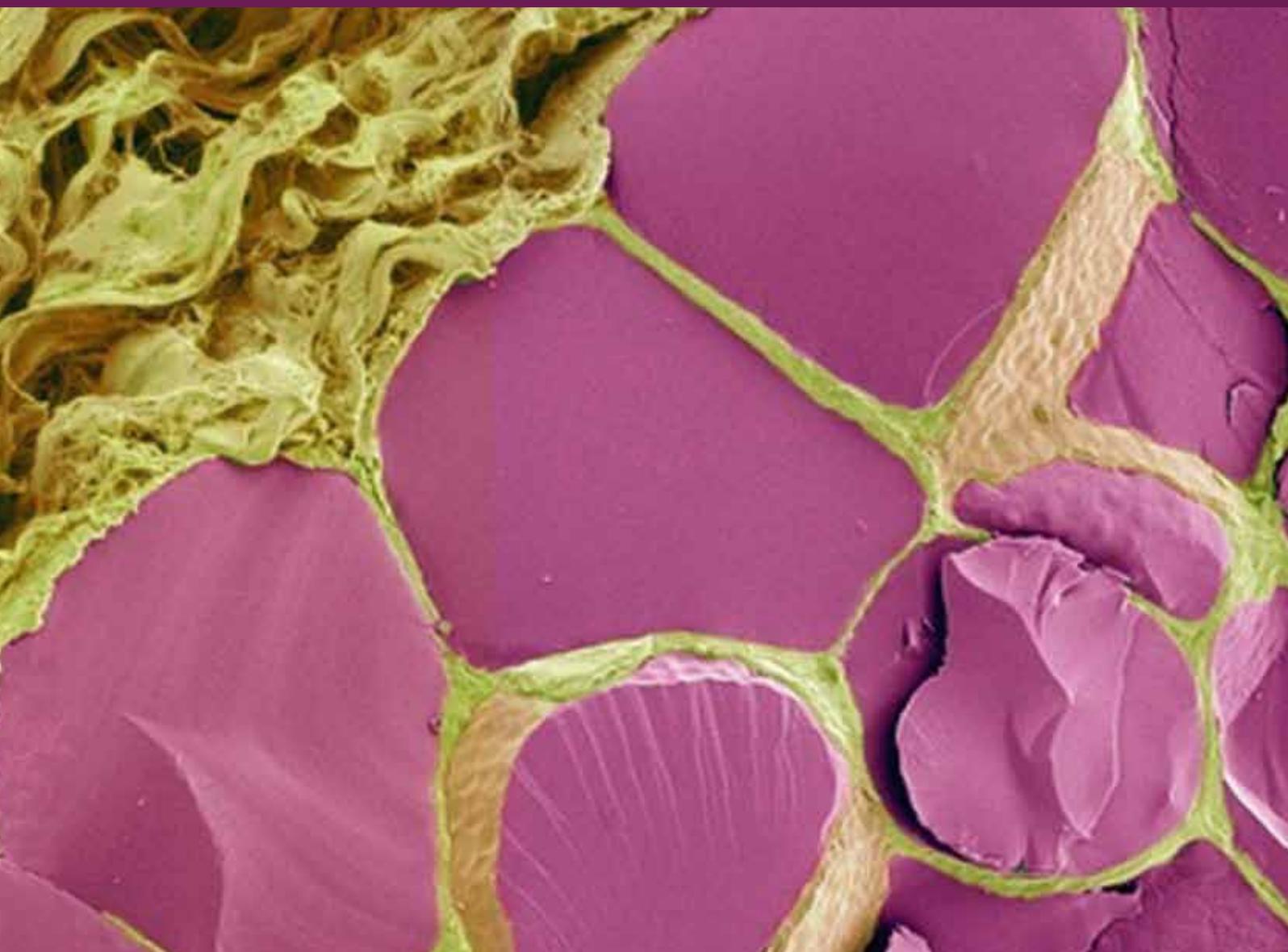


Characterization of Former Gestational Diabetes Mellitus: Prognostic, Therapeutic, and Predictive Aspects

Guest Editors: Andrea Tura, Alexandra Kautzky-Willer, Graziano Di Cianni, and Yariv Yogev





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Editorial

Characterization of Former Gestational Diabetes Mellitus: Prognostic, Therapeutic, and Predictive Aspects

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1. Introduction

This special issue was focused on original research and review articles regarding the pathophysiology of glucose and lipid metabolism in women with previous gestational diabetes mellitus, as well as the identification of early markers for the risk of developing type 2 diabetes and related cardiovascular complications in that category of women. In addition, the study of the metabolic condition during pregnancy, that is, during overt gestational diabetes, was also of interest for the special issue. Some reports on educational programs for behavior modification and improved awareness of the disease were also included. In fact, it is known that the risk of developing type 2 diabetes is increased when the condition of previous gestational diabetes is accompanied by other factors, which predispose to diabetes also in different categories of subjects, like improper diet habits, sedentary lifestyle, obesity, or weight gain. The following paragraphs briefly present the articles included in this special issue. In the first section, we summarize the articles related to basic studies of pathophysiology, with special focus on the relationships between metabolic, endocrine, and cardiovascular parameters. The second section refers to articles more directly related to the disease care, including possible approaches for prevention and for improved clinical care and home support. In the last section some review articles are reported.

2. Pathophysiology/Metabolic, Endocrine, Cardiovascular Interactions

The study by S. Farhan et al. investigated the possible relationships between fetuin-A and gestational diabetes. Fetuin-A is calcification inhibitor, also interacting with insulin receptor tyrosine kinase, thus possibly causing insulin resistance. In fact, in human studies fetuin-A was found to be related to insulin resistance and metabolic syndrome, and linked to incident diabetes mellitus. In the women with gestational diabetes analyzed by S. Farhan et al., significant relationships between fetuin-A and parameters of insulin sensitivity or metabolic control were not observed, but a strong relationship was found with the early postpartum body mass index.

The study by E. Vitacolonna et al. investigated the prevalence of thyroid dysfunction and autoimmunity in women with gestational diabetes. They report that increased incidence of organ-specific autoimmunity towards endocrine cells other than beta-cells was described in type 1 diabetes, but recently some studies also reported increased incidence of thyroid autoimmunity in type 2 diabetes, thus suggesting that diabetes can trigger the onset of the thyroid autoimmune disorder. However, few studies evaluated the prevalence of thyroid dysfunction and autoimmunity in women with gestational diabetes. E. Vitacolonna et al. found that maternal hyperglycemia is a risk factor for later development of

thyroid autoimmunity. In fact, during pregnancy they did not observe different prevalence in thyroid dysfunction and autoimmunity between gestational diabetic and normally pregnant women, but a significant increase in thyroid autoimmunity was seen after gestation in the women previously affected by gestational diabetes.

In the study by A. Sokup et al., the hypothesis that in women with a history of gestational diabetes, early endothelial dysfunction (2 to 24 months after delivery) is associated with the development of glucose dysregulation and other atherosclerosis risk factors was explored. A. Sokup et al. analyzed several parameters, with special focus to some parameters of endothelial dysfunction, such as the concentration of soluble E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1). They found that soluble VCAM-1 and some parameters of atherogenic dyslipidemia were associated with previous gestational diabetes independently of body mass index, insulin resistance, and fasting glucose, whereas soluble E-selectin correlated with metabolic syndrome components, but it was not independently associated with abnormal glucose regulation. They concluded that the atherogenic dyslipidemia may be a key metabolic predictor of cardiovascular risk in women with previous gestational diabetes.

The study by A. Ghio et al. aimed to assess if changes in insulin action and secretion during pregnancy are related to 1-hour plasma glucose concentration during an oral glucose tolerance test. According to the new international criteria of gestational diabetes (IADPSG criteria) each glucose value, that is, fasting, 1 hour, and 2 hours are equally but independently important and may present different pathophysiologic conditions. A. Ghio et al. found that for each 20 mg/dL increase in 1-hour plasma glucose concentration there was a concomitant impairment in insulin action and reduction in insulin secretion. In the authors' opinion, the alterations in insulin action and secretion are more apparent for elevations of the 1-hour plasma glucose than for other time samples of the oral glucose tolerance test. Thus, it is suggested that 1-hour value may provide a better parameter for risk stratification in pregnant women.

In the study by G. Pacini et al., the incretin effect was assessed in a group of women with a history of gestational diabetes who underwent both intravenous and oral glucose tolerance test immediately after partum. Since it is known that the incretin hormones contribute to insulin secretion after oral administration of glucose, the incretin effect was assessed by comparing in each individual the possible differences in beta-cell function between intravenous and oral glucose tolerance tests. G. Pacini et al. found that normotolerant women with previous gestational diabetes exhibit an incretin effect similar to that of healthy women, who had a normal pregnancy. At contrast, compromised incretin effect, proper of obese and type 2 diabetic subjects, characterizes women with previous gestational diabetes and current condition of impaired glucose tolerance. Prevention studies in women with a history of gestational diabetes using incretin-based therapies will clarify if these drugs may exert a specific benefit in this subgroup of subjects at increased risk of type 2 diabetes.

3. Improved Prevention, Clinical and Home Care Approaches

The study by A. M. Ramos-Leví et al. proposed a model for the assessment of risk of developing gestational diabetes. A. M. Ramos-Leví et al. analyzed women attending prenatal care that were screened for gestational diabetes. Women completed a questionnaire on sociodemographic, anthropomorphic, and behavioral characteristics, and reproductive and medical history. The authors found that acting on modifiable factors, that is promoting healthy lifestyle habits such as moderate intake of coffee, low intake of biscuits, sugared drinks and red meats, and regular physical activity may represent a promising approach for the prevention of gestational diabetes.

The article by E. Korpi-Hyövähti et al. describes a study aimed to the increase of postpartum testing, based on oral glucose tolerance tests and other associated factors, in women at risk for gestational diabetes. E. Korpi-Hyövähti et al. found that the most important explaining factor for compliance to the postpartum testing program was a special call or reminder from the central hospital. They concluded suggesting that communication between primary care providers, obstetrics and gynecology care providers, and endocrinologists should be encouraged, and a reminding system for primary care should be developed.

The report from A. Lapolla et al. presented some results from the DAWN (Diabetes Attitudes, Wishes and Needs) study. The DAWN study is a survey promoted by the International Diabetes Federation to recognize needs, perceptions, and feelings of people affected by diabetes mellitus, and identify possible areas of concern. Within the DAWN framework, A. Lapolla et al. performed a survey specifically addressed to women with gestational diabetes. The survey included questions on several topics, such as the general characteristics of the women, the evolution of gestational diabetes, the diet and lifestyle regime during gestation, the relationships with the gestational diabetes specialist and the other medical doctors, the quality of family support, and the feelings related to the diagnosis of gestational diabetes. It was found that the large majority of women were satisfied with the quality of care, though the degree of cooperation between diabetes specialists and gynecologists was considered sometimes unsatisfactory. In addition, some women reported troubles in following the prescribed program of glucose self-monitoring and the suggested dietary regimen.

The study by H. D. McIntyre et al. proposed a home-based exercise program with telephone support for the early postpartum period in women with recent gestational diabetes. H. D. McIntyre et al. found that the physical activity (walking being the predominant type) was increased in the women included in the educational program compared to women receiving usual care support. However, it was acknowledged that significant changes were not observed in body weight and composition, or in the main parameters of glucose metabolism, probably due to the relatively short duration of the study.

The study by B. Valentini et al. suggests that when a diet for immigrant women with gestational diabetes is prescribed, their different traditional eating habits should be considered. To this purpose, B. Valentini et al. proposed a food plan including dishes typical of the foreign women's original countries (the "ethnic meal plan"). They compared the ethnic meal plan to a standard meal plan in immigrant women with gestational diabetes. They found that the ethnic approach to diet has positive effect on the outcome of pregnancy, as the ethnic meal plan group had babies with a lower birth weight, and no cases of macrosomia were observed. They concluded that the ethnic method could be considered a valid approach to the nutritional management of immigrant pregnant women with gestational diabetes.

The study by X. Jian Yun et al. assessed to what extent short-term use of corticosteroids for fetal lung maturation affects fasting blood glucose and insulin levels in normal singleton pregnancies, normal twin pregnancies, and pregnancies with impaired glucose tolerance condition but not requiring insulin treatment. Specifically, X. Jian Yun et al. investigated whether glucose and insulin levels differ after the administration of dexamethasone. They found that the degree of modification of the maternal fasting plasma glucose and insulin levels determined by dexamethasone was correlated with the basic maternal glucose metabolic condition, and may be correlated with twin pregnancy status. They concluded that blood glucose levels of twin pregnancies, and those with impaired glucose tolerance, should be closely monitored during the use of dexamethasone.

4. Review Articles

The article by G. E. Rice et al. is a review on the benefits of screening for type 2 diabetes in women with a previous history of gestational diabetes. In fact, it is known that women with previous gestational diabetes are at a greater risk of developing type 2 diabetes within 10 to 20 years of their index pregnancy. However, there is still the need for better early detection of predisposition to the development of the disease. G. E. Rice et al. discuss some screening approaches, with special attention to preconception screening strategies. Furthermore, as regards preconception programs in women already with overt diabetes, the authors report that several studies have documented evidence of positive financial returns for preconception counseling, mainly based on savings in hospitalisation costs.

One article by N. Vrachnis et al. is a review on the role of adipokines and other inflammatory mediators in gestational diabetes. Previous gestational diabetes has been associated with future development of both type 2 diabetes and metabolic syndrome. N. Vrachnis et al. report that the pathogenesis and risk factors implicated in the later development of these conditions are not as yet fully understood, but scientific research has recently focused on a group of substances produced mainly by adipose tissue called adipokines (such as adiponectin, leptin, retinol-binding protein-4 (RBP-4), and resistin). These substances as well as

other inflammatory mediators (CRP, IL-6, PAI-1, and TNF- α) seem to play an important role in glucose tolerance and insulin sensitivity dysregulation in women with previous gestational diabetes. In fact, such women are characterized by chronic subclinical inflammation, which is associated with insulin resistance and abnormality in glucose metabolism.

Another review article by N. Vrachnis et al. revised the role of possible markers of risk for cardiovascular diseases in women with a history of previous gestational diabetes. In fact, it is known that previous gestational diabetes can increase the risk of developing not only type 2 diabetes, but also cardiovascular diseases independently of a diagnosis of diabetes. N. Vrachnis et al. report and discuss several markers for cardiovascular diseases risk, such as insulin resistance, hyperlipidemia, increased levels of ICAM-1, VCAM-1, E-selectin, and low levels of adiponectin.

5. Conclusions

Several topics have been addressed in the studies included in this special issue dedicated to gestational diabetes. The focus of these studies was not limited to the assessment of the main parameters in glucose metabolism, such as insulin sensitivity, insulin secretion, and beta-cell function, but also to the analysis of the interactions between such metabolic variables and different hormones and parameters, such as fetuin-A, thyroid parameters, and cardiovascular markers. In addition, several studies were specifically focused on different aspects of care, including strategies to enhance adherence to prescriptions in terms of glucose testing, appropriate diet, and improved general lifestyle, for prevention of both type 2 diabetes and cardiovascular diseases. The variety of the presented studies shows that gestational diabetes is a metabolic condition of primary clinical interest. In our opinion, some other research topics not specifically addressed in this special issue, which will gain increasing relevance, are the study of offspring of women with a history of gestational diabetes, fetal programming research for improved understanding of intrauterine environment influences on both short and long-term fetal outcomes, and epigenetic studies to identify the processes that drive the evolution of an individual phenotype from the genome, and investigate to what extent the intrauterine environment determines how these genes act. In summary, we believe that in the next future several new interesting studies will come in the field of gestational diabetes.

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Review Article

Gestational Diabetes Mellitus: A Positive Predictor of Type 2 Diabetes?

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The aim of this paper is to consider the relative benefits of screening for type two diabetes mellitus in women with a previous pregnancy complicated by gestational diabetes mellitus. Recent studies suggest that women who experience GDM are at a greater risk of developing type 2 diabetes within 10–20 years of their index pregnancy. If considered as a stand-alone indicator of the risk of developing type 2 diabetes, GDM is a poor diagnostic test. Most women do not develop GDM during pregnancy and of those that do most do not develop type 2 diabetes. There is, however, a clear need for better early detection of predisposition to disease and/or disease onset to significantly impact on this global pandemic. The putative benefits of multivariate approaches and first trimester and preconception screening to increase the sensitivity of risk assignment modalities for type 2 diabetes are proposed.

1. Introduction

The keystone to improving disease management and health outcomes remains the early and accurate diagnosis of the predisposition to, or onset of, disease. Early detection of disease risk and onset is the first step in implementing efficacious treatment and improving patient outcomes. In the context of screening for prediabetic and diabetic conditions in asymptomatic individuals, early detection may allow the implementation of dietary, lifestyle, and/or pharmacologic interventions that limit or prevent the development of disease-specific pathophysiologies. The rationale for seeking to develop predictive tests for diabetes and other metabolic disorders, thus, is clearly evident.

Recent studies suggest that women who experience gestational diabetes mellitus (GDM) are at a greater risk of developing type 2 diabetes mellitus (type 2 diabetes) within 10–20 years of their index pregnancy [1]. Monitoring glycemic control and intervention strategies to delay or prevent disease onset have been advocated in such women. Type 2 diabetes, however, is a disease of heterogeneous aetiology and GDM is but one risk factor. If considered as a stand-alone indicator of the risk of developing type 2 diabetes, GDM is a poor

diagnostic test. Most women do not develop GDM during pregnancy and of those that do most do not develop type 2 diabetes. Postpartum monitoring of women who developed GDM during pregnancy, nevertheless, may be of clinical utility in this higher risk cohort. There is, however, a clear need for better early detection of predisposition to disease and/or disease onset to significantly impact on this global pandemic.

For women (and their partners), pregnancy represents a period of increased interaction with the healthcare system and a period where changes in lifestyle may have significant impact not only on the parents but also on the disease susceptibility of the next generation. This period represents an opportunity to implement more comprehensive educational, lifestyle, and disease susceptibility or onset screening initiatives, either during first trimester or, perhaps more effectively, in the setting of a preconception clinic. The objectives of preconception care are to promote the health of women (and their partners) before conception and thereby improve pregnancy-related outcomes (both current and future) and to reduce the risk of adult-onset disease (e.g., cardiovascular and metabolic) in their children. Over the next decade, the combined effects of an increase in the

incidence of type 2 diabetes in younger women [2] and an increased maternal age at first delivery will result in an increased number of pregnancies exposed to the potentially adverse effects of undiagnosed diabetic conditions. Globally, the number of live births each year is estimated to be more than 134 million. Preconception programs, thus, have the potential to specifically target more than 360 million individuals per year (i.e., the parents and the offspring).

The aim of this paper is to consider the relative benefits of screening for type 2 diabetes in women with a previous pregnancy complicated by GDM. The putative benefits of multivariate approaches and preconception screening to increase the sensitivity of risk assignment modalities for type 2 diabetes are proposed.

2. Diabetes Mellitus

2.1. Epidemiology. Diabetes mellitus is one of the most common chronic diseases. The number of adults with diabetes has more than doubled over the past 30 years. Recent reports by Danaei et al. [3] and Shaw et al. [4] estimate the global prevalence of diabetes to be between 285 and 347 million people. By 2030, diabetes is expected to affect 552 million people. The prevalence of this disease is increasing in most countries as changing lifestyles lead to reduced physical activity and increased obesity [4]. Since 1980, the age-standardised fasting plasma glucose concentration has increased by 0.07 mmol/L per decade for men and 0.09 mmol/L per decade for women [3]. These data are consistent with an overall population-based decline in glycemic control.

The incidence of diabetes has also increased dramatically in women of reproductive age (i.e., 18–44 years). For example, in the USA, the rate of diabetes has increased by 70% in women aged 30–39 years over the past decade. It is estimated that 12.6 million women (or 10.8% of women over the age of 20) have diabetes, and of these women, 90–95% have type 2 diabetes. Death rates for women aged 25–44 years with diabetes are more than 3 times that for women without diabetes [5].

