glycemic index (GI) CH. The glycemic index is a value assigned to foods that defines their impact on postprandial glucose values [34].

The consumption of low GI food is considered to be associated with a lower risk of fetal macrosomia, due to lower postprandial glycemic values. This hypothesis was also the conclusion of a meta-analysis including 5 randomized controlled studies on a total number of 302 pregnant women [35]. Also, a study performed in China on 140 pregnant women [36], which randomized the subjects into a group that followed a diet based on low GI food and another group that followed a diet based on high GI food, with an equal intake of CH in the two groups, showed an extra reduction of fasting glucose (-3.7% in the first group in comparison to -1.2% in the second group) and of postprandial glucose (-19-22% in the first group versus –7-12% in the second group). All these data suggest that diets based on food with a low GI improve the glycemic profile of mothers with GDM and reduce the risk of fetal macrosomia. This aspect could also be used in deciding a menu for breakfast, especially in women who have difficulties in controlling postprandial glucose during this time of the day.

Regarding the fiber intake, ADA recommends an intake of 28 g/day, coming mainly from cereals, fruits, and vegetables, due to their well-known positive effect on the control of postprandial glucose. Studies that investigated the effect of high fiber intake diets (80 g/day) reported a low compliance of pregnant women to this type of diet (40-60%), due

Table 3: The caloric intake of pregnant women with GDM according to DDG-DGGG (German Diabetes Association and German Association for Gynaecology and Obstetrics) [23].

BMI prior to pregnancy (kg/m^2)	Caloric intake (kcals/kg/day)
<18.5 (underweight)	35–40
18.5–24.9 (normal weight)	30–34
25–29.9 (overweight)	25–29
≥30 (obesity)	Maximum 24 kcals/kg/day or a reduction of 30–33% of the prior caloric intake

Table 4: Glycemic index of various foods—adapted after [10].

Low GI (<55)	Medium GI (55-69)	High GI (70-100)
Cauliflower, leek, cabbage, beans, strawberries, peaches, apples, plums, pineapple, milk, yogurt, rye bread, whole grain pasta	Bananas, jam, honey, couscous, pizza, polenta, whole flour bread	Chocolate, donuts, potatoes, white flour, corn flakes

to the gastrointestinal side effects [37]. Recently, a meta-analysis highlighted that the risk for fetal macrosomia was reduced in pregnant women with GDM who had a diet based on low GI foods and high fiber intake, in comparison to those having a diet with low GI foods and low fiber intake [35].

2.3. Protein Intake. During pregnancy, an appropriate protein intake is crucial in order to promote fetal growth and development. There is no evidence from studies indicating a particularity of pregnant women with GDM, neither regarding the protein quantity recommended during pregnancy nor their type. ADA recommends a protein intake of a minimum 71 g/day in pregnant women with GDM for all stages of pregnancy. Recently, a study that used the minimally invasive indicator amino acid oxidation method established the protein requirements to increase from 1.2 g/kg/day at 16 weeks of pregnancy to 1.52 g/kg/day at 36 weeks [38]. Thus, the recommendations regarding the protein and amino acid intake should vary according to the gestational age, in order to adequately fulfill the increasing needs of the mother and the fetus.

The main protein sources are represented by low-fat white and red meat, eggs, soya, nuts, and vegetables. Animal products should be very well and healthy cooked. A special remark should be made regarding the fish consumption. Fish and seafood represent an extremely rich source of proteins, iron, and omega-3, vital for the development of the fetus brain. Nonetheless, these species commonly come from mercury-polluted water, this leading to intoxications, with serious effects on the mother and fetus (neurological damage, cognitive, attention, memory, and language problems) [39, 40].

2.4. Lipid Intake. In GDM, the restriction of the CH intake may lead to the tendency of pregnant women to consume a higher quantity of lipids. This behaviour was shown in numerous studies to have negative consequences for the health of both mother and fetus. First of all, the high level of free fatty acids (FFA) increases insulin resistance. Moreover, a high level of triglycerides (TG) and FFA in the maternal serum was correlated with fetal macrosomia, due to TG hydrolysis and the FFA transport through the placenta to the fetus where it contributes to an excessive fetal growth. A study [41] performed on 34 pregnant women following the diet DASH (Dietary Approaches to Stop Hypertension) (65% CH, 18% lipids) for 4 weeks highlighted the following beneficial effects: decrease of glycated haemoglobin (HbA1c), of systolic blood pressure, of seric lipids, and of oxidative stress and improvement of insulin resistance.

At present, the IOM recommendations indicate a lipid intake of 20-35% of the total caloric intake. German guides recommend that a percentage of 30-35% from the caloric intake should be covered by lipids, specifying that obese women should prefer low-fat food [21]. The saturated FA and Trans FA should be reduced as much as possible, down to 7% from the caloric intake. As such, pregnant women are advised to choose meat with a fat content below 10%, as well as low-fat dairy products. The remaining percentage is divided between monounsaturated fatty acids (MUFA) (olive oil, nuts, peanut, nuts, and avocado), polyunsaturated fatty acids (PUFA), omega-3 (fish, fish oil, and flax oil), and omega-6 (soya oil, sunflower, rape, and corn oil). The report between these three types of FA is not clearly defined in pregnant women with GDM. There were studies highlighting the fact that supplementing the diet with PUFA n-3 reduced fetal macrosomia [42]; still, additional studies are necessary to clearly establish the quantity in which these FA should be found in the diet of pregnant women with GDM (Table 5).

2.5. Vitamin and Mineral Intake. Pregnancy represents a time when women need a high intake of vitamins and minerals, in order to ensure both their needs and the ones of their babies. In a varied and correct diet, all their needs should be covered. In practice, though, most of the time, supplements of vitamins and minerals are used in order to ensure the high necessary intake. Folic acid is essential for the synthesis of nucleic acid, being vital for fetal growth. Supplementing the diet with folic acid before conception and during the first 12 weeks of pregnancy considerably reduced the percentage of pregnancies with neuronal tube defects in children. Supplements with folic acid are recommended in a dose of 5 mg/day, 3 months before conception,

Table 5: Recommended carbohydrate, protein, and lipid intake in GDM.

Macronutrients	% caloric intake
Carbohydrates	35–50% (minimum 175 g/day) (ADA)
Proteins	71 g/day (ADA)
Lipids	20–35% (IOM) 30–35% (DDG)

reducing the dose down to 0.4-1 mg/day starting from the 12^{th} week of gestation [30].

Vitamins C and E are known as strong antioxidants and are very important in the diet of all pregnant women, being the well-known fetotoxic role of the oxidative stress. Although there were theories according to which vitamin C and E supplements could reduce preeclampsia incidence (knowing the role that the oxidative stress plays in this condition), these facts were not clearly defined by studies. Recently, though, a meta-analysis that collected data from the studies performed on a total of 249 975 pregnant women showed a clear relation between the administration of supplements with vitamin D and multivitamins and the reduction of the risk for preeclampsia, a correlation that was not observed in the case of administrating only vitamins C and E [43].

2.6. 25-Hydroxyvitamin D. There are some studies that establish a correlation between vitamin D deficit and the onset of GDM, but none of them can find a clear causality relation. In a randomized study conducted by Asemi et al. [44], 54 pregnant women with GDM received either placebo or 2 doses of 50 000 UI vitamin D for a period of 6 weeks. Pregnant women who received the supplements of vitamin D presented a statistically significant decrease of fasting glucose and insulin resistance assessed through the HOMA IR index. A recent meta-analysis [45] that included 6 randomized studies concluded that the administration of vitamin D supplements led to the improvement of insulin sensitivity, still not to the reduction of fasting glucose or HbA1c. Additional studies are required to clearly establish the connection between vitamin D supplements and the prevention or treatment of GDM. At present, IOM recommends a dose of 5 μg/day while in the North European countries, where the seric concentrations of 25(OH)D are low during winter; 10 μg/day is recommended [46].

During pregnancy, supplements with vitamin A are contraindicated.

The calcium necessary is high during pregnancy. At present, an intake of 900-1000 mg calcium/day is recommended [30].

According to CDC (Centers for Disease Control and Prevention), the iron necessary during pregnancy is of 27 mg/day. This may be ensured through a correct diet, iron supplements being required only in the case of an iron-deficiency anemia. The subject of iron supplementing or of an excess iron intake is a controversial one in GDM. The results of a prospective study performed on 3 158 pregnant women identified a 50% higher risk for GDM in pregnant women who had an excess of heme iron (mainly found in chicken meat and red meat) [47].

2.7. Sugar Substitutes. The intake of sugar substitutes by pregnant women with GDM is allowed, within the limits set by the FDA (Food and Drug Administration) [48], the key word in their case being moderation. Safe sugar substitutes are the following ones: aspartame (except for women with phenylketonuria), sucralose, neotame, advantame, xylitol, sorbitol (may have gastrointestinal side effects), and stevia. Regarding saccharine, although the FDA considers it safe for consumption in the general population, there are countries where it was prohibited as it may cross the placenta and stay for a long time in the fetus tissues, the side effects on the latter one being unknown (Table 6).

Regarding the intake of coffee, alcohol, and smoking, pregnant women with GDM are to follow the general recommendations during pregnancy: alcohol is strictly prohibited (risk for fetal alcoholic syndrome), caffeine intake should be reduced to a maximum of 200 mg/day, and smoking should be discouraged (Table 7).

3. New Research Directions for DM Prevention

3.1. Plant-Based Diets. Scientific evidence suggests that plant-based diets can prevent type 2 diabetes by decreasing gastric emptying, improving insulin sensitivity, and increasing insulin secretion [50]. Lately, there are new evidences that suggest that a diet based on plant-derived food may have a positive impact on GDM also by enhancing antioxidant compounds [51]. Women with GDM have increased levels of oxidative stress and inflammatory markers (tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and C-reactive protein (CRP)) that could be modulated by diets based on plant-derived food such as the Mediterranean Diet. This diet is based on: vegetables, fruits, nuts, seeds, oils, beans, and whole grains.

Taking into consideration the role that some cytokines, especially IL-6, play in the respiratory syndrome of COVID-19 and the fact that the Mediterranean Diet can modulate TNF-α, IL6, and CRP, there are reasons to believe that this diet can reduce the risk of GDM and improve the immune response in COVID-19 pneumonia [52]. This topic is of particular interest for the researchers, but further studies are required.

3.2. Myo-Inositol. Myo-inositol and D-chiro-inositol are the most studied representatives of the inositol family, molecules with an important role played in obtaining and maintaining a healthy pregnancy. These are involved in the cellular energetic metabolism, follicular maturation, and cellular motility. In the last years, myo-inositol was studied in relation to the favorable impact on fertility, as well as for the prevention of certain complications during pregnancy, such as fetal neural tube birth defects or maternal GDM [53]. The role played by the two inositols in the intracellular transmission of the insulin signal was identified for the first time by Larner et al. [54]. Ever since, more and more researchers have been investigating the favorable effects of myo-inositol

Table 6: Sugar substitutes, acceptable daily intake: adapted after [48].

Sweetener	Examples of brand names containing sweetener	Acceptable daily intake (mg/kg body weight/day)
Acesulfame potassium (Ace-K)	SweetOne® Sunett®	15
Advantame		32.8
Aspartame	Nutrasweet® Equal® Sugar Twin®	50
Neotame	Newtame®	0.3
Saccharin	Sweet and Low® Sweet Twin® Sweet'N Low® Necta Sweet®	15
Sucralose	Splenda®	5
Certain high-purity steviol glycosides purified from the leaves of *Stevia rebaudiana* (Bertoni) Berton	Truvia® PureVia® Enliten®	4

Table 7: Caffeine content of different beverages: adapted after [49].

Drink	Average amount of caffeine (mg)
Brewed coffee 220 ml	135 (80-200)
Instant coffee 220 ml	75
Instant tea 220 ml	26-36
Soft drinks (Cola) 330 ml	35

administration in patients with DM and insulin-resistance, with favorable obtained results. Regarding the positive effects of the administration of myo-inositol supplements for GDM prevention, there are already a series of studies including pregnant women or women during their fertile age, special results being obtained both in women with glucidic metabolism disorders and in those with normal glucose tolerance still with risk factors. In 2012, D'Anna et al. [55] published the results of a study performed on 98 women diagnosed with polycystic ovaries, to whom either 4000 mg myo-inositol/day or 1500 mg metformin/day was administered until the pregnancy onset. The GDM incidence in the group of women treated with myo-inositol was 17.4%, compared to 54% in the group treated with metformin. In 2013, Matarrelli et al. [56] published the results of a study on 73 pregnant women or those who wanted to conceive, with FBG values between 92 mg/dl and 126 mg/dl. The results were overwhelming: the GDM incidence was 6% in the group treated with 4000 mg myo-inositol/day, in comparison to 71% in the control group, the necessity of introducing insulin therapy being 3% in the group treated with myo-inositol compared to 21% in the control group, while neonatal hypoglycemia was 0% in the group treated with myo-inositol, compared to 26% in the control group. The reduction of GDM incidence was also quoted by numerous other studies performed on pregnant women with obesity/overweight/family history of DM or glucidic metabolism change, all these results being in favor for the administration of myo-inositol supplements in women at risk, in order to prevent GDM [57–60].

Pregnancy represents a special time in the life of every family, and it should be seen as an opportunity to implement a healthy lifestyle and to break the vicious circle of unhealthy choices and obesity and metabolic syndrome transmitted from one generation to another. In this period of time, families are more motivated and committed to changes and healthy choices. This is the right moment when the medical team may implement efficient prevention strategies for fighting against the epidemics of obesity and DM.

4. Conclusions

GDM is the most common medical complication of pregnancy. Reaching an international consensus regarding the screening, management, and follow-up for women with GDM is of extreme importance in order to prevent the short and long-time complications.

Women with GDM should receive MNT as soon as possible after the diagnosis, but prevention is of utmost importance among pregnant women and women that are trying to conceive.

In this new COVID Era, anxiety and fear of contamination, as well as the limited access to maternal care, led to many cases of undiagnosed GDM, with significant implications for the future.

Further studies are needed in order to evaluate the benefits of different types of diets for women with GDM.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed equally to the publication of this article and share first authorship.

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Research Article

Maternal and Fetal Outcomes in Women with Diabetes in Pregnancy Treated before and after the Introduction of a Standardized Multidisciplinary Management Protocol

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Background. Diabetes in pregnancy is associated with an increased risk to the woman and to the developing fetus. Currently, there is no consensus on the optimal management strategies for the follow-up and the timing of delivery of pregnancies affected by gestational and pregestational diabetes, with different international guidelines suggesting different management options. *Materials and Methods*. We conducted a retrospective cohort study from January 2017 to January 2021, to compare maternal and neonatal outcomes of pregnancies complicated by gestational and pregestational diabetes, followed-up and delivered in a third level referral center before and after the introduction of a standardized multidisciplinary management protocol including diagnostic, screening, and management criteria. *Results*. Of the 131 women included, 55 were managed before the introduction of the multidisciplinary management protocol and included in group 1 (preprotocol), while 76 were managed according to the newly introduced multidisciplinary protocol and included in group 2 (after protocol). We observed an increase in the rates of vaginal delivery, rising from 32.7% to 64.5% (<0.001), and the rate of successful induction of labor improved from 28.6% to 86.2% ($P < 0.001$). No differences were found in neonatal outcomes, and the only significant difference was demonstrated for the rates of fetal macrosomia (20% versus 5.3%, P: 0.012). Therefore, the improvements observed in the maternal outcomes did not impact negatively on fetal and neonatal outcomes. *Conclusion*. The introduction of a standardized multidisciplinary management protocol led to an improvement in the rates of vaginal delivery and in the rate of successful induction of labor in our center. A strong cooperation between obstetricians, diabetologists, and neonatologists is crucial to obtain a successful outcome in women with diabetes in pregnancy.

1. Introduction

Diabetes in pregnancy is associated with an increased risk to the woman and to the developing fetus. Gestational diabetes mellitus (GDM) is characterized by glucose intolerance and insulin resistance recognized for the first-time during pregnancy. GDM is seen to be closely associated with adverse perinatal outcome and with an increased risk of developing

type 2 diabetes mellitus (T2DM) in the future life for both the mother and the fetus [1]. It is estimated that approximately 10-15% of women who have diabetes during pregnancy have pregestational diabetes, either type 1 diabetes mellitus (T1DM) or T2DM [2]. Miscarriage, stillbirth, congenital malformations, preeclampsia, macrosomia, birth injury, perinatal mortality, neonatal hypoglycemia, and neonatal intensive care unit (NICU) admission are more common in women with preexisting diabetes [3]. Women with preexisting diabetes who are planning a pregnancy should be ideally managed by a multidisciplinary team including endocrinologist, diabetologist, maternal-fetal medicine specialist, and nutritionist when available [4].

There is currently no consensus regarding the optimal management strategies, the specific antepartum tests, the frequency of testing, and the timing of delivery of pregnancies affected by GDM and pregestational diabetes, with different international guidelines suggesting different management options. Induction of labor (IOL) is frequently suggested in order to reduce maternal and fetal adverse outcomes.

Diabetes in pregnancy is associated with an increased risk of stillbirth as pregnancy progresses [5–9]. Therefore, the increased neonatal morbidity and mortality associated with delivery before 39 weeks' gestation must be balanced with the increased risk of stillbirth with expectant management [10]. In addition, a policy of IOL at earlier gestational ages might be associated with a higher risk of failed induction and a rising risk of cesarean delivery (CD) [6–8]. Many strategies have been reported to identify women with at high risk of adverse outcomes, which might benefit more from a policy of earlier IOL [10–14]. The suggested gestational age for elective delivery varies between the different guidelines ranging from 36 to 39 for women with pregestational diabetes, whereas planned delivery from 38 to 40 is advised for women with GDM, further demonstrating that there is still lacking consensus to strongly recommend one gestational age over another [2, 15–19]. The aim of the present study is to compare maternal and neonatal outcomes of pregnancies complicated by GDM and pregestational diabetes, followed-up and delivered in a third level referral center before and after the introduction of a standardized multidisciplinary management protocol.

2. Materials and Methods

This is a retrospective cohort study conducted in a tertiary referral centre at the University of Campania "Luigi Vanvitelli." Data were collected from the maternal and neonatal clinical notes of all women with a singleton pregnancy either with GDM or pregestational diabetes and attending our hospital for antenatal care between January 2017 and January 2021. Before July 2019, the management and the timing of delivery of women with diabetes were not codified in a common management protocol, and the decision about the frequency of the antenatal appointments, the timing, and the mode of delivery for each individual case was left to the discretion of the attending physician. In order to standardize the management process and to be consistent with the frequency and type of antenatal care provided to women with diabetes in pregnancy, on July 2019, a standardized multidisciplinary management protocol was written in close collaboration with the diabetologists and neonatologists involved in the antenatal management of women with diabetes and in the postnatal care of their infants. Since the publication and dissemination of the protocol among the hospital staff, all the obstetricians involved in the management of women with diabetes in pregnancy have strictly adhered to it. Women included in the present study were divided into two groups: the first group encompassing all women managed and delivered before the introduction of the multidisciplinary protocol (preprotocol group) and women managed according the multidisciplinary protocol (after protocol group). Maternal baseline characteristics and outcomes and fetal outcomes were compared among the two groups.

2.1. The Standardized Multidisciplinary Management Protocol. Our protocol is currently in use and it includes diagnostic and screening criteria as well as management criteria. The antenatal care, the timing and frequency of testing, and the timing of delivery are discussed and provided separately for women with pre-gestational and gestational diabetes (Table 1).

The diagnosis of GDM and pregestational diabetes is made in accordance to previously published criteria and in line with national guidelines [2, 19]. Women presenting at the first antenatal appointment with a fasting blood glucose ≥ 126 mg/dl (≥ 7.0 mmol/l) or a random blood glucose ≥ 200 mg/dl (≥ 11.1 mmol/l) or a hemoglobin $A_{1C} \geq 6.5\%$ (48 mmol/mol) in two different nonconsecutive measurements before 12 weeks of gestation are classified as "overt diabetes in pregnancy" and therefore managed with the same criteria of pregestational diabetes.

Gestational diabetes is usually diagnosed following the screening performed with a one-step approach [20] using a 75 g, 2-hour oral glucose tolerance test (OGTT). The 75 g, 2-hour OGTT is offered at 16-18 weeks' gestation in women with a particularly high risk of GDM (prior GDM, first trimester fasting glucose 100-125 mg/dL, BMI ≥ 30 kg/m^2), while it is performed at 24-28 weeks' gestation in the remaining women at risk (maternal age ≥ 35 years, BMI ≥ 25 kg/m^2, prior fetal macrosomia, prior GDM with a negative screening at 16-18 weeks, first degree relative with T2DM, high risk ethnicity). GDM diagnosis is made when any single threshold value is met or exceeded (fasting value, 92 mg/dL; 1-hour value, 180 mg/dL; or 2-hour value, 153 mg/dL). The glycemic control during pregnancy was assessed in accordance to the American Diabetes Association criteria [21]. In women with continuous glucose monitoring and T1DM, the glycemic control was considered good if >70% of readings per day were within target glucose range of 63–140 mg/dL, if <4% of readings per day were below target glucose range, and if <25% of readings per day were above target glucose range. In women with continuous glucose monitoring and T2DM or GDM, the glycemic control was considered good if >90% of readings per day were within target glucose range of 63–140 mg/dL, if <5% of readings per day were below target glucose range, and if <5% of

Table 1: The standardized multidisciplinary management protocol details.

	Antenatal care	Delivery criteria
Pregestational diabetes	(i) Counseling regarding the risks and complications associated with diabetes in pregnancy (ii) Baseline evaluation of thyroid function, microalbuminuria, electrocardiogram, and baseline evaluation by ophthalmologist, dietitian, cardiologist, and nephrologist (iii) Regular assessment of blood glucose values and hemoglobin A1c (iv) Ketonemia/ketonuria in case of intercurrent infections/conditions (v) Detailed ultrasound anatomical survey at 16-18 weeks and at 20-22 weeks (vi)Fetal echocardiography at 24-26 weeks (vii) Antenatal appointments (with assessment of fetal growth and amniotic fluid volume) are scheduled monthly until 28 weeks and every 3 weeks afterwards (viii) Weekly cardiotocography is planned from 34 weeks of gestation	In women with a good glycemic control (a) If EFW < 97th centile and AFV is normal, admission at 37^{+6} weeks and IOL or CD is planned from 39^{+0} weeks. Delivery must take place within 40^{+1}weeks (b) If EFW ≥ 97^{th} centile and/or AFV is increased, admission at 36^{+0} weeks for daily monitoring of fetal well-being If there are no concerns about fetal well-being IOL or CD is planned from 37^{+2} weeks, delivery must take place within 38^{+4} weeks. In women with no optimal glycemic control despite increase in the insulin therapy Admission can be considered to optimize glucose control and for close monitoring of fetal well-being, and delivery is planned within 38^{+0} weeks. A conservative management is usually undertaken until 34^{+1} weeks.
Gestational diabetes	(i) Counseling regarding the risks and complications associated with diabetes in pregnancy (ii) Woman is referred to a team of highly experienced diabetologists, and a dietary plan is provided by a nutritionist. Physical activity is encouraged (iii) Antenatal appointments (with fetal growth and amniotic fluid volume) are scheduled monthly, both for obstetrics and diabetologists reevaluation (iv) Cardiac assessment is evaluated in conjunction with the diabetologists to identify women at increased risk of hypertensive disorders (v) Weekly cardiotocography is planned from 36 weeks of gestation (vi) Women are informed on how to monitor glycemia at home which is usually advised from 3 to 10 times per day	In women with a good glycemic control (a) If EFW is <97^{th} centile and AFV is normal, admission is scheduled at 39^{+0} weeks and IOL or CD is planned at 39^{+1} weeks (b) If EFW is ≥97th centile and/or the AFV is increased, admission is scheduled at 38^{+0} weeks for daily monitoring of fetal well-being If there are no concerns about fetal well-being IOL or CD is planned at 39^{+1} weeks. In women with no optimal glycemic control despite insulin therapy Admission is scheduled from 37^{+1} weeks for daily monitoring of fetal well-being, and delivery is planned within 38^{+0} weeks. At earlier gestations, in women with poor glycemic control, hospitalization can be offered to optimize glucose control by improving the dietary compliance and by accurate monitoring of blood glucose levels. A conservative management is usually undertaken until 34^{+1} weeks.

IOL: induction of labor; CD: cesarean delivery; EFW: estimated fetal weight; AFV: amniotic fluid volume.

readings per day were above target glucose range. In women without continuous glucose monitoring, the same percentages of readings per day within target glucose range were roughly applied, on the basis of 3 to 10 readings per day.

2.1.1. Gestational Diabetes. Antenatal care of women with GDM is provided in the high-risk pregnancy antenatal clinic. In addition to the routine pregnancy care and assessment, an intensive counseling regarding the complications of pregnancy associated with GDM is provided. Fetal growth and amniotic fluid volume are evaluated at each antenatal appointment. The woman is referred to a dedicated team of diabetologists highly experienced in the management of pregnant women. A dietary plan tailored on the individual woman BMI, habits, and needs is provided by a nutritionist, and mild to moderate physical activity is encouraged. The need for maternal cardiac assessment is evaluated in conjunction with the diabetologists in order to identify women at increased risk of hypertensive disorders of pregnancy. The following antenatal appointments are usually scheduled every 4 weeks, both for obstetrics and diabetologists' reevaluation. Antenatal fetal monitoring with weekly cardiotocography is planned from 36 weeks of gestation. Women are informed on how to monitor glycemia at home which is usually advised from 3 to 10 times per day. The glycemic goals to be achieved during pregnancy, if compatible with

adequate fetal growth and with no episodes of hypoglycemia, are

(i) <90 mg/dl fasting glucose

(ii) <130 mg/dl 1 hour after meal

(iii) <120 mg/dl 2 hours after meal

(iv) *Hemoglobin* $A_{1c} < 6.5\%$

2.1.2. Delivery Criteria in GDM Women. The delivery criteria applied in the multidisciplinary management protocol take into account both the metabolic control by maternal blood glucose levels (defined by the diabetologists) and two fetal characteristics which have been associated with fetal hyperglycemia: the amniotic fluid volume [6, 16] and the fetal growth centile [22]. The rationale for the inclusion of these criteria in the definition of the optimal time of delivery is based on the fact that in some women; despite the evidence of optimal glycemic values at 3 to 10 daily measurements, a certain degree of hyperglycemia can still be present, with an impact on fetal growth and amniotic fluid production which can be detected at ultrasound assessment. We offer a close monitoring of fetuses presenting with increased amniotic fluid and/or increased growth, which might be at increased risk of adverse outcome [23].

Therefore, in women with a good glycemic control, delivery is planned according to the following criteria:

(i) If estimated fetal weight is $<97^{th}$ centile and amniotic fluid volume is normal (either with a deepest pocket between 2 and 8 cm, or with an *amniotic fluid index* < 24 *cm*), admission is scheduled at 39^{+0} weeks, and IOL or CD is planned at 39^{+1} weeks

(ii) If estimated fetal weight is $\geq 97^{th}$ centile and/or the amniotic fluid is increased (either with a *deepest pocket* ≥ 8 *cm* or with an *amniotic fluid index* ≥ 24 *cm*, *maximum pocket* > 8 *cm*, *AFI* < 24), admission is scheduled at 38^{+0} weeks for daily monitoring of fetal well-being. If there are no concerns about fetal well-being, IOL or CD is planned at 39^{+1} weeks

If the woman has no optimal glycemic control despite insulin therapy, admission is scheduled from 37^{+1} weeks for daily monitoring of fetal well-being, and delivery is planned within 38^{+0} weeks.

At earlier gestations, in women with poor glycemic control despite insulin treatment, hospitalization can be offered in an attempt to safely and aggressively optimize glucose control by improving the dietary compliance and by accurate monitoring of blood glucose levels. A conservative management is usually undertaken until 34^{+1} weeks. After this time, delivery can be considered as the safest mode of management if glycemic control is poor despite insulin therapy and despite admission.

IOL is usually performed with a vaginal prostaglandin pessary (dinoprostone 10 mg). If there is no onset of labor, the vaginal pessary is left in situ for 24 hours. A new pessary is inserted after a 24-hour break. A maximum of three attempts is allowed. At last, feasibility of oxytocin infusion and/or amniorexis is evaluated. Failed induction is diagnosed when there is either no possibility to proceed with oxytocin infusion and/or amniorexis (e.g., unfavorable cervix with a Bishop score < 4) or no cervical changes despite at least 8 hours of oxytocin infusion and regular uterine contractions. In case of failed induction, a CD is performed.

2.1.3. Pregestational Diabetes. At the first antenatal appointment in the high-risk pregnancy clinic, extensive counseling regarding the risks and complications of pregnancy associated with diabetes is performed. Given the evidence of an increased risk of congenital abnormalities, especially anencephaly, microcephaly, and congenital heart disease, directly proportional to hemoglobin A_{1C} during the first 10 weeks of pregnancy, a strict glycemic control is strongly encouraged [24]. Usually, the diabetologists are already informed about the pregnancy, and a close contact with them is ensured in order to plan the following examinations:

(i) Baseline evaluation of TSH, microalbuminuria, and electrocardiogram

(ii) Baseline evaluation by ophthalmologist, dietitian, cardiologist, or nephrologist

(iii) Regular ongoing assessment of blood glucose values and hemoglobin A_{1c}

(iv) Ketonemia/ketonuria in case of intercurrent infections/conditions

Women with T1DM and T2DM are usually already informed on how to monitor glycemia at home. Women with pregestational diabetes are usually prescribed folic acid 5 mg/daily in the first trimester in order to reduce the risk of neural tube defects [17, 25] and low-dose aspirin 100–150 mg/day from the end of the first trimester until 34 weeks' gestation, in order to lower the risk of preeclampsia [26]. A detailed ultrasound anatomical survey is carried out at 16-18 weeks and again at 20-22 weeks. Fetal echocardiography is performed at 24-26 weeks. Fetal growth and amniotic fluid volume are evaluated at each antenatal appointment, which are scheduled monthly until 28 weeks and every 3 weeks afterwards. Antenatal fetal monitoring with weekly cardiotocography is planned from 34 weeks of gestation. The glycemic goals to be achieved during pregnancy are the same reported above for the women with GDM.

2.1.4. Delivery Criteria in Pregestational Diabetes. In women with pregestational diabetes with a good glycemic control, delivery is planned according to the following criteria:

(i) If estimated fetal weight is <97th centile and amniotic fluid volume is normal (either with a deepest pocket between 2 and 8 cm or with an *amniotic fluid index* < 24 *cm*), admission is scheduled at 37^{+6} weeks, and IOL or CD is planned from 39^{+0} weeks. Delivery must take place within 40^{+1}weeks

(ii) If estimated fetal weight is ≥97th centile and/or the amniotic fluid is increased (either with a *deepest pocket* ≥ 8 *cm* or with an *amniotic fluid index* ≥ 24 *cm*, *maximum pocket* > 8 *cm*, *AFI* < 24), admission is scheduled at 36^{+0} weeks for daily monitoring of fetal well-being. If there are no concerns about fetal well-being, IOL or CD is planned from 37^{+2} weeks. Delivery must take place within 38^{+4} weeks

If the woman has no optimal glycemic control despite increase in the insulin therapy, admission can be considered to optimize glucose control and for close monitoring of fetal well-being, and delivery is planned within 38^{+0} weeks. A conservative management is usually undertaken until 34^{+1} weeks. After this time, delivery can be considered if glycemic control is poor despite increase in insulin therapy and despite admission.

2.2. Main Outcome Measures. The maternal baseline antenatal characteristics compared between the two groups of women included in the present study were age, BMI, type of diabetes (pregestational or GDM), and the number of previous vaginal deliveries. The following maternal outcome measures were compared between the 2 groups of women that included the gestational age at the time of delivery, the rates of women undergoing IOL, the rate of response to IOL, the mode of delivery (either vaginal or CD), the rate of operative vaginal delivery, the need for episiotomy, the occurrence of perineal tears, the occurrence of postpartum hemorrhage (PPH), and the length of the first and of the second stage of labor.