2.2. Aetiology of Type 2 Diabetes. The available data are consistent with type 2 diabetes being of genetic origin; however, its precise aetiology remains to be unequivocally established. Behavioural, lifestyle, and environmental factors have all been implicated as modifiers of disease risk. Recent genome-wide screening studies have identified multiple susceptibility variants consistent with type 2 diabetes being of polygenic origin [6–10]. Thus, type 2 diabetes may be a phenotypic manifestation of many different aetiologies that simply share hyperglycemia as a common outcome [11].

A hallmark of the onset of type 2 diabetes is a progressive decrease in insulin-stimulated glucose uptake. High circulating concentrations of glucose induce pancreatic β -cell hypertrophy and/or hyperplasia and increased secretion of insulin. When the capacity of β -cells fails to compensate for the degree of insulin resistance, insulin deficiency and ultimately type 2 diabetes ensue [12]. Pancreatic β -cell failure displays specificity for insulin signaling pathway; while retaining

capacity to respond to challenges (such as, β -adrenergic agonists, amino acids, and sulfonylurea drugs), cells lose their capacity to respond to glucose. If not adequately managed, hyperglycaemia may induce a glucotoxicity and lipotoxicity involving oxidative and endoplasmic reticulum stress, overexpression of proinflammatory autacoids, and increased rates of β -cell apoptosis [12, 13].

It is becoming increasingly evident that inflammation plays a key role in the pathogenesis of type 2 diabetes. It is well established that systemic markers of inflammation, including C-reactive protein, haemoglobin, serum amyloid A, proinflammatory cytokines and chemokines are elevated in the blood of type 2 diabetics. The source of these mediators is of multiorgan origin and, at least in part, in response to elevated concentrations of glucose and fatty acids. Both secretagogues promote proinflammatory conditions in many tissues (including pancreatic islet-7 cells, adipose tissue, liver, and muscle), induce the release of inflammatory autacoids, and alter redox status. Chemokines further promote the recruitment of macrophages to affected tissues and together with T-cell and possibly mast cells may establish a local chronic inflammation that involves the constitutive activation of gene transcription factors such as the nuclear factor κ B (NF- κ B) family.

NF- κ B is a sequence-specific family of transcription factors critically involved in inflammation and innate immune responses. The NF- κ B family comprises at least five proteins, of which the most abundant form in unstimulated cells occurs in the cytoplasm as a heterodimer composed of two proteins, p50 and p65 bound to an inhibitory subunit, $I\kappa$ B α . Upon stimulation, $I\kappa$ B α is phosphorylated by an $I\kappa$ B kinase complex, thus, targeting $I\kappa$ B α for ubiquitin-dependent degradation and liberates NF- κ B dimers to translocate to the nucleus where they bind to the consensus sequence 5-GGGPuNNPyPyCC-3. This κ B motif has been identified in the promoter regions of many proinflammatory mediators, including adhesion molecules (ICAM-1), enzymes (including, inducible nitric oxide synthase, phospholipase A₂s, cyclo-oxygenase-2, urokinase plasminogen activator, metalloproteinases, superoxide dismutase), cytokines (e.g., IL-1 β , IL-6, TNF α), and chemokines (IL-8) [14].

It is noteworthy that recent studies in breast cancer [15] provide data implicating NF- κ B, IL-6, and Let-7 micro-RNA (miRNA) in an epigenetic switch or positive feedback loop that resets inflammatory pathways to a heightened state of responsiveness. In this model, a triggering event induces NF- κ B DNA binding activity and Lin28 expression and represses Let-7 miRNA action. One of the actions of Let-7 is to suppress IL6 formation. Thus, inhibition of Let-7 results in higher levels of expression of IL6 than achieved by NF- κ B activation alone. IL6 activation of the STAT3 transcription factor is necessary for neoplastic cellular transformation, and IL6 activates NF- κ B, thereby completing a positive feedback loop.

We propose that a similar mechanism may induce a sustained inflammatory response, activation of NF- κ B-regulated genes, and insulin resistance in type 2 and gestational diabetes. That is, that a primary pathophysiological

insult (Figure 1, e.g., obesity, metabolic stress, hyperglycaemia, or hyperlipidaemia) may activate NF- κ B-mediated gene expression and proinflammatory regulatory pathways. Subsequent secondary or repetitive insults may induce a persistent, heightened response, the positive feedback loop being established via the induction of the RNA-binding protein Lin28 that suppresses Let-7 microRNA family members. Let-7 miRNA suppresses interleukin-6 expression (binding IL-6 mRNA through its 3' UTR) and other growth and metabolic mediators. The resulting positive feedback loop between NF- κ B and, in particular, IL-6 and the inhibitory effects of Lin 28 and IL-6 on Let-7 is sufficient to result in sustained elevation and responsiveness of these pathways.

In support of this hypothesis, Zhu et al. [16] recently reported that the Lin28/Let-7 axis plays a role in the reprogramming of glucose metabolism in malignancy. Lin28a and Lin28b were reported to promote insulin-sensitivity and resistance to high-fat-diet-induced diabetes. Furthermore, muscle-specific inhibition of Lin28a or overexpression of Let-7 results in insulin resistance and impaired glucose tolerance. These effects were mediated, in part, via Let-7 repression of the insulin-PI3K-mTOR pathway, including the insulin receptor, insulin receptor substrate 2, and insulin-like growth factor 1 receptor. In normal adult individuals, Lin28 expression is low and, thus, Let-7 represses the expression of a cassette of gene pathways associated with growth, cell migration, and catabolic metabolism. Under conditions where Lin28 is induced (e.g., via activation of NF- κ B response pathways), Let-7 repression of these genes is removed. In the case of type 2 diabetes, triggers such as obesity, oxidative stress, and inflammatory mediators may initiate aberrant activation of the NF- κ B pathway and initiate a feedback loop that sustains and progressively increases insulin resistance.

2.3. Management and Intervention. The available data support the contention that the adverse sequelae of diabetes (including microvascular, cardiovascular, and renal disease) can be, at least, ameliorated by adequate glycaemic control [17]. Recent trials have established the benefits of interventions to prevent or delay diabetes and reduce diabetes-related complications and/or associated risk factors [18–21]. Intensive lifestyle modification to promote weight loss and increase physical activity resulted in a 58% reduction in the risk of type 2 diabetes in adults with impaired glucose tolerance [21]. Early diagnosis of predisposition to type 2 diabetes and implementation of effective intervention represent a strategy to abate the incidence of type 2 diabetes and its associated health care burden.

3. Gestational Diabetes Mellitus

3.1. Epidemiology. GDM is glucose intolerance with onset or first recognition during pregnancy [22]. GDM affects ~5% of all pregnancies and its incidence is increasing in parallel with the global increase in obesity and type 2 diabetes. In the USA, GDM affects 135,000 pregnancies per year. GDM has been associated with not only acute increased risk for complications of pregnancy but also long-term disease risks for both mother and baby (Australian Institute of Health and

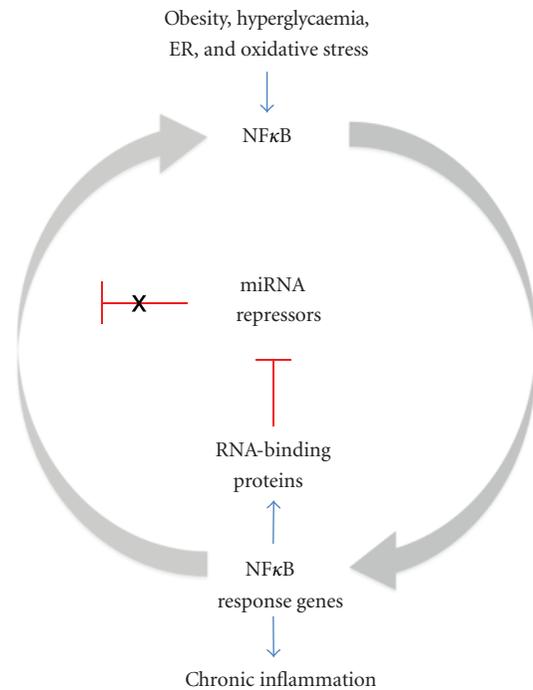


FIGURE 1: Diabetes-related triggers may initiate a positive feedback loop resulting in heightened responsiveness of the NF- κ B by the inhibition of miRNA repressors by NF- κ B-induced RNA binding proteins.

Welfare, 2010). Perinatal morbidity includes hyperinsulinaemia, macrosomia, hypoglycaemia, hyperbilirubinaemia, and respiratory distress syndrome which in turn may generate subsequent complications. Longer-term morbidity for the offspring includes obesity and diabetes independent of genetic factors [23–26]. GDM in the mother is associated with increased risk of overt diabetes later in life. A higher risk of developing metabolic and cardiovascular disease has been reported for women who develop GDM during pregnancy.

In 2011, the American Diabetes Association (ADA) and the International Association of Diabetes and Pregnancy Study Groups (IADPSG) revised recommendations regarding GDM. It is now recommended that patients at increased risk for type 2 diabetes be screened for diabetes using standard diagnostic criteria at their first prenatal visit. High-risk women are defined as having impaired fasting plasma glucose levels of 5.6 mmol/L to 6.9 mmol/L [100 mg/dL to 125 mg/dL] or impaired glucose tolerance (2-hour OGTT values of 7.8 mmol/L to 11.0 mmol/L [140 mg/dL to 199 mg/dL]). Women with an HbA1c of 5.7% to 6.4% are also considered at increased risk. In these patients, confirmed fasting glucose levels of ≥ 7.0 mmol/L (126 mg/dL) or random glucose levels ≥ 11.1 mmol/L (200 mg/dL) are also diagnostic of diabetes. The ADA and the IADPSG recommended that such high-risk women with diabetes diagnosed on the basis of standard diagnostic criteria receive a diagnosis of overt rather than gestational diabetes.

At 24 to 28 weeks of gestation, all women not known to have diabetes (including high-risk women if the initial testing

was normal) should undergo a 75 g OGTT, with diagnosis of GDM based upon the finding of 1 abnormality, rather than the previously recommended 2.

3.2. Aetiology. Normal pregnancy is attended by significant changes in maternal metabolism [27] that are induced, at least in part, by the release of placenta-derived autacoids [28–30]. Early pregnancy is anabolic and associated with the accretion of maternal fat. Late pregnancy is catabolic and characterised by increasing insulin resistance, lipolysis, hyperinsulinaemia, hyperglycaemia, increased postprandial fatty acid concentrations, and declining maternal fat reserves. The net effect of these late gestation changes are increased availability of energy and anabolic substrates to sustain the growth of the feto-placental unit, increased utilisation of glycolytic energy production, and increased free fatty acid availability [31]. Late pregnancy is also associated with a decreased ability to produce glucose via gluconeogenesis, glycogenolysis and lipolysis. This is in part a consequence of an attenuation of hypoglycaemia to induce glucagon, norepinephrine and cortisol.

It has been suggested that GDM and type 2 diabetes may share common pathogenic mechanisms, however, pregnancy may serve to unmask disease in those women who are predisposed and destined to develop type 2 diabetes later in life. Similar to type 2 diabetes, GDM is manifested by the inability of pancreatic β -cell insulin release to compensate for pregnancy-induced insulin resistance resulting in maternal hyperglycaemia and hyperinsulinaemia. Moderate hyperinsulinaemia is considered adaptive during normal pregnancy and a response to increased energy utilisation and demand by the developing fetus. In GDM, the adaptive changes in insulin resistance extend beyond those normally observed [32, 33]. For example, during normal pregnancy, insulin-stimulated glucose transport by skeletal muscle fibers is reduced by ~40%. In women with GDM, glucose transport has been reported to be reduced by up to 65% [34]. Environmental (modifiable) risk factors, including, preconception conditioning, maternal diet and exercise, and other lifestyle factors, may impact on the severity of its manifestation. In most cases, however, symptoms of metabolic dysfunction disappear postpartum following the withdrawal of placental autacoid mediators.

The effects of hyperglycaemia on pregnancy outcome are underpinned by experimental studies that identify putative effector pathways by which exposure to high glucose concentrations may alter placental and maternal adipose tissue phenotype and responsiveness [35–37]. Similar to type 2 diabetes, the role of inflammatory mechanisms in disease progression is evident. Increased biomarkers of oxygen radical damage and an impairment of antioxidant defense have been identified in individuals with type 2 diabetes [38] and in women with GDM [37, 39–41]. Previously, we demonstrated that the placentae of women with GDM display a reduced capacity to respond to oxidative stress in terms of 8-isoprostane and tumour necrosis factor α (TNF α) release [35]. We concluded that GDM placenta may be preconditioned by transient intracellular oxidative stress.

The role of oxidative stress in the aetiology of GDM has recently been reviewed [42].

4. Screening for Diabetes

4.1. Screening for GDM. Currently, GDM is diagnosed in the late second or early third trimester of pregnancy. Any pathology is probably already established by this time and reversal of the potential adverse perinatal outcomes may be limited. The lack of a reliable early test for GDM has hampered the development of useful intervention therapies that may impact not only on the acute but long-term health outcomes (Figure 2). Thus, there is a need to diagnose and predict GDM earlier so that appropriate management can be initiated and tailored to the needs of the patient in order to minimise perinatal complications and their sequelae.

GDM is currently diagnosed by an In Vitro Diagnostic (IVD) Oral Glucose Tolerance Test performed at 24–28 weeks of gestation. A glucose load (75 g) is administered to fasting individuals, and blood glucose concentration is determined at 1 hour and 2 hours [43, 44]. The Third International Workshop-Conference on GDM emphasised the critical importance of developing new diagnostic criteria that are based on the potential to detect pregnancies at risk for adverse perinatal outcome as a result of maternal hyperglycaemia, rather than placing primary emphasis on the identification of mothers at risk for progression to diabetes outside of pregnancy. With the obesity epidemic well entrenched in the Western world and with more women delaying pregnancy and the associated increase in pre-pregnancy body mass index (BMI), the incidence of GDM is increasing irrespective of the diagnostic criteria used.

First trimester pregnancy and preconceptional risk-factors for GDM have been identified including family history of GDM and/or diabetes [45], maternal pregnancy weight gain [46, 47], fasting plasma glucose [48], 1-hour glucose challenge test [49], oral glucose tolerance test [50], and haemoglobin A1c [51] adiponectin [52, 53], C-reactive protein [54], serum triglycerides [55], sex hormone-binding globulin [56], placental growth factor [57] leptin [58], oxidised DNA [59], and follistatin-like-3 levels [60]. Although some have been able to provide a good negative predictive measure for subsequent GDM [61], most tests suffer from poor positive predictive values and are of limited efficacy.

It is now widely acknowledged that single biomarkers are unlikely to deliver significant incremental gain in sensitivity and specificity that will be required for the development of effective screening and classification tests requisite for the implementation of personalised medicine. New approaches based upon the measurement of multiple biomarkers of disease risk afford opportunity to increase diagnostic test sensitivity and specificity. Even the use of two biomarkers can deliver improved performance [62]. The use of modelling algorithms to combine multiple known biomarkers (e.g., candidate-based approaches) similarly may increase diagnostic efficiency and deliver classification models of clinical utility [63–66]. Both candidate-based applications (i.e., in

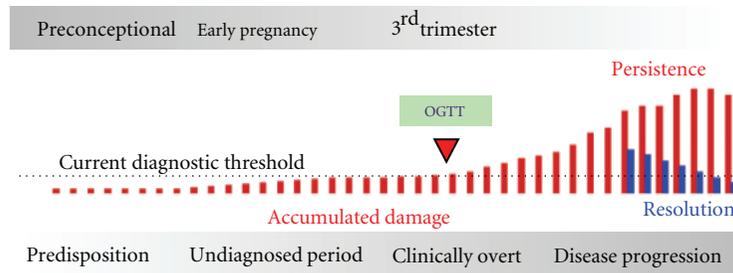


FIGURE 2: GDM disease progression. GDM is currently diagnosed (Clinically Overt) in 3rd trimester (24–28 weeks of gestation) following an oral glucose tolerance test (OGTT). Using this diagnostic threshold, there is no opportunity to prevent pathological changes (accumulated damage) that may occur during 1st and 2nd trimester (Undiagnosed Period). The implementation of screening tests during early pregnancy or the preconception period affords opportunity to identify women at risk of disease and to evaluate intervention strategies on pregnancy outcome and the long-term health of both mother and baby.

which the identity of the analytes being measured are well-established [67]) and signature profiling applications (i.e., in which characteristic patterns or motifs within a signal profile are identified, see [68]) may be utilised in the development of multivariate modelling strategies for the delivery of more informative diagnostic tests [69].

A recent trend in the development of more efficient diagnostic tests has been the use of algorithm-based multivariate index assays (IVDMIA). With the development of this new class of IVD, the discipline has sought new biostatistical approaches for assessing and quantifying incremental gains in diagnostic efficiency. Traditionally, the area under the receiver operator characteristic curve (AUC) has been used as a measure and comparator of diagnostic efficiency. Several investigators have argued that this measure alone may be imperfect and inefficient for comparing the true clinical usefulness of alternative marker panels [70, 71]. These authors reviewed several biomarker studies and observed that when evaluating improvement in risk assignment of biomarkers, very large odds ratios were often associated with very small increases in the AUC. This feature of the receiver operator characteristic curve analysis limits its utility in identifying putative beneficial contributions of new biomarkers to algorithm-based models. Pencina et al. [72] therefore, proposed the use of two new methods for evaluating the diagnostic efficiency of biomarkers. These two methods are (i) Net Reclassification Improvement (NRI) and (ii) Integrated Discrimination Improvement (IDI). The NRI is based on counts of the number of true positives showing an increase in probability of an event and the number of true negatives showing a decrease in probability of an event. The IDI is based on the integral of sensitivity and specificity of all possible thresholds. These new biostatistical approaches may facilitate the development of biomarker panels with improved diagnostic efficiency and aid in the screening and earlier detection of diabetic conditions.

IVDMIA approaches are being developed for risk assignment modalities for use in the first trimester of pregnancy, including the evaluation of multiple candidate-based profiling of blood-borne biomarkers. For example, we measured multiple plasma biomarkers at 11 weeks of gestation in women who subsequently experienced a normal pregnancy

outcome and women who subsequently developed gestational diabetes [73]. Of the biomarkers considered, algorithms that included adiponectin, insulin, and random blood glucose delivered the greatest diagnostic efficiency when compared to individual biomarkers alone. The IVDMIA increased AUC by more than 10%. This simple example demonstrates the putative benefit of a multimarker approach for improving diagnostic efficiency.

4.2. Screening for Type 2 Diabetes after GDM. As discussed above, GDM may unmask a predisposition to type 2 diabetes and, as such, GDM may be diagnostic for type 2 diabetes. A recent meta-analysis [74] reviewed 20 studies conducted between 1960–2009 to estimate the relative risk of developing type 2 diabetes following GDM. The combined cohort involved more than 675,000 pregnancies of which 31,867 cases of GDM were identified (i.e., 4.7% of all pregnancies included in the analysis). Of these cases of GDM, 10,859 incident cases of type 2 diabetes were identified. The relative risk for type 2 diabetes following GDM was estimated to be 7.43 (compared to women who had normoglycaemic pregnancies). Bellamy et al. [74] suggested that increased awareness of the risk of type 2 diabetes after GDM could provide an opportunity to test and use dietary, lifestyle, and pharmacological interventions that might prevent or delay the onset of type 2 diabetes.

While the conclusion that GDM is a risk factor for type 2 diabetes is supported by the available data, it is pertinent to note that more than 95% of women in the cohort did not develop gestational diabetes and that more than 64% of women who have had GDM do not have type 2 diabetes 20 years postindex pregnancy. The prevalence of type 2 diabetes in women 10–20 years after index pregnancy is estimated to be ~5%; thus, in a cohort of 675,000, ~33,700 incidence cases would be expected, that is, 22,841 cases in this cohort were not associated with a previous GDM pregnancy (i.e., a false positive rate of 0.659). The positive predictive value of GDM as a diagnostic test for type 2 diabetes is only 34% (sensitivity = 0.322 and specificity of 0.967).

Similarly, Göbl et al. [75] recently reported results of a small prospective cohort ($n = 110$) with 10-year followup of women who experienced GDM in which 78.7% of women

did not develop type 2 diabetes. Of the 21.3% who did progress to type 2 diabetes, a multivariate analysis identified 2-hour oral GTT concentration, HDL cholesterol, and age as the best predictors. Women with two or more risk factors were at a higher risk than women with only one.

It has further been proposed that women with a previous GDM pregnancy should be followed up by OGTT postpartum and, if positive, intervention and monitoring strategies implemented. While this may be appropriate, it does not assist the ~68% of women who develop type 2 diabetes in the absence of GDM complicated pregnancies. Specifically targeting and monitoring women with a previous GDM pregnancy is an aid in identifying predisposition to type 2 diabetes but is a poor stand-alone diagnostic test for type 2 diabetes in women. Alternative strategies for early detection screening, intervention, and prevention are requisite to reducing the overall burden of type 2 diabetes at a community level.

The objectives of any disease screening program are to

- (1) identify asymptomatic individuals at higher risk of, or predisposition to disease,
- (2) afford the opportunity for treatment or prevention of disease thus limiting severity,
- (3) reduce disease burden in the community.

A number of opportunities for developing screening applications that target women of reproductive age exist, including the development of preconception screening.

4.3. Preconception Conditioning and Screening. The concept that information defining contemporary environmental conditions is coded in maternal physiology and is sensed and informs and adapts the development of the fetus is not novel [76, 77]. This tenet is the basis of epigenetics and the developmental origins of adult disease [78]. Modifiable maternal and environmental factors reported to affect pregnancy outcome and/or disease risk in the offspring include BMI [79, 80], weight gain during pregnancy [47], diet [81–83] physical activity [84, 85], preexisting diabetes [86, 87], and alcohol consumption [88]. The placenta functions as an environmental sensor for the embryo and not only integrates information encoded within the maternal *milieu* but its own ontogenic development and function may be altered by such information. Known modifiers of the placental epigenome in human and animal models include micronutrients [89, 90], diet [91], smoking [92], bacterial infection [93], obesity [94], stress [95], diabetes [96], and hypertension [97, 98].

Based upon the available data, a sound case can be made for the implementation and evaluation of preconception care programs that attempt to optimise or, at least, improve general health, life style, and conditioning of women and their partners before conception. The premise underpinning promoting preconception in addition to prenatal care is that for some maternal conditions and exposures, altered programming and/or damage can occur before prenatal care begins. To promote normal placentation and reduce risks of complications of pregnancy, education and appropriate

interventions must be identified and implemented prior to conception.

In 2006, the Centers for Disease Control and Prevention (CDC) published a report of the CDC/ATSDR Preconception Care Work Group and the Select Panel on Preconception Care [99, 100]. The panel identified four primary objectives:

- (1) improve knowledge, attitudes, and behaviors of men and women related to preconception health,
- (2) assure that all women of childbearing age receive preconception care services that will enable them to enter pregnancy in optimal health,
- (3) reduce risks identified by a previous poor pregnancy outcome through interventions during the inter-conception period, which can prevent or minimize health problems for a mother and her future children,
- (4) reduce disparities in adverse pregnancy outcomes.

The actual benefits of preconception care programs remain to be established as there is a paucity of randomised control data on the health outcomes and economic benefit of attempts to improve preconception maternal (and paternal) health. Several studies, however, have reported some evidence of positive financial returns for preconception counseling for women with diabetes, based on savings in hospitalisation costs [101, 102]. Similarly, case control studies on the health care costs associated with maternal obesity provide further support, reporting that the cost of prenatal care was 5 times higher in mothers who were overweight before pregnancy than in normal-weight control women [103]. More recently, Moos and Bennett [104] reviewed the evidence supporting preconception care for diabetic women.

The implementation of preconception care programs afford opportunity for more broadly based screening for prediabetic conditions and early intervention to reduce the incidence of type 2 diabetes. The efficacy of screening this cohort has yet to be established it would, however, afford opportunity for longitudinal monitoring of biomarkers. Longitudinal monitoring is an approach that has proved effective in increasing the diagnostic performance of oncology diagnostics [105, 106]. It is likely that such an approach would also improve the positive predictive value for the diagnosis of type 2 diabetes.

5. Conclusion

Type 2 diabetes is a “*communicable disease*” that is transmitted between individuals and intergenerationally by the adoption of societal and lifestyle behaviors—behaviours that challenge fundamental energy homeostasis. The juxtaposition of a susceptible genetic background with the ability to access a surfeit of energy dense foods without counterpoise energy expenditure predisposes to obesity and failure of glycaemic control. Modifiable risk factors have been identified that may reduce disease severity. Early identification of individuals at higher risk of developing type 2 diabetes will play a critical role in improving disease management and health outcomes. Of significant promise is the development and

implementation of high performance IVDMIAs and their use in longitudinal monitoring programs.

Abbreviation

IVDMIA: In Vitro Diagnostic Multivariate Index Assay.

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Clinical Study

Gestational Diabetes and Thyroid Autoimmunity

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Background. About 10% of pregnancies are complicated by previously unknown impairment of glucose metabolism, which is defined as gestational diabetes. There are little data available on prevalence of thyroid disorders in patients affected by gestational diabetes, and about their postgestational thyroid function and autoimmunity. We therefore investigated pancreatic and thyroid autoimmunity in gestational diabetic patients and in women who had had a previous gestational diabetic pregnancy. **Methods.** We investigated 126 pregnant women at the time of a 100-g oral glucose tolerance test: 91 were classified as gestational diabetics, and 35 were negative (controls). We also studied 69 women who had delivered a baby 18–120 months prior to this investigation and who were classified at that time gestational diabetics (38 women) or normally pregnant (31 women; controls). **Results.** Our data show no differences for both thyroid function and prevalence of autoimmune disorders during pregnancy; however, a significant increase in thyroid autoimmunity was seen in women previously affected by gestational diabetes. This increased prevalence of thyroid autoimmunity was not associated with the development of impaired glucose metabolism after pregnancy. **Conclusions.** Our data suggest that maternal hyperglycemia is a risk factor for the development of thyroid autoimmunity, a conclusion that should now be confirmed in a larger cohort of patients.

1. Introduction

Gestational diabetes (GDM) is defined as any degree of carbohydrate intolerance that is first diagnosed during pregnancy [1]. The prevalence of GDM ranges between 1% and 14% [1–3], and it is most frequent in women aged ≥ 35 years in the second trimester of pregnancy. In a minority (<10%) of patients, the need for insulin therapy continues after delivery, and in these cases detection of anti-glutamic-acid-decarboxylase- (GAD-) 65 antibodies (GAD65-Ab) is common, and so GDM is considered the onset of type 1 diabetes [4–6]. Increased incidence of organ-specific autoimmunity towards endocrine cells other than β -cells

has been described in type 1 diabetes patients [7], and it is believed to be caused by a genetic propensity to autoimmune disorders. In the majority of patients, however, GDM is believed to be caused by β -cell dysfunction that occurs on a background of chronic insulin resistance. Although in these patients GDM usually resolves after delivery, up to 70% of them develop overt type 2 diabetes mellitus within 10 years [4, 8].

Recent studies have reported increased incidence of thyroid autoimmunity in type 2 diabetes [9, 10], thus implying that diabetes can trigger the onset of thyroid autoimmunity. Few studies have, however, evaluated the prevalence of thyroid dysfunction and autoimmunity in

women with GDM [11–13]. Moreover, little is known about thyroid autoimmunity in post-GDM patients, who usually return to normal glucose control after delivery. In our opinion, GDM offers a good opportunity to study if diabetes and hyperglycemia might predispose to thyroid autoimmunity.

In the present study, we therefore investigated (1) pancreatic and thyroid autoimmunity in GDM patients and (2) pancreatic and thyroid autoimmunity in women with previous GDM.

2. Patients and Methods

2.1. Patients. One-hundred-and-ninety-five women (aged 18 to 51) who were referred to the Diabetic Outpatient Clinic of the “Santo Spirito” Pescara Hospital were evaluated; none of these patients had a hyperglycemic disease before gestation. Initially, 126 consecutive pregnant (group A) women were evaluated at the 14th to 34th weeks of pregnancy at the time of a 100 g oral glucose tolerance test (OGTT), which was interpreted according to the O’ Sullivan and Mahan criteria revised by Carpenter and Coustan [14–16]. According to the OGTT results, these patients were further subdivided into two groups.

- (i) A1: 91 patients (mean age, 33 ± 10 years) with a positive OGTT, considered as women affected by GDM.
- (ii) A2: 35 women (mean age, 29 ± 11 years) with a negative OGTT, considered as normally pregnant women (controls).

The remaining 69 women (group B) had delivered a baby 18 months to 120 months before this study. During their pregnancy, they had been evaluated by an OGTT and were diagnosed accordingly as having been affected, or not, by GDM. For the purpose of this study, these patients were also further subdivided into two groups.

- (i) B1: 38 patients (mean age, 40 ± 11 years) with a positive OGTT during their pregnancy, considered as women previously affected by GDM. At the time of the present study (18–120 months after delivery), they were evaluated again for thyroid function and glucose metabolism by a 75 g OGTT.
- (ii) B2: 31 women (mean age, 40 ± 5.7 years) with a negative OGTT during their pregnancy who did not develop diabetes after delivery, considered as previously normal pregnancy controls; the time elapsed since their delivery was 18 months to 96 months. They were enrolled as mothers whose children were attending the family care clinic of two pediatricians operating in the Pescara area.

Women with a positive history for thyroid diseases and who had taken any drug known to interfere with thyroid function or the immune system were excluded from the study. None of the subjects enrolled in the study were evaluated twice; therefore, none of the patients in group A were also included in group B. All women with elevated TSH

levels in groups A1 and A2 were treated with L-thyroxine; for women of group B1 we individually evaluated the option of treatment. Informed consent was obtained from all of the participants in the study.

2.2. Methods. Commercially available kits were used to determine the levels of free thyroxine (FT4) (immunofluorometric assay; normal range 0.76–1.42 ng/dL; Perkin Elmer Italia spa, Monza, Italy), thyrotropin (TSH) (immunofluorometric assay TSH ultra; normal range 0.4–4.2 μ IU/L; Perkin Elmer Italia spa, Monza, Italy), antiperoxidase antibodies (TPO) (TPO-Ab radioimmunoassay kit; normal value <15 IU/mL; Becton, Dickinson and Co, Franklin Lakes, NJ, USA), antithyroglobulin antibodies (Tg) (Tg-Ab Elisa Kit; normal value <100 IU/mL; Alpco Diagnostics, Salem, NH, USA), and antiglutamic acid decarboxylase-65 antibodies (GAD65-Ab radioimmunoassay; normal value <1 IU/mL; Adaltis Italia spa, Casalecchio di Reno, Italy).

Stimulating TSH receptor antibodies (TSHr-Abs) were calculated using a biological assay, as previously described [17]. Briefly, Chinese hamster ovary (CHO) cells were subjected to a two-step double stable transfection: in the first step, the cells were transfected with a CRE-luc construct, which makes the cells particularly sensitive to changes in cAMP levels. In the second step, the cells were transfected with wild-type human TSHr. These cells were maintained in Ham’s F12 nonessential amino acids supplemented with 10% fetal calf serum and penicillin/streptomycin (1 U/mL/1 mg/mL, resp.) for 24 hours, which was then replaced with starvation media (Hank’s balanced salt solution; no fetal calf serum) for another 24 hours. All of the cells were maintained at 37°C, in 5% CO₂ and at 95% relative humidity. Cut-off of normal values was determined in the following manner: the mean of ≥ 5 samples from normal subjects was calculated. The standard deviation between these normal samples was determined, and this value was multiplied by two and added to the calculated mean. The cut-off value obtained in this way was arbitrarily considered equal to 1 unit (AU). The luciferase activity was determined using the Bright-Glow reagent, by measurement of the light output using a single-tube luminometer. Interassay and intra-assay variability were <5%.

Student’ *T* tests were used with unpaired data, and Fisher’s exact tests and χ^2 tests were performed.

3. Results

The presence of pancreatic autoimmunity in the cohort of patients was evaluated by determination of GAD65-Ab levels. We observed that 3 (3.3%) patients were positive for GAD65-Ab in group A1, 2 of whom had low GAD-65 titers (<2 IU/mL), while the third had a GAD-65 titer of 33 IU/mL. All of the patients in group A2 were negative for GAD65-Ab. In the group of previously gestational women (B1), 2 (5.3%) patients had GAD65-Ab-positive values, 1 of whom had low level positivity (<2 IU/mL), and the second, who had a GAD65-Ab value of 16 IU/mL, was diagnosed as type

TABLE 1: Clinical characteristics and laboratory data of the four groups of women evaluated.

	Groups			
	A1 (n = 91)	A2 (n = 35)	B1 (n = 38)	B2 (n = 31)
Mean age (years)	33 ± 10	29 ± 11	40 ± 11	40 ± 6
Time from delivery (range, months)	—	—	18–120	18–96
BMI at OGTT	29.8 ± 4.0	28.1 ± 4.7	27.2 ± 2.2	—
BMI at follow-up	—	—	24.1 ± 2.8	23.5 ± 3.7
GAD65-Ab positivity [n (%)]	3 (3.3)	0	2 (5.3)	0
Mean FT4 (ng/dL)	0.82 ± 0.13	0.83 ± 0.09	0.93 ± 0.21	0.92 ± 0.17
Mean TSH (mIU/L)	1.46 ± 1.02	1.90 ± 1.4	2.45 ± 4.32	1.44 ± 0.92
TSH < 0.4 mIU/L [n (%)]	8 (8.8)	2 (5.7)	5 (13.1)	2 (6.5)
TSH > 4.2 mIU/L [n (%)]	2 (2.2)	2 (5.7)	4 (10.5)	1 (3.2)
Overall abnormal TSH [n (%)]	10 (11.0)	4 (11.4)	9 (23.7)*	3 (9.7)
Thyroid Ab positivity [n (%)]	16 (17.6)	5 (14.4)	12 (31.6)*	3 (9.7)
Abnormal TSH + thyroid Ab positivity [n (%)]	3 (3.3)	0 (—)	7 (18.4)**	1 (3.2)

* $P < 0.05$ versus groups A1 and B2; ** $P < 0.05$ versus groups B2 and $P < 0.001$ versus group A1. Where indicated, data are means ± SD.

1 diabetic and is on insulin treatment. None of the B2 group (control group) showed positive GAD65-Ab values (Table 1).

The mean TSH values were not significantly different between the four groups (1.46 ± 1.02 versus 1.90 ± 1.4 mIU/L in groups A1 and A2, resp., and 2.45 ± 4.32 versus 1.44 ± 0.92 mIU/L in groups B1 and B2, resp.) (Table 1). No significant differences were seen between the four groups either considering these mean TSH values or separately evaluating the TSH values as below and above the normal range. However, if we consider the overall incidence of abnormal TSH values, a significantly higher incidence ($P < 0.05$) was seen for group B1 versus both groups A1 and B2 (Figure 1).

The FT4 levels were not significantly different when comparing either gestational versus normal pregnancies (0.82 ± 0.13 versus 0.83 ± 0.09 ng/dL for groups A1 and A2, resp.) or postgestational versus controls (0.94 ± 0.21 versus 0.92 ± 0.17 ng/dL for groups B1 and B2, resp.) (Table 1). Our data confirm the significantly lower FT4 values in pregnancy (0.82 ± 0.13 versus 0.93 ± 0.19 ng/dL, resp.; $P < 0.001$); however, FT4 was within the normal range in all of the pregnant women if the values are modified according to pregnancy [18].

Anti-TPO-Ab were detected in 16 (17.6%) patients in group A1, 5 (14.3%) in group A2, 10 (26.3%) in group B1, and 3 (9.7%) in group B2. Anti-Tg-Ab were detected in 6 (6.6%) patients in group A1, 1 (2.8%) in group A2, 6 (15.8%) in group B1, and 1 (3.2%) in group B2. Moreover, 1 patient in group B1 (of previously gestational women) had positive stimulating TSHr-Ab, with a suppressed TSH (< 0.01 μ IU/mL) and negative TPO-Ab. Therefore, the overall incidence of thyroid autoimmunity (12/38, 31.6%) in group B1 was significantly ($P < 0.05$) greater than in groups B2 and A1 (Figure 1). No other subjects were positive for stimulating TSHr-Ab. Only 1 patient (in group A1) had positivity for both GAD65-Ab and TPO-Ab.

When considering the coincident presence of thyroid autoimmunity and abnormal TSH values, it is interesting to note that the combination of both was observed in 3/91

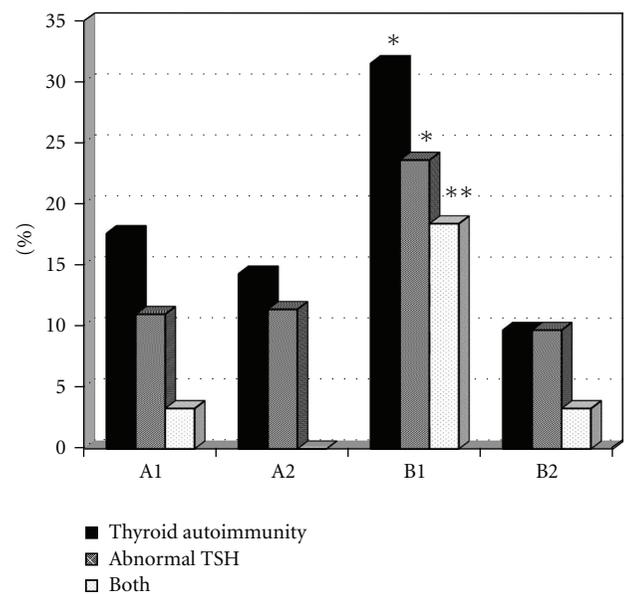


FIGURE 1: Patients (as percentages within each single group) affected by thyroid autoimmunity (TPO-Ab, Tg-Ab, and TSHr-Ab positivity), thyroid dysfunction (TSH < 0.4 mIU/L or >4.2 mIU/L) or the combination. Group A1: GDM pregnant women; group A2: non-GDM pregnant women; group B1: post-GDM women; group B2: healthy mothers. * $P < 0.05$ versus groups A1 and B2; ** $P < 0.05$ versus group B2 and $P < 0.001$ versus group A1.

(3.3%) patients in group A1, 0/35 (0.0%) in group A2, 7/38 (18.4%) in group B1, and 1/31 (3.3%) in group B2. The association of abnormal TSH and TPO-Ab positivity was significantly greater in group B1 versus both groups B2 ($P < 0.05$) and A1 ($P < 0.001$) (Table 1 and Figure 1).

The possibility that thyroid Ab positivity is associated with the onset of permanently impaired glucose metabolism in the previously gestational women (Table 2) was also considered. Here, 18/38 (47.4%) previously gestational women showed hyperglycemic disease at the follow-up;

TABLE 2: Glucose abnormalities and thyroid autoimmunity in post-GDM women (group B1).

Thyroid autoimmunity	Condition	Glucose metabolism [n (%)]
Thyroid Ab positive (n = 12)	Normal	5 (41.7)
	Type 1 diabetes mellitus	1 (8.3)
	Type 2 diabetes mellitus	2 (16.7)
	Impaired glucose tolerance	3 (25)
	Impaired fasting glucose	1 (8.3)
Thyroid Ab negative (n = 26)	Normal	15 (57.7)
	Type 1 diabetes mellitus	1 (3.8)
	Type 2 diabetes mellitus	2 (7.7)
	Impaired glucose tolerance	8 (30.8)
	Impaired fasting glucose	—

these patients were widely distributed between thyroid autoimmune and nonautoimmune patients. Indeed, 7/12 (58.3%) women with thyroid Ab (TPO-Ab and TSHr-Ab) positivity showed hyperglycemia, while 11/26 (42.3%) with negative thyroid Abs had impaired glucose metabolism; no statistically significant differences were detected between these two groups.