The following neonatal outcomes were also compared: the birthweight, the Apgar score at 1 and 5 minutes, the umbilical cord pH, the occurrence of macrosomia (defined as birthweight >4000 gr) and shoulder dystocia, the rates of NICU admission, the length of NICU stay, and the rates of respiratory distress syndrome, sepsis, and asphyxia. We also evaluated the need for hypothermia, the occurrence and length of hypoglycemia, and the need for any kind of respiratory support.

2.2.1. Statistical Analyses. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) v. 20.0 (IBM Inc., Armonk, NY, USA). Data were shown as means ± standard deviation or number (percentage). Chi-square test was performed for categorical variables. Student's *t*-test was used for comparison of means values of the two groups for continuous variables. Mann–Whitney test was used for nonparametric variables. All the analyses were performed using a two-sided model, considering a normal distribution as appropriate. *P* value less than 0.05 was considered statistically significant.

3. Results

During the four-year study period, 133 women with a singleton pregnancy with gestational or pregestational diabetes referred to our institution were managed and delivered at our referral center. Two women with multiple pregnancies (one twin pregnancy and one triplet pregnancy) were excluded from the present study. Therefore, among the 131 women left, 55 were managed before the introduction of the multidisciplinary management protocol and were therefore included in group 1 (preprotocol), while 76 were managed according to the newly introduced multidisciplinary protocol and were therefore included in group 2 (after protocol).

Baseline maternal antenatal characteristics of the women included into the two groups are presented in Table 2. There were no significant differences between the two groups. Importantly, there were no differences in the proportion of women with pregestational and gestational diabetes included into the two groups (*P*: 0.225), allowing the comparison among them.

Maternal outcomes of women included are shown in Table 3. The mean of gestational age at delivery did not differ between the groups. Despite not significant, the rates of women undergoing IOL showed a trend of increase in the group 2 (25.5% versus 38.2%, *P*: 0.137). Interestingly, the only maternal outcomes showing a significant difference between the 2 study periods were the mode of delivery and the response to IOL. Indeed, after introduction of the multidisciplinary protocol, we observed an increase in the rates of vaginal delivery, rising from 32.7% to 64.5% (<0.001). In addition, among women undergoing IOL, the rates of women experiencing a successful vaginal delivery rose from 28.6% to 86.2% ($P < 0.001$).

Neonatal outcomes are shown in Table 4. Data were missing for nine infants of group 2; therefore, the overall number of infants included in this group (*n*: 67) is different from the number of women included in the same group (*n*: 76). There were no differences in the groups (*P*: 0.214). Despite an apparent improvement in several neonatal outcome measures following the introduction of the management protocol, the only significant difference was demonstrated for the rates of fetal macrosomia (20% versus 5.3%, *P*: 0.012). The occurrence of fetal hypoglycemia showed a reduction trend in the group 2 (26.8% versus 14.9%, *P*: 0.119); however, this difference was not significant.

4. Discussion

In this retrospective cohort study, we compared maternal and neonatal outcomes of pregnancies complicated by GDM and pregestational diabetes, delivered at out referral center before and after the introduction of a standardized multidisciplinary management protocol. The main finding of the present study is the significant improvement observed in some of the maternal outcomes after the introduction of the management protocol. Above all, the rates of vaginal delivery rose from 32.7% to 64.5% (<0.001), and the rate of successful IOL improved from 28.6% to 86.2% ($P < 0.001$). At the same time, we found no differences in fetal and neonatal outcomes, apart from a significant reduction in the occurrence of fetal macrosomia (20% versus 5.3%, *P*: 0.012). This is an important finding, proving that the improvements observed in the maternal outcomes did not impact negatively on fetal and neonatal outcomes. This may also suggest that after the introduction of the

Table 2: Maternal antenatal characteristics of women with diabetes in pregnancy who delivered before (group 1) and after (group 2) the introduction of a standardized multidisciplinary management protocol.

	Group 1 Before protocol *n*: 55	Group 2 After protocol *n*: 76	*P*
Number of prior vaginal deliveries	0.4 ± 0.7	0.7 ± 1.2	0.113
Maternal age, years	33.6 ± 5.3	34.1 ± 4.7	0.558
BMI, kg/m^2	29.9 ± 7.9	30.5 ± 45.3	0.633
Type of diabetes			0.225
Gestational diabetes	44 (80)	67 (88.2)	
Pregestational diabetes	11 (20)	9 (11.8)	

BMI: body mass index. Data are given as number (percentage) or mean ± standard deviation.

Table 3: Maternal outcomes of women with diabetes in pregnancy who delivered before (group 1) and after (group 2) the introduction of a standardized multidisciplinary management protocol.

	Group 1 Before protocol *n*: 55	Group 2 After protocol *n* : 76	*P*
Gestational age at delivery, weeks	38.2 ± 1.5	38.5 ± 2.3	0.476
Induction of labor	14 (25.5)	29 (38.2)	0.137
Response to induction*	**4 (28.6)**	**25 (86.2)**	**<0.001**
Mode of delivery			**<0.001**
Vaginal delivery	**18 (32.7)**	**49 (64.5)**	
Cesarean section	**37 (67.3)**	**27 (35.5)**	
Operative vaginal delivery**	4 (22.2)	6 (12.2)	0.439
Episiotomy**	8 (44.4)	11 (22.4)	0.124
Vaginoperineal tears**	8 (44.4)	28 (57.1)	0.439
Postpartum hemorrhage	3 (5.5)	1 (1.3)	0.309
Length of first stage, minutes	164.2 ± 120.9	155.1 ± 140.2	0.799
Length of second stage, minutes	48.6 ± 40.1	44.4 ± 38.6	0.688

Data are given as number (percentage) or mean ± standard deviation. Significant values in bold. *These numbers and percentages refer to women undergoing induction of labor. **These number and percentages refer to women with vaginal delivery.

standardized management protocol, the selection of women undergoing IOL and the choice of the timing of IOL were performed in a more effective way, leading to a reduction in the CS rate and a better response to IOL. This may also suggest that building a multidisciplinary team and ensuring a strong cooperation and interaction between the obstetricians, the diabetologists, and the neonatologists is crucial to obtain a successful outcome in women with diabetes in pregnancy.

One key factor in the management of pregnancies complicated by diabetes is determining at what point in gestation the risk of expectant management outweigh the risk of delivery. Diabetes in pregnancy is associated with and increased risk of stillbirth as pregnancy progresses [16]. Although the risk for stillbirth is particularly increased when glycemic control is poor, this risk is still higher than the general population especially in women with pregestational diabetes even when there is adequate glycemic control [27]. As a result, several attempts have been tried to identify women with a particularly high risk of adverse outcomes, which might benefit more from an intensification of antenatal surveillance or a policy of earlier IOL [10–14].

In view of this, in our multidisciplinary management protocol, we opted to consider as women at higher risk of pregnancy complication not only the ones with poor glycemic control but also the ones with adequate glycemic control showing an increase in the amniotic fluid volume [6, 16] and/or an excessive fetal growth (>97th centile) [22].

One possible explanation to the evidence of excessive fetal growth even in the presence of a good glycemic control is that limited episodes of hyperglycemia have been demonstrated to have similar effects as prolonged hyperglycemia in upregulating glucose and amino acid intake [28].

An additional explanation is provided by a recent study showing a higher risk of delivering an infant large for gestational age (LGA) in women with a poor glycemic control during the first trimester, while glycemic control in later trimesters did not affect this risk [29]. Indeed, the placenta is a vital organ supporting fetal development and ensuring the transport of nutrients to the fetus. It also acts as an

Table 4: Neonatal outcomes of infants delivered by women with diabetes in pregnancy who delivered before (group 1) and after (group 2) the introduction of a standardized multidisciplinary management protocol.

	Group 1 Before protocol n: 55	Group 2 After protocol n: 67	*P*
Birthweight, grams	3453.4 ± 813.3	3311 ± 487.9	0.214
1 min. Apgar score	7.4 ± 1.7	7.6 ± 1.9	0.537
5 min. Apgar score	8.9 ± 1	9.1 ± 0.9	0.110
Umbilical cord pH	7.3 ± 1.1	7.3 ± 1.2	0.105
Fetal macrosomia	**11 (20)**	**4 (5.3)**	**0.012**
Shoulder dystocia*	3 (16.7)	1 (2)	0.056
NICU admission	21 (38.2)	17 (25.4)	0.173
Length of NICU stay (days)	9.5 ± 7	8 ± 12.1	0.642
Respiratory distress syndrome	10 (18.2)	7 (10.4)	0.297
Sepsis	7 (12.7)	3 (4.5)	0.183
Asphyxia	4 (7.3)	5 (7.5)	1.000
Hypothermia	2 (3.6)	2 (3)	1.000
Hypoglycemia	15 (27.3)	10 (14.9)	0.119
Length of hypoglycemia (days)	1.3 ± 0.62	1.1 ± 0.32	0.284
Need for respiratory support	9 (16.4)	9 (13.4)	0.799

NICU: neonatal intensive care unit admission. Data are given as number (percentage) or mean ± standard deviation. Data were missing for 9 infants of group 2; therefore, the overall number of infants included in this group (*n*: 67) is different from the number of women included in the same group (*n*: 76). *These number and percentages refer to women with vaginal delivery (group 1: 18 women–group 2: 49 women). Significant values in bold.

endocrine organ, releasing hormones to promote placental and fetal growth and also influencing maternal metabolism [30]. Placental development occurs during the first trimester; therefore, uncontrolled glycemia during this period might interfere with optimal placental development, and this may explain why the neonatal birthweight has been proven to be mostly affected by glycemic control than in the first trimester of pregnancy.

In comparison with current guidelines our protocol suggests, for women with GDM, elective delivery at 39^{+1} weeks when metabolic control is good. Delivery is planned between 37^{+0} and 38^{+0} weeks in women with no optimal glycemic control despite insulin therapy. In women with pregestational diabetes with a good glycemic control, delivery is advised from 37^{+2} to 39^{+0} weeks. If the woman has no optimal glycemic control despite increase in the insulin therapy, delivery must occur within 38^{+0} weeks. For both women with GDM and pregestational diabetes at earlier gestations, delivery is advised only on an individual basis in cases with a particular high risk of adverse outcome (e.g., fetal growth restriction, preeclampsia, and diabetic complications). The literature and the guidelines regarding timing of delivery of women with diabetes in pregnancy are quite heterogeneous, and there have been few quality studies to assess the optimal management for these patients.

NICE guidelines [2] advise women with GDM to give birth no later than 40^{+6} weeks. While women with type 1 or type 2 diabetes, no other complications are advised to have an elective birth by between 37 weeks and 38^{+6} weeks. The American College of Obstetricians and Gynecologists suggest delivery of women with GDM at 38-39 weeks, while for women with pregestational diabetes early delivery between 36^{+0} to 38^{+6} is indicated in women with particularly high risk. In contrast, women with well-controlled diabetes can be managed expectantly to until 39^{+6} weeks of gestation.

The Canadian Diabetes Association [15] recommends pregnant women with either gestational or pregestational diabetes should be offered induction between 38 to 40 weeks' gestation depending on their glycemic control and other comorbidity factors. The recommendations of the main international societies involved in the care of women with diabetes in pregnancy are summarized in Table 5.

Previous studies have investigated the risks and benefits of elective delivery versus expectant management in women with diabetes. The only randomized controlled trial on induction of labor versus expectant management in women with GDM between 38^{+0} and 39^{+0} weeks found no differences between the 2 groups [31]. However, due to difficulties in the recruitment, the study was ended without achieving the planned sample size. A retrospective study including 193.028 deliveries to women with GDM [32] found that when the risk of planned delivery (as quantified by the risk of infant death at a given gestational age) is compared with the risk of expectant management for one week in women with GDM, the risk of delivery is higher than expectant management at 36 weeks, while at 39 weeks, the risk of expectant management exceeds that of delivery (RR 1.8, 95% CI: 1.2–2.6). Given that neonatal morbidity did not appear to be higher at 39 weeks as compared with 40 weeks, the authors suggested that 39 weeks may be the best timing

Table 5: Comparison of different international guidelines regarding the optimal time of delivery in women with diabetes in pregnancy. GDM: gestational diabetes mellitus.

Authority	Recommendation
National Institute for Health and Clinical Excellence (2015) [2]	Advise pregnant women with type 1 or type 2 diabetes and no other complications to have an elective birth by induced labor or (if indicated) caesarean section, between 37 weeks and 38 weeks plus 6 days of pregnancy.
	Consider elective birth before 37 weeks for women with type 1 or type 2 diabetes who have metabolic or other maternal or fetal complications.
	Advise women with gestational diabetes to give birth no later than 40 weeks plus 6 days. Offer elective birth by induced labor or (if indicated) by caesarean section to women who have not given birth by this time.
	Consider elective birth before 40 weeks plus 6 days for women with gestational diabetes who have maternal or fetal complications.
Canadian Diabetes Association (2019) [15]	Pregnant women with either gestational or pre-gestational diabetes should be offered induction between 38 to 40 weeks gestation depending on their glycemic control and other comorbidity factors.
	In the view that the risk of intrauterine fetal death appears to outweigh the risk of infant death after 39 weeks, induction of labor at 39 weeks could be considered in insulin-treated GDM patients.
	In women with diet-controlled GDM induction by 40 weeks may be beneficial.
American College of Obstetricians and Gynecologists (2018) [16, 17]	Delivery of women with GDM at 38 weeks or 39 weeks of gestation would reduce overall perinatal mortality without increasing cesarean delivery rates.
	For women with pregestational diabetes early delivery (36 0/7 weeks to 38 6/7 weeks of gestation, or even earlier) may be indicated in some patients with vasculopathy, nephropathy, poor glucose control, or a prior stillbirth.
	In contrast, women with well-controlled diabetes with no other comorbidities may be managed expectantly to 39 0/7 weeks to 39 6/7 weeks of gestation as long as antenatal testing remains reassuring.
	Expectant management beyond 40 0/7 weeks of gestation generally is not recommended.
The Royal Australian and New Zealand College of Obstetricians and Gynaecologists (2021) [18]	If well managed with medical nutrition therapy and no fetal macrosomia or other complications, wait for spontaneous labor (unless there are other indications for induction of labor).
	If suspected fetal macrosomia or other complications, consider birth from 38^{+0} to 39^{+0} weeks' gestation.
	Suspected fetal macrosomia alone is not an indication for induction of labor before 39^{+0} weeks' gestation.
	In most cases, women with optimal blood glucose levels who are receiving pharmacological therapy do not require expedited birth before 39^{+0} weeks gestation.
The Australasian Diabetes in Pregnancy Society (2019) [19]	Women with preexisting diabetes should be advised to give birth by the end of 38 completed weeks' gestation, depending on the presence of fetal macrosomia, glycemic levels and any other complicating factors.

at which to plan delivery in order to decrease infant mortality.

There is insufficient evidence on timing of delivery for women with pregestational diabetes. A recent population-based study [33] further supported delivery at 38, 39, or 40 weeks of gestation for women with diabetes. The authors found no maternal benefit and little or no additional neonatal benefit for scheduled delivery at 39 rather than 38 weeks of gestation for women with type 1 or type 2 diabetes. Therefore, as these women have a much greater risk of stillbirth compared with women with GDM and given that a strict glycemic control is challenging to achieve in women with type 1 diabetes, the authors' conclusion is that there is little justification for delaying delivery of women with preexisting diabetes beyond 38 weeks of gestation.

Given the current guidelines, as well as the available evidence, it seems reasonable to consider delivery at 39 weeks' gestation, even in relatively well-controlled women with GDM. This was also the rationale of our management protocol. We reckon that the optimal time of delivery in these women is still matter of debate, and future randomized controlled studies should be conducted to examine this clinical intervention.

IOL is commonly thought to be associated with and increased risk of CD. However, in a Cochrane meta-

analysis of randomized trials, women who were induced were actually proven to have a lower risk of CD [34]. In a specific population of diabetic women, the only randomized controlled trial of induction of labor versus expectant management demonstrated that women induced at 38 weeks' gestation as compared with expectant management had no difference in the rate of CD. In addition, in the expectant management group, there was an increased prevalence of LGA infants (23% vs. 10%) and shoulder dystocia (3% vs. 0%). The available evidence suggests that induction of labor in women with diabetes does not increase the risk of CD. This is particularly true when adequate selection of women is performed. Our findings are in line with the reported evidence. In fact, after the introduction of the multidisciplinary protocol allowing an accurate selection of women for IOL, we observed a huge reduction in the CD rate, which become similar to the total CD rate at our institution (34% in 2019 and 2020). We reckon that all women underwent IOL by vaginal dinoprostone 10 mg and no other methods for IOL were used in our cohort. Therefore, our results may not apply perfectly to different centers with different induction protocols.

The main strength of the present study is the presence of a well-codified and reproducible management protocol, which was strictly applied after its introduction. This is proven by the fact that all the women managed after the introduction of the protocol were delivered according to indications that matched the protocol criteria. Forty-seven women did not undergo IOL due to several reasons. Twenty-four women gave birth spontaneously before the planned time for delivery, and among them, three had a preterm delivery. Twenty-three women underwent a planned CD due to different indications: 13 women because of history of ≥1 prior CDs; 8 women had a fetal or maternal indication (3 for maternal rethinopathy, 2 for fetal growth restriction, 1 for macrosomia, 1 for breech presentation, 1 for poorly controlled diabetes at 34 weeks). Among women undergoing spontaneous labor before the planned time for delivery, two women underwent emergency CD: one for abnormal cardiotocography and one for failure to progress in labor. The women included in the two study groups were homogeneous in terms of their baseline characteristics, in particular, in terms of women affected by pregestational diabetes. We therefore speculate that the improvements seen in maternal and neonatal outcomes were actually due to the introduction of the protocol and to the improvements in the cooperation and interaction between the physicians involved in the multidisciplinary team. One more strength is the fact that we analyzed both maternal and infant complications. This is particularly important, as often, in obstetric decision-making benefits for the infant may increase the chance of harm to the mother, and vice versa [33].

The main limitations of this study are the retrospective nature and the limited sample size, which may have limited the strength of our results. However, the differences in the main maternal outcomes were wide, and they reached statistical significance despite the relatively small numbers. Given the retrospective design in our study, we lacked data on important confounders, like the glycemic control in the first trimester and the hemoglobin A_{1c} values, which are critical to define the level of risk for women with pregestational diabetes. The lower rates of macrosomia in the group of women who delivered after introduction of the study protocol might indicate a higher rate of well compensated women. We can speculate that this was due to the improvements related to the introduction of the study protocol but we cannot rule out a possible higher prevalence of women with poorer glycemic control in the first group. Similarly, we lack data on which diagnostic criteria were used to diagnosis GDM in the first group of women. However, the criteria for the diagnosis of GDM have been included in the national guidelines for the management of low-risk pregnancy in 2011 [35], and we therefore assume that the same criteria were applied also before the introduction of our multidisciplinary protocol. One additional limitation of the present study is the lack of data on the gestational age at diagnosis of GDM. Italian guidelines suggest screening for gestational diabetes at 16–18 or 24–28 weeks of gestation (or both) depending on the personal risk profile. The initial acceleration of fetal growth and fat mass accretion in GDM mothers were demonstrated to be already detectable at 20 weeks of gestation [36]. In addition, women diagnosed with GDM at 16–18 weeks of gestation have been proven to deliver infants with a lower birthweight compared with neonates born to women diagnosed at 24–28 weeks of gestation [37], most likely due to an early and adequate treatment of hyperglycemia. Therefore, the gestational age at the diagnosis and at the initial treatment might have influenced the rates of macrosomia in the two groups.

5. Conclusion

The introduction of a standardized multidisciplinary management protocol led to an improvement in the rates of vaginal delivery and in the rate of successful IOL in our referral centre. At the same time, we found no differences in fetal and neonatal outcomes, apart from a significant reduction in the occurrence of fetal macrosomia. These findings are showing that the improvements observed in the maternal outcomes did not impact negatively on fetal and neonatal outcomes. Our findings demonstrate that building a multidisciplinary team and ensuring a strong cooperation and interaction between the obstetricians, the diabetologists, and the neonatologists is crucial to obtain a successful outcome in women with diabetes in pregnancy. The optimal management strategies and the optimal time of delivery of women with diabetes in pregnancy are still debated. Future randomized trials will have to focus on these important research questions.

Data Availability

All the data used in the present study can be accessed upon request to the corresponding author.

Conflicts of Interest

All the authors declare no conflicts of interest.

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Research Article

Elevated Anthropometric and Metabolic Indicators among Young Adult Offspring of Mothers with Pregestational Diabetes: Early Results from the Transgenerational Effect on Adult Morbidity Study (the TEAM Study)

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Exposure to maternal diabetes *in utero* increases the risk in the offspring for a range of metabolic disturbances. However, the timing and variability of *in utero* hyperglycemic exposure necessary to cause impairment have not been elucidated. The TEAM Study was initiated to evaluate young adult offspring of mothers with pregestational diabetes mellitus. This paper outlines the unique enrollment challenges of the TEAM Study and preliminary analysis of the association between exposure to diabetes in pregnancy and adverse metabolic outcomes. The TEAM Study enrolls offspring of women who participated in a Diabetes in Pregnancy (DiP) Program Project Grant between 1978 and 1995. The DiP Study collected medical and obstetric data across pregnancy. The first 96 eligible offspring of women with pregestational diabetes were age-, sex-, and race-matched to adults from the National Health and Nutrition Examination Survey (NHANES) 2015-2016 with an OGTT. Descriptive and regression analyses were employed to compare TEAM participants to NHANES participants. Among a subset of TEAM participants, we compared the metabolic outcomes across maternal glucose profiles using a longitudinal data clustering technique that characterizes level and variability, in maternal glucose across pregnancy. By comparing categories of BMI, TEAM Study participants had over 2.0 times the odds of being obese compared to matched NHANES participants (for class III obesity, OR = 2.81; 95% confidence interval (CI): 1.15, 6.87). Increasing levels of two-hour glucose were also associated with *in utero* exposure to pregestational diabetes in matched analyses. Exposure to pregestational diabetes *in utero* may be associated with an increased risk of metabolic impairment in the offspring with clinical implications.

1. Introduction

Diabetes mellitus has reached epidemic proportions in the United States and around the world. In some counties in the United States, over 25% of the population has diabetes [1] and 35% of adults 20 years and older have prediabetes [2]. Furthermore, the prevalence of diabetes has increased among women of child-bearing age [3]. Even in regions with the lowest prevalence, nearly one-tenth of the population is affected [1]. Not only is diabetes itself the seventh leading

cause of death but also the consequences of diabetes can be transferred to the next generation.

Exposure to maternal diabetes in utero increases the risk in the offspring for metabolic disturbances, including obesity [4–7], insulin resistance [8–10], type 2 diabetes mellitus [6, 11, 12], and cardiovascular (CV) dysfunction [13, 14]. In addition to metabolic consequences, offspring of mothers with diabetes may be at risk for cognitive and behavioral impairments [15, 16].

While these associations are clear, the timing of hyperglycemic exposure across pregnancy, as well as the level and variability of exposure necessary to cause impairment, has not been elucidated. In addition, it is unknown whether detection of more subtle health consequences, early in the natural history, may provide opportunities for secondary prevention.

In an effort to fill the gap regarding the level and timing of diabetic hyperglycemia in utero, the Transgenerational Effect on Adult Morbidity (TEAM) Study was initiated to evaluate young adult offspring of mothers with pregestational diabetes mellitus, type 1 diabetes or type 2 diabetes, to determine the association between the timing and variability of glucose exposure in pregnancy and risk of obesity, diabetes, and renal and cardiovascular compromise in adult offspring. Building on a Program Project Grant, herein referred to as the Diabetes in Pregnancy (DiP) Study, conducted at the University of Cincinnati Medical Center and Cincinnati Children's Hospital Medical Center between 1978 and 1995, the TEAM Study is enrolling up to 250 young adults from the 454 offspring of women with pregestational diabetes who participated in the DiP Study. The objective of the TEAM Study is to evaluate the association between hyperglycemia in pregnancy and biomarkers, intermediates, and clinical outcomes related to metabolic, cardiac, nephrotic, and both cognitive and behavioral outcomes (Table 1).

The DiP Study examined the effect of the level of maternal diabetic control on major congenital malformations in offspring. This landmark study, along with others [17, 18], demonstrated the benefit of strict glucose control throughout pregnancy resulting in a decreased incidence of congenital malformations and of perinatal mortality from 17% and 16% [19], respectively, to rates which approach those for pregnancies not complicated by diabetes (around 3% and less than 10 per 1,000, respectively) [20–22]. However, exposure to hyperglycemia may result in more subtle and long-term effects to offspring, motivating the initiation of the TEAM Study. The DiP Study collected comprehensive longitudinal clinical, obstetric, and perinatal data throughout pregnancy and delivery (described below), which will be leveraged for the TEAM Study.

In order to enroll offspring of mothers who participated in the DiP Study, it is necessary to identify, locate, and acquire contact information and then contact and enroll individuals with whom the study has had no prior contact and whose mothers have not been contacted in up to 43 years. This is a formidable task but once completed will culminate in an unparalleled research opportunity. Nearly two years into recruiting, over 100 offspring have been enrolled and over 400 have been identified.

This paper describes the successful methods addressing each of the challenges of identifying and enrolling the participants. In addition, we describe the comprehensive TEAM Study procedures, provide a description of the cohort to date, and present preliminary analyses. Preliminary analyses presented here compare anthropometric and metabolic outcomes of TEAM participants (exposed to pregestational diabetes *in* utero) to an age-, sex-, and race-matched cohort from the National Health and Nutrition Examination Survey (NHANES). In addition, among a subset of participants, we examine associations in the mean level and variability of glucose across pregnancy, characterized by maternal glucose profiles.

2. Methods

2.1. Diabetes in the Pregnancy Program Project Grant (DiP). The DiP Study was a clinical trial conducted at the University of Cincinnati between 1978 and 1995, which enrolled women preconceptionally or during pregnancy prior to 10-week gestation for randomization and later in gestation for observation. Participants were diagnosed with diabetes prepregnancy including women with both type 1 and type 2 diabetes. The purpose of the study was to determine if more strict glucose targets combined with more frequent clinic visits early in pregnancy would have an impact on pregnancy outcomes. Women participating in the clinical trial were randomized to receive either strict glycemic control or customary glycemic control [22]. Treatment groups were defined by fasting and 90-minute targeted levels of glucose control. Fasting and 90-minute postprandial blood glucose targets for strict glycemic control were <100 mg/dL and<120 mg/dL, respectively, and those for customary glycemic control were <120 mg/dL and <140 mg/dL, respectively [23]. Blood glucose was monitored at clinic visits and daily by participants (after 1981). At the clinic visit, both pre- and 90-minute postprandial blood glucose concentrations were measured. At home (after 1981), reflectance blood glucose meters (Ames Dextrometer; Miles Inc., Diagnostics Division, Elkhart, IN) were employed for women to self-monitor glucose levels four to six times daily.

Complete medical and obstetric histories were obtained from each participant. Ongoing medical data related to diabetes and pregnancy were obtained at regular clinic visits, which were required for study participation. During the first trimester, clinic visits occurred every week for the strict glycemic control group and every 2 weeks for the standard control group. For the rest of pregnancy, all participants had weekly clinic visits. Care at each visit was provided by a team of specialists including a dietician, a diabetes nurse educator, a maternal-fetal specialist, and an endocrinologist with additional care available by an ophthalmologist, neonatologist, and geneticist. Women were provided intensive diabetes education, and their glucose control and insulin requirements were monitored throughout gestation to optimize management of their diabetes. Infants, whether liveborn or stillborn, were examined by both a neonatologist and geneticist/dysmorphologist immediately after birth.

Table 1: The TEAM Study visit procedures, methods, lab tests, and assessments.

Assessment	Measurement
Pregnancy test	Urine pregnancy testing (for females)
Questionnaires	Health history, sociodemographic, physical activity, and sleep questionnaires
Cardiovascular	Endothelial & vascular function (Endo-Pat, FMD), blood pressure, brachial artery distensibility, augmentation index (AiX) and pulse wave velocity (PWV), carotid ultrasound, echocardiography
Renal	Creatinine, cystatin C, albumin, hepatic panel
Metabolic, diabetogenic	Oral glucose tolerance test (glucose, insulin), glucose-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), glucagon, C-peptide, hemoglobin A1c (HbA1C), lipids, islet cell antibodies (ICA), adiponectin, leptin, phospholipids, free fatty acids, high-sensitivity C-reactive protein (hsCRP), vitamin D, thyroid-stimulating hormone (TSH)
Nutrition	24-hour food recall (followed by 2 postvisit recalls) & block food frequency V3
Anthropometric	Hip, waist (iliac and midpoint) and sagittal abdominal diameter (SAD), dual X-ray absorptiometry (DXA)
Neurocognitive	Brief Symptom Index (BSI-18); Conners' Adult ADHD Ratings Scale (CAARS); Wechsler Abbreviated Scale of Intelligence, 2nd edition (WASI-II); Social Responsiveness Scale (SRS-2); PedsQL

2.2. The TEAM Study. The TEAM Study is aimed at applying innovative statistical approaches to associate the timing, level, and variability of *in utero* glucose exposure to morbidity in the adult offspring of women with pregestational diabetes mellitus. The specific aims of the TEAM Study are as follows:

(1) To demonstrate the transgenerational effect of the hyperglycemic intrauterine environment on metabolic health of adult offspring of women with pregestational diabetes. Specific gestational periods of hyperglycemia predictive of specific metabolic morbidities in the adult offspring will be identified

(2) To demonstrate the transgenerational effect of the hyperglycemic intrauterine environment on cardiac and peripheral vascular structure and function in adult offspring of women with pregestational diabetes. We will determine if cardiovascular compromise in the adult offspring may be predicted by the gestational glycemic profile

(3) To determine the effect of the hyperglycemic intrauterine environment on cognition in young adult offspring of women with pregestational diabetes

(4) To determine the effect of exposure to a hyperglycemic intrauterine environment on behavioral outcomes in young adult offspring of women with pregestational diabetes

2.3. The TEAM Study Procedures. The TEAM Study includes one clinical study visit that takes place at the Cincinnati Children's Hospital Medical Center (CCHMC) William K. Shubert Clinical Research Center. Participants are asked to fast for 9 hours prior to the visit and provide first morning urine using a collection kit that was provided in advance. Upon arrival, participants provide a second urine sample to assess renal function and to test for pregnancy in female participants. If they are pregnant, they are required to reschedule at least 3 months following the pregnancy outcome. Participants undergo anthropometric measures and cardiovascular tests of structure and function including left ventricular mass (LVM), pulse wave velocity (PWV), augmentation index, and carotid intima-media thickness (cIMT) to enable detection of subclinical abnormalities of cardiac and peripheral vascular structure and function; a fasting blood sample and a frequently sampled oral glucose tolerance test (OGTT) (glucose measured by an enzymatic assay) and c-peptide were interpreted using the minimal model developed by Gower et al. [24, 25] to assess beta cell function; dual-energy X-ray absorptiometry (DXA) and sagittal abdominal diameter (SAD) to assess visceral and total body fat, measurement of hip, and iliac and midpoint waist; measures of nutrition, physical activity, and a sleep survey, as well as neurocognitive and behavioral testing (the fasting blood sample also provides measures of metabolic, cardiac, and renal indices).

2.3.1. Potential Participant Identification and Contact Process. The TEAM Study sampling base was limited to the 454 offspring of DiP pregnancies. We aim to enroll 250 of these offspring for the TEAM Study. The youngest enrolled participant will be 22 years old and the oldest up to 43 years old at the time of study participation. To ensure an unbiased order of recruitment, the list of the 435 offspring (all eligible offspring except 19 who participated in a pilot study in 2008/2009) was randomized using simple randomization to determine the order of contact. The identity of most offspring was unknown as infant names were not recorded in the DiP Study database. We therefore employed two approaches to identify and contact the offspring: (1) contacting their mothers and (2) a comprehensive Internet search for names and contacts using methods described below.