4. Discussion

The prevalence of pancreatic autoimmunity in GDM has been widely investigated [3, 5, 6, 19], and it has been shown to differ for racial and geographic reasons. In the present study, GAD65-Abs were detected in 3.3% of our population, a level that is in agreement with several previous reports [2, 3, 5, 6, 19]. In our study we have chosen to determine only anti-GAD, because GAD autoantibodies are markers with the highest diagnostic sensitivity in LADA, so they should be used to identify such patient [20].

Fewer studies have investigated the prevalence of thyroid autoimmunity during GDM: most of these did not show a significant increase [13, 21], although few reports [11, 22] showed a higher risk of thyroid autoimmunity in women with a family history of diabetes and thyroid diseases. The present study also shows no significant differences.

The mean TSH value of the GDM patients was similar to that seen in normally pregnant women, and no differences were seen relating to the prevalence of abnormal TSH values between these two groups. At the same time, the FT4 levels were not significantly different. In summary, it can be concluded that no differences in thyroid function and autoimmunity were detected in the present study in GDM patients, as compared with normally pregnant women.

To our knowledge, little data are available on the prevalence of thyroid autoimmunity in women with previous GDM. We found an increased frequency of thyroid antibodies in patients with previous GDM; indeed, 31.6% of

our patients were positive for TPO-Ab, Tg-Abs or TSHr-Ab, as compared with 9.7% for women with previously normal pregnancies. We would also underline that in one patient, thyroid autoimmunity was only revealed by the TSHr-Ab assay. The presence of the TSHr-Ab as the only marker of thyroid autoimmunity has been described previously for a population of type 1 diabetes patients [23]; however, GAD65-Abs were negative in our patients, and there is no need at present for insulin therapy. The TSHr-Ab assay has therefore to be considered as a useful tool to reveal subclinical autoimmune hyperthyroidism whenever TSH is below the normal range, even if TPO-Abs are negative and there is no other sign of endocrine autoimmunity.

An association of thyroid dysfunction and Ab positivity was detected more frequently in the previously gestational women (7/38 [18.4%] in group B1, and 1/31 [3.3%] in group B2). On the basis of our data, it can be speculated that an increase in thyroid autoimmunity occurs in post-GDM women and that this phenomenon is relevant enough to cause subclinical thyroid dysfunction.

An association between autoimmune diabetes (type 1 and latent autoimmune diabetes) and other organ-specific autoimmune disorders has been widely described [7, 24]; however, in the present study, only 1 woman showed positivity for both pancreatic and thyroid autoimmunities. A common (pancreatic and thyroid) autoimmune trait appears therefore an unlikely explanation for the increased prevalence of thyroid autoimmunity in our post-GDM patients. Igawa et al. [25] suggested that the clinical association between chronic autoimmune thyroiditis and type 2 diabetes is related to a common antigen that is shared by pancreatic β -cells and thyroid follicular cells. More recently, we have shown that a 10 mM increase in glucose levels in cultured thyroid cells can upregulate major histocompatibility complex (MHC) class I expression [26]. We therefore hypothesized that this phenomenon causes the thyrocyte to become an antigen-presenting cell and possibly to overcome self-tolerance. It has indeed been shown that elevated levels of MHC molecules, which increase thyroid antigen presentation, can trigger thyroid autoimmunity in animal models [27, 28].

In accordance with this observation, we speculate that hyperglycemia at the time of pregnancy or immediately after delivery triggered the autoimmune disorder in our patients. If this is the case, GDM represents a unique chance to evaluate progression of thyroid autoimmunity from its onset.

It has to be emphasized, however, that the present study and our speculative explanation should still be considered as a preliminary step. In our study the diagnosis of GDM was carried out by OGTT with 100 g of glucose, and as is known new diagnostic criteria suggested by IADPSG [29] and ADA have been recently introduced. We cannot say whether the conduct would have been the same with new criteria. In fact the new criteria for diagnosing GDM identified a group of women previously classifiable as normal according to the 4th International Workshop Conference criteria, but revealing metabolic characteristics and pregnancy outcomes resembling those of women who would have been considered to have gestational diabetes by the previous criteria [30].

A major limit of this study is indeed the relatively small number of subjects evaluated. Therefore, observations on a larger population are needed to confirm our data.

In this context, we also cannot rule out the possibility that progression towards hyperglycemic disease after delivery can further facilitate the onset of thyroid autoimmune disease; the small number of patients did not allow the reaching of statistical significance, even if a higher percentage of patients with impaired glucose metabolism showed thyroid Ab positivity (58.3% versus 42.3%). In a 20-year follow-up, Männistö and colleagues [31] showed that instead of thyroid antibodies presence of overt hypothyroidism poses risk of diabetes. On the other side, the presence of TPO-Ab is not predisposing to the development of GDM [32]. Indeed, in a large study that included more than 600 pregnant women, GDM was seen in 8.1% of the women with TPO-antibody positivity, as compared to 6.8% without the TPO antibody, and this difference was not significant.

In summary, the main and new finding from our study is the higher prevalence of thyroid autoimmunity in women who have had previous GDM; in the same group, thyroid dysfunction is also more prevalent. We speculate that gestational hyperglycemia can trigger thyroid autoimmunity.

Conflict of Interests

The authors declare that they have no competing financial interests.

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Clinical Study

Quality of Life, Wishes, and Needs in Women with Gestational Diabetes: Italian DAWN Pregnancy Study

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The DAWN (Diabetes Attitudes, Wishes and Needs) study is a survey promoted by the International Diabetes Federation to recognize the perceptions and attitudes of people suffering from diabetes mellitus. In this context, we evaluated the quality of life of Italian and immigrant women with gestational diabetes mellitus (GDM). Information was gathered using a structured questionnaire for patients' self-compilation. In a 3-month period, a 51-item questionnaire was submitted to 198 Italians and 88 immigrants (from 27 different foreign nationalities). Italian women were older and had higher education than the immigrants. 60% of the Italians and 38% of the immigrants had a family history of diabetes mellitus. In both groups, the diagnosis of GDM caused anxiety; one-third of women feared their child could contract diabetes at delivery and/or have congenital malformations. Some women had trouble in following treatment regimens: the major concern being dietary advice and blood glucose testing. Most women were satisfied (34%) or highly satisfied (60%) with the quality of care, although the degree of cooperation between diabetes specialists and gynaecologists was considered sometimes unsatisfactory. In order to optimize maternal and foetal outcomes, educational projects and improved communication between patients and the healthcare provider team are recommended.

1. Introduction

The DAWN study (Diabetes Attitudes, Wishes and Needs) is a survey promoted by the International Diabetes Federation, with the aim of recognizing the feelings and attitudes of patients suffering from diabetes mellitus and the healthcare professionals responsible for patient management and care. Up to now the results of the DAWN study have shown that about half of the people with diabetes have a lower quality of life. The most important factors predicting discomfort or worse quality of life among diabetic patients are the country of residence and, subsequently, the type of healthcare system adopted there (i.e., the presence or absence of a specialized

team dedicated to the care and assistance for diabetic patients and their chronic complications) [1, 2].

Inside the Italian DAWN study framework, the Italian DAWN Study Group on Pregnancy performed this survey to evaluate the wishes and needs of Italian and immigrant women affected by GDM.

2. Materials and Methods

2.1. Study Design. The research was conducted in 10 Italian centres specialized in the care of pregnant women with diabetes. Each centre supplied 20 questionnaires submitted

to pregnant women with GDM by nurses who had previously been trained in this task during a specific briefing session. Overall, 198 questionnaires were collected and analyzed.

The project was also targeted at immigrant women with GDM. The questionnaire used was based on the same criteria as those ones used for Italian women, with the addition of seven questions about the specific condition experienced by immigrants. A Cultural Mediator helped immigrant pregnant women with language difficulties. Overall, 88 questionnaires were collected. The two surveys were conducted in the same period.

The 51 questions covered the following topics:

- (i) the general characteristics of women with GDM,
- (ii) the feelings related to the diagnosis of diabetes during pregnancy,
- (iii) the evolution of diabetes,
- (iv) the diet in pregnancy,
- (v) the family support,
- (vi) the gestational diabetes specialist centre,
- (vii) the relationship with medical doctors.

The study was approved by the local ethics committee and the women gave their informed consent to participation in accordance with the Declaration of Helsinki.

GDM was diagnosed according to the Carpenter and Coustan's criteria [3]. Women with GDM were helped to achieve good metabolic control: fasting plasma glucose (FPG) <5.3 mM and 2 h postprandial plasma glucose (PPG) <6.7 mM. Patients were given dietary advice about the nutritional requirements of pregnancy [1], and nurse educators showed them how to self-monitor blood glucose. They attended the centres every two weeks for specialist consultations. Insulin treatment was started when FPG was higher than 5.3 mM and/or 2 h PPG higher than 6.7 mM [4]. All women with GDM were monitored for metabolic and obstetric purposes until delivery.

2.2. Statistical Evaluation. Data are expressed as absolute values, percentages, or mean \pm Standard Deviation (SD). Differences among groups were tested using the chi square or F-test. Statistical significance was set at $P < 0.05$. Significant differences obtained using the F-test were confirmed by the Mann-Whitney *U*-test if the assumptions of the F-tests were not met. All statistical analyses were performed using SPSS statistical package, version 16.0 (SPSS, Chicago, IL).

3. Results

3.1. General Characteristics. General characteristics of Italian and immigrant women with GDM are reported in Table 1.

The immigrant respondents belonged to as many as 27 different foreign nationalities; the countries of origin with the highest representation were Romania and Morocco, followed by Bangladesh, Albania, and Nigeria. 39% of the immigrant women with GDM lived in Italy for 5–9 years, 30% for 3–4 years, 22% for 1–2 years, and 12.5% for 10–14 years.

TABLE 1: General characteristics of women with GDM.

	Italians	Immigrants
Cases (<i>n</i>)	198	88
Age (years)	34.2 \pm 5	32.5 \pm 4*
Primiparous (%)	50.5	44*
Married (%)	92	95
Single/Divorced (%)	8	5
<i>School level</i>		
University/High School (%)	72	24*
Primary/Middle (%)	28	76*
<i>Occupation</i>		
Office workers (%)	34	—
Housewife (%)	20	52
Factory workers (%)	18	10
Shop assistant (%)	14	—
Housemaid (%)	2	16
Caregiver for elderly (%)	—	5
Unemployed (%)	6	12
Other occupations (%)	6	5

* $P < 0.05$.

3.2. Pregnancy and Diabetes. A previous pregnancy complicated by GDM was reported in 25% of Italian women and 42% of Immigrant ones ($P < 0.005$). In 88.5% of immigrant cases, the previous GDM was diagnosed in Italy.

The time of diagnosis of GDM in current pregnancy was made mostly (58.5%) between the 25th–29th week of gestation, although an early diagnosis (before 20 weeks of gestation) was performed in 40.5% of the immigrant women and 27% of the Italian women ($P < 0.05$). Like the Italian respondents, about 90% of the immigrant women were advised to undergo tests to diagnose gestational diabetes by their gynaecologist.

3.2.1. Feelings Arising from the Diagnosis of Diabetes during Pregnancy. The diagnosis of diabetes resulted in the development of anxiety in the same way as in both immigrant women and Italian women (90% versus 87%, $P = ns$). The most predominant feeling among pregnant women was the fear of possible consequences for their child (66%), while a significant minority (28.9%) was also worried about possible malformations in their babies.

Although worried about their general health, 52% of Italian respondents were moderately optimistic throughout their pregnancy. In fact, the frequency of respondents concerned about the consequences for their child (including malformation) decreased, being the mother's main care that of a serious illness or a possible failure in her pregnancy. In contrast, a higher number of immigrant respondents were anxious about their general conditions and about the consequences for their child, and fewer of them were moderately optimistic, ($P < 0.05$), (Figure 1).

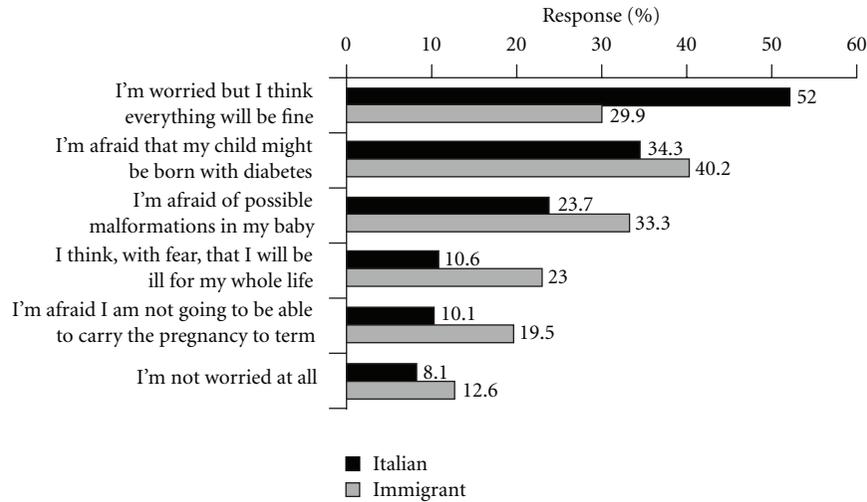


FIGURE 1: Patients' current concerns in relation to pregnancy. (The sum exceeds 100% because multiple answers were possible). The differences between Italian and Immigrant pregnant women are significant ($P < 0.05$).

TABLE 2: Difficulties and anxiety related to diabetes monitoring.

	Italians	Immigrants
<i>Difficulty in following:</i>		
Dietary advice (%)	58	43.5*
Home blood glucose (%)	63	36.5°
Physical activity (%)	50.5	61
Increase in anxiety for insulin therapy	31.5	40

* $P < 0.02$; ° $P > 0.01$.

3.2.2. *Difficulties Related to Diabetes Monitoring.* A considerable number of respondents claimed that they had experienced some difficulties in following the dietary advice, home blood glucose, and physical activity recommendations. The initiation of insulin therapy, that occurred in 31.5% of Italian and 40% of immigrant women, increased women's anxiety because they believed that insulin might cause problems for their unborn child (Table 2).

In all respondents, the treatment costs were not a load for the family budget.

The great majority of pregnant women with diabetes did not think that their diet was different from that of pregnant women without diabetes; however, a significantly higher proportion of immigrant women with GDM thought that the diets were indeed different (31% versus 16.7%; $P < 0.02$), (Figure 2). The reasons for this belief can be ascribed to their own experience and comparison with pregnant friends without diabetes, as well as to opinions held within their family, traditional beliefs, and a certain degree of perceived "suffering" from having to follow strict eating patterns and to abstain completely from sugar and sweets. In this respect, it is noteworthy that 78% of the immigrant respondents changed their eating habits nonetheless, and even though 80% of them said they had easy access to shops selling ethnic foods, the majority (58%) had a mixed diet that combined

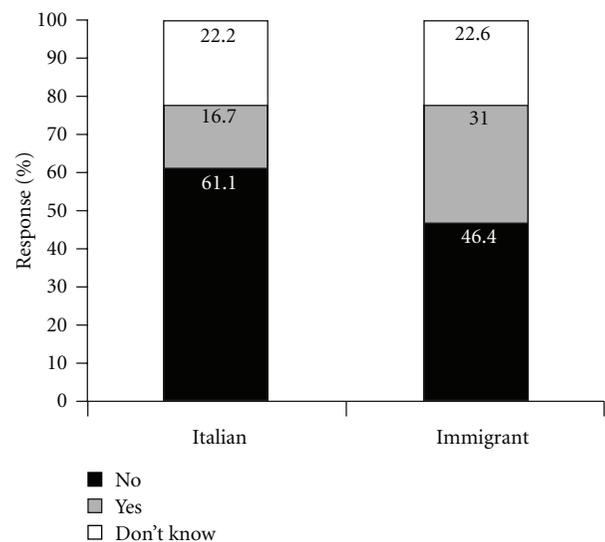


FIGURE 2: Patients' opinions about whether diet was different from that of pregnant women without diabetes. The differences between Italian and immigrant pregnant women are significant ($P < 0.02$).

the cuisine of their country with Italian eating habits. 25% of the immigrant women, mostly Muslim, said that there are religious recommendations about diet during pregnancy, but only one third of these women actually put these ones into practice.

95% of Italian women and only 29% of immigrants ($P < 0.001$) felt that their husband and other family members (parents, parents-in-law, sisters, etc.) helped them to deal with the problems related to their pregnancy. Among immigrant women, 40% said that they were helped by other family members, 30% by friends of the same country, 13% by Italian friends, 10% by Cultural Mediator, and 7% by a social worker.

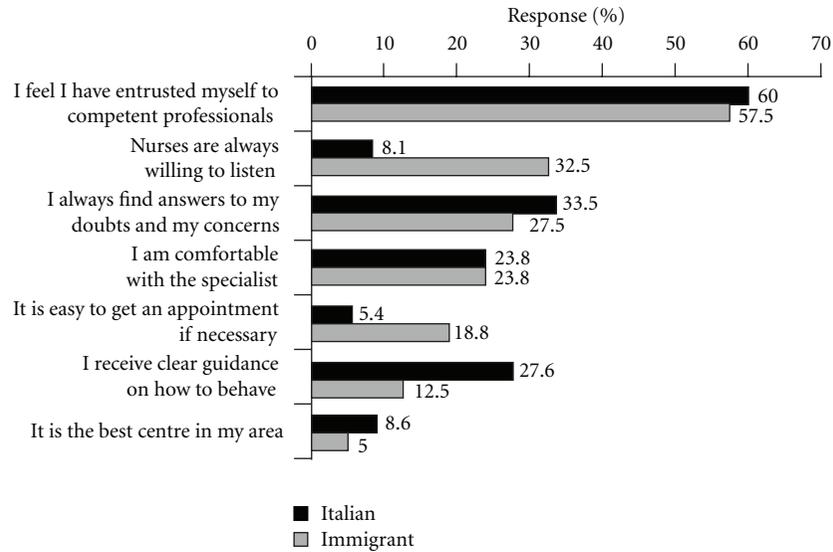


FIGURE 3: Relationship between patients and diabetes centre. (The sum exceeds 100% because multiple answers were possible).

3.3. The Gestational Diabetes Specialized Centre and the Relationship with Medical Doctors. The majority of respondents (83%) said they had heard about the Diabetes Centre from their gynaecologist; only 3.5% of Italian women and 23.5% of immigrant women received this information from their general practitioner. Most respondents were satisfied (34%) or highly satisfied (60%) for the quality of care provided by Diabetes Centres (Figure 3).

Pregnant women were usually comfortable talking to their gynaecologist and diabetes specialists. Among immigrant respondents, 8% declared some difficulties to have an appointment for gynaecologist consultancy and 13% did not have a gynaecologist they could trust. Gynaecologists and diabetes specialists cooperated in only 25-26% of cases, and most pregnant women (73%) felt that better cooperation between these practitioners is the best way to improve the care available to pregnant women with diabetes.

4. Discussion

The data from this survey, which is the first conducted in the field of gestational diabetes, has highlighted several areas of concern.

The diagnosis of a chronic disease, for example, diabetes mellitus, may generate anxiety [5]; this is especially true for women who received the diagnosis of diabetes during their pregnancy [6, 7]. An effective and satisfactory communication between pregnant women with gestational diabetes and healthcare providers can be fundamental to reduce their level of anxiety. These women need information about the disease, the potential risks for mother and child, the management strategy, and a treatment plan to avoid maternal and foetal complications [8].

In our study population, all women received structured information from a healthcare team comprising diabetologists, experienced nurses and dieticians. This results in

relatively few fears related to GDM, and in a moderate degree of optimism in many women. Thus, the role of the care team is crucial for immigrant women with GDM, particularly when considering their possible language difficulties, as well as cultural and religious differences [9–11].

Most women were satisfied with the treatment received at the Diabetic Centre for the competence of the healthcare team and the availability of the experienced nurses to listen to their doubts and worries and to help them to overcome their concerns. Nevertheless, it should be emphasized that a great number of women regarded the degree of cooperation between diabetes specialists and gynaecologists as unsatisfactory. We found that gynaecologists and diabetes specialists cooperated in about 25% of cases. Therefore, it was not surprising to find that most pregnant women indicated greater contact between these practitioners as the main way through which to improve the care of women with GDM. If you consider the difficulties that immigrant women encountered in contacting or being visited by their gynaecologist, it is essential that general practitioners should play a more crucial role in the care of pregnancy complicated by diabetes throughout the gestation period, starting with the diagnostic procedures and, finally, in the preconception period.

This survey shows that even in centres that have reached a good standard for treatment of pregnant women, GDM is often diagnosed at a late stage of pregnancy, confirming recently published Italian data [12]. Finally, the presence of an increasing number of women from foreign countries contacting diabetes centres during their pregnancies suggests a need for dedicated practices, with specially trained personnel, to ensure that treatment regimens are well adopted [10].

The limitations of this study are that the maternal and foetal outcomes of pregnant women were not considered and the results were obtained only from selected centres specializing in the care of diabetes during pregnancy in Italy

(most small diabetes centres are not included in this survey). Nevertheless, the strength of the survey is that, to the best of the authors' knowledge, no other similar study has been published so far. The data have allowed us to identify some care aspects that need to be examined in order to improve the quality of life of women with GDM and reduce the related foetal-maternal morbidity. To achieve this outcome, it is essential to carry out information campaigns directed to young women, using the press and other mass media, to raise awareness of the issues such as screening, diagnosis, and postpartum followup of diabetes mellitus.

Furthermore, immigrants make up a significant proportion of the pregnant population in Italy, as well as in other European countries [13], so it is necessary to promote training initiatives for the establishment of regional networks to improve communication between patients, cultural mediators, and healthcare professionals. In conclusion, measures to improve communication and cooperation between the various healthcare professionals will be essential if local and immigrant women with GDM are to deliver healthy, full-term babies with minimal risk to themselves.

Abbreviations

GDM: Gestational diabetes mellitus
 DAWN: Diabetes Attitudes, Wishes and Needs
 GCT: Glucose challenge test
 OGTT: Oral glucose tolerance test
 FPG: Fasting plasma glucose
 PPG: Postprandial plasma glucose
 SD: Standard deviation.

Conflict of Interests

The authors declare no conflict of interests.

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Clinical Study

Elevation of sE-Selectin Levels 2–24 Months following Gestational Diabetes Is Associated with Early Cardiometabolic Risk in Nondiabetic Women

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Objective. We hypothesised that the endothelial dysfunction is associated with early glucose dysregulation and/or atherosclerosis risk factors in nondiabetic women with a previous history of gestational diabetes (pGDM). **Material/Methods.** Anthropometric parameters, glucose regulation (OGTT), insulin resistance (HOMA), lipids, biomarkers of endothelial dysfunction, and inflammation were evaluated in 85 women with pGDM and in 40 controls 2–24 months postpartum. **Results.** The pGDM group consisted of 67% normoglycemic women (pGDM-N) and 33% with prediabetic state (pGDM-P). The BMI, waist circumference, fasting and 2 h glucose (OGTT), soluble adhesion molecules, tissue plasminogen activator antigen, high sensitivity C-reactive protein, total-, LDL-cholesterol, and triglycerides/HDL-cholesterol ratio were higher in the pGDM women compared with the controls. After adjustment for BMI and fasting glucose, only higher triglycerides, higher TG/HDL and lower HDL-cholesterol were associated with pGDM. The pGDM-P differed from pGDM-N for only higher triglycerides and TG/HDL. The plasma level of sE-selectin was not independently associated with glucose concentration in pGDM group. sE-selectin level correlated with triglycerides, TG/HDL, plasminogen activator inhibitor-1 antigen, and sICAM-1. **Conclusions.** sE-selectin level correlated with components of metabolic syndrome, but only the atherogenic lipid profile was independently associated with a previous history of GDM in nondiabetic women 2–24 months postpartum.

1. Introduction

Women with the previous history of gestational diabetes mellitus (pGDM) have a significantly increased risk of type 2 diabetes and cardiovascular disease in the next years after delivery [1]. Very important problem is both GDM and its future consequences are drastically increasing public health and global problem which requires specific preventive strategies.

In the years postpartum, women with pGDM have an increased cardiometabolic derangement, including vascular risk factors and early vascular dysfunction [2–4]. Several studies performed early (6 weeks–2 years) after delivery have

found the association of cardiovascular risk factors with glucose intolerance, and in particular with type 2 diabetes [5, 6].

Shah et al. [1] have observed that there is an increased prevalence of metabolic syndrome in the third month postpartum among women with even mild glucose intolerance during pregnancy. Thus, it is possible that different stages of glucose dysregulation diagnosed during pregnancy are associated with early cardiovascular risk postpartum [7]. It has been suggested that the association between prediabetic state or diabetes and cardiovascular disease may be mediated through endothelial dysfunction [8, 9].

Soluble forms of adhesion molecules are released from shedding or proteolytic cleavage from the endothelial cell surface and may reflect overexpression of their respective membrane-bound forms [10]. Circulating levels of some endothelial-derived factors, soluble cell adhesion molecules, von Willebrand factor (vWF), tissue plasminogen activator (t-PA), and plasminogen activator inhibitor type1 (PAI-1) have been linked to the risk of type 2 diabetes in populations without pGDM [8, 11–13]. There are few controversial data concerning the associations between levels of soluble adhesion molecules and glucose regulation status in women with the pGDM. Studies simultaneously have assessed the associations with soluble adhesion molecules and hemostatic endothelial activation biomarkers with prediabetic and proatherogenic state in women with the pGDM [14–16].

In current study we hypothesized that early (2–24 months) endothelial dysfunction is associated with the development of early glucose dysregulation and/or the other atherosclerosis risk factors and may be the potential target included in preventive strategies in women with the pGDM. The aim of current study was to assess selected parameters of dysfunction of endothelium such as concentration of soluble E-selectin (sE-selectin), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble intracellular adhesion molecule-1 (sICAM) in women with pGDM 2–24 months postpartum.

2. Material and Methods

2.1. Patients and Subjects. The research was conducted at the Diabetes and Pregnancy Unit at the Dr. J. Biziel University Hospital in Bydgoszcz, Poland, between 2005 and 2007.

A total of 85 Caucasian women with single gestations complicated by previous GDM (pGDM) and 40 women with normal glucose status during pregnancy (previous normal glucose tolerance—pNGT) as the control group were evaluated in the period of 2–24 months after delivery.

All subjects ingested at least 150 g carbohydrates a day and refrained from exercise for at least 3 days before the study. None of the participants had renal, hepatic, or cardiovascular disease. None of them were taking medications that affected lipid or carbohydrate metabolism. Women with a family history of diabetes mellitus, in addition of infectious processes, under stress as well as, those who were smoking, were not included.

GDM was diagnosed according to the modified criteria of the World Health Organization [17] after performing a fasting and 2 h 75 g oral glucose tolerance test (OGTT). These modified criteria are commonly used in Poland and are based on taking fasting glucose concentration of <5.6 mmol/L as a norm. This test was carried out in all pregnant women with a screening test (GCT-50-g oral glucose challenge) showing 2 h glucose value equal to or above 7.8 mmol/L. The screening test was performed during the first prenatal visit in all study groups and was repeated in the 24–28 weeks of gestation.

At the postpartum assessment, all participants underwent a physical examination that included anthropometric measurements. Waist circumference was measured at the

smallest circumference between the ribs and iliac crest. BMI was calculated as weight in kilograms divided by the squared height in meters.

The standard 75 g 2 h OGTT was performed in order to assess glucose tolerance after delivery. The current status of glucose regulation was defined according to the modified WHO recommendations.

Venous blood (4.5 mL) for endothelial markers tests was collected in a fasting state into cooled tubes (Becton Dickinson Vacutainer System, Plymouth, UK) containing 0.13 mol/L trisodium citrate (the final blood-anticoagulant ratio was 9:1) after 30 min of rest between 7.00 and 9.00 am and after a 12 h overnight fast. The blood samples were immediately mixed and centrifuged at $3000 \times g$ at $+4^\circ\text{C}$ for 20 min. The obtained platelet-poor plasma was divided into 200 μL Eppendorf-type tubes, and then samples were frozen at -86°C until assayed, but no longer than six months. Blood for serum lipids; insulin concentration, and high sensitivity C-reactive protein (hsCRP) was collected in a tube containing no anticoagulant (Becton Dickinson Vacutainer 17490, Plymouth, UK), and the serum was separated by centrifuging at $2500 \times g$ for 15 min and kept at $+4^\circ\text{C}$ until analyzed.

Serum glucose levels in samples from the OGTT were analyzed using the glucose hexokinase method on the automatic analyzer (Olympus Diagnostica GmbH, Ireland). Serum insulin concentration was determined using an immunochemical assay (8K41 Architect Insulin, Abbott Diagnostics, Denka Seiken, Japan) performed in the Architect Insulin analyzer (Abbott Diagnostics, Denka Seiken, Japan). Serum concentrations of triglycerides and total LDL and HDL cholesterol were measured by enzymatic techniques (Olympus Diagnostica GmbH, Ireland) on the Olympus automatic analyzer (Olympus Diagnostica GmbH, Ireland).

The concentration of t-PA:Ag was determined by Enzyme-Linked Immunosorbent Assay (ELISA)-ASSER-ACHROM t-PA (Diagnostica Stago, Asnieres, France), and PAI-1:Ag was determined by ELISA-ASSERACHROM PAI-1 (Diagnostica Stago, Asnieres, France), vWF:Ag was assessed by ELISA-STA-Liatest VWF:Ag (Diagnostica Stago, Asnieres, France), C-reactive protein (CRP) was measured with a high-sensitivity assay by test IMUCLONE CRP (hs) ELISA, C-Reactive Protein-ADI, using reagents from the American Diagnostica. Soluble sICAM-1 and sVCAM-1 were measured by using reagents from Bender Medsystems (Biomedica, Poland), and soluble E-selectin was determined by testing E-selectin (ELAM-1) antibody using reagent from IBL (Hamburg, Germany).

The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as a sensitivity index of the insulin sensitivity of the whole body. The HOMA index was calculated by multiplying the fasting glucose level (millimoles per liter) by the fasting insulin level (microunits per milliliter) and dividing the product by 22.5 [18].

2.2. Statistical Methods. The statistical analysis was carried out using Statistica 8,0 (StatSoft, Cracow, Poland).

The analyzed parameters were tested for normal distribution using the Shapiro-Wilk test. Variables were not normally distributed; *U* Mann Whitney test was used. Results shown are median (interquartile range) or frequencies (percentages). These results were further adjusted for BMI and fasting glucose. The *P* values <0.05 were considered statistically significant. Spearman's correlation coefficients were calculated to determine if there were associations between measured parametric values and sE-selectin level in the group with the history of GDM. To investigate the association between glucose level and plasma levels of biomarkers of endothelial dysfunction, all pGDM women were divided into two subgroups in accordance with low and high-glucose concentration within the prediabetic range. The high-glucose subgroup comprised of women with fasting glucose equal or above 6.4 mmol/L and <7.8 mmol/L at 2 h after 75 g glucose challenge (OGTT), women with fasting glucose <5.6 mmol/L and >8.9 mmol/L at 2 h, and women with fasting glucose equal to or above 6.4 mmol/L and >8.9 mmol/L at 2 h. Other pGDM women were included into the low-glucose subgroup. We compared plasma levels of endothelial dysfunction biomarkers in these subgroups. Additionally, the association of sE-selectin concentration with fasting glucose was examined by multiple logistic regression analysis.

2.3. Results. At the postpartum assessment of 85 women with pGDM and 40 women with normal glucose regulation during pregnancy (previous normal glucose tolerance—pNGT group), 7 women (8.24%) in pGDM group had impaired fasting glucose with normal glucose tolerance at 2 h OGTT (IFG/NGT), 16 (18.82%) normal fasting glucose with impaired glucose tolerance at 2 h OGTT (NFG/IGT), 5 (5.88%) impaired fasting glucose with impaired glucose tolerance at 2 h OGTT (IFG/IGT), and 57 women (67.06%) with normal glucose regulation (NFG/NGT). In the pNGT group all women were classified as NFG/NGT.

Women in pGDM group had significantly higher levels of fasting glucose, 2 h (OGTT) glucose, HbA1c, sVICAM-1, sICAM-1, sE-selectin, tPA:Ag, BMI, waist circumference, total cholesterol, LDL-cholesterol, triglycerides, and triglycerides/HDL-cholesterol ratio and lower HDL-cholesterol. After adjustment for BMI and fasting glucose, the pGDM was associated only with higher sVICAM-1, higher triglycerides, lower HDL-cholesterol, and higher triglycerides/HDL-cholesterol ratio (Tables 1 and 2).

Women in pGDM-P subgroup differed from pGDM-N group for higher fasting, 2 h (OGTT) glucose and higher triglycerides and triglycerides/HDL-cholesterol ratio (Table 2).

Only sE-selectin levels were significantly higher in high-glucose subgroup compared with the low-glucose subgroup, but this result was eliminated after further adjustment for BMI (Table 3). In multivariate regression analysis fasting glucose was associated only with insulin ($P = 0.0087$ and HOMA-IR ($P = 0.0013$).

In pGDM group sE-selectin was significantly correlated with triglycerides ($r = 0.32$), triglycerides/cholesterol-HDL

ratio ($r = 0.2910$), PAI-1:Ag ($r = 0.2647$), tPA:Ag ($r = 0.2622$), and sICAM-1 ($r = 0.2504$).

3. Discussion

Among the 85 apparently healthy women with the previous history of gestational diabetes (pGDM), 33% showed prediabetic state; 19% impaired glucose tolerance, 8% impaired fasting glucose and 6% impaired fasting glucose with impaired glucose tolerance at two hours of oral glucose tolerance test. These results are in accordance with previous reports [5].

Our study shows that these nondiabetic women with pGDM have higher concentrations of cardiovascular risk factors at average 10 months assessment after delivery in comparison with women with normal glucose regulation during pregnancy. The pGDM population was characterized by higher values of body fat indexes as well as levels of endothelial dysfunction parameters (sICAM-1, sVICAM-1, sE-selectin, tPA:Ag) and low-grade inflammation (hsCRP) which coexisted with dyslipidemic lipids profile and comparable insulin resistance. After adjustment for BMI and fasting glucose, only higher triglycerides, higher sVICAM-1, lower HDL-cholesterol, and higher triglycerides/HDL-cholesterol ratio were associated with pGDM.

Thus, our results suggest that the atherogenic dyslipidemia may be specific and important metabolic feature of nondiabetic women with pGDM during first years postpartum. It is well known and generally accepted that this metabolic abnormality is associated with endothelial dysfunction and insulin resistance [19–21]. Interestingly, our current study shows that atherogenic lipids profile in pGDM nondiabetic women is independent of both insulin resistance and BMI.

Our results are inconsistent with one previous report suggesting elevated sE-selectin levels during 12–26 months after delivery only in pGDM women with abnormal glucose regulation and with other two studies presenting higher sE-selectin levels irrespectively of glucose regulation status [14–16] and metabolic abnormalities related to insulin resistance [16]. Moreover, results of these studies suggest that elevated level of sE-selectin in post-GDM women is independent of time since delivery [14, 16].

The current study is the first in which we observed higher plasma levels of sICAM-1, sE-selectin, and tPA:Ag levels with simultaneously normal levels of PAI-1:Ag and vWF:Ag in nondiabetic pGDM women with both abnormal and normal glucose regulation.

Several studies have shown that circulating levels of ICAM-1, E-selectin [8, 11], tPA:Ag [12, 13], vWF:Ag, and PAI-1:Ag [12] have been linked to the risk of type 2 diabetes in populations without the previous history of GDM.

In our study we have found that in the case of nondiabetic women with pGDM, high-glucose concentrations within the prediabetic range were associated with higher sE-selectin levels. This association was eliminated after further adjustment for BMI. Additionally, in multivariate regression analysis, fasting glucose was associated only with insulin

TABLE 1: Characteristics of women with previous history of GDM (pGDM group) and control participants with normal glucose regulation during pregnancy (pNGT group).

Variables (units)	pGDM (<i>n</i> = 85)	pNGT (<i>n</i> = 40)	<i>P</i> -values
Age (years)	29.00 (26.00, 35.00)	27.00 (25.00, 35.50)	0.2040
BMI (kg/m ²)	23.68 (20.96, 27.54)	22.00 (20.31, 24.33)	0.0098
Waist (m)	0.80 (0.73–0.92)	0.74 (0.69–0.78)	0.0007
HbA ₁ C (%)	5.50 (5.40–5.70)	5.40 (5.30–5.40)	0.4399*
Fasting plasma glucose (mmol/L)	4.50 (4.61–5.33)	4.72 (4.50–4.78)	0.0001*
2-h (OGTT) plasma glucose (mmol/L)	6.16 (5.55–7.66)	4.84 (4.77–5.44)	0.1655*
Insulin (pmol/L)	54.87 (41.67–80.56)	63.20 (54.87–71.53)	0.1755
sVCAM-1 (ng/mL)	1113.53 (419.60–1616.70)	672.05 (455.70–1113.46)	0.00018*
sICAM-1 (ng/mL)	294.89 (238.73–364.19)	140.40 (121.34–179.90)	0.5053*
sE-selectin (ng/mL)	28.13 (19.17–43.95)	20.90 (17.11–26.01)	0.0791* 0.4791**
tPA:Ag (ng/mL)	4.89 (3.53–7.66)	3.50 (2.66–4.64)	0.4058*
vWF:Ag (%)	104.30 (96.20–122.30)	104.87 (74.96–125.67)	0.4405
PAI-1:Ag (ng/mL)	57.09 (46.28–79.43)	71.36 (50.54–81.80)	0.1371
hsCRP (μg/mL)	1.22 (0.66–2.76)	0.41 (0.22–0.79)	<0.0004* 0.1939**
Total-cholesterol (mmol/L)	5.04 (4.55–5.51)	4.56 (4.27–5.09)	0.9940*
HDL-cholesterol (mmol/L)	1.53 (1.28–1.73)	1.73 (1.56–1.82)	0.0027* 0.0449**
LDL-cholesterol (mmol/L)	3.10 (2.79–3.54)	2.57 (2.30–3.05)	0.4022*
Triglyceride (mmol/L)	0.97 (0.78–12.83)	0.86 (0.67–1.05)	0.0006* <0.0001**
Triglyceride/HDL cholesterol	1.52 (1.06–2.63)	1.11 (0.89–1.50)	<0.0001* <0.0001**
HOMA-IR (%)	1.67 (1.22–2.80)	1.89 (1.58–2.37)	0.3973
NFG/IGT <i>n</i> (%)	16 (18.82)	—	—
IFG/NGT <i>n</i> (%)	7 (8.24)	—	—
IFG/IGT <i>n</i> (%)	5 (5.88)	—	—
NFG/NGT <i>n</i> (%)	57 (67.06)	40 (100)	—

Values are median (IQR) or *n* (%). *P* values were from *U*-Mann Whitney test. *adjusted for body mass index, **adjusted for fasting glucose.

concentration and insulin resistance assessed by HOMA-index. Thus, our results showed that endothelial dysfunction evaluated by soluble forms of its specific biomarkers was not independently associated with glucose dysregulation in nondiabetic women with pGDM.

In the whole population of pGDM women, sE-selectin levels correlated positively with other markers of endothelial dysfunction, tPA:Ag, sICAM-1, and with surrogate markers of insulin resistance, triglycerides, triglycerides/HDL-cholesterol ratio, and PAI-1:Ag level. Recently, triglycerides/HDL cholesterol ratio has been suggested to be a predictor of insulin resistance and of the proportion of small and dense LDL particles, which is characteristic of atherogenic phenotype of the metabolic syndrome [22].