To contact mothers, starting with the last known contact, a letter was sent describing the TEAM Study and asking for contact information for their offspring. However, for some mothers, only mothers' name and both her date of birth and that of the offspring were available, so a comprehensive Internet search was conducted to find her current contact information employing many of the methods listed below. In addition, if mothers were found to be deceased, a search was conducted to locate an obituary which could potentially include the offspring names.

To determine the identity of offspring directly, the following procedures were employed in order:

(i) The CCHMC electronic medical record (EPIC) was searched using the infants' date of birth and sex. Each date of birth/sex search resulted in approximately 30 records to be reviewed for matches with the mothers' name

(ii) Accurint LexisNexis was searched for the mother using her name and date of birth. Though LexisNexis does not track relatives, this provided information about the mothers' current city

(iii) Using the mother's current name and city, a search of https://fastpeoplesearch.com/ was employed which results in a current age (additional confirmation of correct individuals) and family members. These family members were reviewed to identify the offspring with an age or date of birth and sex matching those of the offspring in question

(iv) The offspring name was searched in LexisNexis to confirm age and date of birth or determine if they are incarcerated or deceased

(v) If still unable to identify and locate participants, additional websites were searched including http://familytreenow.com/ (which lists all possible relatives and year they were born) and Google searches

Once potential participants were identified, contact was initiated to introduce the study and confirm interest in participation. First, letters were sent with a contact information sheet, business reply, and refusal opt out. The study team then waited six weeks until additional communications were attempted. After six weeks, participants were called using phone numbers identified through online searches. The frequency of calls was every 8-15 days. After four voice mails were left with no return call, the frequency was reduced to once per month or ceased for a period of time to focus on the next batch. If only an email was identified online, an email was sent every two weeks for about six weeks and then likewise ceased for a period of time to focus on the next batch. If no phone number or email address was available, then a follow-up contact attempt from the letter was not possible. Post cards were also sent to 88 potential participants who never made verbal contact or for whom the study team had sent information and had been attempting contact for more than three months.

To date these methods have been successful for identifying our first 107 participants who completed a study visit prior to the March 2020 COVID-19 shutdown. However, to contact the next set of potential participants, additional methods will be employed, including additional contact via phone calls and emails to the original DiP participants, the mothers. It was a priori determined to concentrate on contacting offspring of mothers with type 1 diabetes first and then mothers with type 2 diabetes, and this was included in the randomization scheme. Thus, the first 96 (after excluding 11 participants who were taking insulin or other oral or injectable medications for diabetes in order to replicate exclusion criteria used for the NHANES OGTT) included in the analyses are offspring of mothers with type 1 diabetes only. All participants provided written informed consent.

2.4. Preliminary Analysis

2.4.1. NHANES. Analyses were conducted using the 2015-2016 NHANES cohort, the most current data available at the time of analysis. NHANES participants were eligible for inclusion in the present analyses if they provided fasting and 2-hour samples for the oral glucose tolerance test (OGTT). In addition, NHANES participants included for matching were restricted with the same age range as TEAM participants (24-43 years). In total, 719 individuals were included from the NHANES cohort. For NHANES, the fasting blood tests were performed on all participants who were over 12 years of age following a nine-hour fast. For the OGTT, after initial venipuncture, participants consumed a 75 g dose of glucose (Trutol™). After two hours, a second venipuncture was performed. Participants were excluded from the OGTT if they had hemophilia, were on chemotherapy, had fasted less than nine hours, and were taking insulin or other oral or injectable medications for diabetes, if they had self-reported weight loss or bariatric surgery, and if they refused phlebotomy, were pregnant, or were unable to consume the Trutol™ in the allotted time (5 minutes). Glucose was measured employing an enzymatic method using the Roche C311 (https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/OGTT_I.htm), and HbA1c was measured using the Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 for quantitative measurement of the percent HbA1c in whole blood (https://wwwn.cdc.gov/nchs/data/nhanes/2015-2016/labmethods/GHB_I_MET.pdf).

2.4.2. Matching of TEAM Study Participants with NHANES Participants. TEAM Study participants were age- (within 1 year), sex-, and race-matched up to 1 : 3 to NHANES participants using the gmatch macro for SAS, which employs a greedy matching algorithm, also known as the local optimal method [26]. Using the greedy method, after randomly sorting each group, a match is selected once a participant is identified meeting the matching criteria and is not broken, even if more optimal matches could be found across the sample. The "distance" between TEAM Study and comparison participants (D_{ij}) was determined by identifying the NHANES participant (j) closest to the TEAM participant (i) based on the weighted sum of the absolute difference between the matching factors. This process is repeated until no more matches can be found up to the preselected case to the comparison ratio within the program.

2.4.3. Outcomes. The primary outcomes included body mass index (BMI, kg/m^2), obesity class (normal: BMI < 25; overweight: 25 ≤ BMI < 30; class I: 30 ≤ BMI < 35; class II: 35 ≤ BMI < 40; and class III: BMI ≥ 40), iliac waist circumference (mean centimeters of three measurements), systolic blood pressure (SBP, mean mmHg of three measurements), diastolic blood pressure (DBP, mean mmHg of three

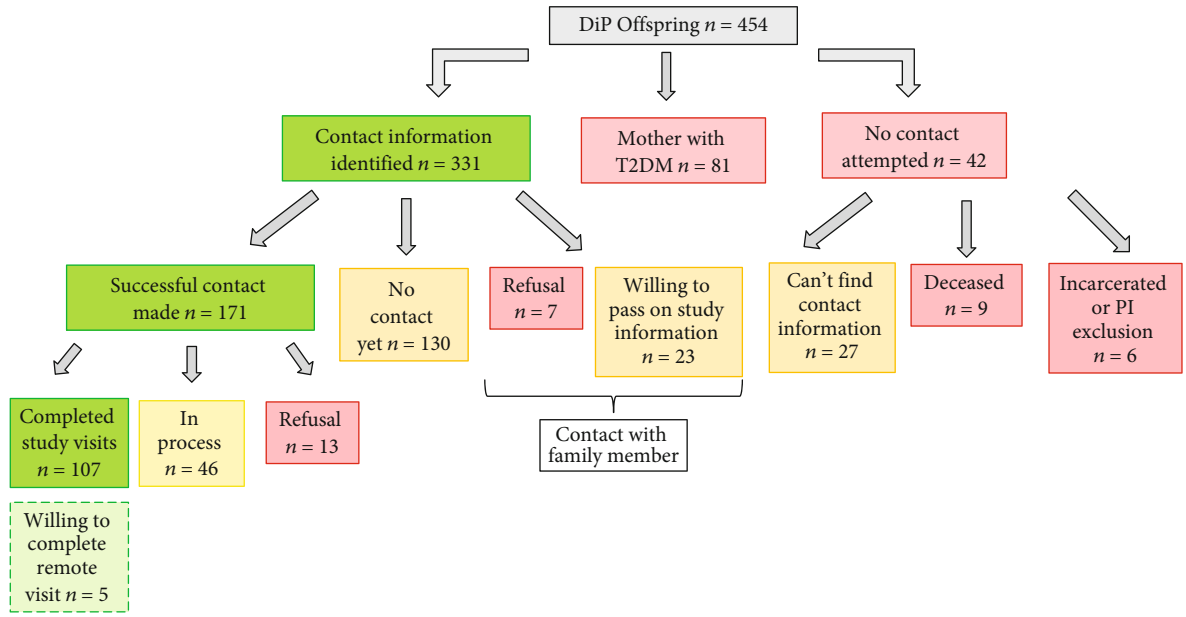

Figure 1: Eligible participant identification, contact attempts, and enrollment in the TEAM Study.

measurements), fasting glucose (<100 mg/dL, 100-<126 mg/dL, and ≥126 mg/dL), and 2-hour glucose (<140 mg/dL, 140-<200 mg/dL, and ≥200 mg/dL) and HbA1C (<5.7%/39 mmol/mol, 5.7-6.4%/39 mmol/mol–46 mmol/mol, and ≥6.5%/48 mmol/mol).

2.4.4. Statistical Analyses. Data were summarized using *n* (%) for categorical variables and means (standard deviations) and medians (25^{th}-75^{th} percentile) for continuous variables. Differences were evaluated using linear regression, accounting for the matched sets by employing a random effect of an identity variable for each matched cluster and for continuous variables and a Friedman test for categorical variables. Logistic regression employing GEE to the matched sets generated odds ratios and 95% confidence intervals describing the odds of metabolic impairment in TEAM participants versus the comparison (NHANES) participants.

Among a subset of TEAM participants whose mothers provided up to six daily glucose measures, glucose profiles representing longitudinal patterns of control across pregnancy were evaluated [27]. Profiles of temporal glucose were estimated utilizing cubic B-splines. Sparse functional principal component analysis (fPCA) for longitudinal data was used to obtain univariate scores based on the first fPC. Each mother's score was used to assign her profile into exactly one of three groups. Based on these scores, those below the first quartile of scores were classified as group 1 and represented high mean and variability; those between the first and third quartile of scores were classified as group 2 and represented moderate mean levels with moderate variability; scores exceeding the third quartile were considered to be in group 3 and represented low mean and variability across pregnancy [27]. ANOVA and chi-square tests determined whether differences in continuous and categorical variables, respectively, varied across glucose profile groups.

3. Results

The first mailing was initiated on February 15, 2018, and the 107^{th} participant was enrolled on February 11, 2020. Contact information was identified for 331 of the 454 offspring through either contact with the original DiP participant (mother) or directly searching for the participants. Of the 331, there has been successful contact with 171 participants (no successful contact yet with 130 individuals). For the remaining 30 individuals, we have successfully contacted a family member. For seven of the 30, there was a refusal by proxy (unwilling to share information) while 23 were willing to either pass along study information or provide the participant's information. In total, 107 study visits have been completed, 46 are in-process (scheduled or will be recontacted for scheduling), and 13 individuals refused study participation. There have been 5 participants willing to schedule a remote visit, which is planned to take place in the coming year. There are 123 offspring with whom no contact has been attempted, 81 of whom are offspring of women with type 2 DM. For the remaining 42 offspring of mothers with type 1 DM, we have been unable to find their name or contact information online for 27; however, of the other 15, nine are deceased and six are either incarcerated or were excluded at the PI discretion, but no other exclusion criteria were applied (Figure 1).

The first 96 Team Study participants were matched to the NHANES comparison cohort at least 1 : 2 (mean matches per TEAM participant was 2.13) after excluding 11 of the 107 who were taking diabetes medications to match NHANES eligibility criteria (including 4 with T1DM, 3 with T2DM, 3 with GDM, currently still on diabetes medication, and 1 with MODY). After matching, TEAM and NHANES participants were not appreciably different by age at screening, race, or sex. Groups did differ by several metabolic indicators (Table 2). While 32% of NHANES participants had

Table 2: Demographic and glycemic measures comparing TEAM participants to NHANES participants matched on age, race, and sex.

	NHANES participants ($N = 213$)	TEAM participants ($N = 96$)	P value*
Age			
Mean (standard deviation)	31.8 (5.0)	32.0 (4.5)	0.78
Median (25^{th}–75^{th} percentile)	32.0 (28.0-36.0)	32.2 (28.0-35.5)	
Race			
White	175 (82.2)	85 (88.5)	0.16
Black	38 (17.8)	11 (11.5)	
Male sex	118 (55.4)	52 (54.2)	0.84
BMI (kg/m^2)			
Mean (standard deviation)	29.1 (7.5)	31.8 (8.1)	0.01
Median (25^{th}–75^{th} percentile)	27.4 (23.8-33.0)	30.4 (26.4-35.1)	
Normal	68 (31.9)	20 (20.8)	0.04
Overweight	68 (31.9)	24 (25.0)	
Class I obesity	39 (18.3)	28 (29.2)	
Class II obesity	19 (8.9)	10 (10.3)	
Class III obesity	19 (8.9)	14 (14.6)	
Iliac waist circumference (cm)			
Mean (standard deviation)	98.3 (17.8)	102.2 (18.6)	0.09
Median (25^{th}–75^{th} percentile)	95.9 (85.5-108.5)	98.7 (88.8-112.1)	
Systolic blood pressure (mmHg)			
Mean (standard deviation)	117.6 (12.4)	119.6 (11.5)	0.18
Median (25^{th}–75^{th} percentile)	116.0 (109.3-124.7)	117.2 (111.8-126.2)	
Diastolic blood pressure (mmHg)			
Mean	69.4 (9.3)	75.2 (10.6)	<0.0001
Median	69.3 (62.7-76.0)	74.0 (68.0-83.0)	
Fasting glucose (mg/dL)			
Mean (standard deviation)	100.6 (23.9)	90.8 (23.0)	0.001
Median (25^{th}–75^{th} percentile)	98.0 (92.0-103.0)	85.7 (80.2-96.2)	
<100	121 (56.8)	79 (82.3)	<0.0001
100-<126	86 (40.4)	13 (13.5)	
≥126	6 (2.8)	4 (4.2)	
Two-hour glucose (mg/dL)			
Mean (standard deviation)	100.8 (37.9)	133.5 (49.2)	<0.0001
Median (25^{th}–75^{th} percentile)	95.0 (82.5-116.0)	124.3 (102.3-144.8)	
<140	171 (92.9)	69 (71.9)	<0.0001
140-<200	10 (5.4)	21 (21.9)	
≥200	3 (1.6)	6 (6.3)	
HbA1C (%)			
Mean (standard deviation)	5.3 (0.8)	5.4 (0.8)	0.24
Median (25^{th}–75^{th} percentile)	5.2 (5.0-5.5)	5.3 (5.1-5.6)	
<5.7%	188 (88.7)	80 (83.3)	0.34
5.7-6.4%	20 (9.4)	12 (37.5)	
≥6.5%	4 (1.9)	4 (4.2)	

*For continuous variables, difference between means and for categorical variables, chi-square.

normal BMI (<25 kg/m^2), only 21% of TEAM participants had normal BMI (overall $P = 0.04$). Similarly, morbid obesity (≥40 kg/m^2) was about 1.7 times as high among TEAM participants compared with NHANES participants (15% versus 9%). Both fasting glucose and two-hour glucose differed between NHANES and TEAM participants, though the results were somewhat less consistent. A normal two-hour glucose was present in 93% of NHANES participants and only 72% of TEAM participants and elevated among 2% versus 6% for NHANES and TEAM, respectively ($P < 0.0001$). For fasting glucose, three times the number of NHANES participants had impaired fasting glucose

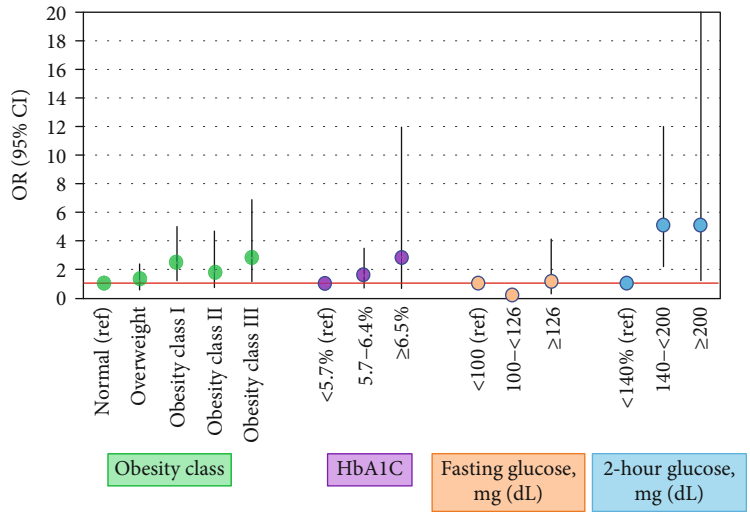

Figure 2: Odds ratios (95% confidence intervals) for adverse anthropometric and metabolic outcomes comparing offspring of mothers with pregestational diabetes (the TEAM Study) to age-, sex-, and race-matched NHANES 2015-2016 participants.

between 100 and 126 mg/dL (40% versus 14%), and a higher percentage of TEAM participants had normal fasting glucose < 126 mg/dL (82% versus 57%, $P < 0.0001$). In bivariate comparisons, diastolic blood pressure also differed significantly between NHANES and TEAM participants. Comparable findings were observed in multivariable analyses with and without adjustment for age (Figure 2).

Representations from the three groups of glucose profiles based on quartiles of fPC scores could be characterized as follows: group 1: both high mean and variability in glucose control across pregnancy; group 2: moderate mean levels with moderate variability; and group 3: low mean and variability across pregnancy. Mean levels of adult offspring BMI varied across profiles (35.6, 31.2, and 28.0 kg/m^2, respectively, P value 0.05). However, their fasting and 2-hour plasma glucose as well as HbA1C did not vary by maternal glucose profiles (Table 3).

4. Discussion

We described the identification, recruitment, enrollment, and study completion of the first 107 participants of the TEAM Study. In addition, we observed that adult offspring born to mothers with type 1 diabetes during pregnancy were more likely to be obese and have impaired glucose metabolism as indicated by elevated two-hour glucose compared to an age-, sex-, and race-matched cohort. Finally, a profile of maternal glucose in pregnancy representing a high mean level with high variability of glucose across pregnancy was associated with obesity in a subset of participants. Overall, these results align with prior studies that have identified an association between exposure to glucose impairment *in utero* and adverse offspring metabolic outcomes. In addition, these results ideally frame the context for completing the TEAM Study with the aim of determining the timing in pregnancy that is most detrimental to development of metabolic impairment and how variability in the level of glucose exposure across pregnancy contributes to this impairment.

The importance of the fetal environment for adult health outcomes was popularized by the work of Barker who demonstrated that women and men whose own birth weights were low had an increased risk for coronary heart disease [28]. Following the "Barker hypothesis," additional findings were found that not only low birth weight but also increased weights at birth were associated with adverse childhood and adult metabolic outcomes. For example, longitudinal studies in Pima Indians identified an association between small for gestational age, large for gestational age, and exposure to diabetes in pregnancy with type 2 diabetes later in life [29, 30]. Both obesity [31] and hyperglycemia in pregnancy [32, 33] have been associated with neonatal adiposity [34]. Research in this area was additionally guided by the Pedersen hypothesis, which suggested that fetal overgrowth was driven by placental transfer of maternal glucose, leading to the release of fetal insulin and, in turn, fetal macrosomia [35]. Evidence of fetal macrosomia and other short-term consequences of exposure to type 1 diabetes, such as still birth, major malformations, perinatal mortality, and preterm birth, have been demonstrated and broadly reproduced [21, 36–39].

Studies of the long-term offspring metabolic consequences of exposure to type 1 diabetes *in utero* are more sparse. However, results of existing studies are generally in line with our findings. For example, a study in Denmark of 160 offspring aged 18-27 years of women with type 1 diabetes identified a two-fold increased risk for overweight and 2.5-fold increased risk for metabolic syndrome compared with the background population [40]. Most striking is the background level of overweight (≥25 kg/m^2) in each population, which was around 24% in Denmark and 65% in NHANES. Therefore, relative to background, differences between TEAM and NHANES participants were most evident at the highest levels of obesity with a nearly 2- and 3-fold increased risk for class II and class III obesity, respectively. In the same Danish cohort, comparisons of fasting glucose and two-hour

Table 3: Metabolic outcomes by maternal glucose clusters representing glucose control across pregnancy for 56 TEAM Study participants.

Covariate		Maternal glucose clusters			
		1 ($N = 9$)	2 ($N = 32$)	3 ($N = 15$)	Parametric P value*
BMI categories	Normal	1 (11.11)	6 (18.75)	2 (13.33)	0.25
	Overweight	0 (0)	6 (18.75)	6 (40)	
	Obesity class I	2 (22.22)	12 (37.5)	4 (26.67)	
	Obesity class II	2 (22.22)	3 (9.38)	1 (6.67)	
	Obesity class III	4 (44.44)	5 (15.63)	2 (13.33)	
		9	32	15	
BMI (kg/m^2)	Mean	40.0	32.5	31.5	0.05
	Median	35.6	31.2	28.0	
		9	32	15	
HbA1C	Mean	5.4	5.7	5.3	0.26
	Median	5.5	5.5	5.3	
		9	32	15	
Fasting plasma glucose (mg/dL)	Mean	90.7	95.2	88.0	0.68
	Median	86.1	89.1	86.0	
		9	32	15	
2-hour plasma glucose (mg/dL)	Mean	125.3	141.9	123.7	0.49
	Median	124.5	132.4	114	

*The parametric P value is calculated by ANOVA for numerical covariates and the chi-square test for categorical covariates.

glucose were also comparable to those of the TEAM Study cohort. As with the Danish study (5.2 versus 5.1 mmol/L; for offspring of women with type 1 diabetes versus control), we did not see appreciably higher levels of fasting glucose among offspring of type 1 diabetes; in fact, we observed lower levels among our offspring of type 1 diabetes compared to NHANES participants (5.0 versus 5.6 mmol/L; note: values are converted from mg/dL in Table 2 to mmol/L in order to compare with the Danish study). However, both studies observed larger differences compared with pregnancies without diabetes with mean two-hour glucose of 5.8 versus 5.3 mmol/L for the Danish study and 7.4 versus 5.6 mmol/L for the TEAM Study [6]. Despite several studies with comparable findings, we did identify one small study ($n = 21$) of young adult offspring aged 16 to 23 years born to women with type 1 diabetes which found no increase in blood glucose or anthropometric measures compared with no maternal history of diabetes [41]. The reasons for these findings are unclear but may be due to differences in exclusion criteria, for example, offspring with type 1 diabetes were excluded in the TEAM Study, due to comparison with the NHANES participants or due to variations in participation rates, potentially affecting their results.

The findings associating maternal glucose profiles in pregnancy with obesity in the offspring introduce the potential for identifying the critical windows and type of exposure (constant high exposure versus glucose excursions, for example) that are most detrimental to the developing fetus. Future analyses among the entire cohort will allow us to identify specific timing and variability associated with adverse metabolic and cardiac and nephrotic outcomes and refine these clinically relevant phenotypes.

A few limitations of the present analyses should be noted. First, it is unknown whether the NHANES participants were exposed to diabetes *in utero*. However, we can expect only a minority of the pregnancies complicated by diabetes, especially due to the age of the participants under study, and therefore, it would not have a strong effect on the results. In addition, any effect would likely underestimate the relative effect of *in utero* exposure for TEAM participants compared with NHANES participants. Also, for NHANES participants, we do not have detailed information on maternal blood glucose in pregnancy.

Overall, the results of the present analyses were in line with both our hypotheses and with the existing research. In addition, the results emphasize the need for future work that will elucidate the impact of timing and variability of maternal glycemia across pregnancy (the primary objectives of the TEAM Study). In addition to metabolic outcomes, the TEAM Study will identify risks for a wide range of cardiac, microvascular, cognitive, and nephrotic outcomes in these offspring, including subtle outcomes early in their natural history that may be amenable to secondary prevention. Diabetes in pregnancy affects more than 10% of pregnancies and is increasing in prevalence in the United States and therefore presents a considerable opportunity for prevention of these long-term consequences. With multiple daily measures of maternal glucose across pregnancy, the TEAM Study is uniquely positioned to answer these questions in the coming years.

Data Availability

The TEAM Study data used to support the findings of this study may be released upon the application of the TEAM

Study data and specimen request form. Contact Dr. Jane Khoury for details at jane.khoury@cchmc.org.

Ethical Approval

This study has been approved by the Institutional Review Board of Cincinnati Children's Hospital Medical Center.

Consent

All participants provided written informed consent.

Disclosure

This work has been presented in abstract form at the Society for Epidemiologic Research Annual Meeting in 2020 (virtual) and in part at the North America Diabetes in Pregnancy Meeting in 2019, Washington D.C. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

KB analyzed the data and drafted the manuscript, SE contributed to the study design and critically reviewed the manuscript, LMD provided clinical insight and critically reviewed the manuscript, RG critically reviewed the manuscript, MA provided statistical support and critically reviewed the manuscript, NJO critically reviewed the manuscript, RS critically reviewed the manuscript, PC critically reviewed the manuscript, ES developed and administered contact methods and critically reviewed the manuscript, and JK conceived the idea, analyzed the data, and critically reviewed the manuscript. The authors are responsible for the scientific content of this manuscript.

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Research Article

Continuous Glucose Monitoring in Women with Normal OGTT in Pregnancy

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Continuous glucose monitoring (CGM) might be an effective tool to improve glycemic control in gestational diabetes mellitus (GDM). Few data are available about its utilization as a diagnostic tool to find potential alterations of glycemia in subjects with normal oral glucose tolerance test (OGTT). In this preliminary prospective real-life observational study, we aimed to analyze the glycemic pattern in normal and gestational diabetes mellitus (GDM) women by continuous glucose monitoring (CGM) in order to detect potential differences between the two groups and glycemic alterations despite a normal OGTT. After the screening for GDM, subjects were connected to a CGM system for seven consecutive days. The areas under the curve of the first 60 minutes after each meal and 60 minutes before breakfast were analyzed. Women with normal OGTT that during CGM showed impaired glycemic values (more than 95 fasting or more than 140 one hour after meals or more than 120 two hours after meals) performed one week of self-monitoring of blood glucose (SMBG). After OGTT, 53 women considered normal and 46 affected by GDM were included. CGM parameters did not show any differences between the two groups with impaired glycemic excursions found in both groups. After CGM period, 33 women with normal OGTT showed abnormal glycemic patterns. These 33 women then performed one week of SMBG. After evaluation of one week of SMBG, 21 required diet therapy and 12 required insulin treatment and were followed until the delivery. An increase in gestational weight gain was observed in normal women with normal OGTT but this was not significant. No significant data were found regarding neonatal outcomes in the two groups of women. In conclusion, CGM use in pregnancy might help to detect glycemic fluctuations in women with normal OGTT, improving their treatment and outcomes.

1. Introduction

Gestational diabetes mellitus (GDM) is a complex widespread condition and is increasingly present in approximately 7.5-27.0% of all pregnancies [1]. It is defined by any degree of glucose intolerance recognized during pregnancy in women who do not have a previous diagnosis of diabetes [2, 3]. It represents a risk factor for short- and long-term maternal and fetal complications, including, for the mother, hypertensive disorders and delivery concerns (failure to progress in labour, caesarean section, preterm or instrumental delivery), and for the fetus, macrosomia, dystocia, neonatal hypoglycemia, and perinatal death, and for both mother and fetus, obesity, metabolic syndrome, type 2 diabetes mellitus (T2D), and cardiovascular disease [4, 5]. Macrosomia for the fetus and type 2 diabetes for the mother are the main

adverse outcomes in GDM. Maternal blood glucose significantly affects fetal growth, and glycemic control is essential for adequate diabetes management [6]. Therefore, after diagnosis, patients begin a diet and exercise program, together with the self-monitoring of blood glucose (SMBG). Drug therapy is started when the recommended SMBG goals are not achieved [3]. However, there is still no agreement on GDM screening type (universal versus selective), timing, and diagnostic methods. Early pregnancy screening is recommended, but no agreement has been reached on the methods and interpretation of results between different guidelines [7, 8]. Regarding diagnosis, the current WHO statement applies the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria [9], performing a "one-step" 75 g oral glucose tolerance test (OGTT) at 24-28 weeks of gestation, but alternative "two-step" methods are recommended by other guidelines committees [2, 10–12], by screening with a 50 g glucose load test (50 g GLT) followed by diagnosis by 100 g OGTT. The IADPSG criteria endorsed the results of the "Hyperglycemia and Adverse Pregnancy Outcomes" (HAPO) [13] study, a large-scale international cohort study involving 25505 pregnant women in nine countries. In the absence of treatment, this study shows a strong continuous relationship between any maternal glucose levels and primary outcomes, including birth weight. For the first time, glucose levels below the diabetic threshold were included in the analysis, and for most complications, no threshold for risk was found. Therefore, the debate has begun on the diagnostic-therapeutic management of those pregnant who do not fall under the GDM criteria, but belong to the category of hyperglycemia called "mild gestational diabetes," in which the fetal maternal outcome is often adverse [14]. Furthermore, it is known that both the OGTT test at diagnosis and the self-monitoring of blood glucose during follow-up are not always reliable in terms of accuracy and reproducibility [15–17]. For these reasons, literature data suggests the use of CGM during pregnancy [18]. CGM seems superior to SMBG in detecting hypoglycemia and hyperglycemia incidents in impaired glucose tolerance and overt GDM in pregnancy, leading to more accurate decision-making during follow-up [19–21].

However, no CGM data concerning comparison between women with normal OGTT and GDM pregnant women are available. Therefore, using CGM, our aim was to compare glycemic patterns between women with normal OGTT and women with GDM diagnosed by OGTT in order to detect differences between the two groups and potential alterations of glycemia despite a normal OGTT.

2. Materials and Methods

2.1. Patients. We performed a prospective real-life observational study, recruiting a cohort of consecutive pregnant women attending our outpatient clinic from September 2018 to December 2019. Each patient was screened for GDM, which was diagnosed by OGTT between 24 and 28 weeks of pregnancy, according to the IADSPG guidelines [22]. The Italian health service uses a risk-based selective screening approach and only women with one or more risk factors for GDM (high-risk ethnicity, family history of diabetes, previous macrosomia/GDM, and advanced maternal age) were screened. Inclusion criteria were a single pregnancy and the absence of fetal malformation and/or chromosomal pathologies. The exclusion criteria were steroid treatment, previous metformin/inositol-based insulin sensitizer treatment, and forced sedentary life due to chronic neurological and/or orthopedic pathologies. The study was approved by the local ethics committee and conducted according to the principles of the Declaration of Helsinki.

All women were provided with a glucometer for measuring blood glucose on finger capillary blood until delivery. Measurements were made four times a day: in the morning after night fasting, one hour after breakfast, one hour after lunch, and one hour after dinner. The values were reported by the women either on a paper diary delivered at the time of their visit or on their smartphone, after installing the iPro2 application. The values of fasting glucose < 95 mg/dl (5.3 mmol/l) and either one-hour postprandial glucose < 140 mg/dl (7.8 mmol/l) or two-hour postprandial glucose < 120 mg/dl (6.7 mmol/l) represent the optimal control during follow-up. Each woman was also given a specific food intake of 2000 kcal, drawn up by the unit for diabetes in pregnancy, so that during the measurements, there will be a caloric and nutritional intake as homogeneous as possible between the two groups. All patients were aware of the OGTT result. The time interval between OGTT and CGM was on average one week. After OGTT, all patients (both normoglycemic and diabetic pregnant women) were submitted to CGM. All subjects during the CGM period performed SMBG as it was necessary for the calibration of the CGM. If subjects with normal OGTT showed glucose values more than 95 fasting or more than 140 one hour after meals or more than 120 two hours after meals during CGM, an intensive SMBG and diet were prescribed for at least 1 week. If during this week of SMBG, at least 20% of the values were higher than the target glucose levels (<95 mg/dl fasting, <140 mg/dl 1 hour after meal, and <120 mg/dl 2 hours after meal), insulin therapy was started. The data concerning anamnesis, anthropometric characteristics, and obstetric and neonatal outcomes have been obtained from the outpatient and inpatient medical records. Prepregnancy BMI was calculated at the first appointment before 14 gestational weeks based on the study by Fattah et al. [23], which demonstrated that there were no changes in mean maternal weight and body composition during the first trimester in a cohort of nondiabetic women. Gestational weight gain (GWG) was calculated as the difference between the maximum-recorded weight gain during pregnancy and the body weight recorded at the first visit prior to 14 weeks of gestation. The newborn population parameters evaluated were neonatal weight (NBW), neonatal weight percentile, Apgar score at 1 and 5 minutes, cordonal pH, need for admission to neonatal intensive care unit (NICU), mode, and complications of birth.

2.2. Continuous Glucose Monitoring. After the screening for GDM, subjects were connected to a continuous glucose monitoring (CGM) system for seven consecutive days. The women were instructed to record the time of each meal during the study period. For each meal, the area of the first 240

minutes was analyzed. Time per day within the target glucose range (TIR, between 63 and 140 mg/dl), time below the target glucose range (TBR, <63 mg/dl), and time above the target glucose range (TAR, >140 mg/dl) were also assessed (expressed in %).