Results of our study suggest also that endothelial dysfunction at assessment 2–24 months (average 10 months) after delivery in nondiabetic women with pGDM is characterized by higher plasma levels of circulating ICAM-1, sE-selectin, and tPA:Ag in women with normal glucose regulation as well as pGDM women with prediabetic state, matched for age, body fat indexes values, inflammation, and

PAI-1:Ag level. These observations agree with the previous studies of Bo et al. performed 6.5 years after delivery in pGDM women. In this study, they obtained higher levels of sE-selectin and sICAM-1 and higher intima media thickness (IMT) values characterized pGDM women, even those without any components of the metabolic syndrome except for glycaemia [16]. In all pGDM women, sE-selectin, sICAM-1, interleukin-6, and hsCRP values were significantly associated with IMT, thus supporting associations of these biomarkers with pathogenesis of atherosclerosis [16].

Our present findings contrast with the results of previous study of Lawrence et al. [15]. These authors have found clinical and biochemical markers of insulin resistance but have not shown any associations between sE-selectin levels and these markers [15]. Additionally, higher levels of sE-selectin in post-GDM women were restricted to women with abnormal glucose regulation. Thus, discrepant results of current study and the study of Lawrence et al. may suggest pathophysiological differences in populations subject to examinations [15].

TABLE 2: Clinical and laboratory data for participants in studied groups: pGDM-P, pGDM-N and pNGT as a control group.

Variable (units)	pGDM-P <i>n</i> = 29	pGDM-N <i>n</i> = 56	pNGT <i>n</i> = 40	<i>P</i> -values
Age (years)	31.50 (27.50–35.50)	30.00 (26.00–35.00)	27.00 (25.00–35.50)	0.1145
BMI (kg/m ²)	25.12 (21.83–29.04) ^b	24.44 (21.26–27.22) ^a	22.00 (20.31–24.33)	0.0022
Waist circumference (cm)	80.00 (73.00–96.00) ^b	82.00 (73.00–90.00) ^a	74.50 (69.00–78.00)	0.0010
Fasting plasma glucose (mmol/L)	5.16 (4.77–5.88) ^{b,c}	4.77 (4.44–5.13)	4.72 (4.50–4.78)	0.0002
2-h (OGTT) plasma glucose (mmol/L)	8.27 (8.05–8.77) ^{b,c}	5.88 (5.44–6.66) ^a	4.84 (4.77–5.44)	<0.0001
Insulin (pmol/L)	57.44 (42.36–95.84) ^{cc}	54.17 (41.67–69.45)	63.20 (54.87–71.53)	0.0353
sVCAM-1 (ng/mL)	960.30 (353.73–1638.03)	1127.63 (466.68–1582.58) ^a	672.05 (455.70–1113.46)	0.0553
sICAM-1 (ng/mL)	295.90 (238.80–363.38) ^b	299.28 (243.46–364.19) ^a	140.40 (121.34–179.90)	<0.0001
sE-selectin (ng/mL)	35.80 (26.37–43.65) ^b	28.57 (20.35–42.54) ^a	20.90 (17.11–26.01)	0.0022
tPA:Ag (ng/mL)	6.01 (4.09–7.51) ^b	4.68 (3.25–7.24) ^a	3.50 (2.66–4.64)	0.0005
vWF:Ag (%)	107.79 (96.20–131.59)	104.30 (95.36–117.22)	104.87 (74.96–125.67)	0.6498
PAI-1:Ag (ng/mL)	57.67 (49.44–83.75)	57.09 (42.85–85.12)	71.36 (50.54–81.80)	0.2178
hsCRP (μg/mL)	1.30 (0.81–2.86) ^b	1.32 (0.66–2.76) ^a	0.41 (0.22–0.79)	<0.0001
Total-cholesterol (mmol/L)	5.33 (4.84–5.92) ^b	5.02 (4.50–5.48) ^a	4.56 (4.27–5.09)	0.0022
HDL-cholesterol (mmol/L)	1.45 (1.29–1.73) ^b	1.55 (1.27–1.73) ^a	1.73 (1.56–1.82)	0.0048
LDL-cholesterol (mmol/L)	3.26 (2.97–4.06) ^b	3.03 (2.77–3.54) ^a	2.57 (2.30–3.05)	<0.0001
Triglyceride (mmol/L)	1.21 (1.02–2.22) ^{b,c}	79.00 (63.00–116.00)	74.50 (58.50–91.00)	0.0004
Triglyceride/HDL cholesterol	1.83 (1.35–4.06) ^{b,c}	1.33 (0.95–2.30)	1.11 (0.89–1.50)	0.0003
HOMA-IR (mmol/L)	2.32 (1.27–3.49) ^{ccc}	1.64 (1.22–2.15)	1.89 (1.58–2.37)	0.0499

P-values for testing over the three groups (ANOVA): a: *P* < 0.05 controls versus previous GDM with normal glucose regulation; b: *P* < 0.05 controls versus previous GDM with prediabetic state; c: *P* < 0.05 previous GDM with normal glucose status versus previous GDM with prediabetic state, cc: *P* = 0.05, ccc: *P* = 0.06.

TABLE 3: Plasma concentrations of endothelium markers according to low and high-glucose concentration within the prediabetic range.

Variable (units)	Low glucose (<i>n</i> = 58)	High glucose (<i>n</i> = 27)	<i>P</i> -values
sVCAM (ng/mL)	849.78 (550.70–1285.55)	669.18 (419.60–13.90)	0.3610
sICAM (ng/mL)	222.44 (152.66–317.08)	247.79 (212.59–338.68)	0.1066
sE-selectin (ng/mL)	25.90 (19.03–40.17)	37.75 (27.93–46.15)	0.0314 0.0791*
vWF:Ag (%)	101.72 (91.76–119.04)	107.10 (86.68–136.34)	0.3457
tPA:Ag (ng/mL)	4.05 (2.63–6.05)	4.92 (3.69–6.68)	0.0838
PAI-1:Ag (ng/mL)	61.85 (47.42–85.18)	55.47 (44.93–83.75)	0.7013

Values are median (IQR), and *P* values were from *U* Mann Whitney test. * Adjusted for body mass index.

4. Conclusion

Higher body fat indexes, glucose during OGTT, levels of endothelial dysfunction biomarkers, low-grade inflammation (hsCRP), and atherogenic dyslipidemia characterized nondiabetic women with pGDM and normal glucose regulation as well as women with pGDM and prediabetic state assessed 2–24 months after delivery. Our findings indicated that only parameters of atherogenic dyslipidemia and sVCAM-1 level were associated with pGDM independently of BMI, insulin resistance, and fasting glucose. sE-selectin levels correlated with metabolic syndrome components but were not independently associated with abnormal glucose regulation. Thus, the atherogenic dyslipidemia may be the key metabolic feature and a predictor of cardiovascular risk in women with pGDM and thus needs early and regular monitoring and special therapeutic interventions after an index pregnancy.

Abbreviations

pGDM: Previous gestational diabetes
sVCAM-1: Soluble vascular cell adhesion molecule-1
sICAM: Soluble intracellular adhesion molecule-1
PAI-1:Ag: Plasminogen activator inhibitor-1 antigen
tPA:Ag: Tissue plasminogen activator antigen
hsCRP: High sensitivity C-reactive protein.

Conflict of Interests

The authors declare that they have no conflict of interests.

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Clinical Study

Incretin Effect in Women with Former Gestational Diabetes within a Short Period after Delivery

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Background and Aims. Women with former gestational diabetes (fGDM) are characterized by impaired beta-cell function (BC). Incretin hormones contribute to insulin secretion after oral administration of glucose. We aimed to assess the possible role of incretins on altered insulin release in fGDM. **Materials and Methods.** We studied 104 fGDM women within 6 months after delivery and 35 healthy women after normal pregnancy (CNT) with a 75 g oral (OGTT) and a 0.33 g/kg intravenous (IVGTT) glucose test, both lasting 3 h. The ratio of suprabasal areas under the concentration curves for glucose (dAUC_{GL}) and C-peptide (dAUC_{CP}) evaluated BC during OGTT (BC_{OG}) and IVGTT (BC_{IV}). Incretin effect was computed in all fGDM and in fGDM with normal tolerance (fGDM_{NGT}) and with impaired glucose regulation (fGDM_{IGR}). **Results.** dAUC_{GL} of fGDM was higher ($P < 0.0001$) than CNT for both tests; while dAUC_{CP} were not different. BC_{OG} and BC_{IV} were lower in fGDM versus CNT ($1.42 \pm 0.17 \text{ nmol}_{\text{CP}}/\text{mmol}_{\text{GLUC}}$ versus 2.53 ± 0.61 , $P = 0.015$ and 0.41 ± 0.03 versus 0.68 ± 0.10 , $P = 0.0006$, respectively). IE in CNT ($66 \pm 4\%$) was not different from that of all fGDM (59 ± 3) and fGDM_{NGT} (60 ± 3), but higher than that of fGDM_{IGR} (52 ± 6 ; $P = 0.03$). IE normalized to BMI was $2.77 \pm 0.19\% \text{ m}^2/\text{kg}$ in CNT, higher than that of fGDM_{IGR} (1.75 ± 0.21 ; $P = 0.02$) and also of fGDM_{NGT} (2.33 ± 0.11 ; $P = 0.038$). **Conclusion.** Compromised IE characterizes fGDM_{IGR}. In both fGDM categories, regardless their glucose tolerance, IE normalized to BMI was reduced, signifying an intrinsic characteristic of fGDM. Therefore, the diminished IE of fGDM seems to reflect an early abnormality of the general beta-cell dysfunction in the progression toward type 2 diabetes.

1. Introduction

The incretin effect is the potentiation of the glucose-mediated insulin secretion due to the gut hormones, namely, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). They are released by the intestine in the blood stream after oral glucose load, such as during an oral glucose tolerance test or a meal [1]. The incretin effect has been demonstrated by comparing insulin concentration during oral and intravenous glucose administration that yielded the same peripheral glucose concentration; however, insulin concentration was significantly higher after the oral load. Since this happened despite the same glucose levels than with the intravenous load [2], the difference in insulin concentration was ascribed to the effect of the incretin hormones. In type 2 diabetic patients,

a reduction of this effect has been reported [3, 4] and pharmacological agents have been developed to restore this effect, important for maintaining a good glucose homeostasis [5].

Women with former gestational diabetes (fGDM) exhibit markedly increased risk for the later development of type 2 diabetes and related complications. They often present metabolic abnormalities in insulin sensitivity compared to control subjects, but even greater differences have been observed for beta-cell function. In a previous study on fGDM, we have assessed beta-cell function by the analysis of two independent tests, intravenous (IVGTT) and oral (OGTT) glucose tolerance tests and found that the impairment in beta-cell function was observable only with OGTT [6]. We hypothesize that the incretin effect may play a pivotal role

in the subtle derangement of beta-cell function observed in fGDM.

Therefore, the aim of this study was to evaluate the incretin effect in a larger group of fGDM, who underwent both IVGTT and OGTT immediately after partum. They were also divided according to their glucose tolerance to evaluate if it plays some role on the possible changes of the incretin effect in fGDM.

2. Materials and Methods

A total of 104 fGDM were studied within 6 months after delivery and compared to 35 healthy women after normal pregnancy (CNT). Table 1 shows the main characteristics of the two groups; body mass index (BMI) of fGDM was borderline higher, but in general still in the overweight range. Every subject randomly underwent a 75 g OGTT with sampling at 10, 20, 30, 60, 90, 120, 150, 180 min and a 0.33 g/kg frequently sampled intravenous glucose test (IVGTT) with insulin (0.03 IU/kg, Humulin R; Eli Lilly, Indianapolis, IN, USA) intravenous infusion at time 20 for 5 min. Less than 3 weeks elapsed between the two tests, without any diet or habit changes in between. Both tests lasted 3 h; glucose and C-peptide were determined in duplicate by commercially available radioimmunoassay kits with an interassay coefficient of variation <5%. Further details on the performance of the two tests were reported previously [7, 8].

Out of 104 fGDM, 77 resulted with normal glucose tolerance (fGDM_{NGT}) according to the OGTT criteria of the American Diabetes Association, 6 with type 2 diabetes and 21 with impaired glucose regulation (fGDM_{IGR}), which included both impaired fasting glucose and impaired glucose tolerance. All CNT exhibited normal glucose tolerance.

For the purpose of this study, we calculated the area under the concentration curves (AUC) for glucose (AUC_{GL}) and C-peptide (AUC_{CP}) for 3 h in both tests, by using the trapezoidal rule. The suprabasal, dynamic AUC (dAUC) were computed by subtracting from the AUC the basal area (i.e., fasting value \times 180 min). AUC_{CP} represents the absolute insulin release at the beta-cell level. The beta-cell function describes the ability of glucose to stimulate insulin release from the beta-cell. Beta-cell function during OGTT (BC_{OG}) and that during IVGTT (BC_{IV}) were calculated according to the respective ratio dAUC_{CP}/dAUC_{GL} in both tests: units nmol_{CP}/mmol_{GLUC}. The incretin action occurs only during an oral administration of glucose; thus, it can be estimated by subtracting the glucose stimulated secretion during the IVGTT from that evaluated during the OGTT: the formula $100 \times (BC_{OG} - BC_{IV})/BC_{OG}$ yields therefore the percent incretin effect [3]. This approach has been already successfully exploited in previous studies [9, 10].

In order to evaluate whether different glucose tolerance within the fGDM plays a role in the assessment of beta-cell function and incretin effect, these parameters were also computed in the single subgroups of normotolerant and impaired metabolism.

Data are expressed as mean \pm SE; means have been compared with the Student's *t*-test.

3. Results

Fasting and total AUC glucose in both tests were markedly higher in fGDM (Table 1); fasting C-peptide was not different, while the dynamic C-peptide response to glucose stimulation only tended to be higher in fGDM, but only with borderline significance. Suprabasal AUC of glucose (dAUC_{GL}), which represents the main stimulus to the secretory response of the beta-cell, was higher in fGDM than that of CNT for both tests (338 ± 20 mmol/L 3 h versus 189 ± 21 , $P = 0.0001$ for OGTT; 206 ± 13 versus 119 ± 16 , $P = 0.0002$ for IVGTT). Dynamic insulin secretion, dAUC_{CP} was not different between fGDM and CNT in both tests, but much higher ($P < 0.00001$) during OGTT (285 ± 9 nmol/L 3 h versus 257 ± 15 , $P = 0.13$ fGDM versus CNT for OGTT; 61 ± 3 versus 55 ± 5 , $P = 0.36$ for IVGTT).

Beta-cell function and incretin effect are shown in Table 2, where fGDM was also divided into fGDM_{NGT} and fGDM_{IGR}, the latter presenting with higher BMI. From these subgroups the 6 type 2 diabetics were excluded, since their small number allowed no statistical power for any possible comparison. BC_{OG} was markedly higher than BC_{IV} in both fGDM and CNT ($P = 0.003$); both BC_{OG} and BC_{IV} were lower in fGDM (all together) compared to CNT; and were lower in fGDM_{IGR} compared to fGDM_{NGT}.

When comparing the normotolerant fGDM_{NGT} to CNT, both BC_{OG} and BC_{IV} were not different, while beta-cell functions of fGDM_{IGR} were significantly lower than those of CNT. Incretin effect resulted similar between fGDM all together and CNT; that of fGDM_{NGT} was not different from that of CNT (P value ranging in both cases 0.2–0.8); while that of fGDM_{IGR} was significantly lower than that of women with normal pregnancy (Table 2). When incretin effect was normalized to BMI, to take into account that the main determinant of IGR was the increased BMI, the differences in the incretin effect were even more substantial. The value for CNT was $2.77 \pm 0.19\%$ m²/kg, still higher than that of fGDM_{IGR} (1.75 ± 0.21 ; $P = 0.019$), but also the difference with that of fGDM_{NGT} (2.33 ± 0.11) became significant ($P = 0.038$). Incretin effect normalized to BMI was 2.21 ± 0.10 ; $P = 0.006$ versus CNT).

4. Discussion

Within a short period after partum, beta-cell function, evaluated both with the oral and with the intravenous glucose tests, was reduced in a general population of overweight women who exhibited gestational diabetes mellitus during pregnancy [7]. Indeed, in both tests, fGDM exhibited C-peptide release only slightly increased despite markedly higher glucose. Results of this study show that incretins do not play a fundamental role in this observed reduced beta-cell function characterizing fGDM: in fact, the surrogate index of incretin effect used here did not differ from the same index evaluated in a population of healthy women, who had a normal pregnancy, studied within the same period after partum. In both CNT and fGDM, the beta-cell function obtained with the OGTT was much higher than that with IVGTT, showing that incretins have a potent effect in

TABLE 1: Main characteristics and area under the concentration curve (AUC) of glucose and C-peptide, in the two tests, for women with former gestational diabetes (fGDM) and women who had normal pregnancy (CNT).

	fGDM	CNT	<i>P</i> value
<i>N</i>	104	35	
Age (time of the study) (years)	33.5 ± 0.5	31.8 ± 0.9	0.076
Body mass index (kg/m ²)	27.3 ± 0.5	25.3 ± 1.0	0.048
Fasting glucose (mmol/L)	5.08 ± 0.08	4.62 ± 0.06	0.0008
Fasting insulin (pmol/L)	60 ± 4	57 ± 5	0.668
Fasting C-peptide (pmol/L)	646 ± 31	582 ± 31	0.258
AUC glucose (OGTT) (mol/L 3 h)	1.26 ± 0.03	1.02 ± 0.02	0.00001
AUC C-peptide (OGTT) (nmol/L 3 h)	402 ± 13	353 ± 19	0.048
AUC glucose (IVGTT) (mol/L 3 h)	0.98 ± 0.02	0.85 ± 0.02	0.0001
AUC C-peptide (IVGTT) (nmol/L 3 h)	150 ± 8	122 ± 9	0.055

TABLE 2: Beta-cell function from OGTT (BC_{OG}) and IVGTT (BC_{IV}) and incretin effect (IE) in all fGDM and in the two subgroups of fGDM characterized by normal glucose tolerance (fGDM_{NGT}) and impaired glucose metabolism (fGDM_{IGR}), compared to women who had normal pregnancy (CNT).

	fGDM _{IGR}	fGDM _{NGT}	<i>P</i> ^a	CNT	<i>P</i> ^b	all fGDM	<i>P</i> ^c
<i>N</i>	21	77		35		104	
BMI	30.2 ± 1.0	26.2 ± 0.5	0.001	27.3 ± 0.5	0.001	25.3 ± 1.0	0.048
BC _{OG}	0.67 ± 0.06	1.70 ± 0.21	0.014	2.53 ± 0.61	0.023	1.42 ± 0.17	0.016
BC _{IV}	0.29 ± 0.17	0.47 ± 0.04	0.011	0.61 ± 0.10	0.020	0.41 ± 0.03	0.012
IE	51.5 ± 5.8	60.0 ± 2.8	0.171	65.8 ± 3.6	0.030	58.9 ± 2.5	0.146

BMI: body mass index (kg/m²); units for BC_{OG} and BC_{IV}: nmol_{CP}/mmol_{GLUC}; for IE: %; all fGDM include also 6 fGDM type 2 diabetic women; *P* values: ^afGDM_{IGR} versus fGDM_{NGT}; ^bfGDM_{IGR} versus CNT; ^call fGDM versus CNT.

both groups. The elevated beta-cell response during OGTT could mask possible significant differences in the beta-cell sensitivity to glucose between fGDM and CNT; however, no difference is also highlighted by the IVGTT, which provides the evaluation independent on incretins.