We evaluated the following parameters extrapolated from the CGM: the average of all glycemic values in six days per single patient; average prebreakfast area under the curve (one hour before breakfast); average area under the postmorning curve (60minutes after breakfast); average area under the postafternoon curve (60minutes after lunch); average area under the postevening curve (60 minutes after dinner).

The glycemic monitoring used in our study is iPro™ 2 Professional Continuous Glucose Monitoring (CGM), Medtronic Minimed Inc. The instrument can detect up to 288 values in 24 hours, equal to one every 5 minutes, providing continuous, complete, and reliable glycemic profiles throughout the day. The data is collected in a CGM retrospective mode, i.e., after the woman has used the sensor, and the data were transferred by specialized medical personnel at the next check-up after 7 days using the CareLinkTM iPro™ software. Monitors were calibrated against capillary blood glucose measurements as per the manufacturer's instructions.

2.3. Statistical Analysis. The statistical analysis was carried out using the program "Statistical Package for Social Science (SPSS)", version 15.0. Continuous variables are expressed as means ± standard deviation (SD) and categorical variables are represented as frequencies. The normal distribution of the data was verified using the Kolmogorov-Smirnov test. The appropriate statistical, parametric, and nonparametric test (Student's T or Mann-Whitney's U test, ANOVA, repeated measures ANOVA, Kruskal-Wallis or Friedmann's ANOVA, χ^2 or Fisher test) was used for the analysis of results. All tests for statistical significance were two-sided. A p value of less than 0.05 indicated a significant difference.

3. Results

We included 46 consecutive women with diagnostic OGTT for gestational diabetes (GD) and 53 subjects with normal OGTT (Normal N).

The two groups had similar characteristics in terms of age and BMI at the time of CGM positioning (Table 1); however, at first prenatal visit, a slight larger fraction of overweight/obese women was present in the GD cohort with respect to normal glucose tolerance cohort (25% vs. 36%; $p = 0.07$). Furthermore, patients in the GD group had significantly higher rates of family history of type 2 diabetes or obesity compared to the control group. Concerning gestational weight gain (GWG), the greatest increase in average weight is observed in the group of women with normal OGTT, but these differences do not reach a statistical significance (Figure 1).

Before treatment, the time interval between the evaluation of OGTT and the positioning of the CGM was on average one week.

Comparing the average daily glucose levels during CGM period in the two groups, no statistically significant differences were found ($p = 0.145$).

In all assessments, we observed that the average glucose levels were higher in group N than in the GD group, without a significant difference (Table 2).

TIR, TBR, and TAR were similar in both groups, without any significant difference (Table 2), although N showed a TAR slightly higher.

Glycemic excursions were present in both groups considering the similar food intake.

During the CGM period, were found in group N 33 women with abnormal glycemic patterns. Among these 33 women, 21 required diet therapy and 12 required insulin treatment after evaluation of one week SMBG and were followed until the delivery (Table 2).

We have subclassified normal pregnant women into 2 subgroups according to CGM results: CGM+: women with normal OGTT showing impaired glycemic control during CGM; CGM-: women with normal OGTT showing normal glycemic pattern during CGM. Significant differences were observed in plasma glucose-AUC after breakfast, time below target glucose range (<70 mg/dl), and time above target glucose range (>140 mg/dl) (Table 4 supplementary material).

The data collected after delivery showed that newborns had no major complications at birth and in the first days of life, with the exception of two cases in the GDM group: (a) one case, which presented an Apgar score of 3-6 at 1 and 5 minutes and a cordonal pH of 7.05 with an ominous neonatal outcome; it was a case with a highly premature delivery at 26 weeks; (b) a second case, who presented an Apgar score of 5-7 at 1 and 5 minutes, respectively, and a pH of 7.10, for which admission to the Neonatal Intensive Care Unit was necessary, with a following positive outcome. The majority of women ($n = 55$) delivered vaginally, 4 of which by instrumental vaginal delivery, and the remaining 44 had a caesarean section (CS). The onset of labor and the route of delivery were similar in both groups, and no differences were observed regarding the indication for CS in terms of elective CS vs. fetal concerns. There were no maternal deaths (Table 3). Finally, we compared the week of birth, the weights of newborns at the time of delivery, and the percentiles of birth weight in the two groups of women, and no statistically significant correlations were found (Table 3).

4. Discussion

From the criteria proposed by O'Sullivan and Mahan of 1964, gestational diabetes mellitus diagnostic criteria has evolved. While the original purpose of these criteria was primarily to assess the risk of type 2 diabetes (T2D) in the mother, subsequent studies have been designed to analyze and attempt to quantify both the possibility of adverse pregnancy and offspring outcomes [2]. The HAPO study showed that the presence of maternal hyperglycemia less severe than GDM diagnostic values is associated with an increased risk of adverse pregnancy outcomes [13]. Our study confirms the need for an improvement in the knowledge of the GDM spectrum disease. It seems that we do not have all the

Table 1: Baseline features in normal women and women with gestational diabetes mellitus (GDM); BMI: body mass index; DM: diabetes mellitus; OGTT: oral glucose tolerance test; Significant p values are in bold.

	Normal ($n = 53$)	GDM ($n = 46$)	p
Mean age of mothers (yrs.)	34 ± 5	33 ± 6	0.528
BMI (kg/m^2)	24.0 ± 3.3	24.7 ± 3.1	0.253
Smokers n (%)	2 (3.6%)	2 (3.7%)	0.628
Family history n (%) for type 2 DM/obesity	9 (17%)	25 (54.3%)	**<0.001**
Plasma glucose (OGTT 0') (mg/dl)	79 ± 7	79 ± 5	0.930
Plasma glucose (OGTT 60') (mg/dl)	130 ± 23	186 ± 26	**<0.001**
Plasma glucose (OGTT 120') (mg/dl)	116 ± 20	171 ± 29	**<0.001**
HbA1C			
%	4.8 ± 0.49	5.2 ± 0.51	0.114
Mmol/Mol	29 ± 2.9	33 ± 3.2	

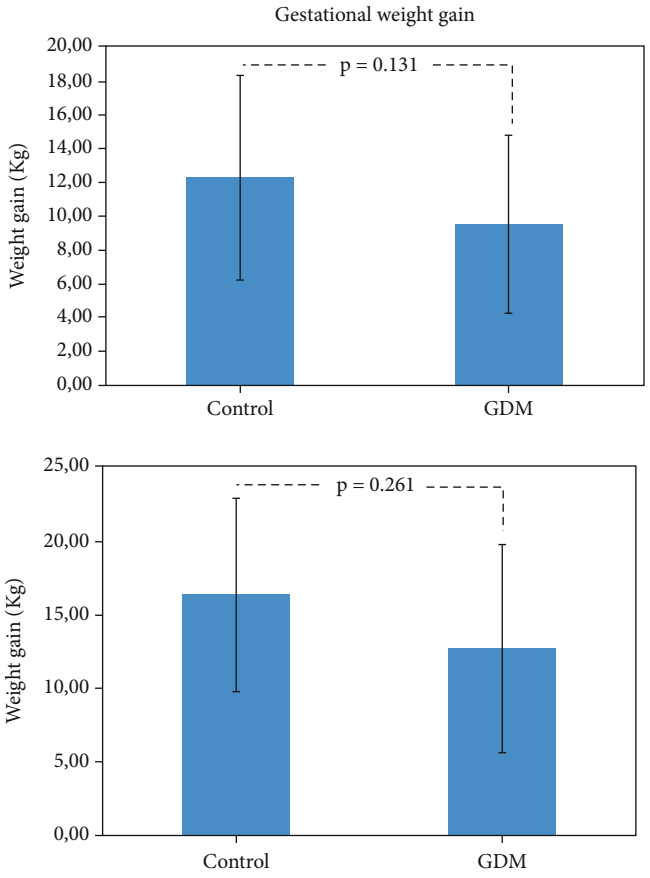

Figure 1: Gestational weight gain in normal women and women with gestational diabetes mellitus (GDM) expressed in Kg (a) and percentage (% (b)).

diagnostic tools to recognize dangerous blood sugar levels in pregnant women and the so-called "mild" gestational diabetes mellitus. A multicenter trial has shown that the treatment of mild gestational diabetes mellitus significantly reduces perinatal outcomes such as high birth weight rate, large babies for gestational age, macrosomia, and preeclampsia [24, 25]. Another study demonstrated that pregnant women, who remained untreated after negative GDM testing, developed a late-pregnancy dysglycemia related to uncontrolled weight gain which may contribute to the development of an overweight child and maternal diabetes [26]. The reproducibility and accuracy of the OGTT is questioned by some authors [15, 16]. Different studies showed that the use of fasting glycemia could be a reliable screening and diagnostic method of GDM as much as OGTT, both alone or with the postprandial plasma glucose levels [27–29]. Furthermore, a recent research showed that a single fasting plasma glucose measurement, such as OGTT, can provide a valid and predictive tool for the occurrence of unfavorable neonatal outcome [30]. Finally, a recent interesting Canadian prospective study is aimed at comparing 75 g OGTT and the SMBG in defining hyperglycemic status, and outcomes in pregnant women concluded that combining OGTT and SMBG is really effective in detecting hyperglycemic women who do not exceed GDM threshold values under OGTT alone [31]. CGM has proven to be a reliable and accurate method of glycemic control, superior to the SMBG in the recognition of episodes of hyper- and hypoglycemia during the follow-up of the GDM [18]. Law and coworkers [32] observed that GDM mothers of LGA infants have significantly higher glucose overnight compared with mothers without LGA infants. Furthermore, in pregnant women before the screening test for GDM, CGM parameters (duration and magnitude of hyperglycemic excursions measured by AUC above different thresholds) correlate with birth weight percentile [33]. In our study, CGM is applied for the first time immediately after the OGTT execution in order to detect impaired glucose levels (more than 95 fasting or more than 140 one hour after meals or more than 120 two hours after meals) despite a normal OGTT. CGM showing the blood glucose patterns after the meals mimics what happens during a mixed-meal tolerance test is as effective as the OGTT in diagnosing impaired glucose tolerance and is even more sensitive [34]. Interestingly, our data highlight the lack of difference in the percentage of TBR between normal and GDM pregnant women, according to literature data [35]. In our opinion, the absence of differences between subjects with gestational diabetes diagnosed

Table 2: Continuous glucose monitoring (CGM) parameters and treatments in normal women and women with gestational diabetes mellitus (GDM). TIR: time per day within target glucose range (between 70 and 140 mg/dl); TBR: time below target glucose range (<70 mg/dl); TAR: time above target glucose range (>140 mg/dl); AUC: area under the curve; Significant p values are in bold.

	Normal ($n = 53$)	GDM ($n = 46$)	p
Mean plasma glucose (mg/dl) (all values in six days)	98 ± 9	95 ± 8	0.145
Plasma glucose-AUC before breakfast (mg/dl/min.)	6623 ± 771	6400 ± 1003	0.223
Plasma glucose-AUC after breakfast(mg/dl/min.)	7277 ± 1096	7073 ± 1405	0.427
Plasma glucose-AUC after lunch (mg/dl/min.)	7444 ± 1183	7241 ± 1389	0.439
Plasma glucose-AUC after dinner (mg/dl/min.)	7481 ± 1510	7145 ± 1460	0.263
TBR (time <63 mg/dl, %)	5.3 ± 5.3	7.6 ± 8.0	0.091
TIR (time 63-140 mg/dl, %)	89.6 ± 5.2	88.8 ± 7.6	0.534
TAR (time >140 mg/dl, %)	5.1 ± 4.6	3.6 ± 3.0	0.065
New impaired glycemic control women, n (%)	33 (62%)	—	
Treatment			
Nutritional therapy, n (%)	21 (39.0%)	46 (100%)	$\mathbf{p \leq 0.001}$
Insulin, n (%)	12 (22.6%)	35 (76.1%)	$\mathbf{p \leq 0.001}$

Table 3: Delivery features and neonatal outcomes in normal women and women with gestational diabetes mellitus (GDM).

	Normal ($n = 53$)	GDM ($n = 46$)	p
Gestational age at delivery (yrs.)	37.7 ± 5	37.8 ± 3	0.913
Delivery modality			
Vaginal, n (%)	27 (51%)	28 (61%)	0.321
Caesarean, n (%)	26 (49%)	18 (39%)	0.236
Weight (kg)	3147 ± 891	2951 ± 710	0.498
Weight percentile	56.2 ± 23	51.1 ± 30	0.514
Large weight for gestational age, n (%)	—	2 (4.6%)	0.539
Small weight for gestational age, n (%)	4 (7.5%)	2 (4.6%)	0.821

through OGTT and pregnant women with normal OGTT is due to the greater CGM ability to detect glycemic excursions. After the subclassification of women with normal OGTT, we did not find significant differences about risk factors for gestational diabetes in these two subgroups although this analysis might be limited by the small sample size. These results can further support the relevance of weight gain in the pathogenesis of glycemic fluctuations during the pregnancy. Concerning the pattern found during CGM, it should be underlined that there was a significant difference between these two subgroups especially in the area under the curve 1 hour postbreakfast. This could mean that some particular dietary modifications such as the utilization of a breakfast with low glycemic index could be useful to prevent it, for example, when an important weight gain is present.

The absence of differences in neonatal outcomes is probably also due to the management of patients led by CGM, which allowed a more aggressive approach on a subject considered nondiabetic after OGTT. Moreover, an interesting result of our study, although not significant, is the increase in body weight of subjects considered nondiabetic after OGTT. The trend of an increased weight gain in the group with normal OGTT could be linked to the lack or delay in the offering of focused lifestyle counseling for the group not known to have GDM, compared to the group known to have GDM after OGTT. According to the real-life study design, the patients were aware of the GDM diagnosis based on OGTT, and therefore, they may have paid attention to diet more strictly than the group with normal results. On the other hand, the 2009 Institute of Medicine (IOM) guidelines have different recommendations on gestational weight gain for overweight and normal-weight women that could also explain the findings of a slightly greater gain in weight in normal women than GDM women in the present manuscript [35].

We cannot rule out that the impaired glycemic values of many of the subjects during CGM is the consequence of the gestational weight gain (GWG). The HAPO study showed that maternal BMI is an independent risk factor for maternal blood glucose levels [13]. A recent paper confirms these data and showed that excessive gestational weight gain (eGWG) is a "synergic risk factor" for poor outcome in both obesity and in GDM [36, 37]. However, a study published by Kong et al. [38] concluded that maternal diabetes under insulin treatment appears to be associated with a marked risk of LGA and preterm birth, while maternal obesity associated with

type 2 diabetes has only a moderately increased risk. In the study design, however, the authors did not consider the weight gain that the enrolled women had during pregnancy and its important role in fetal macrosomia [39, 40].

Our data seem large enough to suggest that the management of glucose levels, after CGM results, makes the 2 groups, controls and GDM subjects, completely similar for fetal outcomes. Concerning this field, our data seem to confirm recent publications on this topic that did not show differences in fetal growth and birth weight percentiles of neonates born to GDM mothers (classified as medium or low risk) and NGT women [41, 42].

The fact that 44% of our patients needed a cesarean is quite high and requires an explanation. The high rate of cesarean delivery may be associated with the increased CS rate found in untreated mild hyperglycemia, as in the HAPO study, and with the known increased CS rate in our country [43]. Limitations: our study undoubtedly has limitations, the greatest of which is the low number of participants and the lack of data concerning hypertensive disorders. For this reason, a subclassification between normal subjects and subjects with impaired glycemia after CGM has not been included in the paper. We do not think the results of this study lead to actionable conclusions without further substantial analysis or additional studies and the consideration of the feasibility of actually doing CGM on a large scale for pregnant women. Realistically, in order to avoid excessive medicalization of pregnancy, the widespread use of technology requires more robust evidence, and therefore, a long-term large controlled clinical trial on this topic is mandatory.

5. Conclusion

The diagnosis of gestational diabetes mellitus based on current WHO criteria could be insufficient to identify all pregnant women with abnormal glycemic excursions although it remains the chosen tool. The addition of a CGM period could be a good tool to detect glycemic fluctuations and improve the management of these patients. We are aware that, currently, CGM cannot replace OGTT as diagnostic tool, especially from a cost-effective point of view. However, in the future, a holistic approach to mild GDM, through the use of continuous glucose monitoring, probably as an integral part of a metabolic gestational score involving maternal and fetal anthropometric parameters could really distinguish which pregnant women should be followed by the caregivers in terms of more intensive management, to counteract the short- and long-term maternal and fetal complications.

Data Availability

A reformulation of our data statement is not necessary and data ara available upon reasonable request to the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Dario Pitocco and Antonio Lanzone conceived the project. Linda Tartaglione, Enrico di Stasio, and Dario Pitocco wrote the manuscript. Enrico Di Stasio, Mauro Di Leo, and Dario Pitocco analyzed the data. Linda Tartaglione, Alessandro Rizzi, Angelo Sirico, Agnese Caneschi, and Sara De Carolis collected the information. Dario Pitocco, Salvatore Caputo, and Antonio Lanzone contributed to the data interpretation and gave critical comments on the first draft. All authors edited the final version of the manuscript. All authors read and approved the final manuscript. Linda Tartaglione, Enrico di Stasio, Dario Pitocco, and Antonio Lanzone contributed equally to this work. The last two authors (Dario Pitocco and Antonio Lanzone) should be considered co-seniors.

Supplementary Materials

This file includes Table 4, in which we provide the subclassification between normal glucose tolerant women and those with impaired glycemia after CGM. We did not find significant differences about risk factors for gestational diabetes in these two subgroups although this analysis might be limited by the small sample size. These results can further support the relevance of weight gain in the pathogenesis of glycemic fluctuations during the pregnancy. Concerning the pattern found during CGM, it should be underlined that there was a significant difference between these two subgroups especially in the area under the curve 1 hour postbreakfast. It might mean that some particular dietary modifications to prevent it could be useful, for example, the utilization of a breakfast with low glycemic index, in particular, when an important weight gain is present. *(Supplementary Materials)*

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Review Article

Adherence to Antidiabetic Medications among Women with Gestational Diabetes

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Background. Optimal adherence to prescribed medications in women with gestational diabetes is relevant for perinatal outcomes. *Objective*. To summarize available information on the prevalence and factors contributing to medication adherence in women with gestational diabetes from the biological and psychosocial perspectives. *Methods*. A literature search on adherence in gestational diabetes was conducted in PubMed/MEDLINE, CINAHL, Scopus, and the Directory of Open Access Journals for studies published on the topic. The Arksey and O'Malley framework for scoping reviews was used to explore and summarize the evidence. *Results*. A total of 2395 studies were retrieved of which 13 fully met the eligibility criteria. The studies were reported in Zimbabwe ($n = 5$), Iran ($n = 1$), Mexico ($n = 1$), South India ($n = 1$), the United States of America ($n = 4$), and one multinational study covering Australia, Europe, North and South America. The main types of antidiabetic medications used were insulin ($n = 6$), metformin ($n = 4$), and glyburide ($n = 2$). The prevalence of adherence ranged from 35.6% to 97%, with the assessment tool being self-report measures ($n = 8$). The main factors associated with nonadherence included worsening pregnancy symptoms, side effects of medications, perceived risks, mental health symptoms, poor social support, and socioeconomic status. Recommendations that evolved from the studies to improve adherence included education, counselling, improved support networks, and social interventions, while the main reported interventional study employed continuous education on the impact of adherence on perinatal outcomes. *Conclusion*. Medication nonadherence in gestational diabetes seems to be influenced by multiple factors with some educational interventions positively impacting adherence behaviours. Thus, future research in women with gestational diabetes could consider interventions from a multifactorial perspective to improve therapeutic outcomes.

1. Introduction

Gestational diabetes is defined as the onset of glucose intolerance during the period of pregnancy [1]. It is associated with diabetes initially recognized in pregnancy and usually resolves when the pregnancy ends [2]. Gestational diabetes is a major public health problem affecting approximately 15.1% of people globally with severe implications on both maternal and neonatal outcomes when left untreated [3–5]. Research on gestational diabetes suggests a longer-term risk of developing Type II diabetes in mothers compared with those without pregnancy-related blood glucose problems [3]. Aside from the conventional effects of diabetes, there have been reports of long-term postpartum diabetes in mothers mainly due to diet and obesity [5]. The risk for gestational diabetes has been linked to women with psychotic disorders during pregnancy and those using specific antipsychotic agents as well [6, 7]. Despite the effects on the mother, gestational diabetes is also associated with adverse outcomes for the baby including neonatal hypoglycemia, jaundice, and

respiratory distress syndrome with long-term effects on their health [5].

Gestational diabetes is managed with conventional medications like insulin and oral antidiabetics such as metformin, in addition to diet and exercise [5, 8]. Due to the risks of adverse consequences in pregnancy, management of gestational diabetes requires adequate adherence to these medications and regular clinical appointments [9]. However, poor adherence has been reported and reasons such as mistrust in the safety of medications during pregnancy and fear of birth defects have been implicated [10]. Poor adherence to medications is common and is associated with high morbidity and mortality rates, as well as threats to high economic and logistical burden on public health systems through poor maternal and neonatal outcomes [11, 12].

Previous research or reviews have however focused on the role of diet and weight management in gestational diabetes [13] or on treatment strategies and guidelines [14], while a recent review, for example, has documented only studies relating to a plant-based diet and their impact on gestational diabetes [8].

This scoping review therefore aimed to provide an overview of medication adherence in gestational diabetes to inform future research and provide direction to healthcare professionals, patients, and policymakers on how to increase adherence and health outcomes.

2. Methods

A scoping review was conducted following the framework by Arksey and O'Malley, to explore and summarize evidence on medication adherence in gestational diabetes [15]. The process followed the six-stage methodological framework on the identification of research question, identification of relevant studies, selection of studies, data charting, data synthesis, collating, summarising, and reporting. The sixth stage which involved stakeholder consultations was however not utilized in this review.

The review protocol was registered in Open Science Framework (https://osf.io/vfp7n), and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for Scoping Reviews (PRISMA-ScR) was adopted in the reporting [16].

2.1. Step 1: Research Questions. The scoping review focused on identifying the area of adherence-related issues in the management of gestational diabetes and was guided by the research question, "What is known about medication adherence and associated factors in women with gestational diabetes?". Four specific areas of relevance are based on the concept of adherence [17]. (i) What is the rate of medication nonadherence in women with gestational diabetes? (ii) What are the assessment tools used to estimate medication adherence in women with gestational diabetes? (iii) What are the factors associated with medication nonadherence in women with gestational diabetes from the biological and psychosocial perspectives? (iv) What interventions have been utilized in improving medication adherence in women with gestational diabetes?

2.2. Step 2: Search Strategy. A comprehensive literature search in MEDLINE (PubMed), CINAHL, and Scopus was performed for all studies published on medication adherence in gestational diabetes. The Directory of Open Access Journals was also searched for grey literature which may not be indexed in the databases listed. The search terms related to adherence to antidiabetic medications and gestational diabetes using keywords, synonyms, and MeSH terms: Adherence, Non-adherence, Non adherence, Compliance, Non-compliance, Non compliance, AND Gestational Diabetes, Diabetes, Pregnancy Induced, Gestational Diabetes Mellitus, Pregnancy-Induced Diabetes, Diabetes Mellitus, Gestational, Diabetes, Pregnancy-Induced, AND Medications, Drugs, Antidiabetic.

2.3. Step 3: Screening and Study Selection. Studies were included if they described the prevalence, factors, and/or interventions for medication adherence in gestational diabetes and recommendations. Studies that did not meet these eligibility criteria, as well as reviews, commentaries, and guidelines, were excluded. The eligibility criteria included the mention of adherence to antidiabetic medication in the study. Titles and abstracts of the publications were independently screened by two members of the review team (M.A.D. & I.A.K.). Full-text articles after the initial screening were read. To reduce the potential for selection bias, the screening process was undertaken in duplicate by two reviewers working independently. Disagreements on the eligibility of articles were resolved through discussions.

2.4. Step 4: Data Charting. The data were organized based on information on the authors of the publication, year of publication, the title of publication, country of study, study type, objectives, adherence measures, other outcome variables, and key findings.

2.5. Step 5: Collating, Summarizing, and Reporting Results. After charting the data, results were summarized in line with the research questions where information on prevalence, adherence assessment, associated factors, and interventions relating to medication adherence in women with gestational diabetes and recommendations were noted. In reporting the results, the pharmaceutical care, clinical, and policy implications were also suggested.

3. Results

The initial search of electronic databases yielded 2395 citations of which 1946 remained after removing 449 duplicates. After reading through the titles and abstracts of the 1946 citations, 32 were selected for full-text review and assessment for eligibility. A total of 13 journal articles were deemed eligible and were included in this review. Most of the studies that were excluded at the full-text review were studies that focused on the medication used in diabetes but did not measure adherence ($n = 9$), focused on adherence to lifestyle therapy ($n = 5$), or could not be retrieved ($n = 5$). Figure 1 presents the PRISMA-ScR diagram indicating the selection of the publications.

The papers covered Europe, Asia, Africa, North and South America, and Australia (Table 1). 12 papers were published in the last 7 years (between 2014 and 2020), and

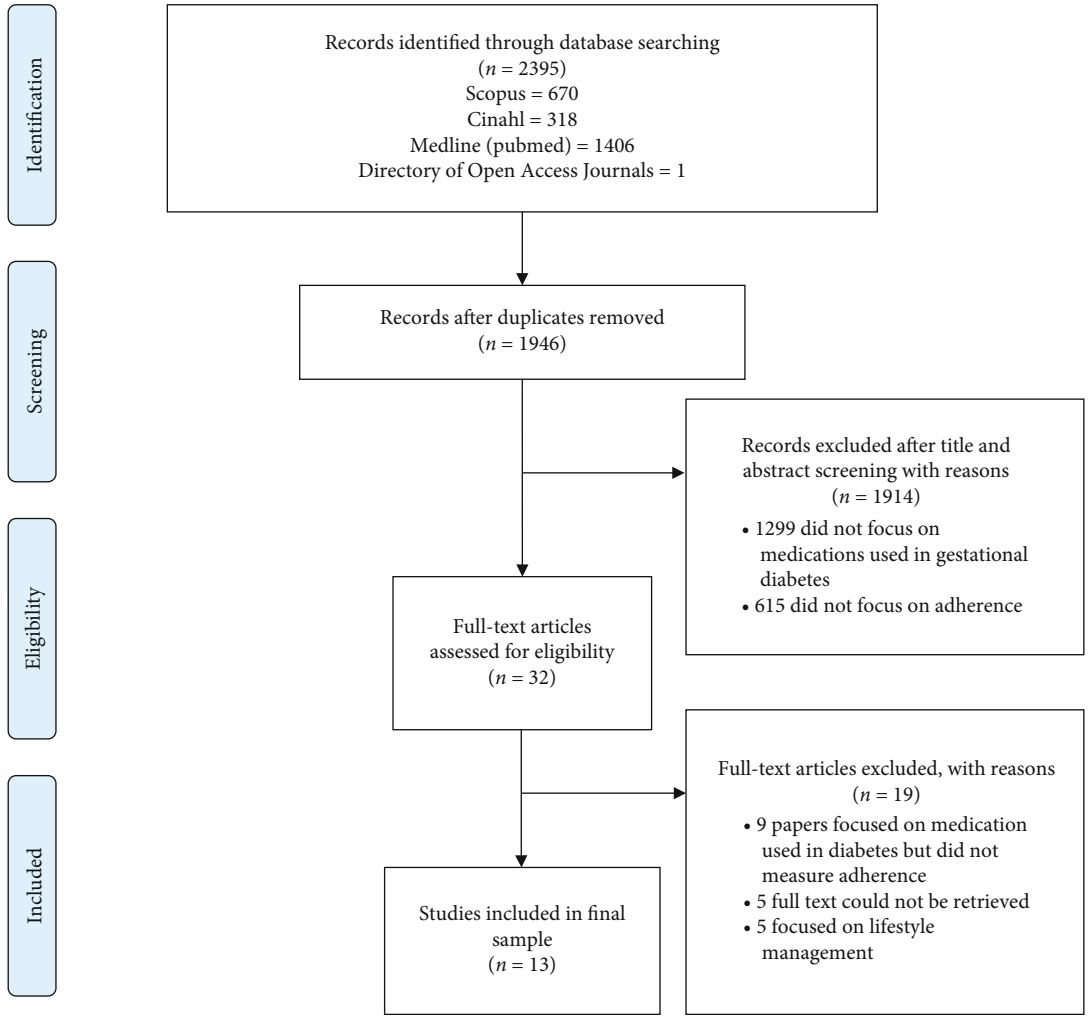

Figure 1: Study selection flow chart.

only one was published in 1990. All the studies were situated in specialist settings such as antenatal clinics or diabetic clinics. Two studies were interventional, focusing on analysing the association between adherence to antidiabetic therapy (diet, physical activity, and medications) and perinatal outcomes [18] and the association between antidiabetic therapy and glycaemic control [19].

3.1. Types of Medications and Adherence Rates. The dominant medications used in the management of gestational diabetes according to the papers reviewed were insulin [18, 20–24] and metformin [19, 21, 22, 25]. Besides medications, adherence to lifestyle/behaviours like self-monitoring of blood glucose levels, dietary therapy, and physical activity were considered [18, 20, 22–28]. Self-report measures were the recorded means of estimating adherence [18–26, 28, 29]. Medication adherence ranged between 35.6% and 97% (Table 2).

3.2. Factors Associated with Medication Adherence in Gestational Diabetes. From this scoping review, the factors associated with medication adherence in gestational diabetes were categorised under biological and psychosocial factors.

3.2.1. Biological Factors. Biological factors that impacted medication adherence included pathophysiology of diabetes [18], effects of pregnancy such as vomiting, loss of appetite, unusual discomfort [18, 26, 30], complicated medication regimen [26, 30], the type of medications used [24], and medication side effects [25].

3.2.2. Psychosocial Factors. Psychosocial factors reported to negatively impact medication adherence included patients' beliefs [20], fear of disease and medication complications [21], beliefs in abstaining from medication while pregnant despite being ill and belief in the use of herbal remedies when pregnant [29], concerns for the fetus health and wellbeing [21, 23, 29], poor socioeconomic status, lack of support from significant others and peers [22–24, 26, 28], poor health information [27, 30], and financial barriers [26] (Table 3).

4. Discussion

The review identified 13 papers that reported on medication adherence in gestational diabetes. The prevalence of

Table 1: Summary of studies on medication adherence in patients with gestational diabetes.

Study	Title	Country	Study type	Outcome
Mokena et al. (2018) [18]	Association between adherence to anti-diabetic therapy and adverse maternal and perinatal outcomes in diabetes in pregnancy	Zimbabwe	Cohort study (intervention)	Perinatal outcomes
Mokena et al. (2017) [26]	Barriers of adherence and possible solutions to non-adherence to antidiabetic therapy in women with diabetes in pregnancy: patients' perspective	Zimbabwe	Descriptive qualitative study	N/A
Haghdoost et al. (2019) [20]	The impact of socioeconomic factors on the adherence of patients with gestational diabetes mellitus to medical recommendations	Iran	Prospective study	N/A
Chávez García et al. (2019) [21]	Gestational diabetes adherence to treatment and metabolic control	Mexico	Cross-sectional Study	Glycemic control
Lupattelli et al. (2014) [29]	Adherence to medication for chronic disorders during pregnancy: results from a multinational study	Europe, North and South America, and Australia	Multinational, cross-sectional study	N/A
Krishnakumar et al. (2020) [19]	Impact of patient education on KAP, medication adherence and therapeutic outcomes of metformin versus insulin therapy in patients with gestational diabetes: a hospital based pilot study in South India	South India	Prospective observational (intervention)	Glycemic control and knowledge, attitude, and practice of medication adherence
Mukona et al. (2017) [27]	Barriers and facilitators of adherence to antidiabetic therapy in pregnant women with diabetes: Health care workers' perspectives	Zimbabwe	Descriptive study	N/A
Mukona et al. (2017) [30]	Development of an adherence promotion framework for women with diabetes in pregnancy to improve adherence to anti-diabetic therapy and perinatal outcomes	Zimbabwe	Mixed methods sequential dominant status	Perinatal outcomes
Mukona et al. (2017) [28]	Adherence to anti-diabetic therapy in women with diabetes in pregnancy	Zimbabwe	Descriptive study	Perinatal outcomes
Refuerzo et al. (2015) [25]	The effects of metformin on weight loss in women with gestational diabetes: a pilot randomized, placebo-controlled trial	United States of America	Randomized controlled trial	Gestational weight gain
Ruggiero et al. (1990) [23]	Impact of social support and stress on compliance in women with gestational diabetes. Diabetes care	United States of America	Cross-sectional	Adherence
Sperling et al. (2018) [24]	Prenatal care adherence and neonatal intensive care unit admission or stillbirth among women with gestational and preexisting diabetes mellitus	United States of America	Retrospective cohort	Perinatal outcomes
Carter et al. (2020) [22]	Pilot randomized controlled trial of diabetes group prenatal care	United States of America	Randomized controlled trial	Perinatal outcomes

N/A: not available.

adherence ranged from 35.6% to 97% with worsening pregnancy symptoms, side effects of medications, perceived risks, poor social support, and socioeconomic status as the reported factors associated with nonadherence.