Our results on beta-cell function seem to be in contrast with a previous study [11], where it is reported an unchanged beta-cell function in fGDM. However, in that study, the authors used the 30 min insulinogenic index with insulin, which is known to be not fully reliable [12], while the present investigation exploited C-peptide evaluated during the whole 3 h duration of the test: a more reliable figure of the incretin-mediated sensitivity of the beta-cell to the glucose stimulation. To quantify this process, we have used the percent (normalized) difference of the beta-cell function between the tests, which gauges the relative contribution of the incretins in fostering glucose-dependent insulin release. We have used the beta-cell function instead of the simple AUCs as previously done [3, 9, 10], because the OGTT and the IVGTT did not yield isoglycemic patterns; thus, we had to normalize the insulin release to glucose with the ratios $dAUC_{CP}/dAUC_{GL}$. The incretin effect we estimated is a kind of general measurement and cannot discriminate between the real effect of the incretins and the possible reduction in fGDM of the incretins production [13]. In fact, our measurement is an indirect surrogate, since we have not measured the incretin hormones concentration. This is the main weakness of our study. Having for instance the pattern

of GLP-1, we could apply another straightforward method [14] that quantifies the direct incretin action, independently on the actual hormone secretion. Some investigators found a mildly reduced GLP-1 response to oral glucose only during the first 30 min, while the entire 2 h GLP-1 AUC did not differ from that of the control subjects [13]. This was substantiated by other studies that reported no differences in the secretion of incretin hormones after oral glucose ingestion in women with a history of gestational diabetes [11]. Therefore, we are quite confident that the incretin effect we observed in our study is prevalently due to the action of incretins in sensitizing the beta-cell.

A recent report [10] showed that incretin effect is reduced with obesity, which is known to be characterized by insulin resistance and reduced glucose tolerance. Another study related incretin effect to various degrees of glucose intolerance [15]. These findings in a general population were then verified in our fGDM, who were divided into normo- and impaired tolerant. To the best of our knowledge, this is a novelty of our study. We have found a clear reduction in the incretin effect in fGDM with IGR. Incretin effect was instead similar between former GDM with NGT and CNT. The reason may be in the fact that IGR have a slightly higher BMI, though not reaching overt obesity yet. This fact corroborates more our point, since it is known that obesity is accompanied by hyperinsulinemia before the onset of overt diabetes. Thus the circumstance that fGDM_{IGR} still presents reduced beta-cell sensitivity to glucose, despite higher BMI, appears to

be an intrinsic defect of this population. In fact, when the incretin effect was normalized to BMI, fGDM_{NGT} too had a lower index, despite similar BMI than CNT. Since the majority of fGDM return normal after delivery, in general, we can claim that a reduced incretin effect likely remains a characteristic of this condition.

In conclusion, normotolerant women with previous gestational diabetes exhibit an incretin effect similar to that of healthy women, who had a normal pregnancy. Compromised incretin effect, proper of obese and type 2 diabetic subjects, characterizes instead fGDM with impaired glucose tolerance, probably related to their, though slightly, augmented body weight. When the incretin effect was evaluated after normalization to BMI, it resulted in reduced in both categories, giving the impression of an intrinsic characteristic of fGDM, regardless of their glucose tolerance. The diminished incretin effect of fGDM seems therefore to reflect an early abnormality of the general beta-cell dysfunction in the progression toward type 2 diabetes.

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Clinical Study

Risk Factors for Gestational Diabetes Mellitus in a Large Population of Women Living in Spain: Implications for Preventative Strategies

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The aim of this study is to establish a risk appraisal model for GDM by identifying modifiable factors that can help predict the risk of GDM in a large population of 2194 women living in Spain. They were recruited between 2009-2010 when screening for GDM was performed. Participants completed a questionnaire on socio-demographic, anthropomorphic and behavioral characteristics, and reproductive and medical history. A total of 213 (9.7%) women were diagnosed as having GDM. Age, pregestational body weight (BW) and body mass index (BMI), and number of events of medical, obstetric and family history were significantly associated with GDM. After logistic regression model, biscuits and pastries intake <4 times/week, red and processed meats intake <6 servings/week, sugared drinks <4 servings/week, light walking >30 minutes/day, and 30 minutes/day of sports at least 2 days/week, compared with opposite consumption, was associated with less GDM risk. Our study identified several pregestational modifiable lifestyle risk factors associated with an increase in the risk of developing GDM. This may represent a promising approach for the prevention of GDM and subsequent complications. Further intervention studies are needed to evaluate if this appraisal model of risk calculation can be useful for prevention and treatment of GDM.

1. Introduction

Gestational diabetes mellitus (GDM) has been defined for many years as any degree of glucose intolerance with onset or first recognition during pregnancy [1]. According to the hyperglycemia and adverse pregnancy outcomes (HAPO study) [2], a large-scale multinational epidemiologic study, the risk of adverse maternal, fetal, and neonatal outcomes continuously increases as a function of maternal glycemia at 24–28 weeks of gestation. There was no threshold for most of these complications, but preventing and early identifying GDM is a growing health concern.

Because the prevalence of GDM is increasing in a similar way to the ongoing epidemic of obesity and type 2 diabetes

in women of child-bearing age [3], understanding the significance of risk factors becomes of highlighted importance. Nonmodifiable risk factors such as past history of GDM and increasing maternal age have been identified [4, 5]. However, the impact of diet and lifestyle modifiable risk factors has not yet been systematically synthesized [6, 7]; they can be different in countries of the Mediterranean area, and only preliminary data are known [8].

The aim of this study is to establish a risk appraisal model for GDM by identifying modifiable factors that can help predict the risk of GDM in a large population of women living in Spain and target early intervention, at the time of screening for GDM, with the potential of preventing the development of or ameliorating GDM.

TABLE 1: Demographic and anthropomorphic characteristics of the 2194 women enrolled in the study, according to diagnosis of GDM.

Ethnicity		N	GDM				P value	
			NO Mean	SD	Yes N	Mean		SD
Caucasian Spanish	Age (years)	1068	32.7	5.0	134	35.0	4.3	0.000
	Pregestational body weight (kg)		60.9	10.5		68.5	14.7	0.000
	Pregestational BMI (kg/m ²)		22.7	3.7		25.7	5.2	0.000
	Gestational body weight (kg)		65.8	10.6		71.4	14.8	0.000
	Gestational BMI (kg/m ²)		24.6	3.7		26.9	5.0	0.000
Caucasian non-spanish	Age (years)	121	29.0	5.1	13	30.5	4.6	0.264
	Pregestational body weight (kg)		60.8	10.3		73.5	23.2	0.038
	Pregestational BMI (kg/m ²)		22.4	3.5		27.9	8.1	0.016
	Gestational body weight (kg)		67.2	12.6		72.7	13.3	0.114
	Gestational BMI (kg/m ²)		24.5	4.5		27.9	5.0	0.041
Hispanic	Age (years)	692	29.1	5.9	54	34.0	4.8	0.000
	Pregestational body weight (kg)		61.3	12.3		68.8	12.4	0.000
	Pregestational BMI (kg/m ²)		24.3	4.8		27.6	4.9	0.000
	Gestational body weight (kg)		66.5	12.0		70.0	11.1	0.014
	Gestational BMI (kg/m ²)		26.3	4.6		28.4	4.1	0.001
African	Age (years)	42	29.4	5.7	8	34.0	5.5	0.053
	Pregestational body weight (kg)		67.1	11.6		63.6	8.0	0.436
	Pregestational BMI (kg/m ²)		25.7	3.4		24.1	2.1	0.324
	Gestational body weight (kg)		70.5	12.1		69.5	8.2	0.900
	Gestational BMI (kg/m ²)		26.8	3.8		26.0	2.2	0.559
Asian	Age (years)	28	28.2	5.9	2	31.5	0.7	0.588
	Pregestational body weight (kg)		52.5	7.7		.	.	—
	Pregestational BMI (kg/m ²)		19.9	2.5		.	.	—
	Gestational body weight (kg)		59.1	8.1		52.5	3.5	0.193
	Gestational BMI (kg/m ²)		22.5	2.3		22.9	.	0.931
Other	Age (years)	30	29.4	7.0	2	31.0	8.5	0.700
	Pregestational body weight (kg)		58.2	8.1		.	.	—
	Pregestational BMI (kg/m ²)		21.6	2.1		.	.	—
	Gestational body weight (kg)		65.8	10.6		.	.	—
	Gestational BMI (kg/m ²)		25.0	3.7		.	.	—

2. Population, Research Design, and Methods

Participants were recruited from women attending prenatal care at our Hospital during the years 2009-2010, at 24–28 weeks of gestation, when screening for GDM was performed. A total of 2194 women were invited to participate and gave their written informed consent. The study was approved by the Ethical Committee of the Hospital Clínico San Carlos and was carried out following the principles expressed in the Helsinki Declaration. Table 1 shows a summary of the demographic and anthropomorphic characteristics of the study population, according to the positive or negative diagnosis of GDM.

Women were screened for GDM based on the two-step American Diabetes Association recommendations: at week 24–28 of gestation, women with no previous history of diabetes mellitus were assessed, after a 12-hour fasting and no diet restriction on previous days, via the O'Sullivan test

(OS). When plasma glucose levels one hour after glucose load were ≥ 7.2 mmol/L (≥ 140 mg/dL), a further 100-gram OGTT was performed, and new glucose levels were measured while fasting and 1, 2, and 3 hours after the intake. GDM was diagnosed according to Coustan and Carpenter criteria.

At enrollment, participants completed a questionnaire with supervision of a trained nurse. These questionnaires were used to gather information on sociodemographic, anthropomorphic and behavioral characteristics, and reproductive and medical histories. The following were stratified in a semiquantitative way: history of smoking habit (never, smoke—to 6 months before pregnancy,—to pregnancy,—currently), pregestational and gestational physical activity (walking frequency, climbing up stairs per day and at least 30-minute moderate intensity sports per week), and pregestational and gestational weekly intake of vegetables, salads, fruit, dried fruits, nuts, blue fish, whole wheat bread, legumes, skimmed dairy products, red and processed meats,

TABLE 2: Number of women with gestational, personal and family medical history for the 2194 women enrolled in the study, according to the diagnosis of GDM.

Ethnicity	History	Events	GDM				P value
			No		Yes		
			N	%	N	%	
Caucasian Spanish	Gestational history	None	815	76.4	88	66.2	0.002
		One	233	21.8	37	27.8	
		More than one	13	1.2	7	5.3	
		Unknown	6	0.6	1	0.8	
	Personal Medical history	None	955	89.5	110	82.7	0.028
		One	101	9.5	19	14.3	
		More than one	5	0.5	3	2.3	
		Unknown	6	0.6	1	0.8	
	Family Medical history	None	310	37.5	30	36.1	0.101
		One	276	33.4	19	22.9	
		More than one	236	28.5	33	39.8	
		Unknown	5	0.6	1	1.2	
Caucasian non-Spanish	Gestational history	None	76	62.8	8	61.5	0.158
		One	41	33.9	3	23.1	
		More than one	1	0.8	1	7.7	
		Unknown	3	2.5	1	7.7	
	Personal medical history	None	110	90.9	12	92.3	0.871
		One	6	5.0	1	7.7	
		More than one	1	0.8	0	0	
		Unknown	4	3.3	0	0	
	Family medical history	None	53	54.6	8	72.7	0.399
		One	29	29.9	1	9.1	
		More than one	11	11.3	2	18.2	
		Unknown	4	4.1	0	0	
Hispanic	Gestational history	None	394	57.0	24	44.4	0.024
		One	250	36.2	21	38.9	
		More than one	19	2.7	5	9.3	
		Unknown	28	4.1	4	7.4	
	Personal medical history	None	574	83.1	35	66.0	0.008
		One	80	11.6	10	18.9	
		More than one	7	1.0	2	3.8	
		Unknown	30	4.3	6	11.3	
	Family medical history	None	333	56.1	16	43.2	0.296
		One	156	26.3	12	32.4	
		More than one	75	12.6	5	13.5	
		Unknown	30	5.1	4	10.8	
African	Gestational history	None	23	54.8	5	62.5	0.791
		One	17	40.5	3	37.5	
		More than one	2	4.8	0	0	
		Unknown	0	0	0	0	
	Personal medical history	None	39	92.9	5	62.5	0.015
		One	3	7.1	3	37.5	
		More than one	0	0	0	0	
		Unknown	0	0	0	0	
	Family medical history	None	20	57.1	1	14.3	0.052
		One	13	37.1	4	57.1	
		More than one	2	5.7	2	28.6	
		Unknown	0	0	0	0	

TABLE 2: Continued.

Ethnicity	History	Events	GDM				P value
			No	Yes	No	Yes	
			N	%	N	%	
Asian	Gestational history	None	20	71.4	2	100	0.377
		One	8	28.6	0	0	
		More than one	0	0	0	0	
		Unknown	0	0	0	0	
	Personal medical history	None	26	92.9	2	100	0.696
		One	2	7.1	0	0	
		More than one	0	0	0	0	
		Unknown	0	0	0	0	
	Family medical history	None	15	65.2	0	0	—
		One	4	17.4	0	0	
		More than one	4	17.4	0	0	
		Unknown	0	0	0	0	
Other	Gestational history	None	18	64.3	0	0	0.082
		One	7	25.0	2	100	
		More than one	0	0	0	0	
		Unknown	3	10.7	0	0	
	Personal medical history	None	20	71.4	2	100	0.677
		One	5	17.9	0	0	
		More than one	0	0	0	0	
		Unknown	3	10.7	0	0	
	Family medical history	None	10	47.6	0	0	—
		One	3	14.3	0	0	
		More than one	6	28.6	0	0	
		Unknown	2	9.5	0	0	

saucers, sugared drinks and sodas, juices, biscuits, pastries, alcohol, coffee and water. This questionnaire has been previously reported [9, 10].

Regarding medical history, three groups were analyzed: obstetric history, which included previous abortion, GDM and hypertension; personal medical history, which considered overweight, dyslipidemia, hypertension and altered glucose metabolism; family history, which grouped diabetes, obesity, dyslipidemia and hypertension. Answers were reorganized according to the number of events in each category, and the differences between the GDM and non-GDM groups were analyzed (Table 2).

IBM SPSS program version 19.0 was used for data processing. The relationship between the nonmodifiable factors age, pregestational weight, ethnicity, and personal and family past medical history, and the independent variable GDM, was assessed. The population's lifestyle habits were evaluated, and the chi-square test was used to investigate the existence of possible differences in their association to the characteristic of GDM/non-GDM.

To analyze the effects of the different items of lifestyle habits, a generalized lineal model of binary logistic type was performed. The dependent variable was the diagnosis or not of GDM; reference category was taken as value 0, meaning non-GDM, whilst diagnosis of GDM was taken as value 1. Seventeen items were selected as predictors

of GDM: ten related to pregestational intake of nutrients (biscuits and pastries, red and processed meats, fruit, dried fruit and nuts, skimmed dairy products, legumes, whole wheat bread, blue fish, sauces, vegetables and salad); four regarding pregestational consumption of beverages (water, alcohol, sugared drinks and juices and coffee); three related to pregestational physical activity (sports, light walking and climbing up stairs). For each of the previous indicators, intensity was graded according to an ordinary increasing scale with three category levels; low, medium, and high. Missing values were excluded from the analysis. A model of the principal effects of each of the factors considered was chosen, including the intercept. Hybrid method was selected to estimate the different parameters. Type III analysis was used because of the fact that the items considered do not follow any specific arrangement.

To be able to refine and improve the interpretation of the results of the generalized lineal model of binary logistic type, an automatic lineal regression analysis of the model was elaborated. The characteristic of GDM was again considered the outcome, and all of the items previously referred to regarding lifestyle habits were taken as entry predictors. The option of automatic preparation of data was selected; this option may group some of the categories of the items so that the procedure's predictive capacity can be optimized.

TABLE 3: Logistic regression equation for GDM = 1 using pregestational lifestyle habits.

$$\begin{aligned}
& -0,3862 * [\text{biscuits and pastries} = <2/\text{week}] + -0,2925 * [\text{biscuits and pastries} = 2-4/\text{week}] + \\
& -0,3664 * [\text{red and processed meats} = <3/\text{week}] + -0,4235 * [\text{red and processed meats} = 3-6/\text{week}] + \\
& -0,2434 * [\text{fruit} = <6/\text{week}] + -0,2750 * [\text{fruit} = 6-12/\text{week}] + \\
& -0,0780 * [\text{dried fruit and nuts} = <0/\text{week}] + -0,2132 * [\text{dried fruit and nuts} = 1-3/\text{week}] + \\
& -0,07478 * [\text{skimmed dairy products} = <3/\text{week}] + 0,1928 * [\text{skimmed dairy products} = 3-6/\text{week}] + \\
& 0,1409 * [\text{legumes} = <1/\text{week}] + 0,1305 * [\text{legumes} = 1-2/\text{week}] + \\
& 0,0580 * [\text{blue fish} = <3/\text{week}] + 0,3042 * [\text{blue fish} = 3-6/\text{week}] + \\
& 0,1638 * [\text{whole wheat bread} = <1/\text{week}] + -0,3230 * [\text{whole wheat bread} = 1-3/\text{week}] + \\
& -0,2706 * [\text{sauces} = <2/\text{week}] + 0,3943 * [\text{sauces} = 2-4/\text{week}] + \\
& 0,3967 * [\text{vegetables and salads} = <6/\text{week}] + 0,3068 * [\text{vegetables and salads} = 6-12/\text{week}] + \\
& -0,1582 * [\text{water} = \text{no}] + 0,0288 * [\text{water} = \text{shared}] + \\
& 0,2084 * [\text{alcohol} = 1-4/\text{day}] + 0,0998 * [\text{alcohol} = 4-6/\text{day}] + \\
& -0,2761 * [\text{sugared drinks} = <2/\text{week}] + -0,01169 * [\text{sugared drinks} = 2-4/\text{week}] + \\
& -0,2931 * [\text{coffee} = 0-1/\text{day}] + -0,4721 * [\text{coffee} = 2-3/\text{day}] + \\
& 0,2078 * [\text{light walking} = <30 \text{ minutes}/\text{day}] + 0,0530 * [\text{light walking} = 30-60 \text{ minutes}/\text{day}] + \\
& -0,1544 * [\text{climbing up stairs} = <4/\text{day}] + -0,1006 * [\text{climbing up stairs} = 4-16/\text{day}] + \\
& 0,6758 * [\text{sports} = <2 \text{ days}/\text{week}] + 0,3991 * [\text{sports} = 2-3 \text{ days}/\text{week}] + -2,357
\end{aligned}$$

3. Results

Mean age of the pregnant women enrolled in the study was 31.35 years old [range 13–47]. Diagnosis of GDM was confirmed in 213 (9.7%) women (Table 1). Differences in GDM rate were observed between ethnicities ($P \leq 0.05$), although it must be pointed out that the number of African and Asian women in the sample was small.

Age, pregestational BW and BMI did not follow a normal distribution (Kolmogorov-Smirnov test, $P < 0.001$ in all cases). Independent samples of Mann-Whitney U Test used to compare the effect of these variables on the diagnosis of GDM showed significant differences between the GDM and non-GDM groups. However, some of these differences were not observed when comparisons according to the different ethnicities were made (Table 1).

The distribution of the number of events related to medical history, according to the different ethnicities, for the GDM and non-GDM groups is shown in Table 2. The chi-square test showed significant differences for some of the comparisons and these were more evident when cases for which events were unknown were excluded. Again, it is worth mentioning the fact that, in some cases, the number of observations was small.

Figure 1 displays the results of lifestyle patterns according to the diagnosis of GDM. The colored bar represents the distribution of the intake levels or practice frequency of each lifestyle item. Significant differences in the intake of red and processed meats ($P = 0.023$), sugared drinks ($P = 0.035$), and coffee ($P = 0.022$) were found. We observed a trend for differences in biscuits and pastries ($P = 0.068$) and lifestyle habits related to sports and light walking ($P = 0.073$ and $P = 0.088$, resp.). Differences among the remaining factors were not found.

When comparing distributions by pairs, the following significant differences were found: high intake of biscuits

and pastries ($>4/\text{week}$), red and processed meats ($>6/\text{week}$), sugared drinks ($>4/\text{week}$), and coffee ($>3/\text{day}$), and low level of practice of sports ($<2 \text{ days}/\text{week}$) and light walking ($<30 \text{ minutes}/\text{day}$) was greater amongst GDM. Low intake ($<2/\text{week}$) of sugared drinks was greater in non-GDM.

Logistic regression model is shown in Table 3. The significant coefficients observed were red and processed meats = 3–6 times/week ($B = -0.423$; $P = 0.015$); coffee = 2–3 times/day ($B = -0.472$; $P = 0.051$); sauces = 2–4/week ($B = 0.394$; $P = 0.058$); practice of sports = less than 2 times/week ($B = 0,676$; $P = 0.038$).