Although self-reported measures were the main tools for estimating medication adherence in this review [18–26, 28, 29], some studies assessed fasting blood glucose or glycated haemoglobin as a means of confirming adherence and predicting disease outcomes [18, 22, 28]. Generally, adherence to oral antidiabetic medications and insulin has been found to range between 36-93% and 62-64%, respectively [31, 32]. This review observed consistent levels of adherence to oral hypoglycaemic medication and insulin which averaged 86% and 64%, respectively [18–22, 24, 25]. These reported levels could be associated

Table 2: Adherence levels per study and recommendations/outcomes recorded.

Study	Type of measure	Level of adherence	Interventions made	Study recommendations to improving adherence
Mokena et al. (2018) [18]	Self-report	68.79%	Continuous education of patients	Advocacy for strict adherence to healthy lifestyle habits to control diabetes mellitus particularly in developing countries like Zimbabwe where access to health care and quality of health care are huge problems.
Mokena et al. (2017) [26]	Self-report	N/A	N/A	Fostering family, peer, and community support, getting financial support, and improvement of service at the hospital
Haghdoost et al. (2019) [20]	Self-report	48.90%	N/A	Educating target groups and doing social interventions.
Chávez García et al. (2019) [21]	Self-report	90% for metformin cohort and 71% for insulin cohort	N/A	Training patients with diagnosis of gestational diabetes and emphasize the appropriate adherence to the treatment established.
Lupattelli et al. (2014) [29]	Self-report	37%	N/A	Adequate counselling and proper teratogenic risk communication to potentially attenuate women's negative beliefs about medication and heighten medication adherence during pregnancy.
Krishnakumar et al. (2020) [19]	Self-report	5.6+/-1.15	Continuous patient education	Continuous patient education to positively impact on the knowledge, attitude, practice, and medication adherence patterns of pregnant women with gestational diabetes.
Mukona et al. (2017) [27]	N/A	N/A	N/A	Subsidizing healthcare costs, collaboration among health care workers, and establishment of a unit dedicated to care of pregnant women with diabetes
Mukona et al. (2017) [30]	N/A	35.6%	N/A	Utilization of the framework model designed will improve adherence to antidiabetic therapy and help to reduce incidence of adverse perinatal outcomes.
Mukona et al. (2017) [28]	Self-report	80%	N/A	Customizing health education to suit individual patient needs.
Refuerzo et al. (2015) [25]	Self-report	97%	N/A	Medication side effects and dissatisfaction were the greatest inhibitor of medication adherence.
Ruggiero et al. (1990) [23]	Self-report	71%	N/A	Social support is a particularly important variable to assess when evaluating regimen compliance in pregnant women with gestational diabetes
Sperling et al. (2018) [24]	Self-report	N/A	N/A	Factors that improve prenatal care should be encouraged as it improved perinatal and neonatal outcomes
Carter et al. (2020) [22]	Self-report	6.4+/-1.5	Group care meetings	Most patient's needs can be managed in the group setting with additional individual visits, as needed.

N/A: not available.

with the population used in the study where women have been shown to be less adherent to medication than men in diabetes management. Furthermore, pregnant women are less adherent to medication due to worsening pregnancy symptoms, side effects of medications, perceived risks to the unborn child, and mental health issues such as anxiety and depression [18, 24–26, 30]. These documented factors are consistent with observations from the review. Other biological barriers to adherence from the review include complications and complex medication regimen [26, 30]. These factors may have reduced patient tolerance to medication and led to women forgetting to take their medications or decrease their motivation to take their medications, further reducing adherence [33, 34].

The main psychosocial factor influencing adherence in the papers reviewed was social support [22–24, 26, 28]. The role of support from family and significant others in providing monitoring, reassurances, and coping avenues for patients to deal with their health-related concerns and its impact on adherence cannot be overlooked in diabetes management [35]. Some papers reviewed described supportive behaviours such as peer groups for pregnant women with diabetes, spousal accompaniment to antenatal clinics, and understanding from family members [22–24, 26, 28]. Supportive behaviours such as these have been reported to positively impact medication adherence in diabetes management [35, 36]. Financial support especially from friends also plays a huge role in improving adherence

Table 3: Description of factors associated with medication adherence based on the biopsychosocial perspective.

ID	Biological factors	Psychosocial factors
Mokena et al. (2018) [18]	Unusual pregnancy discomfort	Information overload from health professionals in a short time
Mokena et al. (2017) [26]	Pathophysiology of diabetes, effects of pregnancy, complicated therapeutic regimen	Poor socioeconomic status; lack of family, peer, and community support; cultural and religious beliefs; and poor health care system.
Haghdoost et al. (2019) [20]	N/A	Fear of medication and disease complication, financial barriers, high workload
Chávez García et al. (2019) [21]	N/A	Patient acceptance of route of administration, educational level attained
Lupattelli et al. (2014) [29]	N/A	Personal beliefs (belief in abstaining from medication while pregnant despite being ill, belief in the use of herbal remedies when pregnant)
Krishnakumar et al. (2020) [19]	N/A	Low knowledge levels about the risk factors for gestational diabetes and the course of gestational diabetes. Low knowledge on the increased risk for future type2 diabetes after a previous diagnosis.
Mukona et al. (2017) [30]	N/A	Lack of finances, lack of health education, inadequate expertise of staff
Mukona et al. (2017) [18]	Complications of pregnancy (loss of appetite, nausea), complicated medication regimen	N/A
Mukona et al. (2017) [28]	N/A	Financial challenges, lack of spousal support
Refuerzo et al. (2015) [25]	Medication side effects (diarrhea, nausea, and hypoglycemia), medication intolerance	N/A
Ruggiero et al. (1990) [23]	N/A	Concern for fetus health, social support, stress
Sperling et al. (2018) [24]	Medication used	Previous psychiatric history, previous addictions (tobacco or alcohol use), intimate partner violence, socioeconomic status (health insurance, employment, married or single)
Carter et al. (2020) [22]	N/A	Peer support; reassurance from women on a particular care plan served to encourage those newly rolled on and were apprehensive to adhere to treatment, also, accounts from other women set expectations for medication and lifestyle modification challenges

N/A: not available.

[37]. Papers reviewed showed a similar trend. Pregnant women with poor financial support were less adherent compared to women with better financial support. This is because women with poor financial support could not attend antenatal clinic regularly or purchase medications to ensure their availability and adherence [30, 38]. Financial support from family and friends is vital especially in low-income settings where poor adherence rates have been reported because patients could not afford their medications [38–40]. The impact of social support and the potential to the use of a support network in improving diabetes medication adherence among pregnant women especially in low- and middle-income settings is highly recommended based on findings from this review.

Some papers also showed that patients with high socioeconomic status (SES) had higher adherence rates [20, 25, 27], while another study showed the opposite [20]. Women with low socioeconomic status were often nonadherent due to financial constraints. Thus, when they received medication subsidies and improved access to healthcare for instance, through insurance schemes, they were more likely to be adherent [25, 30, 41]. This correlates with literature which demonstrates an increased adherence behaviour with health insurance [40, 42]. Meanwhile, according to Haghdoost et al. [20], women with higher SES were nonadherent due to lifestyle concerns such as having a demanding job which negatively impacted on their adherence behaviour. These women were often burdened with work responsibilities and fixed schedules that either made them forget to take their medications or decide to skip them. On the other hand, those with low SES often had more flexible and less demanding jobs and could make time to take their medications. Again, the women with high SES had better health literacy and were not concerned with their diagnosis while those with low SES were very disturbed about their diagnosis and the likely financial costs of disease complications due to nonadherence [20]. These findings are however contrary to available literature on medication adherence and socioeconomic status among persons with diabetes mellitus generally [43, 44]. The association between socioeconomic status and medication adherence among women with gestational diabetes needs to be further studied to identify peculiarities that might be useful for improving medication adherence.

Poor information retention was cited as a cause of medication nonadherence by Mukona et al. [18]. Poor

information retention was reportedly caused by health professionals who overload patients with too much health information on a visit. Poor communication from the health professional included failure to communicate clearly, inept health advice due to lack of expertise with the disease, and long waiting times due to inadequately qualified staff [27, 30]. This finding is consistent with literature [45, 46]. Thus, the need for continuous education from qualified health professionals cannot be overemphasized based on these findings.

In terms of interventions for adherence, some studies demonstrated the positive associations between medication knowledge and adherence and improved disease outcome [19, 21, 22, 27, 30]. Some studies instituted continuous adherence training through health professionals, while others were through peer support groups or community champions [18, 19, 22, 27, 30]. Providing disease and medication knowledge improved health literacy, dispelled myths and negative beliefs, set medication side effect expectations, and allayed fears and concerns with medication use. These findings have been corroborated in literature [47–49]. These studies demonstrated the importance of continuous education of patients especially in the management of chronic diseases.

Identifying the factors for adherence behaviour can be leveraged to designing policies and frameworks to manage gestational diabetes and increase adherence among pregnant women. Medication adherence is complex, requiring multifactorial strategies to improve and promote it. Thus, the application of nonresource intensive interventions from multiple perspectives could be used to enhance medication adherence. This will be useful in the proper and effective management of gestational diabetes and improve the perinatal and neonatal outcomes of patients with gestational diabetes. In terms of clinical practice and policy, patient counselling and education could be targeted towards patients based on their health and social groupings. This will be key to tailoring patient counselling to meet the patient type and hence enhance adherence. Also, policies designed could consider addressing patient barriers to adherence.

Despite the above, this review acknowledges the limitation that some studies and grey or unpublished literature may have been missed, because they may not be indexed in the databases that were utilised. Second, since the aim of this review was to scope available evidence on medication adherence in gestational diabetes, quality appraisal of the included studies was not conducted. In addition, most reported studies in literature focused on adherence to nutritional and physical exercise regimens for pregnant women, and few studies have specifically reported on medication adherence in women with gestational diabetes. Thus, this scoping review observes the gap in gestational diabetes medication adherence research and the opportunity to address barriers to improve medication adherence.

5. Conclusion

Medication adherence in gestational diabetes seems to be influenced by factors from a biopsychosocial perspective with some educational interventions positively impacting adherence behaviours. The review observed complex factors that influence patients' medication adherence in gestational diabetes. Thus, future research in women with gestational diabetes could consider interventions from a multifactorial perspective to improve therapeutic outcomes.

Conflicts of Interest

All authors have no conflict of interest to declare with respect to the research and publication of this article.

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Research Article

The Potential Role of Chemerin, Lipocalin 2, and Apelin in the Diagnosis and Pathophysiology of Gestational Diabetes Mellitus

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The exact role of adipokines in the pathogenesis of gestational diabetes mellitus (GDM) still remains not fully clear, and multiple studies have analyzed their potential contribution to the pathophysiology of this pregnancy complication. This study is aimed at evaluating serum chemerin, lipocalin 2, and apelin concentrations in GDM and healthy pregnant patients, assessing the correlation between these adipokines, and suggesting the potential role of these cytokines in the diagnosis and pathophysiology of GDM. The study comprised 237 pregnant women: 153 with GDM and 84 with physiological pregnancy. Serum concentrations of chemerin, lipocalin 2, and apelin were obtained at 24–29 weeks of gestation. The mean concentrations of chemerin and lipocalin 2 were significantly higher in the GDM group. The concentration of apelin was slightly higher in the GDM group, but not statistically significant. The strong positive correlation between chemerin and lipocalin 2 concentrations was noticed in both groups. Our data suggest that maternal chemerin and lipocalin 2 may play a significant role in the pathophysiology of GDM. We imply that these adipokines could potentially be established as novel biomarkers for the early identification of GDM. However, more studies are needed to analyze the effect of these adipokines on glucose metabolism during early pregnancy.

1. Introduction

Gestational diabetes mellitus (GDM) is the most frequent medical and metabolic complication characterising pregnant women. GDM affects from 5 to 20% of all pregnancies, depending on the ethnicity, screening method employed, and the diagnostic tests used [1]. GDM is associated with a higher risk of fetal a maternal adverse outcomes (macrosomia, hypertensive disorders, cesarean section, asymmetrical intrauterine growth retardation, stillbirth, neonatal, hyperbilirubinemia, hypoglycemia, hypocalcemia, polycythemia, and neonatal respiratory distress) [2, 3]. It should be emphasized that GDM patients have also a significantly increased risk for the development of type 2 diabetes mellitus (T2DM) and cardiovascular morbidity and mortality in future life [4]. Their offspring are at higher risk of fostering obesity and impaired glucose metabolism in later life. During pregnancy, an adaptation of maternal metabolism with increased nutritional requirements to support growth is observed [4]. Pregnancy is also characterized by decreased insulin sensitivity [5]. Decreased maternal prepregnancy insulin sensitivity and preconception insulin resistance, impaired insulin response during the pregnancy, and insulin-producing β-cells dysfunction are believed to be the most important components of the pathophysiology of GDM development [5]. However, insulin resistance is considered a physiological metabolic change during pregnancy, which provides a suitable concentration of glucose for the metabolic needs of the rapidly growing fetus.

Although our research was performed before the COVID-19 pandemic outbreak, at present, we have to remember the influence of this situation on the prevalence of GDM [6, 7]. The pandemic lockdown because of a decrease in physical activity and modifications in patients' dietary habits, increased consumption of snacks, unhealthy foods, and sweets, may influence body weight. The metabolic

changes include an increase in insulin resistance, total body fat, abdominal fat, and inflammatory cytokines. These factors have been shown to correlate with the higher risk of GDM. It is suggested that the Mediterranean diet could be considered, especially during a pandemic, as a useful dietary option during pregnancy to decrease the risk of maternal-fetal complications [7]. Another possible mechanism for the increased number of women with GDM may be the greater anxiety associated with the COVID-19 lockdown. The stress that pregnant women have experienced during the lockdown could initiate a cascade of endocrinological and immunological alterations that affect the delicate equilibrium necessary to maintain a physiological pregnancy and can cause the development of pregnancy complications. It is suggested that excessive activity of circulating cortisol may increase insulin resistance, a typical feature in the pathogenesis of GDM [6].

Numerous metabolic changes observed during the pregnancy appear to be influenced by adipokines [8]. It has been described that adipokines may play a key role in maternal-fetal metabolic adaptations and are involved in numerous metabolic processes. They modulate placental function and may have a significant impact on fetal development. Abnormal production or secretion of adipokines is observed in insulin resistance [8]. The significance of adipokines in the pathogenesis of GDM is still not well known. The dysregulation of several adipokines metabolism and/or placental function may play a crucial role in the pathophysiology of GDM [9].

Different adipokines have been analyzed as biomarkers for GDM; however, no marker has been reported for GDM screening so far [5].

Chemerin is a novel chemoattractant 14 kDa protein, described as retinoic acid receptor responder protein 2 (RARRES2), secreted as a prochemerin. This inactive precursor is changed into the active molecule by coagulation and inflammatory serine proteases [10]. Chemerin and the receptor of chemerin, chemokine-like receptor 1 (CMKLR1, also known as ChemR23) are almost exclusively expressed and synthesized in white adipose tissue [11]. Swensson et al. confirm that adipokines such as chemerin are also produced in several tissues apart from adipose tissue including human serum albumin [12].

Chemerin plays an important role in adipocyte differentiation, and insulin signaling results in an impact on the regulation of inflammation and major metabolic processes [10]. Its elevated levels are observed in obesity and metabolic syndrome [10]. The increased level of chemerin that occurs with obesity is hypothesized to play a substantial role in the development of T2DM as a result of dysregulation of the essential pathophysiological processes modified by chemerin [10]. It has been also described that chemerin might be an independent predictor of T2DM and cardiovascular events [13]. Recent studies have also postulated that chemerin may play an essential role in the pathophysiology of GDM [11]. Some authors notice that markedly increased circulating chemerin levels in peripheral blood are observed in GDM patients [14, 15]. It has been also suggested in the first and second trimester of pregnancy logistic multivariate regression analysis that chemerin concentrations are positively correlated with the increased risk of GDM, and together with other factors, chemerin can be used as an independent risk factor of gestational diabetes mellitus [14, 15].

Lipocalins are a superfamily of proteins characterized by a range of different molecular-recognition features [16]. Lipocalin 2 (LCN2), also known as neutrophil gelatinase-associated lipocalin (NGAL), was first found in human neutrophils and is also expressed in adipose tissue, liver, and kidneys [17]. Numerous inflammatory stimuli, such as lipopolysaccharides and interleukin-1, can significantly induce lipocalin-2 expression and secretion [18]. LCN2 plays a crucial role in the protection of matrix metallopeptidase 9 (MMP-9) from degradation and is upregulated in pathological situations as well as cancer [18, 19]. LCN2 is one of the transcripts, which are expressed in the pregnant myometrium [20]. It is suggested that LCN2 is a possible mediator that joins obesity with chronic low-grade inflammation [21]. LCN2 has also been proved to be an inflammatory marker closely associated with insulin resistance and hyperglycemia [21].

Apelin is the natural ligand of the orphan G-protein coupled APJ receptor [22]. Apelin is produced as prepropeptide consisting of 77 amino acids and shorter biologically active forms with 12, 13, 16, 17, and 36 amino acids. The most active biological fragment is probably apelin-13. Apelin acts at peripheral tissues and the central nervous system, where it takes part in glucose metabolism [23], immune system responses, inotropy, brain signaling pathway, hemodynamic homeostasis, angiogenesis, vasodilation [24], and oxidative stress-linked atherosclerosis [25]. The presence of apelin and its receptor has also been identified in adipose tissue, where their production is regulated by nutritional status. Its expression is decreased by fasting and upregulated by refeeding [26]. The increased levels of apelin are observed in obesity-associated hyperinsulinemia [23]. Animal studies have revealed that apelin can improve glucose metabolism; therefore, it has been suggested that apelin could be a promising therapeutic target in the treatment of insulin resistance [26]. The presence of apelin has been described in human placental tissue, suggesting a crucial role of this peptide in pregnancy [27].

The role of chemerin, lipocalin 2, and apelin in the pathogenesis of GDM still remains not fully clear, and the relationship between circulating concentrations of these adipokines and risk of GDM is not well known.

We aimed to investigate serum chemerin, lipocalin 2, and apelin levels in patients diagnosed with gestational diabetes and healthy pregnant patients, to analyze the relationship between these adipocytokines, and to discuss the potential role of these cytokines in the diagnosis and pathophysiology of GDM.

2. Materials and Methods

The prospective study was conducted on 153 pregnant patients with diagnosis of gestational diabetes and 84 patients with uncomplicated pregnancy and was performed in the Chair and Department of Obstetrics and Perinatology, Medical University of Lublin, Poland. Patients signed informed

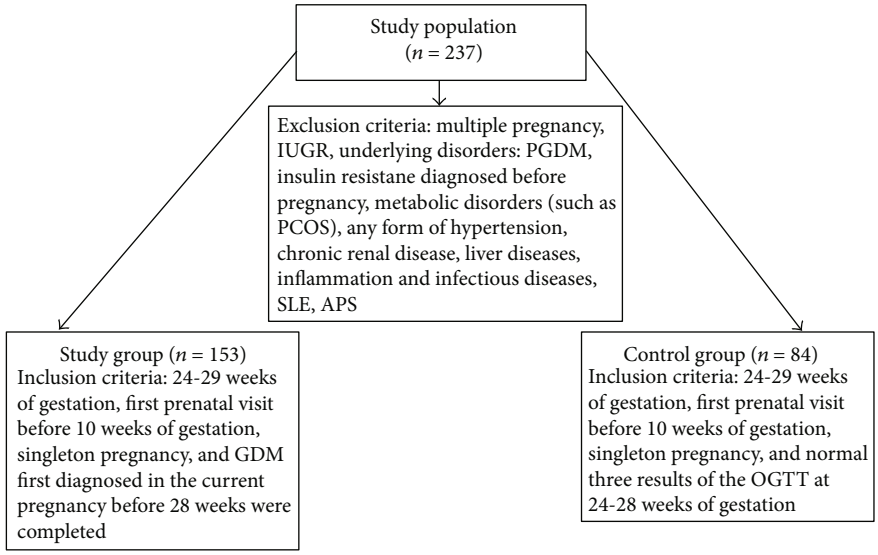

Figure 1: Flow chart of study population.

decision about participation in the clinical investigation. Approval for the trial was obtained from the Bioethical Review Board of the Medical University of Lublin (No. KE-0254/117/2018). The research was conducted in accordance with the principles published in the Declaration of Helsinki.

Inclusion criteria for the study group were the following: gestational age between 24 and 29 weeks, first prenatal visit before 10 weeks of gestation, singleton pregnancy, and gestational diabetes first recognized in the present pregnancy before 28 weeks of gestation.

Inclusion criteria for the control group were the following: gestational age between 24 and 29 weeks, first prenatal visit before 10 weeks of gestation, singleton pregnancy, and normal three results of the oral glucose tolerance test (OGTT) at 24–28 weeks of gestation (Figure 1).

Those patients with multiple pregnancy, intrauterine growth restriction (IUGR), concomitant disturbances: pregestational diabetes mellitus (PGDM), insulin resistance diagnosed before pregnancy, metabolic disorders (such as polycystic ovary syndrome—PCOS), hypertensive disorders, chronic renal and liver diseases, inflammatory and infectious diseases, systemic lupus erythematosus (SLE), and antiphospholipid syndrome (APS) were excluded from the study.

All participants had undergone screening for GDM with a 75 g OGTT at 24–28 weeks' gestation, according to WHO standards. GDM was diagnosed if at least one of the threshold values was met: fasting glucose level 5.1–6.9 mmol/L (92–125 mg/dL) at 1st hour ≥10.0 mmol/l (180 mg/dL) and at 2nd hour 8.5–11.0 mmol/L (153–199 mg/dL) [28].

Data on present pregnancy and history of previous pregnancies, maternal and family history, maternal age, and infant outcome were received by analyzing medical records.

Prepregnancy body mass index (BMI) was computed as reported weight prior to pregnancy (kg) divided by square of measured height (m). Height was measured at baseline by trained research assistants, with a wall-mounted stadiometer and shoes taken off. Weight was measured on a digital scale with 100 g resolution and capacity of 150 kg. The participants were wearing light clothing and no shoes. BMI was recalculated when the blood samples were taken.

The blood specimens for research analysis were taken at the same time when the blood specimens have been taken for routinely performed laboratory analysis. Serum levels of chemerin, lipocalin, and apelin were analyzed at 24–29 weeks of pregnancy. The samples were allowed to sit for at least 30 minutes and then centrifuged at 2000 gravitational units (g) for 20 minutes. Afterwards, serum was removed and then stored at −70°C. The chemerin level assay was performed with ELISA kit (Human Chemerin, BioVendor R&D Products, Czech Republic), as well as the lipocalin level (Human Lipocalin-2/NGAL, BioVendor R&D Products, Czech Republic), and apelin concentration (Human Apelin, Cloud-Clone Corp., USA). The limit of chemerin detection was 0.1 ng/ml. The intra- and interassay coefficients of variation (CVs) were 5.1% and 8.6%, respectively. The limit of lipocalin detection was 0.02 ng/mL, while the intra-assay and interassay coefficients of variation were 7.0% and 9.8%, respectively. The limit of apelin detection was 8.25 pg/ml. The intra- and interassay coefficients of variation (CVs) were <10% and <12%, respectively.

The patient's age, gravidity, gestational age at baseline, pregestational BMI, BMI at blood collection, estimated fetal weight (EFW) at sampling, and OGTT hourly glucose levels, as well as chemerin, lipocalin 2, and apelin levels were investigated. Correlations between chemerin, lipocalin, apelin and BMI, maternal age, gravidity, EFW, and OGTT hourly glucose levels were analyzed.

All statistical analyses were performed using STATISTICA, v. 12.0 (StatSoft, Inc., Tulsa, OK, USA). For variables of normal distribution and homogenous variances, difference significances were determined using a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. The Shapiro-Wilk test for normal distribution of data and one-tailed Student's *t*-test, or (in unequal variance) the Cochran-Cox test (absence of normal distribution and non-parametric data), and the Mann–Whitney *U* test, were

Table 1: Baseline clinical characteristics (mean and standard deviation; median and range 25-75 percentile for gravidity).

	GDM group ($n = 153$)	Uncomplicated pregnancy group ($n = 84$)	p value
Maternal age (years)	27.59 (4.87)	27.23 (4.67)	NS
Gravidity	2 (1-2.5)	2 (1-3)	NS
Pregestational BMI (kg/m^2)	23.71 (2.64)	22.81 (2.05)	$p < 0.05$
BMI at blood collection (kg/m^2)	26.63 (2.11)	26.13 (1.71)	NS
EFW at blood collection (g)	920.0 (187.8)	961.2 (168.5)	NS
Weeks of gestation at blood collection	26.54 (1.41)	26.81 (1.26)	NS

GDM: gestational diabetes mellitus; BMI: body mass index; EFW: estimated fetal weight; p: statistical significance; NS: statistically not significant.

Table 2: Chemerin, lipocalin 2, apelin, and glucose concentrations in both groups (mean and standard deviation).

	GDM group ($n = 153$)	Uncomplicated pregnancy group ($n = 84$)	p
Chemerin (ng/mL)	259.55 (63.24)	211.00 (49.38)	$p < 0.0001$
Lipocalin 2 (ng/mL)	40.49 (15.73)	20.63 (7.48)	$p < 0.0001$
Apelin (pg/ml)	10816.45 (7329.52)	9988.24 (5056.90)	$p = 0.71$
Glucose (mmol/L)			
0’	5.20 (0.38)	4.46 (0.39)	$p < 0.00001$
60’	10.06 (1.12)	7.59 (1.41)	$p < 0.00001$
120’	8.71 (1.05)	6.72 (1.19)	$p < 0.00001$

GDM: gestational diabetes mellitus; p: statistical significance; NS: statistically not significant.

all done. Results with normal distribution were presented as the means ± standard deviation (SD). The correlation analysis was conducted using Pearson’s and Spearman’s correlation tests. Significance was set at $p < 0.05$. Univariate and multivariate logistic regression analyses were performed for calculations odds ratios (ORs) with 95% confidence intervals (CIs) predicting gestational diabetes mellitus based on chemerin and lipocalin serum levels. The diagnostic value of the dependent variables—chemerin and lipocalin serum level, as the predictors of the GDM, was assessed using multiple linear regression analysis. The model was performed including independent variables such as BMI before and during the pregnancy, maternal age, gestational age, and the estimated fetal weight at the day of the sample collection. As the serum level of the apelin has not differed significantly between the GDM and healthy patients groups, it was not included in the univariate and multivariate logistic regression models.

3. Results

There were no significant differences between the GDM group and the control group with regard to maternal age, gravidity, EFW, gestational age, and BMI at blood collection. Pregestational BMI was significantly higher in GDM patients as compared with uncomplicated pregnancy group (23.71 ± 2.64 vs. 22.81 ± 2.05 kg/m^2, $p < 0.05$) (Table 1). The highest prepregnancy BMI value was 27.2 kg/m^2 in the GDM group and 24.6 kg/m^2 in the control group.

In oral fasting glucose tolerance test, at 1st and 2nd hour of test, the glucose concentrations were markedly higher in the GDM group than in the control group (Table 2).

The mean chemerin concentration was significantly higher in the GDM group than in the control group (259.55 ± 63.24 vs. 211.00 ± 49.38 ng/mL, $p < 0.0001$). The mean lipocalin 2 concentration was also significantly higher in the GDM group as compared with the control group (40.49 ± 15.73 vs. 20.63 ± 7.48 ng/mL, $p < 0.0001$). The concentration of apelin was slightly higher in the GDM patients but the difference was not statistically significant (10816.45 ± 7329.52 vs. 9988.24 ± 5056.90, $p = 0.71$) (Table 2, Figure 2).

The strong positive correlation between chemerin and lipocalin 2 levels was observed in the GDM group ($R = 0.631$, $p < 0.0001$) and in the control group ($R = 0.635$, $p < 0.0001$) (Table 3). There was no correlation between chemerin and apelin levels and lipocalin 2 and apelin levels.

The correlations between chemerin, lipocalin 2 and apelin levels, and demographic and clinical features (patient’s age; gravidity; pregestational BMI and BMI at blood collection; weeks of gestation and EFW at blood collection; OGTT hourly glucose concentrations) were evaluated for the GDM group and control group.

Chemerin level was positively associated with pregestational BMI, and BMI at blood collection in the GDM patient group ($R = 0.775$, 0.693, respectively), and in the control one ($R = 0.500$, 0.493, respectively) (Table 3).

There was a significant positive correlation between lipocalin 2 levels and pregestational, and at blood collection BMI in GDM patient group ($R = 0.467$ and 0.394, respectively), and in the control one ($R = 0.311$, 0.276, respectively)

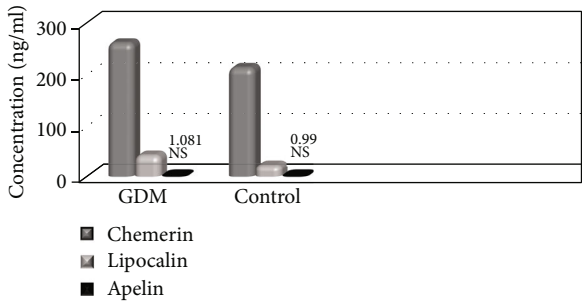

Figure 2: Chemerin, lipocalin 2, and apelin in GDM and control groups.

(Table 3). No correlation between apelin and BMI was observed.

A statistically significant relationship between chemerin levels and all values of OGTT hourly glucose concentrations were noticed in GDM patients ($R = 0.528$, 0.731, and 0.503, respectively) and in the control group ($R = 0.817$, 0.740, and 0.707, respectively). A correlation between lipocalin 2 levels and OGTT hourly glucose levels was also observed in the GDM group: $R = 0.266$, 0.425, and 0.491, respectively, and in the control one: $R = 0.553$, 0.511, and 0.423, respectively. No correlations between apelin levels and OGTT values were observed. Lipocalin 2 levels were also correlated with maternal age in the GDM group and gravidity in the control group.

The univariate linear regression model which was performed for chemerin and lipocalin has shown that the growth of each substance serum level similarly increases the likelihood of the GDM incidence in the analyzed group of patients—18% for each 10 ng/ml of chemerin and 20% for each 1 ng/ml of lipocalin (CI 95%, OR: 1,180 vs. 0.200, respectively).

In the multiple linear regression analysis of the patients with gestational diabetes, we have established that the adjusted R-square for chemerin was significantly elevated as compared to lipocalin (46.10 vs. 20.60, respectively).

4. Discussion

Our data demonstrate that pregnant women with GDM are characterized by a significantly higher concentration of chemerin and LCN2 and not significantly higher level of apelin. However, the role of these adipokines as pro- or anti-inflammatory factors is controversial.

One of the disadvantages of our study is that we did not analyze the cord blood or placenta tissue for adipokines which would have also been useful in coming to a better understanding of GDM. The clinical utility of our findings has remained limited due to the relatively small number of patients. Thus, the analysis of outcomes may be underpowered.

Another disadvantage is that maternal obesity may also influence the expression of several adipokines in the adipose tissue and the placenta. In our study, the mean prepregnancy BMI was 23.71 in the GDM group and 22.81 in the control one and the next studies should be conducted in patients with higher BMI.

Numerous adipokines have been studied during pregnancy, and their concentrations have been suggested as biomarkers of pregnancy complications, some of them with pathophysiological signification. There are controversies in the literature about the concentrations of different adipokines and their role during pregnancy. The discrepancies may be caused by the time of maternal blood sampling, laboratory methods used for analysis, sample size, and population differences.

In our study, we focused on adipokines, which are dysregulated in GDM, and three of them have been analyzed: chemerin, lipocalin 2, and apelin. To the best of our knowledge, this is the first study to compare circulating levels of chemerin, lipocalin 2, and apelin in the same group of women with gestational diabetes and those with a normal pregnancy. We also evaluated the serum levels of these adipokines in GDM and uncomplicated pregnancy patients in comparison to clinical and demographic parameters.

We noticed that the mean chemerin level was significantly higher in the GDM group than in the control one. Our results are found to be compatible with a previously published studies [29–33]. Additionally, in the study presented by Li et al., the concentrations of chemerin in all GDM groups were increased in comparison to the normal-weight-NGT group, but the chemerin level in the obese-GDM group was significantly lower than in the normal-weight-GDM and overweight-GDM group [30]. The significantly higher levels of chemerin in the third trimester in comparison to the first trimester of pregnancy were also revealed [29, 31, 34]. It is postulated that it can be associated with proinflammatory conditions because of increased levels profile of mediators of inflammation such as TNF-α, resistin, or IL-6 [34]. Interestingly, Yang and colleagues reported that the level of chemerin in the third trimester in the GDM group was markedly higher than in the NGT group, but the serum concentration of chemerin in the first trimester was lower in the GDM group than in the NGT group. The limitation of the study was small groups: 19 patients with GDM and 20 NGT women [31]. We also found that chemerin levels were correlated with pregestational BMI, BMI at sampling in the GDM group, and in the control group. Kasher-Meron et al. presented results, which are in line with our findings [29]. In the study conducted by Ademoglu et al., in multiple linear regression analyses they noticed that chemerin level was markedly correlated not only with BMI but also with HDL-cholesterol, triglyceride, HbA1c, insulin concentrations, and homeostasis model assessment of insulin resistance (HOMA-IR) [32]. Interestingly, fasting insulin level was comparable in both groups.

However, the HOMA-IR tended to be higher in patients with GDM but did not reach statistical significance. In the presented study, we were not able to obtain the data on the insulin concentration of all patients, but in the smaller groups (55 GDM patients and 23 controls), no correlations between the chemerin and HOMA-IR were confirmed.

Table 3: Correlation between chemerin, lipocalin, apelin levels, maternal age, gravidity, BMI, EFW, gestational age, and glucose concentrations in OGTT in GDM patients and control group.

	GDM						Control					
	Chemerin		Lipocalin 2		Apelin		Chemerin		Lipocalin 2		Apelin	
	R	p	R	p	R	p	R	p	R	p	R	p
Chemerin			0.631	$p < 0.0001$	0.005	NS			0.635	$p < 0.0001$	-0.048	NS
Lipocalin 2	0.631	$p < 0.0001$			-0.067	NS	0.635	$p < 0.0001$			-0.016	NS
Maternal age (years)	0.157	NS	0.217	$p < 0.01$	-0.108	NS	0.024	NS	0.183	NS	-0.060	NS
Gravidity	0.090	NS	0.130	NS	0.040	NS	0.106	NS	0.216	$p < 0.05$	0.054	NS
Pregestational BMI (kg/m^2)	0.775	$p < 0.00001$	0.467	$p < 0.0001$	-0.105	NS	0.500	$p < 0.0001$	0.311	$p < 0.01$	0.003	NS
BMI at blood collection (kg/m^2)	0.693	$p < 0.00001$	0.394	$p < 0.001$	-0.087	NS	0.493	$p < 0.0001$	0.276	$p < 0.05$	0.054	NS
EFW at blood collection (g)	-0.032	NS	-0.063	NS	-0.145	NS	-0.060	NS	0.091	NS	0.055	NS
Weeks of gestation at blood collection	-0.005	NS	-0.066	NS	-0.156	NS	-0.094	NS	0.084	NS	0.005	NS
Glucose												
0’	0.528	$p < 0.0001$	0.266	$p < 0.01$	0.053	NS	0.817	$p < 0.0001$	0.553	$p < 0.0001$	0.074	NS
60’	0.731	$p < 0.0001$	0.425	$p < 0.001$	0.011	NS	0.740	$p < 0.0001$	0.511	$p < 0.0001$	-0.122	NS
120’	0.703	$p < 0.0001$	0.491	$p < 0.001$	-0.051	NS	0.707	$p < 0.0001$	0.423	$p < 0.001$	-0.047	NS

GDM: gestational diabetes mellitus; BMI: body mass index; EFW: estimated fetal weight; R: Spearman correlation’s coefficient; p: statistical significance; NS: statistically not significant.

In our study, in both groups, there were no obese patients with prepregnancy and at sampling BMI > 30 kg/m^2. It is important that the highest prepregnancy BMI value was 27.2 kg/m^2 in the GDM group and 24.6 kg/m^2 in the control group. We excluded from our study the patients with prepregnancy diabetes mellitus, insulin resistance diagnosed before pregnancy, metabolic disorders (such as polycystic ovary syndrome), and any form of hypertension. So, the risk of markedly higher insulin resistance in our study group as compared to the control group was relatively small. Our study results showed a statistically significant correlation of chemerin levels and the OGTT hourly glucose levels in both groups. These observations are partially in line with the study presented by Fatima et al. [33]. In this analysis, chemerin level was positively associated with fasting glucose level, and additionally with HOMA-IR, and EFW.

The chemerin concentrations of both venous and arterial umbilical cord blood in newborns were also sampled and analyzed. Increased chemerin level in arterial cord blood in GDM group as compared that in control one was found but the concentrations in venous cord blood were comparable in both groups. Chemerin concentration in venous cord blood was increased in newborns of obese patients. Arterial and venous chemerin values were correlated with maternal chemerin values at the time of delivery. It has been noticed that chemerin value in arterial blood was associated with gestational diabetes status [35]. The opposite observations have been described by Barker and colleagues. In this study, no effect of GDM on maternal and cord chemerin levels was noticed as well as no change in the release of chemerin from the placenta and adipose tissue [36]. Because in our study, we did not analyze the chemerin values in arterial and venous umbilical cord blood, and we cannot compare our findings with these observations.

In a meta-analysis performed by Zhou et al., they revealed that the higher levels of circulating chemerin were correlated with GDM, and, according to the authors, this suggests that chemerin might play an essential role in the pathophysiology of GDM. They noticed that the increased chemerin levels were found in the second trimester of pregnancy as compared to women in the third trimester of pregnancy. This could be explained by the fact that serum albumin concentrations usually decrease during late pregnancy, and chemerin is released from human serum albumin. However, according to Zhou et al., these results should be interpreted with caution due to essential heterogeneity between studies, and further prospective cohort studies are needed to determine these observations [37].

Despite the growing evidence supporting a link between chemerin and GDM, the details of the mechanisms involved are unknown. The altered chemerin concentrations in GDM patients may cause insulin resistance, and an increased concentration in physiological pregnancy may have a protective role to decrease pregnancy-related insulin resistance [28]. Chemerin influences on the production of proinflammatory cytokines, chemokines, and matrix metalloproteinases (MMPs) [35, 38]. It has been also described that the administration of chemerin reduces glucose tolerance, decreases serum insulin levels, and lowers basal glucose uptake in diabetic mice *in vivo* [31]. As a result, the abnormalities in chemerin concentrations may be correlated with the development of GDM through lower insulin sensitivity and impaired anti-inflammatory capacity.

Increased concentrations of lipocalin 2 were described in metabolic diseases such as T2DM, preeclampsia, and PCOS [39, 40]. There have been published several studies describing pregnancy-related LCN2. However, we have found very few articles analyzing LCN2 in relation to GDM [41–44].

In the presented study, we have found higher LCN2 concentrations in the GDM group than in the control one. Similar observations have been published by Edelstam and colleagues, who noticed that LCN2 levels were elevated during the third trimester of pregnancy and additionally significantly increased postpartum [43].

In the study published by Lou et al., LCN2 concentration in GDM overweight and nonoverweight women were markedly higher in comparison to NGT women. LCN2 level was also markedly higher in GDM overweight than in GDM nonoverweight group. There were also positive correlations between LCN2 and parameters of insulin resistance: fasting plasma glucose (FGP), HOMA-IR, fasting plasma insulin (FPI), high-sensitivity C-reactive protein (hs-CRP), total cholesterol, and triglyceride. Furthermore, the expression of LCN2 mRNA and protein in subcutaneous adipose tissue (SAT) was higher in obese women. The researchers suggested that LCN2 could act in the development of insulin resistance in GDM, and its expression in subcutaneous adipose tissue may be associated with obesity in GDM women [42]. These results coincide with our outcomes. We found a significant correlation between LCN2 levels and pregestational BMI, and BMI at sampling, and additionally with OGTT glucose levels in both groups. However, as in chemerin results, we were not able to obtain the data on the insulin concentration of all patients, but in the smaller groups (55 GDM patients and 23 controls), no correlations between the LCN2 and HOMA-IR were revealed.

Additionally, in our study, in the GDM group, the weak positive correlation between LCN2 levels and maternal age at the blood collection has been noticed. The relationship between age and adipokine levels is ambiguous [44]. There appear to be no data in the literature on the possible mechanisms of such correlation and clinical implications, and we cannot compare our findings with other publications. This observation may suggest that the increase of maternal age could be potentially associated with the developing of insulin resistance in GDM patients. However, elderly pregnant women have also a higher BMI index, and it could indicate that adipose tissue may also have an influence on LCN2 levels. But we did not observe a similar correlation in chemerin and apelin analysis in the same group of patients.

A few studies have been published analyzing the role of LCN2 as a predictor of GDM [41, 45]. D'Anna et al. revealed that in women who developed GDM in the previous 12 months, in the first trimester of pregnancy, circulating LCN2 level was markedly higher in patients who subsequently developed GDM. Median serum LCN2 concentrations were positively correlated with HOMA-IR. However, they failed to demonstrate a correlation between LCN2 and pregestational BMI, maternal age, or birth weight [45].

Sweeting et al. in their study tried to find the best risk prediction model for GDM. The authors observed higher LCN2 levels in women who developed GDM. They observed a 10% increase in median MoM LCN2 values in women with GDM and suggested that so small differences, as compared to D'Anna et al. study, presumably reflect the impact of ethnicity on biomarker associations with GDM [41].

In our study, we observed in both groups the positive correlation between chemerin and LCN2 levels. However, the potential physiological and pathological importance of our observations need further explanation. The univariate linear regression model which was performed for chemerin and LCN2 has shown that the growth of each substance serum level similarly increases the likelihood of the GDM incidence in the analyzed group of patients—18% for each 10 ng/ml of chemerin and 20% for each 1 ng/ml of LCN2 (CI 95%, OR: 1.180 vs. 0.200, respectively). In the multiple linear regression analysis of the women with gestational diabetes, we have noticed that the adjusted R-square for chemerin was markedly increased as compared to lipocalin (46.1 vs. 20.60, respectively). However, it is important to remember that the main goal of this research was not to describe the cut-off levels of these adipokines in women at 24-28 weeks of gestation when the OGTT is performed.

Gestational diabetes is considered to be an inflammatory disease. Expression of LCN2 in adipose tissue and liver can be induced by lipopolysaccharides, suggesting that LCN2 may be an acute-phase protein. It is suggested that LCN2 may be a significant key to the pathogenesis of inflammation, leading to insulin resistance, followed by an increase in fasting plasma glucose and fasting plasma insulin [42, 46]. However, further studies are needed to evaluate the role of LCN2 in the pathogenesis and prediction of GDM.

In our study, a positive correlation between chemerin and lipocalin 2 levels was observed in both groups. There is a lack of data in the literature regarding the possible explanation of such relationship and clinical importance. We can only hypothesize that our observations can also confirm the possible role of these adipokines in the pathophysiology of gestational diabetes. Thus, the presented results suggest that chemerin serum level evaluation appears to be a more reliable independent predictor of the GDM in future analysis.

The physiological role of apelin is not well known. Animal studies showed that apelin had a glucose-lowering effect correlated with stimulation of glucose utilization in adipose tissue and skeletal muscle from normal and obese insulin-resistant mice [47]. An increased plasma concentration of apelin was noticed in animal models of obesity correlated with hyperinsulinemia. Boucher et al. confirmed that in the obese men and mice, both plasma apelin and insulin values were markedly increased, suggesting that apelin homeostasis is impaired in obesity and indicating that the higher value of plasma insulin could support an increase in blood levels of apelin. Thus, apelin overproduction by adipose tissue may be involved in several obesity-related disturbances [23].

Higher levels of apelin were noticed in patients suffering from T2DM [48]. It has been suggested that apelin secretion can be modulated by proinflammatory adipocytokines, the levels of which are higher in insulin resistance. Daviaud et al. reported a positive correlation between apelin and TNF-α expression in adipose tissue and revealed a direct upregulation of apelin expression in both human and mouse adipocytes by TNF-a [49].

There are also some controversies in the literature regarding apelin levels during physiological pregnancy and pregnancy complicated by GDM, and the data are very

limited. In the study performed by Kourtis et al., in non-GDM patients, apelin levels were significantly lower in pregnant women than in nonpregnant [50]. In our study, the apelin levels were not statistically significantly higher in the GDM group as compared to the control one. Similar observations have been described by Aslan et al. [51]. However, they measured the apelin levels at the time of delivery. Telejko et al. observed no significant differences in plasma apelin concentrations between the GDM and non-GDM women [52]. The decreased levels of apelin have been revealed by Boyadzhieva et al. and Oncul et al. [53, 54]. In Boyadzhieva et al's study, apelin concentration was significantly lower in the GDM group during the pregnancy. However, there were no statistically significant differences in postpartum groups and no significant correlations between apelin levels and metabolic parameters [53]. In the study conducted by Oncul et al., they also analyzed the maternal and cord blood apelin levels [54].

The cord blood apelin concentrations were significantly lower in GDM women than in the control group. They suggest that GDM appears to modify fetoplacental apelin metabolism, but apelin cannot directly regulate maternal insulin sensitivity [54]. The opposite results of cord blood apelin have also been published [46]. In the study of Aslan et al., the cord blood apelin levels were comparable in the GDM group and in the control one. They also noticed that levels of apelin in the serum of the mothers had a positive correlation with their respective cord blood concentrations. However, in our study, we did not investigate the concentrations of apelin in cord blood, and a full comparison of our results with these observations cannot be performed. Aslan et al. observed the negative association between serum and cord blood apelin values and the gestational age and birth weight [51]. No correlations between serum and cord blood apelin values, maternal age, fasting glucose and insulin levels, BMI, and HOMA-IR were revealed. In our study, we also found no correlations between apelin, clinical, and demographic parameters, but we measured parameters at 24-29 weeks of gestation and, as in chemerin and LCN2 results, we did not analyze the insulin levels and HOMA-IR index.

In our study, there was also no correlation between chemerin and apelin levels and LCN2 and apelin levels. Thus, our results suggest that GDM has no impact on circulating apelin levels.

The differences between our findings and those published in the literature could be explained by the differences in the study protocols and selection process of patient including the week of gestation at blood sampling—the second or third trimester, the type of gestational diabetes—a dietary treatment or insulin treatment which may suggest the severity of metabolic disturbances, the week of pregnancy at the diagnosis of GDM—the first (possible prepregnancy impaired glucose tolerance) or second trimester ("typical" gestational diabetes), the pregestational BMI value or at enrolling to the studies.

The significance of adipokines in the pathogenesis of GDM is still not well known, and none of them have been used as an early predictor for the development of GDM.

Screening for GDM with a 75 g oral glucose tolerance test at 24–28 weeks' gestation and diagnosing GDM in this period of pregnancy have been questioned due to the potential delay in accomplishing the positive effects of pharmacological therapy, diet, and lifestyle modifications. Identifying patient at risk for GDM is essential in the first trimester of pregnancy to minimize maternal and neonatal mortality and morbidity. A limited number of publications have prospectively analyzed the correlation of the HOMA-IR, glycosylated hemoglobin, sex hormone-binding globulin, and cholesterol panel values as a marker for prediction subsequent GDM in low-risk pregnancies during the first trimester of pregnancy but they have low sensitivity and positive predictive value, especially in overweight and obese women. None of these markers have proven adequate to be used in the clinical screening. Mainly, increased HOMA-IR values have been suggested to be associated with GDM. However, the range of HOMA-IR values is wide in women with GDM, and cut-off level is ambiguous [55–57].

5. Conclusion

Gestational diabetes mellitus is a widespread condition observed in a large population of pregnant patients. The precise role of adipokines in the pathogenesis of GDM is still not well known. To the best of our knowledge, we did not find in the literature the comparison of circulating levels of chemerin, lipocalin 2, and apelin in the same group of patients with GDM and healthy pregnant women.

We can speculate that these adipokines could potentially be established as novel biomarkers for the early diagnosis of GDM. We hope that our findings will be useful to determine guidelines, in which adipokines may become a novel biomarker in GDM prediction, especially when early pregnancy is concerned. However, further prospective studies are required to evaluate chemerin and lipocalin 2 in the first trimester of pregnancy as a marker of GDM, before the period of pregnancy when the OGTT is performed. It should be remembered that maternal obesity influences the expression of several adipokines in the placenta and in the adipose tissue. Due to these reasons, the correlations between the investigated adipokines and pregnancy-related conditions should be interpreted separately referring to maternal pregestational BMI and pregnancy weight gain, and this problem should be considered in further studies.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflict of interests.

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Research Article

The Relationship between Fetal Abdominal Wall Thickness and Intrapartum Complications amongst Mothers with Pregestational Type 2 Diabetes

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Objectives. To evaluate the utility of fetal abdominal wall thickness (AWT) for predicting intrapartum complications amongst mothers with pregestational type 2 diabetes. *Methods*. This was a historical cohort study of pregnant mothers with pregestational type 2 diabetes delivering at a Canadian tertiary-care center between January 1, 2014, and December 31, 2018. Delivery records were reviewed to collect information about demographics and peripartum complications. Stored fetal ultrasound images from 36 weeks' gestation were reviewed to collect fetal biometry and postprocessing measurement of AWT performed in a standardized fashion by 2 blinded and independent observers. The relationship between fetal AWT was then correlated with risk of intrapartum complications including emergency Caesarean section (CS) and shoulder dystocia. *Results*. 216 pregnant women with type 2 diabetes had planned vaginal deliveries and were eligible for inclusion. Mean maternal age was 31.3 years, and almost all were overweight or obese at the time of delivery (96.8%). Overall, the incidence of shoulder dystocia and emergency intrapartum CS was 7.4% and 17.6%, respectively. There was no difference in mean fetal AWT between those having a spontaneous vaginal delivery (8.2 mm (95% CI 7.9-8.5)) and those needing emergency intrapartum CS (8.1 mm (95% CI 7.4-8.8); $p = 0.71$) or shoulder dystocia (8.7 mm (95% CI 7.9-9.5); $p = 0.23$). There was strong interobserver correlation of AWT measurements ($r = 0.838$; $p < 0.00001$). The strongest association with intrapartum complications was birthweight ($p = 0.003$): with birthweight > 4000 grams, the relative risk of shoulder dystocia or CS is 2.75 (95% CI 1.74-4.36; $p < 0.001$). *Conclusions*. There was no obvious benefit of AWT measurement at 36 weeks for predicting shoulder dystocia or intrapartum CS amongst women with type 2 diabetes in our population. The strongest predictor of intrapartum complications remained birthweight, and so studies for improving estimation of fetal weight and evaluating the role of intrapartum ultrasound for predicting risk of delivery complications are still needed.

1. Introduction

Diabetes complicates ~5-7% of pregnancies worldwide, and numbers continue to increase in parallel with worsening rates of obesity [1, 2]. In our province, the prevalence of pregestational type 2 diabetes is amongst the highest in Canada, and with increasing rates, the number of affected pregnancies has also increased [1]. Pregestational diabetes increases the risk of perinatal complications for the mother, fetus, and newborn, including a higher risk of developing other medical complications of pregnancy such as preeclampsia. Another specific concern is the 4-5 times higher rate of stillbirth for mothers with pregestational type 2 diabetes, which has prompted increased efforts to improve antenatal surveillance and maternal glycemic control [3, 4]. Around the time of delivery, diabetes increases the risk of almost all peripartum complications of childbirth: induction of labor, Caesarean section (CS), operative vaginal delivery, high-degree lacerations, shoulder dystocia and related newborn injuries including asphyxia, postpartum hemorrhage, and prolonged hospital stay

[3–5]. However, our ability to predict which patients with type 2 diabetes are most at risk of these intrapartum complications remains limited [3–8].

Shoulder dystocia complicates 1% of all births (even higher in those affected by diabetes) and can result in significant injury to newborns and mothers, and is also an independent risk factor of perinatal mortality [9]. Unfortunately, there is almost no way to further risk stratify these patients for individualized prediction of intrapartum shoulder dystocia or emergency intrapartum CS. Traditionally, fetal macrosomia has been the main risk factor of intrapartum complications: it is also the basis for several professional organizations recommending elective primary CS for large fetal size in pregnancies with or without diabetes [10]. However, studies from our center have highlighted the safety of vaginal delivery in the setting of fetal macrosomia, and thus, we have no current policy of elective primary CS for fetal macrosomia alone in the general population [11, 12]. Yet, local pregnant patients with uncontrolled diabetes plus fetal macrosomia are frequently induced around 36 to 37 weeks' gestation due to concerns about potential risk of stillbirth [1].

With advances in fetal ultrasound usage to predict intrapartum labor progress and success of vaginal delivery, there is the potential for its use to enhance prediction of specific intrapartum complications for women with pregestational diabetes as well. Fetal macrosomia is a major risk factor of intrapartum complications and birth trauma, including shoulder dystocia; however, there are concerns regarding performance of fetal ultrasound during late pregnancy to accurately predict postnatal weights [12–15]. Novel ultrasound techniques are being developed to improve antenatal prediction of macrosomia in order to prevent intrapartum birth complications: soft tissue measurements and other anthropometric markers as well as fetal volumes using three-dimensional ultrasound have all been suggested as ways to improve diagnosis of fetal overgrowth before delivery [16–18]. Cranial shape, ratio of abdominal-to-head circumferences, and biacromial measurements are proposed methods to enhance prediction of shoulder dystocia specifically [19–22]. Fetal abdominal wall thickness has also been proposed as a potential marker of shoulder dystocia or failed labor progress: however, the studies published thus far have been limited by small sample size and timing of ultrasound relative to delivery [21, 22]. To date, there have been some reports of an association between fetal abdominal wall thickness at midpregnancy ultrasound and prediction of gestational diabetes later in pregnancy; however, such findings need to be interpreted cautiously given the inherent difficulty of ensuring that women diagnosed with diabetes for the first time in pregnancy are truly those with gestational diabetes and not cases of undiagnosed type 2 diabetes which might otherwise explain the increased thickness of subcutaneous fetal fat [23]. In other preliminary work from our group, there does appear to be a difference in the abdominal wall thickness of fetuses exposed to pregestational type 2 diabetes: fetuses exposed to diabetes in utero have significantly thicker subcutaneous abdominal wall fat than those born to healthy controls [24]. The goal of this study was to evaluate the utility of fetal abdominal wall thickness (AWT) in the third trimester for predicting intrapartum complications amongst mothers with known pregestational type 2 diabetes.

2. Materials and Methods

This was a historical cohort study conducted at the Health Sciences Centre Women's Hospital in Winnipeg, Canada, over a 5-year period between January 1, 2014, and December 31, 2018. This tertiary-care hospital serves as one of two regional referral sites for a total population of 1.3 million inhabitants and a geographic region which includes urban, rural, and northern/remote communities: it also represents the highest concentration of diabetes in pregnancy in the region. There are approximately 5000 to 5500 deliveries per year at the study hospital and over 10,000 ultrasounds performed within its Fetal Assessment Unit annually. Research ethics approval was obtained from the University of Manitoba Health Research Ethics Board. Because this project was retrospective in nature and did not require any direct patient contact, individual consents were not required by our institution.

All pregnant patients with a diagnosis of pregestational type 2 diabetes and delivering at the study hospital during the 5-year period were eligible for inclusion. Potential study subjects were identified using delivery record books and the diagnosis of type 2 diabetes cross-validated with the maternal diagnosis entered in the stored fetal assessment record. Cases of multiples, congenital anomalies, planned postnatal palliation, planned delivery by Caesarean section, and those delivering prior to 36 weeks were excluded. Cases were also excluded if they did not have stored fetal ultrasound images from a 35- to 36-week scan, noting that it is the local standard of care to perform a fetal assessment scan for all patients with type 2 diabetes during that time period.

Hand searches of delivery record books were performed by experienced research personnel to identify potential cases of pregestational type 2 diabetes and information regarding basic maternal demographics, pregnancy and delivery information, and early postnatal outcomes abstracted using standardized data collection sheets. Postprocessing review of stored ultrasound images and fetal assessment reports was also performed to obtain data about fetal biometry and measurements of abdominal wall thickness. Abdominal wall thickness measurements were performed in a standardized fashion as described by Higgins et al. in 2008 [25] and utilize the standard, transverse axial section view of the fetal abdomen commonly obtained for measurement of the abdominal circumference [26]: in this plane and at the level of the stomach bubble and portal umbilical venous complex, the thickest area of the subcutaneous layer in the near-field anterior abdominal wall within 45 degrees of the cord insertion is measured (Figure 1). Written consent was obtained by the individual patient for use of this ultrasound image. A second blinded observer performed repeated measurement of fetal abdominal wall thickness in a random selection of 25% of cases to ensure interobserver reliability. Where multiple scans were performed during this time frame, the scan closest to delivery was chosen and used to obtain the measurements of interest. Biometry and abdominal wall thickness were then correlated with intrapartum complications (shoulder

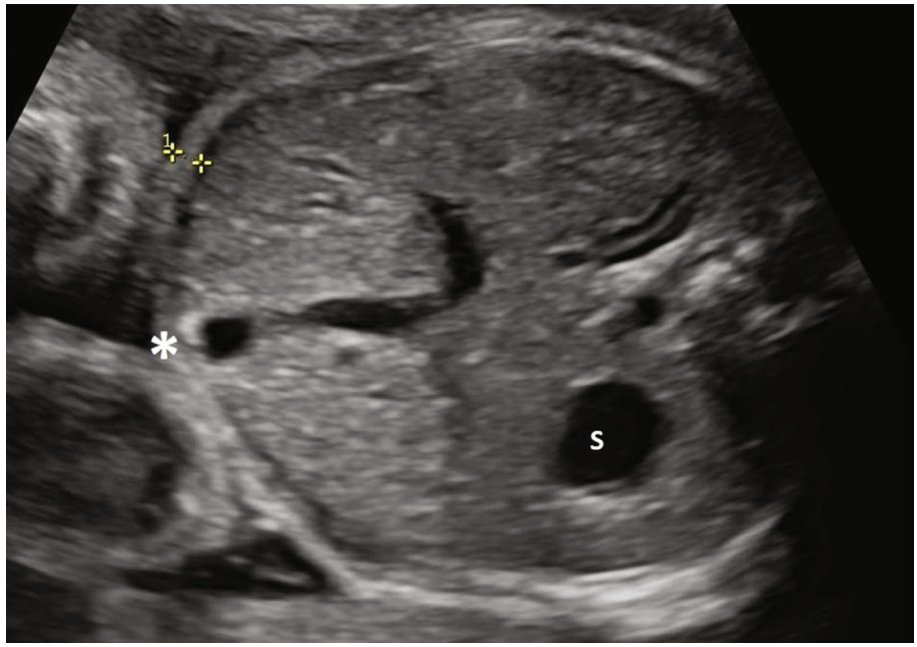

Figure 1: Anterior abdominal wall thickness measurement (calipers) as obtained from the standard abdominal circumference view. S = stomach bubble; *area of cord insertion near origin of portal umbilical vein complex.

dystocia and emergency intrapartum Caesarean section). Macrosomia in the fetus was defined as estimated fetal weight above the 90th percentile for gestational age on fetal growth curves standardly used in our unit; neonatal macrosomia was defined separately as birthweight above 4500 grams and as greater than the 90th percentile at birth by the newborn growth curves used locally [26–28]. All patients in the cohort had adequate follow-up until delivery.

Statistical analysis was performed using Stata v.14.2 (StataCorp LLC, College Station, TX) software, with a p value less than 0.05 used to denote statistical significance. Continuous variables were presented as means with 95% confidence intervals (or standard deviations) if normally distributed or as medians with interquartile ranges if nonparametrically distributed. Dichotomous and categorical variables were described as proportions. Student's t-, chi-square, Wilcoxon rank-sum, Kruskal-Wallis, and analysis of variance tests were used to compare outcomes between groups depending on data type and distribution. Linear regression analyses were performed to evaluate the relationship between fetal ultrasound measurements of abdominal wall thickness and abdominal wall circumference, as well as estimated fetal weight: logistic regression was then used to evaluate the crude odds of intrapartum complications by individual ultrasound measurements and birthweight (given the inherent error of estimated fetal weight measurements [ref]). The Spearman correlation coefficient was used to evaluate interobserver reliability of abdominal wall thickness measurements.

3. Results and Discussion

There were 216 patients that met study criteria and included in the analysis. In our cohort, pregnant women with pregestational type 2 diabetes had a mean age in years of 31.3 (SD 6.5) and most were multiparas (77.6%) (Table 1). The mean body mass index (BMI) at delivery was high at 36.6 kg/m^2: only 3.2% of the entire cohort had a normal BMI and 96.8% were considered overweight or obese, including one-third that were categorized as class 3 obesity with a BMI $\geq$ 40 kg/m^2. 34.4% of these pregnancies were complicated by additional medical conditions, including 18.8% with hypertensive disorders of pregnancy, although there was only 1 documented case of preeclampsia. According to fetal ultrasound findings antenatally, 21.8% of cases were suspected to have fetal growth abnormalities prior to delivery: 21.3% were diagnosed with fetal macrosomia > 90th percentile for gestational age along with 0.5% diagnosed with fetal growth restriction < 10th percentile for gestational age (Table 1). The mean abdominal wall thickness of fetuses exposed to pregestational type 2 diabetes was 8.2 mm (95% CI 8.0-8.4).

The majority of patients in our cohort were induced (81.9%) (Table 1). 32.1% required some form of cervical ripening, either by chemical or mechanical means (Table 1). Almost half of patients (41.6%) required oxytocin at some point during the process of induction. Most patients had spontaneous vaginal deliveries (71.3%), whereas 9.7% required operative vaginal deliveries and another 19% had CS deliveries (with an overall prevalence of "emergency" intrapartum CS equal to 17.6%) (Table 1 and Figure 2). 7.4% of vaginal deliveries were complicated by shoulder dystocia. The median gestational age at delivery was 37 + 1 weeks' gestation [IQR 36 + 0 to 38 + 3]. Apgar scores were 8 [IQR 6 to 9] and 9 [IQR 9 to 9] at one and five minutes, respectively: fewer than 3% of deliveries were complicated by a 5-minute Apgar score less than 7. About half of the newborns in the cohort were female. Mean birthweight was

Table 1: Maternal characteristics and peripartum outcomes associated with pregnancies affected by pregestational type 2 diabetes.

Variable of interest	Total cohort ($n = 216$)
Maternal age in years, mean (SD)	31.3 (6.5)
Gravidity, median [IQR]	3 [2 to 6]
Gravidity > 1 (%)	86.1%
Parity, median [IQR]	2 [1, 3]
Parity > 0 (%)	77.6%
Body mass index[a], mean (SD)	36.6 kg/m^2
BMI < 18.5, underweight (%)	0
BMI 18.5-24.9, normal (%)	3.2%
BMI > 25 – 29.9, overweight (%)	13.7%
BMI 30-34.9, class 1 obesity (%)	25.9%
BMI 35-39.9, class 2 obesity (%)	24.9%
BMI > / = 40, class 3 obesity (%)	32.3%
Other medical complications of pregnancy (%)	34.4%
Hypertensive disorders	18.8%
Other maternal complications	11.6%
Fetal growth abnormalities on US (%)	21.8%
Macrosomia > 90th %ile for GA	21.3%
IUGR < 10th %ile for GA	0.5%
Induction of labor (%)	81.9%
Prostaglandin gel	13.1%
Prostaglandin insert	14.9%
Foley catheter or cervical ripening balloon	4.1%
Artificial rupture of membranes	21.6%
Oxytocin	46.3%
Gestational age at delivery, median [IQR]	37 + 1 [36 + 0 to 38 + 3]
Mode of delivery (%)	
Spontaneous vaginal delivery	71.3%
Assisted vaginal delivery	9.7%
Caesarean section	19%
1 min Apgar	8 [6, 9]
5 min Apgar	9 [9, 9]
5 min Apgar < 7 (%)	2.8%
Birthweight in grams, mean (SD)	3529.8 (655.3)
>4500 grams (%)	6.0%
>90th %ile for GA (%)	32.9%
Female fetus (%)	52.1%

Notes: [a]calculated for $n = 189$ with available BMI data.

3529.8 grams (95% CI 3440-3620), and only 6% of deliveries were designated as macrosomic at birth using the definition of >4500 grams: however, by using greater than the 90th percentile for gestational age to define macrosomia, 32.9% of newborns in the cohort were considered macrosomic at birth.

Regarding perinatal characteristics differentiating pregnancies with and without delivery complications, cases with shoulder dystocia or intrapartum CS had significantly higher BMIs than those with spontaneous vaginal deliveries ($p = 0.026$) (Table 2). Pregnancies resulting in emergency intrapartum CS were more likely to have other comorbid medical complications but a trend towards fewer inductions of labor. One-minute Apgar scores were significantly lower amongst those deliveries complicated by shoulder dystocia ($p = 0.013$), but there was no difference in 5-minute Apgar scores between the three groups ($p = 0.788$) (Table 2). There was no significant difference in mean fetal abdominal wall thickness between those having spontaneous vaginal deliveries (8.2 mm (95% CI 7.9-8.5)) and those requiring emergency intrapartum CS (8.1 mm (95% CI 7.4-8.8); $p = 0.71$) or those deliveries complicated by shoulder dystocia (8.7 mm (95% CI 7.9-9.5); $p = 0.23$) (Figure 2). There was moderate positive correlation between abdominal circumference and abdominal wall thickness ($r = 0.548$; $p < 0.0001$) and strong

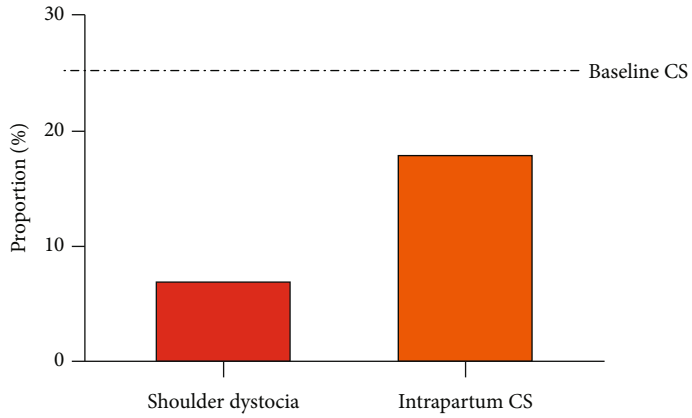

Figure 2: Proportion of deliveries complicated by shoulder dystocia and intrapartum Caesarean section (CS), compared to the baseline CS risk in the population (25.4%) [20].

Table 2: Perinatal characteristics and birth outcomes associated with intrapartum complications.

	Spontaneous vaginal delivery ($n = 154$)	Shoulder dystocia ($n = 13$)	Caesarean section ($n = 38$)	p value
Multiparous (%)	81.6%	76.9%	73.8%	0.511
Body mass index[a], mean (SD)	36.1 (7.3)	39.3 (7.7)	39.3 (6.6)	0.026
BMI < 18.5, underweight (%)	0	0	0	—
BMI 18.5-24.9, normal (%)	3.7%	0	0	—
BMI > 25 – 29.9, overweight (%)	17%	0	5.9%	—
BMI 30-34.9, class 1 obesity (%)	25.9%	0	17.6%	—
BMI 35-39.9, class 2 obesity (%)	23.7%	41.7%	26.5%	0.470
BMI > / = 40, class 3 obesity (%)	29.7%	58.3%	50%	0.011
Other medical complications of pregnancy	16%	15.4%	32.6%	0.092
Hypertensive disorders	15.8%	15.4%	16.3%	0.713
Other maternal conditions	8.2%	0	23.2%	—
Induction of labor (%)	91.4%	91.7%	79.3%	0.076
Gestational age at delivery, median [IQR]	37 [36 to 38]	37 [36 to 38]	37 [36 to 38]	0.899
1 min Apgar	8 [6.5 to 9]	6 [6 to 7]	8 [4.5 to 9]	0.013
5 min Apgar	9 [9 to 9]	9 [9 to 9]	9 [9 to 9]	0.788
Birthweight in grams, mean (SD)	3469.4 (627.38)	3992.9	3679.6 (805.3)	0.008
Birthweight > 4500 grams (%)	5.1%	(276.8) 30.8%	10.5%	0.001
Birthweight > 90 %ile for GA (%)	43.4%	84.6%	71.1%	0.0004
Female fetus (%)	55.7%	38.5%	44.2%	0.110

Notes: [a]calculated for $n = 189$ with available BMI data.

interobserver correlation of AWT measurements ($r = 0.838$; $p < 0.00001$). The strongest association with intrapartum complications was birthweight ($p = 0.003$): with birthweights > 4000 grams, the relative risk of shoulder dystocia or CS is 2.75 (95% CI 1.74-4.36; $p < 0.001$).

4. Discussion

Incidence of pregestational type 2 diabetes mellitus in pregnancy is steadily increasing across the world and along with it the associated antenatal and intrapartum complications. As evidenced by our study, the frequency of shoulder dystocia in our cohort of women with pregestational type 2 diabetes of 7.4% is much higher compared to that of the general obstetric population of 0.2-3.0% [9]. However, the risk of CS amongst women with pregestational type 2 diabetes was lower than the baseline population risk of CS at our center (17.6% versus 25.4%) [29]: this finding might be reflective of local practice patterns whereby pregnancies complicated by poorly controlled diabetes plus fetal macrosomia are routinely induced around 36-37 weeks' gestation due to concerns about stillbirth risk [1, 6, 10] (Figure 3). In addition to the lower CS rate, the overall risk of immediate newborn complications was also low in this cohort: fewer than 3% of newborns had a 5-minute Apgar less than 7 (incorporated as a proxy for fetal asphyxia), and there were no cases of

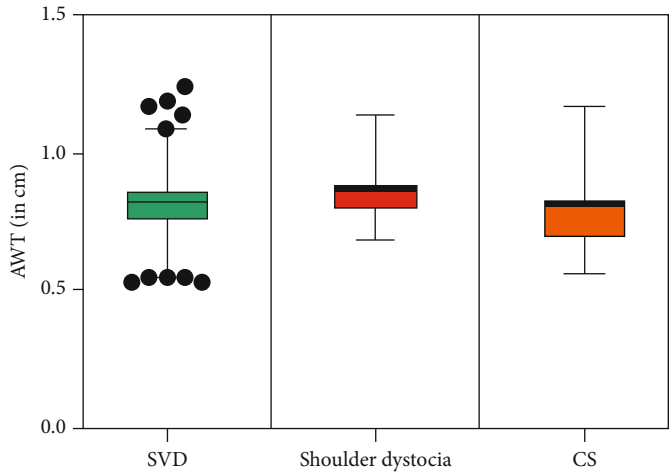

Figure 3: Abdominal wall thickness (AWT) by intrapartum outcome (spontaneous vaginal delivery (SVD), shoulder dystocia, and Caesarean section (CS)).

intrapartum birth injuries or fractures amongst these neonates. This particular pregnancy cohort (women with type 2 diabetes) was chosen as the study group of interest given the existing evidence regarding frequency of intrapartum complications and an assumption that if there was a true association between fetal AWT and shoulder dystocia or intrapartum complication, the best chance of finding a relationship would be in this restricted high-risk population: it also eliminated any possibility of bias that might occur when including women diagnosed with gestational diabetes who may in fact represent women with previously undiagnosed type 2 diabetes.

While our study results did not show any benefit of fetal AWT measurement at 36 weeks' gestation in the prediction of shoulder dystocia or emergency intrapartum Caesarean section, this again could be reflective of our local practice of inducing women with poorly controlled type 2 diabetes between 36 to 37 weeks' gestational age: most AWT measurements were taken within one week of delivery, but AWT might be more significant if taken upon admission to hospital in labor and/or if interpreted relative to other measurements of fetal biometry (i.e., head circumference) instead of as an isolated marker. Because this study is unable to determine if fetal AWT might be influential in centers without such a high frequency of late preterm inductions for women with poorly controlled pregestational type 2 diabetes, additional studies are needed to explore AWT and other potential ultrasound markers to predict risk or success of a vaginal delivery in pregnancies both with and without diabetes: concurrently, evaluation of policies regarding timing of induction of labor which directly compare the risk/benefits of late preterm delivery on stillbirth prevention versus neonatal sequelae is also needed to ensure optimal care for pregnant women with diabetes. There is heightened interest for use of intrapartum ultrasound particularly since the inception of new professional guidelines for use of ultrasound on the labor floor as well as individual studies which have highlighted the utility of ultrasound to evaluate likelihood of successful vaginal delivery [30–33]. In our cohort of patients with high rates of labor induction, the strongest relationship between intrapartum complications (shoulder dystocia or emergency intrapartum CS) remained birthweight. Those deliveries requiring emergency intrapartum CS tended to have lower rates of induction of labor compared to those resulting in spontaneous vaginal delivery, thus dispelling potential concerns about a risk of CS due to induction of labor which is consistent with the literature. It was notable that fetal ultrasound in our center tended to underdiagnose fetal macrosomia compared to postnatal diagnosis using birthweights over the 90th percentile for gestational age: this finding was consistent with another preliminary work by our team with a similar population and likely impacted by the high rates of morbid obesity in this group as well as the inherent limitations of fetal ultrasound to accurately predict newborn weight during late pregnancy [5, 15]. Diagnostic thresholds that use a cut-off of 4500 grams to designate macrosomia in the newborn are also likely to underestimate the frequency of fetal overgrowth in this population or for other populations where delivery before term is undertaken [10]. The need to explore improved models of estimated fetal weight or novel markers of fetal body composition, particularly amongst women with pregestational type 2 diabetes, is necessary to better refine risk prediction of intrapartum complications in this high-risk group [32, 34, 35].

The global diabetes epidemic closely parallels trends in rising obesity, and the rates of obesity in this study population cannot be understated: with almost 97% of pregnant women with pregestational type 2 diabetes in our cohort classified as overweight or obese at the time of delivery, enhanced efforts to improve preconceptional health and weight management as well as strategies to address appropriate weight gain during pregnancy are urgently needed. There is also evidence that the current COVID-19 pandemic, particularly the restrictions on daily activities, has further exacerbated problems of inactivity and weight gain in pregnancy [36]. Given what is known in the literature about the effects of multiparity on weight gain and likelihood of long-term obesity and health risks following postpartum weight retention, the fact

that two-thirds of mothers in our cohort were multiparas may have been contributory to our findings of high BMI [37–40]. In our study, women with higher BMIs were significantly more likely to have intrapartum complications (shoulder dystocia and emergency intrapartum CS). The increased risk of shoulder dystocia with maternal obesity is consistent with what is described in the literature, as is the heightened risk of CS: however, we are unable to determine with certainty if the frequency of emergency intrapartum CS in our population was exclusively driven by maternal obesity leading to intrapartum dystocia or failure of labor progress or if there is confounding by indication—could obstetricians have a lower threshold for recommending intrapartum CS earlier or more frequently in women with type 2 diabetes and high BMIs due to concerns about an inability to perform a crash CS if one became indicated? Both diabetes and obesity are associated with hypertensive disorders of pregnancy, and almost 1 in 5 women in our cohort had this complication of pregnancy as well. It was notable that there was only one case of preeclampsia diagnosed in this high-risk group; however, this might also reflect a potential impact of earlier induction of labor on reducing the development of preeclampsia in this high-risk group. In modern maternity care, strategies regarding appropriate weight gain and postpartum weight loss are counselled and managed at the individual patient level, although this study highlights the importance of considering broader public health policies to improve BMI amongst reproductive age women and particularly those with comorbidities such as diabetes [41–43]. With evidence that adherence to a Mediterranean diet during COVID-19 is protective against gestational diabetes during the pandemic and other virtual weight loss technologies are effective at supporting postpartum weight loss, these tools offer innovative solutions for mothers of young children and newborns, even through times of physical distancing and pandemic quarantines [44–46]. At a minimum, achievement of a healthy BMI for women with pregestational diabetes specifically will reduce diabetes-related morbidity in addition to improving perinatal outcomes by reducing intrapartum complications [39, 42, 43].

The relationship between fetal AWT and long-term health of offspring remains unknown. With evidence to support increasing prevalence and disease severity of type 2 diabetes with each successive generation affected [46–48], there is question as to whether or not a thicker fetal subcutaneous fat layer might represent an early marker of future metabolic disease. Overall, fetuses in our study had thicker subcutaneous fat layers than described in other studies (8.2 mm at 35 to 36 weeks versus 5.4 mm at 35 to 39 weeks in the Higgins study) [25]: this difference may be related to the restriction of our study population to only those mothers with confirmed pregestational type 2 diabetes or it may be a consequence of a poorer underlying maternal metabolic environment of mothers in our cohort including higher rates of morbid obesity. However, with an AWT of less than 4 mm proposed as the "normal" cut-off for fetuses between 36 and 38 weeks' gestational age, the subcutaneous fat thickness of offspring in this cohort remains considerably higher by comparison as well [23]. While there was not either an obvious relationship between fetal AWT and intrapartum asphyxia or birth trauma, we were underpowered to comment on these risks definitively given the rarity of these complications in our study population. Ongoing work is needed to elucidate any potential linkage between subcutaneous fat thickness in offspring and possible fetal origins of future metabolic disease, particularly given the worsening prevalence of childhood-onset diabetes in our health region and around the world [1, 47, 49, 50]: if a relationship between fetal AWT and long-term metabolic disease exists, this could offer considerable lead time and an opportunity for interventions to improve health and reduce chronic diseases in children exposed to maternal type 2 diabetes in utero.

Benefits of this study include a large sample size and incorporation of a novel fetal biometric measurement (AWT) using existing ultrasound images taken at the time of routine 36-week ultrasound. With excellent interobserver reliability, our study showed that fetal AWT measurement can easily and practically be incorporated at the time of third trimester ultrasound and using the standard images already obtained during measurement of the fetal abdominal circumference, without requiring any additional healthcare resources or costs. Since we restricted our study population to women with known, pregestational type 2 diabetes, we ensured a universal exposure of the entire study population: as previously mentioned, one risk of including all patients with diabetes in pregnancy without restriction is that it is difficult to know with certainty if women diagnosed with gestational diabetes have true hyperglycemia with onset only in pregnancy versus misclassified women with previously undiagnosed type 2 diabetes. As a retrospective cohort study, there are inherent limitations such as information and misclassification bias and missing data. We were also unable to evaluate the influence of individual-level glycemic control or ethnicity on fetal abdominal wall thickness. Future research is needed to evaluate the role of additional ultrasound predictors of intrapartum complications within the general obstetric population beyond diabetes, including fetal AWT at later gestational ages closer to delivery, and considering the relative influence of AWT combined with other fetal measurements (i.e., head circumference or biparietal diameter) for intrapartum risk stratification. The relationship between fetal AWT and long-term health of offspring exposed to maternal type 2 diabetes in utero also remains unknown.

5. Conclusions

There was no obvious benefit of adding fetal AWT measurement at 36 weeks for predicting shoulder dystocia or intrapartum CS in a population of women with pregestational type 2 diabetes in a setting where routine induction of labor is undertaken for those with poor glycemic control and high risk of stillbirth. The strongest predictor of intrapartum complication remains birthweight, and so studies evaluating improved methods for estimating fetal size (weight) and the role of intrapartum ultrasound for enhancing prediction of delivery complications are still needed. The potential

relationship between fetal AWT and long-term health in offspring also requires further investigation.

Data Availability

Data may be available upon reasonable request.

Disclosure

Preliminary findings were presented as a trainee poster abstract at the 2020 World Congress of the International Society of Ultrasound in Obstetrics and Gynecology.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Research Article

Adipocyte-Specific Fatty Acid-Binding Protein (AFABP) and Chemerin in Association with Gestational Diabetes: A Case-Control Study

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Background. Adipocytokines participate in regulating the inflammatory response in glucose homeostasis and type 2 diabetes. However, among these peptides, the role of adipocyte-specific fatty-acid-binding protein (AFABP), chemerin, and secreted protein acidic and rich in cysteine (SPARC) in gestational diabetes (GDM) has not been fully investigated. *Method*. The maternal fasting level of adipocytokines of 53 subjects with GDM and 43 normal pregnant (NGDM) was measured using multiplex immunoassay at 24–28 weeks, before delivery, immediate postpartum, and 2–6 months postpuerperium. *Results*. Higher levels of AFABP were associated with a 3.7-fold higher risk of GDM. Low chemerin levels were associated with a 3.6-fold higher risk of GDM. Interleukin-10 (IL-10) was inversely associated with the risk of GDM. SPARC had no association with GDM. AFABP was directly correlated to interleukin-6 ($r = 0.50$), insulin resistance index ($r = 0.26$), and body mass index ($r = 0.28$) and inversely correlated to C-reactive protein ($r = -0.27$). Chemerin levels were directly and strongly correlated with IL-10 ($r = 0.41$) and interleukin-4 ($r = 0.50$) and inversely correlated to insulin resistance index ($r = -0.23$) in GDM but not NGDM. In the longitudinal assessment, there were no significant differences in AFABP and chemerin concentrations of both studied groups. *Conclusion*. AFABP and chemerin were associated with a higher risk of GDM. These adipocytokines were related to insulin resistance, body mass index, and inflammation in pregnant women diagnosed with GDM.

1. Background

Adipose tissue plays a significant role in the pathophysiology and development of type 2 diabetes (T2DM) and gestational diabetes (GDM) [1–4]. GDM is a condition of abnormal maternal glucose tolerance that occurs for the first time in pregnancy and can be prevented by using insulin sensitizers [5]. GDM is associated with an increased risk for subsequent abnormal glucose tolerance later in life [6]. Adipose tissue is a metabolically dynamic tissue secreting adipocytokines (or adipokines) that possess endocrine and paracrine properties, which are involved in energy homeostasis, immune response and systemic inflammation, reproduction function, and blood pressure regulation [7]. Adipocytokines chemerin, AFABP (adipocyte-specific fatty acid-binding protein), interleukin-4 (IL-4), and interleukin-6 (IL-6) are enlisted in the regulation of insulin resistance and inflammation [8]. An increase in the level of several cytokines and in particular IL-6 has been reported in coronavirus disease-19 (COVID-19) infection and increased the risk of adverse prenatal outcomes such as gestational diabetes [9, 10]. The role of chemerin is a novel adipocytokine that plays a role in adipogenesis, energy metabolism, and inflammation [11]. Studies have shown that expression of both chemerin and its receptor was upregulated in fat tissue of animal model with obesity and T2DM, where its circulation was

considerably related to characteristics of metabolic syndrome and obesity (e.g., circulating triglycerides, blood pressure, body fat content, and insulin resistance) in normoglycemic individuals [12]. Chemerin elevation has been shown to lead to insulin resistance in *in vitro* studies of human myocytes and to glucose reduction in *in vivo* studies of animals in obese mice [13]. Furthermore, vitamin D deficiency has been associated with higher levels of circulating inflammatory marker chemerin and low insulin sensitivity [14] and increase the risk of GDM [15, 16]. The recent finding reported the protective effect of vitamin D3 supplementation on GDM via improving the antioxidant and inflammatory status and decreasing circulating chemerin level [17]. In serial measurements in early, mid, and late pregnancy, chemerin levels have been related to a noticeably increase in late pregnancy compared to early and midpregnancy stages [18]. However, there are conflicting results regarding chemerin circulation during GDM with its level being shown to be either elevated [19–22], unchanged [23–26], or reduced [27].

Secreted protein acidic and rich in cysteine (SPARC), a multifunctional matricellular peptide of 43 kDa, is related to the extracellular matrix and expressed largely in the basal lamina [28]. SPARC manages cell functions including cell adhesion, differentiation, and proliferation [29], where the protein has a broad range of biological effects [30]. SPARC is highly expressed in subcutaneous fat, and its production and excretion in adipose tissue are affected by fat mass, insulin, and glucose [31]. SPARC with profibrotic effects participate in metabolic dysregulation in obesity [31]. High SPARC expression in adipose tissue leads to insulin resistance [32]. Furthermore, higher levels of this peptide have been linked to T2DM, diabetic retinopathy, and nephropathy [32, 33]. In a recent cross-sectional study on normal pregnancy and GDM, SPARC levels were significantly correlated with inflammation and dyslipidemia [32]. This recent study showed that SPARC independently represented insulin resistance in late pregnancy, suggesting its possible role in the pathophysiology of GDM. AFABP belongs to the fatty acid-binding protein family which is highly expressed in adipose tissue [34]. AFABP is the largest cytosolic protein of mature adipocytes, accounting for roughly 6% of total cellular proteins [35]. This protein acts as a significant regulator of systemic insulin sensitivity and lipid and glucose metabolism [36–38], where AFABP concentrations are directly associated with indicators of metabolic syndrome and vascular disease [34]. High fasting AFABP serum levels have been shown to predict the risk of metabolic and vascular morbidity and mortality in T2DM [34, 39]. It has also been reported that AFABP secretion promotes insulin resistance in male mice, where AFABP mediates the degradation of peroxisome proliferator-activated receptors (PPARγ) in adipose tissue and consequently reduces the expression of insulin-sensitizing adiponectin [40]. AFABP has been correlated with insulin resistance and inflammation in T2DM and associated obesity [41]. Upregulation of AFABP in GDM mothers has been described in previous cross-sectional studies which may be linked with obesity and insulin resistance in pregnancy [42, 43]. A study on pregnant women has stated that fetal tissues are the main source of cord arterial serum AFABP [42]. The same study also suggests that in fetuses of pregnancy with GDM, AFABP values correlate with adiposity indicators [42].

To the best of the authors' knowledge, no study has defined the predictive value of chemerin, SPARC, and AFABP concentrations in the development of GDM. Therefore, this study has been designed to assess the association between the serum concentration of these adipocytokines and the development of GDM and to evaluate the circulation of these peptides throughout pregnancy and after delivery.

2. Method

The protocol of this study has been approved by the Scientific Review Committee of University of Malaya Medical Centre (UMMC) (reference number 1052.8, MEC ID 201402-0725), and written informed consent was obtained from all participants. The protocol of this study was published elsewhere [3, 4, 44]. Briefly, as the authors have described recently [3, 4, 44], pregnant women aged between 18 and 45 years, gestational age between 24 and 28 weeks, a singleton pregnancy, and intending to participate in our longitudinal study and delivery at UMMC were initially eligible to participate in this cohort study. Those women with multifetal pregnancy, history of pregestational diabetes or previous GDM, drug, smoke, and/or alcohol abuse, hypertension, heart disease, renal or liver disease, uncontrolled endocrine disease, or any medical conditions that would affect lipid and glucose metabolism were deemed not eligible to enter the study. At the end of the longitudinal study, pregnancy outcomes were abstracted by extracting information from medical records. Subjects from whom it was not possible to collect a fasting blood sample or those who developed any pregnancy complications such as preeclampsia, eclampsia, pregnancy-induced hypertension (PIH), preterm labour (<36 weeks), or post-term labour (>41 weeks and 6 days) were also excluded from the study. The maternal fasting samples were collected on four occasions:

Examination 1 (E1): 24–28 weeks of pregnancy (at the time of GDM screening).

Examination 2 (E2): prior to caesarean/vaginal delivery.

Examination 3 (E3): early postpartum; within 24 hours after delivery.

Examination 4 (E4): postpuerperium; within 2–6 months after delivery.

Screening for the diagnosis of GDM was performed by the 2-hour 75 g oral glucose tolerance test; fasting plasma glucose level greater than or equal to 5.6 mmol/L or 2-hour plasma glucose level greater than or equal to 7.8 mmol/L [45]. Serum levels of SPARC, chemerin, AFABP, IL-4, IL-6, IL-10, and CRP were measured using Magnetic Multiplex Sandwich ELISA assay (LXSAHM, R&D, USA). Based on the manufacturer's report the intra-inter-assay coefficient of variation (CV%) of the assays were as follows: adiponectin (5.6-9.2), chemerin (6.7-13.7), AFABP (4.7-13.8) and SPARC (5.0-12.0), IL-4 (6.3-10.27), IL-6 (5.2-9.6), IL-10 (5.4-10.73), and CRP [9–13]. Homeostasis model assessment index (HOMA-IR) was computed using the following formula: fasting serum glucose (mmol/L)/22.5 × fasting serum insulin

(mIU/L). Data regarding HOMA-IR has been reported previously [3].

2.1. Statistical Analysis. Descriptive statistics were applied for qualitative variables (mean ± standard error (SE)). The parametric Student's t-test or nonparametric Mann–Whitney U test was used to compare differences between two independent groups. Binary and multivariate logistic regression was performed to explore associations between the studied peptides and GDM risk (odds ratio (OR; 95% confidence interval (CI)). A paired sample t-test or two-related samples Wilcoxon test was performed to assess longitudinal changes between different time points (using Bonferroni correction). The Spearman/Pearson correlation coefficient described the correlation between peptides and metabolic markers. The p value of <0.05 was considered as statistically significant. All statistical analyses were performed using IBM SPSS 26.0.

3. Results

Ninety-six pregnant participants comprised of 53 who were diagnosed with GDM and 43 with normal pregnancy (NGDM) were recruited in this study. The demographic characteristics of the subjects are presented in Table 1. At the time of Examination 1, no significant differences were observed for participants' age ($p > 0.24$), pregestational BMI ($p = 0.40$), gestational BMI ($p > 0.88$), gestational weeks ($p = 0.72$), family history of diabetes ($p = 0.07$), and parity ($p = 0.88$) between both the GDM and NGDM groups. As expected, the GDM group presented a higher fasting blood glucose level ($p = 0.003$) and 2 hours postprandial glucose tolerance test ($p = 0.006$), as compared to NGDM subjects. Those diagnosed with GDM presented lower IL-10 ($p < 0.001$) and IL-4 ($p = 0.04$) compared to the normal group. No significant difference was observed in the level of IL-6 and CRP.

Using logistic regression, there was no association between SPARC, IL-4, IL-6, and CRP concentration and GDM risk (Table 2). IL-10 was inversely associated with the risk of GDM (0.18 (95% CI: 0.07-0.45)). Serum AFABP was directly associated with GDM risk. Participants with the higher levels of AFABP (>10.09 ng/mL) had a 3.7-fold higher risk of developing GDM compared with the lowest level. However, this relationship was attenuated but remained significant after adjustment for confounders including maternal age, gestational weeks, and BMI (aOR 1.1, 95% CI: 1.00-1.22). Serum chemerin (OR 0.85 (95% CI: 0.73-0.98)) was inversely associated with GDM. In the crude analysis, participants with chemerin (<8.03 ng/mL) presented a 3.6-fold higher risk of GDM compared to participants with the >10.2 ng/mL (95% CI: 1.3-10.4). After adjustment for maternal age, gestational age, and BMI, the lowest tertile of the chemerin value remained a strong predictor for the diagnosis of GDM (aOR 4.5; 95% CI: 1.4-14.0) (Table 2).

Using Spearman/Pearson correlation coefficient, AFABP was directly correlated to IL-6 ($r = 0.50$), HOMA-IR ($r = 0.26$), BMI ($r = 0.28$), and CRP ($r = 0.27$). Serum chemerin level was directly and strongly correlated with IL-10 ($r = 0.41$) and IL-4 ($r = 0.50$) and inversely correlated to HOMA-IR ($r = -0.23$) in GDM but not NGDM.

Table 1: Demographic characteristics of subjects, mean (SE).

	GDM	NGDM
Participants' age (year)	33.2 (0.6)	32.1 (0.8)
Gestational week	25.8 (0.2)	25.9 (0.2)
Pregestation BMI (kg/m^2)	27.0 (0.8)	25.2 (0.7)
FBG (mmol/L)	5.0 (0.2)	4.2 (0.1)*
2 hrs OGTT (mmol/L)	10.8 (1.5)	5.9 (0.1)*
IL-10 (pg/mL)	1.2 (0.10)	4.0 (0.32)*
IL-4 (pg/mL)	6.5 (0.31)	8.8 (1.67)*
IL-6 (pg/mL)	2.8 (0.41)	2.6 (0.34)
CRP (ng/mL)	2.8 (0.86)	2.8 (0.33)

*p value < 0.05 significant difference between the two pregnancy groups.

The intra- and intergroup comparisons of adipocytokines are shown in Table 3. Over the pregnancy and postpuerperium, no significant difference was observed in the SPARC level between both studied groups. Levels of AFABP concentration were high in GDM just in E1 ($p = 0.04$) compared to NGDM. AFABP was in the lowest level in the first examination; however, with advancement in gestational age, its levels increased ($p < 0.0001$) and reached a peak in late pregnancy in both GDM and NGDM. Immediately after delivery, the level of AFABP decreased ($p < 0.002$) and this reduction continued slightly in postpuerperium. Serum chemerin was significantly low in GDM compared to NGDM (E1: $p = 0.02$). There was no significant difference between chemerin concentration of GDM and NGDM groups in late pregnancy and after delivery. In the longitudinal assessment, chemerin levels of the normal pregnant group decreased with pregnancy development ($p < 0.05$). This reduction continued slightly and reached to its lowest level in postpuerperium (E4). In contrast, there were no significant changes in chemerin concentration of GDM with progress in gestational age. However, its concentrations reduced slightly ($p > 0.05$) in E3 and reached to its lowest level ($p < 0.05$) in E4.

4. Discussion

In this study, we showed that higher circulation of fasting serum AFABP concentrations in the second trimester, at the time of GDM screening, was associated with an increased risk for the development of GDM. When we categorized AFABP concentration to the four quartiles, we noticed that participants of upper quartile with the AFABP level higher than 10.09 ng/mL were at risk of GDM about 3.7-fold higher than pregnant women in the lowest quartile (AFABP < 4.90 ng/mL). This relationship is attenuated and reduced to about 1.1 but remained significant when we adjusted for confounders including maternal age, gestational week, and BMI. Similarly, a study on pregnant women in their first trimester of pregnancy reported an association of GDM risk with higher quartile AFABP concentration [42, 43, 46]. AFABP is highly expressed in adipocytes and contributes to insulin sensitivity and energy metabolism. We

Table 2: Association between adipocytokines and GDM risk (24-28 weeks).

		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
IL-10 (pg/mL)		0.18 (0.07-0.45)*	0.17 (0.06-0.48)*
°p value		<0.001	0.001
IL-4 (pg/mL)		0.98 (0.96-1.01)	0.98 (0.95-1.00)
IL-6 (pg/mL)		1.03 (0.82-1.30)	1.03 (0.79-1.33)
CRP (ng/mL)		1.0 (1.0-1.01)	1.0 (1.0-1.01)
SPARC (μg/mL)		0.44 (0.16-1.20)	0.12 (0.16-1.24)
AFABP (ng/mL)			
Quartile 1	$X < 4.90$	Referent	Referent
Quartile 2	$4.90 < X < 7.57$	2.02 (0.62-6.55)	1.92 (0.55-6.67)
Quartile 3	$7.57 < X < 10.09$	0.93 (0.29-3.0)	0.92 (0.27-3.11)
Quartile 4	$X > 10.09$	3.68 (1.06-12.77)*	1.11 (1.00-1.22)*
p value		0.04	0.03
Chemerin (ng/mL)			
Tertile 1	<8.03	3.6 (1.3-10.4)	4.5 (1.4-14.0)
Tertile 2	$8.0 \leq X < 10.2$	1.2 (0.4-3.2)	1.1 (0.4-3.0)
Tertile 3	≥10.2	Referent	Referent
p value		<0.001*	0.001*

OR (95% CI) adjusted for maternal age, gestational age, and BMI. *p value < 0.05.

Table 3: Between- and within-group comparisons of adipocytokine.

	Examination (E1)	Examination (E2)	Examination (E3)	Examination (E4)
SPARC (μg/mL)				
GDM	1.23 (0.07)	1.09 (0.06)[a]	1.08 (0.06)	1.03 (0.07)[a]
NGDM	1.37 (0.05)	1.11(0.05)[a]	1.06 (0.05)	1.00 (0.05)[a]
p value	0.11	0.80	0.80	0.68
AFABP (ng/mL)				
GDM	9.11 ± 0.69	13.57 ± 1.13[a]	12.47 ± 1.19[a,b]	10.87 (1.00)[b]
NGDM	7.25 ± 0.52	12.28 ± 0.91[a]	10.39 ± 0.79[a,b]	8.94 ± (0.68)[a,b]
p value	0.04	0.39	0.15	0.12
Chemerin (ng/mL)				
GDM	8.70 (0.46)	8.17 (0.38)	7.76 (0.32)	6.18 (0.35)[a,c]
NGDM	10.12 (0.35)	8.14 (0.34)[a]	7.74 (0.33)[a]	5.79 (0.25)[a,b,c]
p value	0.02	0.94	0.97	0.40

[a]p value < 0.05 compared to Examination 1. [b]p value < 0.05 compared to Examination 2. [c]p value < 0.05 compared to Examination 3. *p value < 0.05 compared to NGDM.

further observed that AFABP levels were positively associated with HOMA-IR and BMI. Similar to this finding, AFABP has recently been proposed as a marker of metabolic syndrome in nonpregnancy states [34, 47, 48]. In another study, higher AFABP concentration in GDM was also noted to be an independent risk factor for increased insulin resistance [49]. The role of AFABP in association with insulin resistance, inflammation, and obesity in T2DM has been noted previously [41, 50]. Similarly, in this study, correlations between AFABP level and IL-6 and CRP indicated a proinflammatory role of this adipokine in GDM.

In our longitudinal assessment, we observed that serum AFABP levels in both pregnant groups increased with gestational age and then decreased immediately after delivery. This study is the first to evaluate longitudinal changes in serum AFABP in women with and without GDM during and after their pregnancy period. However, we only found one other study that reported a significant increase in AFABP from the second to the third trimester of pregnancy [43]. Based on a report by Ortega-Senovilla et al. [51], AFABP concentration of cord blood was shown to be higher than maternal level, suggesting that fetal tissues are the main source of AFAPB in cord blood. Hence, increased levels of AFABP in correlation with advanced gestational age and its reduction immediately after delivery implies a significant role of fetal tissues in AFABP production.

In the present study, the second trimester of GDM pregnancy is linked to lower chemerin concentration than normal pregnant controls. Subsequently, we found that chemerin concentrations ≤ 8.0 ng/mL are associated with a 3.3-fold increment in GDM risk compared to levels ≥ 10.2 ng/mL. This result was relatively unchanged after adjusting for BMI and maternal and gestational age. Furthermore, we observed that the chemerin level of GDM was inversely and independently correlated to HOMA-IR. Pregnancy is a complex of metabolic changes in early pregnancy, followed by insulin resistance [52]. It has been determined that GDM develops in the presence of the inability of pancreatic β-cells to induce sufficient insulin secretion and counteract insulin insensitivity of tissues during pregnancy [21]. An animal study has shown that chemerin and its receptors are substantially expressed in β-cells which are necessary for insulin secretion *in vitro* and *in vivo* [53]. Subsequently, chemerin deficiency causes glucose intolerance mainly due to increased hepatic glucose production and impaired insulin secretion [53]. Hence, a reduction in chemerin levels is associated with the development of GDM through decreased insulin sensitivity and attenuated anti-inflammatory capacity [21]. Similar to our study, Hare et al. [27] proposed that low chemerin levels in GDM may lead to insulin resistance and a higher level in normal pregnancy, which may provide a protective effect to decrease pregnancy-induced insulin resistance. Serum levels of chemerin may also be influenced by multiple factors in relation to inflammation and/or metabolic states related to obesity. Lower chemerin level observed in GDM subjects in the current study was formed in correlation with a reduction in IL-10 level. IL-10, an anti-inflammatory cytokine and an immunosuppressive, has been discussed as a key regulator for inflammatory cytokines. Moreli et al. [54], in a study on pregnant women, showed that glycemic mean ≥ 100 mg/dL is associated with a reduction in maternal IL-10 concentrations. Vitamin D level has been associated with circulating inflammatory markers and chemerin and low insulin sensitivity [14] and increase the risk of GDM [15, 16]. The protective effect of vitamin D3 supplementation on GDM through improving the antioxidant and inflammatory status and decreasing circulating chemerin level has been reported recently. In this study, as a limitation, we did not measure the level of vitamin. More studies are warranted to elucidate the relationship between vitamin D level and the inflammatory cytokines specially chemerin in GDM.

Interestingly, we also observed that pregnancy was associated with peak chemerin levels at 24-28 weeks of gestation. When pregnancy progressed, the differences between chemerin levels in both pregnancy groups diminished as serum chemerin levels in women with normal pregnancy decreased significantly, and the corresponding levels in GDM subjects remained relatively unchanged. We further noted an inverse independent correlation between chemerin levels and insulin resistance irrespective of maternal BMI levels. Our finding is in line with previous human and animal studies which revealed that chemerin levels are significantly reduced in circulating serum throughout pregnancy. This reduction has been shown to be inversely associated with a general increase in insulin resistance during gestation [27, 55]. Therefore, unchanged levels of chemerin in GDM subjects in the present study may be due to appropriate managing of disease with diet and/or insulin therapy. This may also explain the comparable serum chemerin levels and normal glucose tolerance in late pregnancy observed in subjects with GDM. In addition, during pregnancy, adipocytokines are produced not only by adipose tissue but also by the placenta [56]. An animal study by Garces et al. [55] has shown that the expression of chemerin mRNA was at its highest level at day 16. This subsequently decreased significantly towards the end of the pregnancy, hence describing an anti-inflammatory environment. Their study also indicated that reductions in levels of chemerin in both the placenta and maternal circulation may be necessary for adequate maternal-fetal immune interaction, essential for the normal progress of pregnancy. Within 24 hours after delivery, chemerin levels insignificantly reduced and remained statistically unchanged in both studied groups. However, there was a significant reduction in chemerin levels postpuerperium in the presence of normal glucose tolerance reestablishment, which challenge the probable role of chemerin in the progression of T2DM after pregnancy.

In summary, the findings of this study suggest that AFABP and chemerin are associated with increased GDM risk, with AFABP and chemerin levels being related to insulin resistance, BMI, and inflammation in women diagnosed with GDM. However, in the nonpregnant state, these peptides had no further contribution to the development of metabolic syndrome when glucose tolerance was achieved. Additional studies with a large sample size are desired to confirm the findings of this study.

Data Availability

Data are available from the authors upon reasonable request.

Conflicts of Interest

There is no conflict of interest.

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Research Article

Predictive Value of First-Trimester Glycosylated Hemoglobin Levels in Gestational Diabetes Mellitus: A Chinese Population Cohort Study

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This study was aimed at exploring the predictive value of first-trimester glycosylated hemoglobin (HbA1c) levels in the diagnosis of gestational diabetes mellitus (GDM). A total of 744 pregnant women registered at the Peking University International Hospital between March 2017 and March 2019 were included in this study. Data on personal characteristics and biochemical indicators of the pregnant women were collected during the first trimester. The International Association of Diabetes and Pregnancy Study Groups has adopted specific diagnostic criteria as the gold standard for the diagnosis of GDM. Receiver operating characteristic (ROC) curve statistics were used to assess the predictive value of first-trimester HbA1c levels in the diagnosis of GDM. HbA1c levels in the first trimester were significantly higher in the GDM group than in the non-GDM group (5.23% ± 0.29% vs. 5.06 ± 0.28%, $P < 0.05$). The first-trimester HbA1c level was an independent risk factor for gestational diabetes. The area under the ROC curve (AUC) of HbA1c for GDM was 0.655 (95% confidence interval 0.620-0.689, $P < 0.001$). The positive likelihood ratio was the highest at HbA1c = 5.9%, sensitivity was 2.78, and specificity was 99.83%. There was no statistical difference in AUC between fasting blood glucose and HbA1c ($P = 0.407$). First-trimester HbA1c levels can be used to predict GDM. The risk of GDM was significantly increased in pregnant women with first-trimester HbA1c levels > 5.9%. There was no statistical difference between first-trimester HbA1c and fasting blood glucose levels in predicting GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is defined as abnormal glucose tolerance with onset or first recognition during pregnancy; however, blood glucose levels in cases of GDM do not reach those indicating obvious diabetes mellitus [1]. With the current global coronavirus disease 2019 (COVID-19) pandemic, local lockdowns have induced an unhealthy diet, physical inactivity, and increased psychological stress [2]. That is an even greater challenge for GDM management. Although pregnant women with GDM followed up as usual during the COVID-19 pandemic lockdown, their diabetes control was lower, with a higher rate of insulin therapy [3]. Pregnant women with GDM have an increased risk of developing preeclampsia, increased rates of cesarean sections, and an increased risk of macrosomia [4]. In addition, pregnant women with GDM have a significantly increased risk of developing type 2 diabetes mellitus later in life [5, 6]. There is a critical period for fetal organ development in the early stages of pregnancy. Abnormal glucose metabolism during this period can result in organ malformation in the developing fetus [7]. Therefore, early screening for GDM is critical. The first-trimester HbA1c level is a reliable predictor of complications during pregnancy, including preeclampsia, fetal macrosomia, and large for gestational age birth weight [8]. Fasting blood glucose (FBG) is used as an early screening tool for gestational diabetes. However, FBG requires fasting, and as FBG has great variability and poor repeatability, it is not effective in the early screening for GDM. Measuring glycosylated hemoglobin (HbA1c) levels has several advantages over

measuring FBG levels [9]: it is more convenient as fasting is not required and more stable and is subject to fewer day-to-day variations due to stress or illness. HbA1c has been widely used in the diagnosis and management of diabetes patients, but its use in the diagnosis of gestational diabetes remains controversial as HbA1c levels fall during the first trimester [10]. This study was aimed at exploring the value of first-trimester HbA1c levels in predicting GDM.

2. Materials and Methods

2.1. Participants. This was a prospective cohort study. A total of 744 pregnant women registered at the Peking University International Hospital in China between March 2017 and March 2019 were included in this study. Inclusion criteria were as follows: pregnant women aged 19–45, resident in Beijing for more than 5 years and registered at this hospital, pregnancy confirmed by ultrasound or blood human chorionic gonadotropin test, and available data on first-trimester HbA1c levels. Exclusion criteria were as follows: absence of HbA1c and routine blood tests in the first trimester; absence of height and/or weight data in the first trimester; a history of prepregnancy diabetes or impaired glucose tolerance; abortion; twin or multiple births; anemia; personal or family history of thyroid disease; use of oral contraceptives or any other drug that may affect thyroid function; and presence of Hashimoto's disease, chronic autoimmune disease malignant tumors, or blood diseases.

The study was approved by the Biomedical Ethics Committee of the Peking University International Hospital (2016-015, 20160710) (2017-021, 20170608). Participants selected for the study gave their informed consent in writing before enrollment.

2.2. Methods. In this study, 744 pregnant women were included for follow-up during pregnancy. All participants underwent blood tests in their first trimester, including evaluation of the red blood cell (RBC) count and hemoglobin (Hb), HbA1c, FBG, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine (Cr), uric acid (UA), thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), total triiodothyronine (TT3), and total thyroxine (TT4) levels. Gestational age was confirmed on the basis of the self-reported date of the last menstrual period or by ultrasound. The nurse recorded each participant's age, number of deliveries, blood pressure, height, and weight. Participants received routine antenatal care throughout their pregnancies, and all participants were screened for gestational diabetes using a 75 g oral glucose tolerance test between 24 and 28 weeks of pregnancy.

2.3. Diagnostic Criteria for GDM. GDM was diagnosed using the IADPSG diagnostic criteria [11], which involves a 75 g oral glucose tolerance test (OGTT). GDM is excluded on the basis of FBG < 5.1 mmol/l, blood glucose 1 hour after glucose load < 10.0 mmol/l, and blood glucose 2 hours after glucose load < 8.5 mmol/l. GDM may be diagnosed if any blood glucose level reaches or exceeds the above limits. These diagnostic criteria were recommended by the American Diabetes Association (ADA) [12] and the Chinese Diabetes Association [13]. The diagnostic criteria for diabetes mellitus were adopted by the World Health Organization in 1999. Prepregnancy diabetes is defined as type 1 diabetes mellitus, type 2 diabetes mellitus, or a special type of diabetes diagnosed before pregnancy.

HbA1c was detected using a G8 automatic HbA1c analyzer with high-performance liquid chromatography. Thyroid function was determined using the Roche COBASE601 automatic electrochemical luminescence method.

2.4. Statistical Analysis. Data analysis was performed using SPSS 23.0. The Kolmogorov–Smirnov test (K–S test) was used to test the normality of distribution, and measurement data were represented as $\overline{x} \pm s$. An independent sample t -test was used for comparison between the two groups according to normal distribution. The rank sum test was used to compare the two groups that did not conform to a normal distribution. Categorical variables were analyzed using the χ^2 test. Logistic regression analysis was used to analyze the risk factors for GDM. MedCalc statistical software was used to analyze the receiver operating characteristic (ROC) curve of HbA1c in diagnosing GDM. The areas under the three ROC curves of FBG, HbA1c, the combination of FBG and HbA1c were compared. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Comparison of General Clinical Data between the GDM Group and the Non-GDM Group. All 744 participants underwent a 75 g OGTT during the second trimester. Among them, 144 participants were diagnosed as having GDM, and 600 participants had normal blood glucose levels. The prevalence of GDM was 19.7%. The average age of the participants diagnosed with GDM was higher than that of the participants with normal blood glucose levels (32.76 years ± 3.91 years vs. 30.62 years ± 3.64 years, $P < 0.05$). First-trimester HbA1c levels were significantly higher in the GDM group than in the non-GDM group (5.23% ± 0.29% vs. 5.06% ± 0.28%, $P < 0.05$). The FBG level in the GDM group was higher than that in the non-GDM group (5.05 mmol/l ± 0.44 mmol/l vs. 4.88 mmol/l ± 0.34 mmol/l, $P < 0.05$). The incidence of gestational diabetes in multiparous participants was 26.4%, which was significantly higher than that in the primiparous participants ($P < 0.05$). Triglyceride, cholesterol, and UA levels in the GDM group were also significantly higher than those in the non-GDM group ($P < 0.05$). There were no statistically significant differences in TSH, TT3, TT4, FT3, FT4, or Cr between the two groups (Table 1).

3.2. Independent Risk Factors for GDM. As shown in Table 2, logistic regression analysis was performed with GDM as the dependent variable and age; BMI ≥ 24 kg/m^2 (dichotomous variable); parity; and first-trimester HbA1c, TC, TG, LDLC, HDLC, TSH, TT3, TT4, FT3, and FT4 levels as independent variables. The results showed that age, BMI ≥ 24 kg/m^2, first-

Table 1: Comparison of general clinical data between the GDM group and the NGDM group ($\bar{x} \pm s$).

	GDM	NGDM	P
n	144 (19.4%)	600 (80.6%)	
Age (year)	32.76 ± 3.91	30.62 ± 3.64	<0.05
Gestational week (weeks)	8.49 ± 2.15	8.66 ± 2.10	0.394
Parity			
Primiparity	72 (15.3%)	399 (84.7%)	<0.05
Multiparity	72 (26.4%)	201 (73.6%)	
SBP (mmHg)	109.63 ± 12.50	109.50 ± 11.43	0.732
DBP (mmHg)	66.14 ± 11.80	65.55 ± 10.83	0.648
BMI (kg/m^2)	22.32 ± 2.87	21.63 ± 2.77	<0.05
BMI < 24 kg/m^2, n/N%	92 (15.6%)	497 (84.4%)	<0.05
BMI ≥ 24 kg/m^2, n/N%	52 (33.5%)	103 (66.5%)	
HB (g/l)	130.92 ± 8.53	130.74 ± 10.76	0.785
HbA1c (%)	5.23 ± 0.29	5.06 ± 0.28	<0.05
FBG (mmol/l)	5.05 ± 0.44	4.88 ± 0.34	<0.05
Cr (mmol/l)	48.34 ± 7.60	48.78 ± 6.96	0.871
UA (mmol/l)	266.07 ± 50.71	213.07 ± 49.38	<0.05
TC (mmol/l)	4.09 ± 0.90	3.93 ± 0.65	<0.05
TG (mmol/l)	1.11 ± 0.77	0.98 ± 0.582	<0.05
LDLC (mmol/l)	2.12 ± 0.55	2.04 ± 0.53	0.155
HDLC (mmol/l)	1.50 ± 0.81	1.42 ± 0.25	0.868
TSH (μIU/ml)	1.91 ± 1.25	2.05 ± 6.74	0.228
FT4 (pmol/l)	16.79 ± 2.27	17.13 ± 2.96	0.317
FT3 (pmol/l)	4.71 ± 0.46	4.77 ± 1.61	0.822
TT4 (nmol/l)	122.70 ± 23.91	121.75 ± 23.88	0.945
TT3 (nmol/l)	2.08 ± 0.40	2.05 ± 0.45	0.393
OGTT			
FBG (mmol/l)	4.89 ± 0.55	4.48 ± 0.36	<0.05
1hBG (mmol/l)	9.67 ± 1.56	7.30 ± 1.34	<0.05
2hBG (mmol/l)	8.40 ± 1.66	6.64 ± 0.98	<0.05

Abbreviations: BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; HbA1c: glycosylated hemoglobin; Cr: creatinine; UA: uric acid; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TSH: thyroid stimulating hormone; FT3: free triiodothyronine; FT4: free thyroxine; TT3: total triiodothyronine; TT4: total thyroxine.

trimester HbA1c level, and FBG level were independent risk factors for GDM.

3.3. ROC Curve of First-Trimester HbA1c Level for Predicting GDM. As shown in Figure 1, the AUC of the first-trimester HbA1c level in the diagnosis of GDM was 0.655 (95% confidence interval, 0.620–0.689), $P < 0.001$. When the HbA1c level was 5.3%, the Jorden index was the highest, and the sensitivity and specificity of the diagnosis of GDM were 33.33% and 89.67%, respectively. When the HbA1c level was 5.9%, the positive likelihood ratio was the highest at 16.35, and the sensitivity and specificity for diagnosing GDM were 2.78% and 99.83%, respectively. When HbA1c was 4.4%, the negative likelihood was the lowest, sensitivity was 100%, and specificity was 1.67%, as shown in Table 3.

Table 2: Logistic regression analysis of influencing factors of GDM.

	B	SE	P	OR	95% CI
HbA1c	1.756	0.408	<0.05	5.787	2.601-12.879
Age	0.072	0.030	<0.05	1.075	1.014-1.140
Parity	0.311	0.221	0.159	1.364	0.885-2.103
BMI ≥ 24 kg/m^2	0.115	0.035	<0.05	1.122	1.047-1.202
FBG	0.888	0.283	<0.05	2.431	1.395-4.236
UA	0.003	0.002	0.180	1.003	0.999-1.007
TC	0.144	0.149	0.334	1.155	0.862-1.546
TG	-0.097	0.167	0.559	0.907	0.654-1.258
Constant	-21.19	2.52	<0.05		

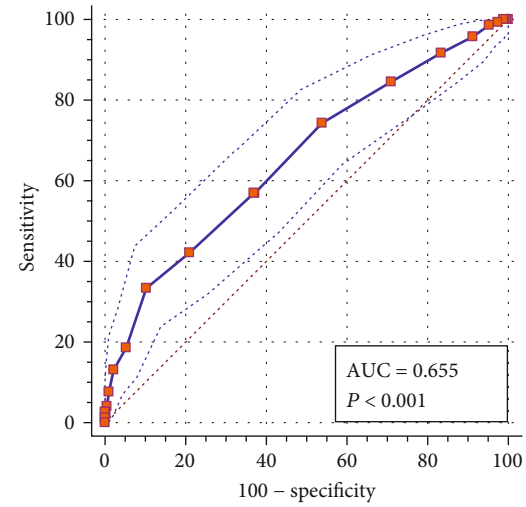

Figure 1: The ROC curve of first-trimester HbA1c level in the diagnosis of GDM in pregnancy.

3.4. Comparisons between the AUCs of HbA1c and FBG. The AUC of the FBG level for the diagnosis of GDM was 0.625 (95% confidence interval, 0.589–0.660, $P < 0.001$). There was no statistical difference in the AUCs between FBG and HbA1c levels ($P = 0.407$). The AUC of the combination of FBG and HbA1c levels for the diagnosis GDM was 0.677 (95% confidence interval, 0.642–0.71, $P < 0.001$). The AUC was not significantly different between the single HbA1c level and the FBG and HbA1c ($P = 0.145$) levels combined, as shown in Figure 2.

4. Discussion

GDM may increase adverse outcomes such as hypoglycemia, fetal death, neonatal respiratory distress syndrome, and giant shoulder dystocia [14, 15]. The rate of cesarean section increases in pregnant women with GDM. The rate of cesarean section does not decrease even when labor is actively induced at 38 weeks' gestation [16]. GDM may increase the risk of type 2 diabetes in both the mother and her child

Table 3: ROC curve values of first-trimester Hba1c in the diagnosis of GDM.

HbA1c	Sensibility (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)
>4.4	100 (97.5-100.0)	1.17 (0.5-2.4)	1.01 (1.0-1.0)	0
>4.5	99.31 (96.2-100.0)	2.67 (1.5-4.3)	1.02 (1.0-1.0)	0.26 (0.03-1.9)
>4.6	98.61 (95.1-99.8)	4.83 (3.3-6.9)	1.04 (1.0-1.1)	0.29 (0.07-1.2)
>4.7	95.83 (91.2-98.5)	8.83 (6.7-11.4)	1.05 (1.0-1.1)	0.47 (0.2-1.1)
>4.8	91.67 (85.9-95.6)	16.67 (13.8-19.9)	1.1 (1.0-1.2)	0.5 (0.3-0.9)
>4.9	84.72 (77.8-90.2)	29 (25.4-32.8)	1.19 (1.1-1.3)	0.53 (0.4-0.8)
>5	74.31 (66.4-81.2)	46.17 (42.1-50.2)	1.38 (1.2-1.6)	0.56 (0.4-0.7)
>5.1	56.94 (48.4-65.2)	62.83 (58.8-66.7)	1.53 (1.3-1.8)	0.69 (0.6-0.8)
>5.12	56.94 (48.4-65.2)	63 (59.0-66.9)	1.54 (1.3-1.8)	0.68 (0.6-0.8)
>5.2	42.36 (34.2-50.9)	78.83 (75.3-82.0)	2 (1.6-2.6)	0.73 (0.6-0.8)
>5.3	33.33 (25.7-41.7)	89.67 (86.9-92.0)	3.23 (2.3-4.5)	0.74 (0.7-0.8)
>5.4	18.75 (12.7-26.1)	94.67 (92.6-96.3)	3.52 (2.2-5.7)	0.86 (0.8-0.9)
>5.5	13.19 (8.1-19.8)	97.67 (96.1-98.7)	5.65 (2.9-11.0)	0.89 (0.8-0.9)
>5.6	7.64 (3.9-13.3)	98.83 (97.6-99.5)	6.55 (2.6-16.6)	0.93 (0.9-1.0)
>5.7	4.17 (1.5-8.8)	99.5 (98.5-99.9)	8.33 (2.1-32.9)	0.96 (0.9-1.0)
>5.8	2.78 (0.8-7.0)	99.67 (98.8-100.0)	8.33 (1.5-45.1)	0.98 (0.9-1.0)
>5.9	2.78 (0.8-7.0)	99.83 (99.1-100.0)	16.67 (1.9-148.0)	0.97 (0.9-1.0)
>6	1.39 (0.2-4.9)	99.83 (99.1-100.0)	8.33 (0.8-91.3)	0.99 (1.0-1.0)
>6.1	0 (0.0-2.5)	100 (99.4-100.0)		1 (1.0-1.0)

Note: NLR: likelihood ratio; PLR: positive likelihood ratio.

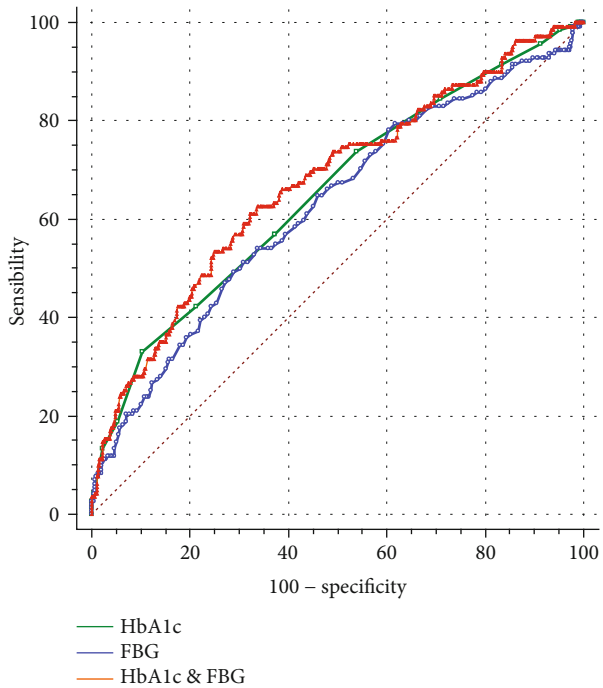

Figure 2: Comparison of the ROC curves of HbA1c, FBG, and the two combined indexes.

[17]. The results of the Hyperglycemia and Adverse Pregnancy Outcome study showed that maternal blood glucose levels were continuously associated with increased birth weight, increased cord blood serum C-peptide levels, and perinatal complications, without corresponding blood glucose turning points [14]. Lifestyle interventions before 20 weeks' gestation in pregnant women at high risk of GDM can reduce the complications of GDM [18]. Therefore, early identification and active management of labor are particularly important in reducing the adverse outcomes of GDM.

Early predictors of GDM include blood glucose indicators, inflammatory markers, insulin resistance indicators, and adipocyte factors [19]; however, the latter has not been widely used in clinical practice. Blood glucose and glycosylated hemoglobin are the most commonly used indicators in clinical practice. The American College of Obstetricians and Gynecologists (ACOG) recommends a two-step GDM screening beginning with the 50 g oral glucose challenge test (OGCT), whereas the ADA recommends 75 g OGTT one-step or two-step screening for GDM [20]. A 50 g OGCT can also be used to predict delivery weight for gestational age [21, 22]. HbA1c reflects the three-month average blood glucose level, which has low individual variability and cannot be affected by time, diet, emotion, and stress responses. However, HbA1c is not recommended for the diagnosis of GDM. This study was aimed at exploring the predictive value of first-trimester HbA1c levels in patients with GDM. Although some studies have discussed the relationship between HbA1c and GDM in early pregnancy, most of these have used the two-step method as the diagnostic standard for GDM [23, 24]. In this study, a one-step method was used as the gold standard for diagnosing GDM. This study found that first-trimester HbA1c levels can be used to predict the occurrence of GDM.

This study showed that the prevalence of GDM was 19.4% and identified maternal age and BMI as risk factors for GDM. With each 1-year increase in age, the risk of

GDM increased by 7.5%. Furthermore, the results of this study showed that the incidence of GDM in primiparous women was significantly lower than that in multiparous women, with a statistically significant difference between the two groups. However, after adjusting for age, it was found that parity was not a risk factor for the occurrence of GDM, which might be related to the older age of multiparous women (33.09 years ± 3.45 years vs. 29.67 years ± 3.19 years, $P < 0.001$).

It was also found that the first-trimester HbA1c level was an independent risk factor for GDM. The higher the first-trimester HbA1c level, the greater the risk of GDM. This finding is consistent with previous research [23, 24]. The reliability of using first-trimester HbA1c levels to diagnose GDM was statistically significant, with a cutoff point of 5.3% ($P < 0.001$). O'Shea et al. [25] studied trimester-specific reference intervals for HbA1c in nondiabetic Caucasian pregnant women and found the normal pregnancy HbA1c-specific reference level to be 4.3%–5.4%, which approaches the 5.3% HbA1c cutoff point. However, if 5.3% HbA1c was used as the diagnostic cutoff point for GDM, the sensitivity was 33.33%. This low sensitivity results in a high rate of missed diagnoses of GDM. Some studies have also shown that the diagnosis of GDM using HbA1c levels in early pregnancy cannot be characterized by both high sensitivity and high specificity [26, 27].

Although first-trimester HbA1c levels cannot be used to diagnose GDM directly, the high specificity of the ROC curve is helpful in predicting the occurrence of GDM. On the basis of this study, the positive likelihood ratio was highest at 5.9% HbA1c in the first trimester, and the sensitivity and specificity of GDM diagnosis were 2.78% and 99.83%, respectively, as shown in Table 3. This indicates that the rate of false diagnosis of GDM was very low in pregnant women with HbA1c > 5.9%. For these women, GDM can be diagnosed in the first trimester without waiting for an OGTT in the second trimester. In Indian pregnant women, HbA1c ≥ 5.9% as the diagnostic cutoff point also showed a low sensitivity of 1.19% and a high specificity of 99.76%[26]. HbA1c ≥ 5.9% in early pregnancy is associated with an increased risk of adverse pregnancy outcomes [28, 29]. This indicates that lifestyle interventions for pregnant women with first-trimester HbA1c levels > 5.9% must be implemented as early as possible to reduce the likelihood of adverse pregnancy outcomes.

In this study, the negative likelihood ratio was the lowest at 4.4%, with sensitivity and specificity of 100% and 1.67%, respectively. This indicates that the risk of GDM at HbA1c < 4.4% was extremely low, and GDM can be excluded in pregnant women with first-trimester HbA1c < 4.4%. Nevertheless, this study showed that OGTT is still recommended for screening for gestational diabetes in pregnant women with HbA1c between 4.4% and 5.9%.

The results of this study showed that the AUC of HbA1c levels did not better predict GDM than did FBG levels and that the combination of FBG and HbA1c levels did not improve the AUC. This may be related to the pathophysiological mechanisms of GDM. The occurrence of GDM is affected by various factors. In contrast to the pathogenesis of diabetes, gestational diabetes is closely related to endocrine function, substance metabolism, and the transport function of the placenta [30]. In this study, HbA1c reflects blood glucose levels before pregnancy and during first-trimester pregnancy, when placental function is still immature. For this reason, the sensitivity of HbA1c in predicting GDM is poor.

This study is a self-sequenced longitudinal prospective study focusing on first-trimester HbA1c levels in China. This study has some limitations. First, HbA1c is associated with ethnicity, and therefore, this study is only representative of eastern Asian pregnant women. Second, the influence of genetic factors and a family history of diabetes on the development of GDM in the research participants is not reflected in this study, as records of family history of diabetes were not available.

5. Conclusions

In conclusion, first-trimester HbA1c levels show low sensitivity and high specificity in the diagnosis of GDM and thus have limited value in diagnosing GDM. However, HbA1c levels show good predictive value for GDM. GDM can be excluded in pregnant women with first-trimester HbA1c levels < 4.4%. However, the risk of GDM increases significantly in pregnant women with first-trimester HbA1c levels > 5.9%. Pregnant women with a first-trimester HbA1c level > 5.9% should be referred for lifestyle interventions in the first trimester to reduce the risk of developing GDM later in pregnancy.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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