Automatic lineal modeling discarded all except the following factors: biscuits and pastries, red and processed meats, sugared drinks, coffee, light walking, and sports. The model reclassified the values as shown in Table 4 in order to optimize the signification level. The direction of the influence of the each factor is sketched in Figure 2. Bearing this fact in mind, the logistic regression using the transformed variables as independent variables was applied. The new equation for GDM = 1 obtained is shown in Table 5. The signification of factors are now biscuits and pastries $<4/\text{week}$ ($P = 0.118$), red and processed meats $<6/\text{week}$ ($P = 0.017$), sugared drinks $<4/\text{week}$ ($P = 0.146$), coffee $<3/\text{day}$ ($P = 0.066$), light walking $<60 \text{ minutes}/\text{day}$ ($P = 0.310$), and sports $<2 \text{ days}/\text{week}$ ($P = 0.026$). The corresponding odds ratio (OR) at 95% CI are represented in Figure 3.

4. Discussion

The prevalence of GDM in different ethnic populations has been reported between 1 and 17.8% [11, 12]. The number is steadily increasing [13], and it is becoming a growing health concern during the last decade. According to data obtained in this study, the prevalence of GDM was between 2–17%, similar to figures previously reported. Considering this number, the purpose of our study gains importance,

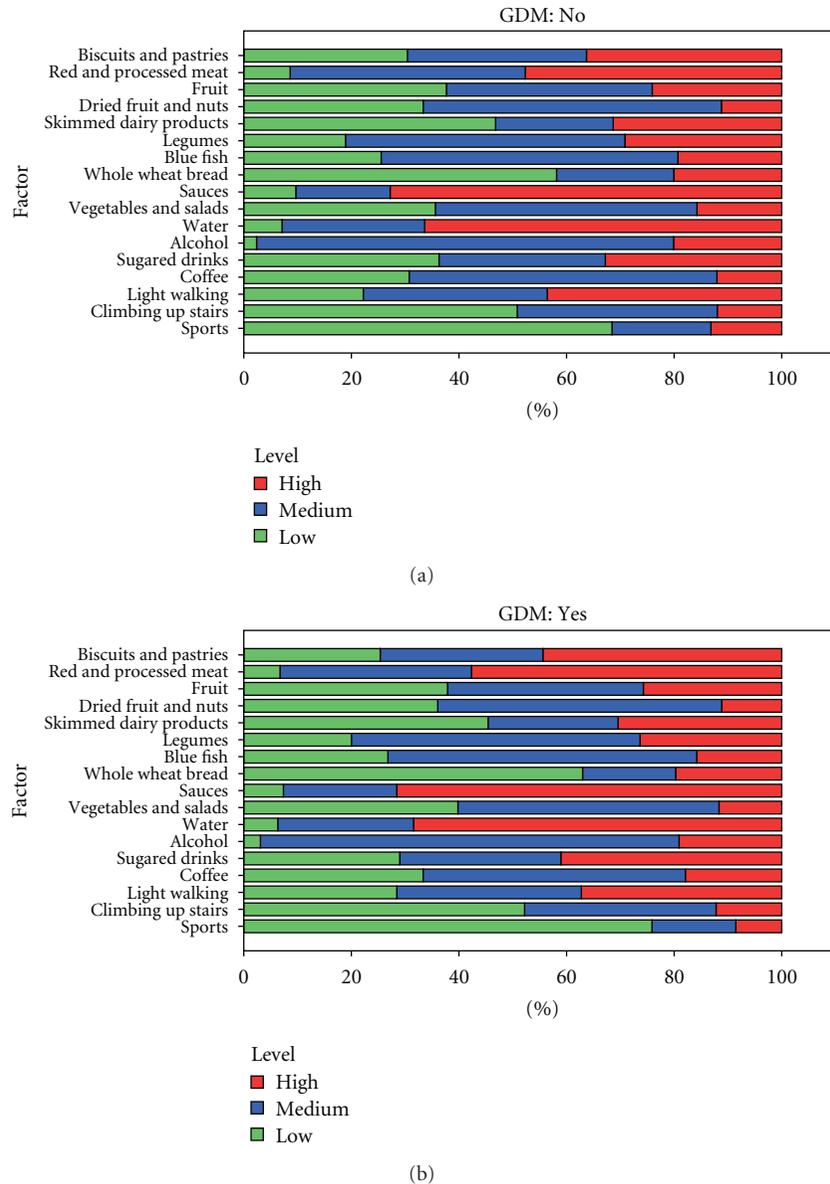


FIGURE 1: Lifestyle patterns of our study population, according to the diagnosis of GDM. For each of the elements considered, for simplifying purposes, a categorical schematic scale of three levels—low, medium, and high—was elaborated to classify the quantity of intake or practice. The limits varied depending on the factor. Biscuits and pastries <2/week, 2–4/week, >4/week; red and processed meats <3/week, 3–6/week, >6/week; fruit <6/week, 6–12/week, >12/week; dried fruits and nuts 0/week; 1–3/week; >3/week; skimmed dairy products <3/week, 3–6/week, >6/week; legumes <1/week, 1–2/week, >2/week; blue fish <3/week, 3–6/week, >6/week; whole wheat bread <1/week, 1–3/week, >3/week; sauces <2/week, 2–4/week, >4/week; vegetables and salads <6/week, 6–12/week, >12/week; water no, shared, exclusive; alcohol 1–4/day, 4–6/day, >6/day; sugared drinks <2/week, 2–4/week, >4/week; coffee 0-1/day 2-3/day, >3/day; light walking <30 minutes/day, 30–60 minutes/day, >60 minutes/day; climbing up stairs <4/day, 4–16/day, >16/day; sports <2 days/week, 2-3 days/week, >3 days/week. (“<” means less than; “>” means more than).

as potential nutritional intervention could be carried out to avoid progression of prevalence.

Previous risk factors established for GDM, including prepregnancy BW and BMI, age and history of GDM [14, 15], were confirmed in this study as independent predictors of glucose intolerance.

Regarding the distribution of eating habits, the results show that intake of certain foods can modify the GDM

risk. Several published case-control and prospective cohort studies have examined associations of diet with GDM [16–21]. They mostly agreed that diet with low fiber, low complex carbohydrates and high glycemic load was associated with an increased risk of GDM. According to data obtained in this study, differences between the GDM and the non-GDM group in the distribution of the intake of biscuits and pastries, red and processed meats, coffee and sugared drinks

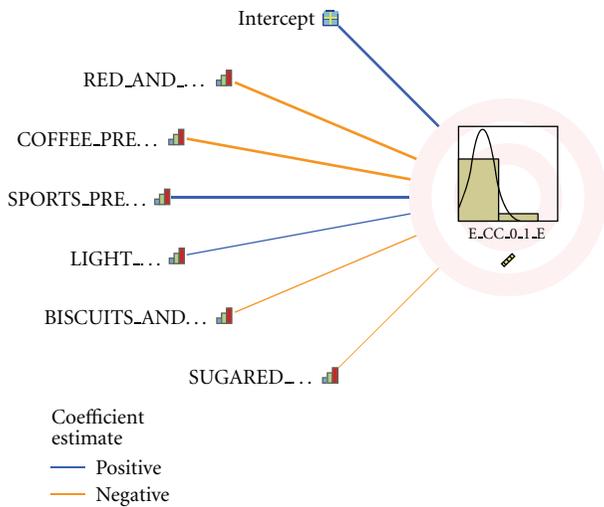


FIGURE 2: Influence in GDM of each factor according to the automatic lineal regression model. “Positive” means lower risk for GDM and “Negative” means greater risk for GDM. The width of the line is directly proportional to the magnitude of the effect and the significance.

TABLE 4: Cutoff points identified by the automatic lineal regression model.

Factor (transformed)	Value = 0	Value = 1
Biscuits and pastries	≤4/week	>4/week
Red and processed meats	≤6/week	>6/week
Sugared drinks	≤4/week	>4/week
Coffee	≤3/day	>3/day
Light walking	≤60 minutes/day	>60 minutes/day
Sports	<2 days/week	≥2 days/week

TABLE 5: Equation for GDM = 1 when applying logistic regression using the transformed variables as independent variables.

$$\begin{aligned}
 & -0,2511 * [\text{biscuits and pastries} = \leq 4/\text{week}] + \\
 & -0,3717 * [\text{red and processed meats} = \leq 6/\text{week}] + \\
 & -0,2351 * [\text{sugared drinks} = \leq 4/\text{week}] + \\
 & -0,3885 * [\text{coffee} = \leq 3/\text{day}] + \\
 & 0,1625 * [\text{light walking} = \leq 60 \text{ minutes}/\text{day}] + \\
 & 0,4025 * [\text{sports} = < 2 \text{ days}/\text{week}] + \\
 & + -1,819
 \end{aligned}$$

were found. The association of these factors to the risk of developing type 2 diabetes is widely known, and because of the increasing prevalence of the analogous GDM, several published studies have begun to study this association. Our data support these previous findings and go one step further in providing new information; they show which types of food are related to higher risk of developing GDM and with what odds. We can then calculate a coefficient of risk based on the level (low, medium, or high) of the factor considered and evaluate how the odds of being diagnosed as GDM are modified.

By using generalized logistic regression and an automatic lineal regression model, we could identify the factors related to GDM. We could further analyze them according to age, weight or ethnicity. This stratification would not be possible for all of the items in the questionnaire because for some of the options data would be insufficient. To be able to assure the influence of these factors despite other circumstances such as age or ethnicity, our study population should be greater, allowing a greater number of women diagnosed with GDM.

If we look at the results regarding coffee intake, although its association with type 2 diabetes has been widely studied [22, 23], there are few papers that consider its role in pregnant women. The systematic review of Van Dam and Hu [23] concluded that habitual coffee consumption was associated with a substantially lower risk of type 2 diabetes, but Wedick et al. [22], on the other hand, could not demonstrate changes in glycemia or insulin sensitivity with coffee consumption. The study by Adeney et al. [24] concluded that moderate prepregnancy caffeinated coffee consumption may have a protective association with GDM. Our study agrees with these previous findings and emphasizes the fact that a higher level of consumption can nullify this protective effect. A possible explanation for this is that, in Spanish population, an increase in coffee intake is associated with an increase in the intake of whole milk and sweets. Prospective studies are still necessary to establish a possible threshold.

Another emerging factor whose influence in GDM is being evaluated is physical activity. Previous data have described that this is one of the strongest predictors contributing to the inverse association to GDM [15, 25–27], and the results of a recent systematic review and meta-analysis demonstrated that greater physical activity before or during early pregnancy is significantly associated with lower risk of GDM, with the magnitude of the association being stronger for prepregnancy physical activity [6]. Our results agree with these previous studies, showing that the less exercise women practiced prior to pregnancy, the more likely they were to develop GDM, and vice versa, the more exercise, the less likely the diagnosis of GDM.

Our study has some limitations that are worth mentioning. Firstly, data regarding information on sociodemographic anthropometric and behavioral characteristics was gathered via self-report in questionnaires. Although these were completed with supervision of a trained nurse, we cannot rule out possible imprecision of pregestational BW and misclassification of food frequency intake and lifestyle habits. This is a common bias in any nutritional epidemiological study, but we assume, however, that bias would occur in a constant and random way, especially because women were unaware of their GDM diagnosis at the time of assessment, and the resulting error would be minimized.

Another possible source of bias is the differential reporting by participant subgroups. For example, women with higher BMI may tend to underreport their food intake, either globally or with specific foods, such as “junk foods.” This introduces a source of confounding that is difficult to account for statistically, although, again, we assume that the

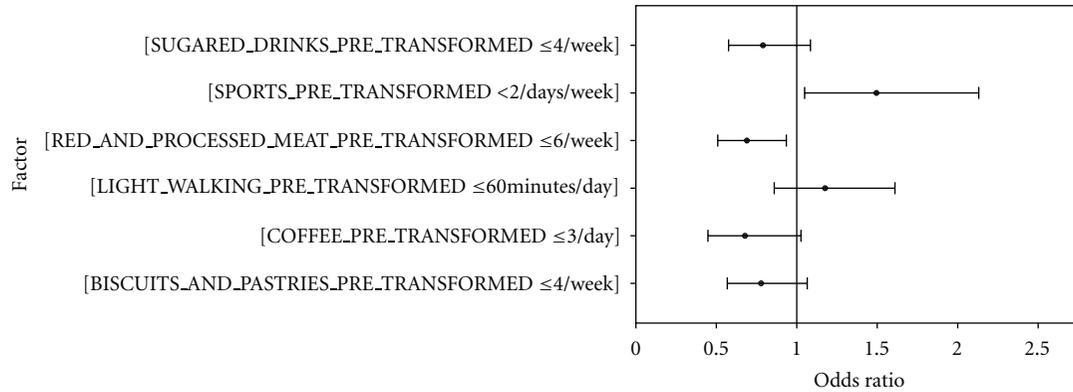


FIGURE 3: Odds ratio (95% CI) of specific cutoff points of lifestyle factors when applying logistic regression.

magnitude of the error is low, because women were unaware of the OGTT result.

Instructions for women attending prenatal care for GDM screening were the same for all participants, all had no previous history of diabetes mellitus and all glucose determinations were made in the same laboratory. This avoids possible measurement errors and misclassification of GDM.

The fact that information on lifestyle habits was reported to be “pregestational” allows a plausible temporal relationship between behavioral characteristics and GDM, which usually develops after the second trimester.

In conclusion, promoting healthy lifestyle habits among women of reproductive age, such as moderate intake of coffee, low intake of biscuits and pastries, sugared drinks and red and processed meats, and regular physical activity, may represent a promising approach for the prevention of GDM and subsequent complications of children born from pregnancies affected by GDM. Moreover, if we know what a woman’s pregestational lifestyle habits are before the screening for GDM, we can predict how the risk is modified, in relation to the population’s prevalence. However, further intervention studies are needed to evaluate if this appraisal model of risk calculation can be useful for prevention and treatment of altered glucose metabolism during pregnancy. It is still unknown whether intervention in dietary habits and beginning an exercise routine before pregnancy results in GDM prevention. Further research and cost-effectiveness studies are warranted to establish the possible risk reduction and economic benefit.

Authors Contribution

All the authors contributed to the interpretation of data, discussion of results and critical review and gave final approval of the last version to be published. None declare duality of interest associated with the paper.

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Clinical Study

1-Hour OGTT Plasma Glucose as a Marker of Progressive Deterioration of Insulin Secretion and Action in Pregnant Women

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Considering old GDM diagnostic criteria, alterations in insulin secretion and action are present in women with GDM as well as in women with one abnormal value (OAV) during OGTT. Our aim is to assess if changes in insulin action and secretion during pregnancy are related to 1-hour plasma glucose concentration during OGTT. We evaluated 3 h/100 g OGTT in 4,053 pregnant women, dividing our population on the basis of 20 mg/dL increment of plasma glucose concentration at 1 h OGTT generating 5 groups (<120 mg/dL, $n = 661$; 120–139 mg/dL, $n = 710$; 140–159 mg/dL, $n = 912$; 160–179 mg/dL, $n = 885$; and ≥ 180 mg/dL, $n = 996$). We calculated incremental area under glucose (AUC_{gluc}) and insulin curves (AUC_{ins}), indexes of insulin secretion (HOMA-B), and insulin sensitivity (HOMA-R), AUC_{ins}/AUC_{gluc} . AUC_{gluc} and AUC_{ins} progressively increased according to 1-hour plasma glucose concentrations (both $P < 0.0001$ for trend). HOMA-B progressively declined ($P < 0.001$), and HOMA-R progressively increased across the five groups. AUC_{ins}/AUC_{gluc} decreased in a linear manner across the 5 groups ($P < 0.001$). Analysing the groups with 1-hour value <180 mg/dL, defects in insulin secretion (HOMA-B: -29.7%) and sensitivity (HOMA-R: $+15\%$) indexes were still apparent (all $P < 0.001$). Progressive increase in 1-hour OGTT is associated with deterioration of glucose tolerance and alterations in indexes of insulin action and secretion.

1. Introduction

Pregnancy is characterized by a complex endocrine-metabolic adaptation process including impaired insulin sensitivity, increased β -cell function, moderate elevation of blood glucose levels, particularly following the ingestion of a meal, and changes in the circulating levels of free fatty acids, triglycerides, cholesterol, and phospholipids [1]. These changes do not reflect a pathological condition; rather, they represent a necessary and indispensable adaptation to meet the energy demand of the foetus and to prepare the maternal organism for delivery and lactation. However, in 3–5% of pregnant women this physiologic adaptation [2] becomes abnormal and gestational diabetes may develop. Therefore, a condition

that should result in healthy growth of the foetus, may turn into a threatening condition for both the mother and her baby. Most likely the development of gestational diabetes reflects individual predisposition [2] because, in spite of common restoration of normal glucose tolerance upon delivery, a large percentage of these women will develop overt type 2 diabetes later in life [3].

The importance of effective and timely diagnosis has been recognized and diagnostic criteria have been established. According to the criteria proposed by Carpenter and Coustan, gestational diabetes is diagnosed whenever, in response to a 3-hour OGTT, two abnormal glucose levels are recorded [4]. These criteria have been recently revised, following the report of the HAPO study [5] because there was

concern that full assessment of risk was not be sufficiently disclosed by old criteria. For instance, we have recently shown that there is no much difference in insulin sensitivity or insulin secretion between GDM women and those who have only one abnormal value (i.e., not diagnostic for GDM) [6]. These alterations were particularly apparent for elevations of the 1-hour plasma glucose than it was for other OGTT time points, suggesting that this value may provide a better parameter for risk stratification. In order to assess this hypothesis, we have reanalysed our cohort of pregnant women by assessing changes in indexes of insulin action and secretion as a function of changes of 1-hour plasma glucose concentration in response to a 100 g OGTT.

2. Research Design and Methods

The study was performed on the same cohort of our previous observation [6]. Briefly, a total of 4,053 pregnant women with positive glucose challenge test (GCT: plasma glucose value ≥ 140 mg/dL 1 hr after a standard 50 g glucose load) carried out around the 27th week of gestation underwent a 3 h 100 g OGTT (samples: 0', 1 hr, 2 hr, 3 hr) for determination of plasma glucose and insulin concentrations.

On the morning of the test, demographic, anthropometric, and clinical data were recorded. Glucose tolerance was defined according to the criteria of Carpenter and Coustan [4]. GDM was diagnosed when two or more plasma glucose levels exceeded the cutoff values; women with a single altered value were classified as having OAV and women not meeting any cutoff values were considered normotolerant.

In the current analysis we have arbitrarily divided the study population based on 20 mg/dL increment of plasma glucose concentration at 1-hour OGTT generating 5 groups (<120 mg/dL, $n = 661$; 120–139 mg/dL, $n = 710$; 140–159 mg/dL, $n = 912$; 160–179 mg/dL, $n = 885$; and ≥ 180 mg/dL $n = 996$).

The study was approved by local ethics committees and women gave their written informed consent to the collection of information, from their medical records.

2.1. Measurements and Statistical Analysis. Plasma glucose levels were determined on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA) based on the glucose oxidase method and plasma insulin concentrations were measured by radioimmunoassay (INSI-CTK Irma; Dia Sorin). The inter- and intraassay coefficients of variation for all parameters were $\leq 5\%$.

Incremental areas under the glucose curve (AUC_{gluc}) and insulin curve (AUC_{ins}) during the OGTT were calculated using the trapezoidal rule. As a measure of insulin secretion, basal insulin and glucose concentrations were used for the estimation of β -cell secretion according to the homeostasis model assessment (HOMA-B) [7]: $(20 \times Ins_0) / (Gluc_0 - 3.5)$. HOMA-R index was calculated [7] to reflect insulin action in a manner independent of OGTT responses. We computed AUC_{ins} / AUC_{gluc} as generalized insulinogenic index.

Data are given as percentages or mean \pm SD. ANOVA with post hoc Bonferroni analysis was used to assess univariate differences among continuous variables; for qualitative

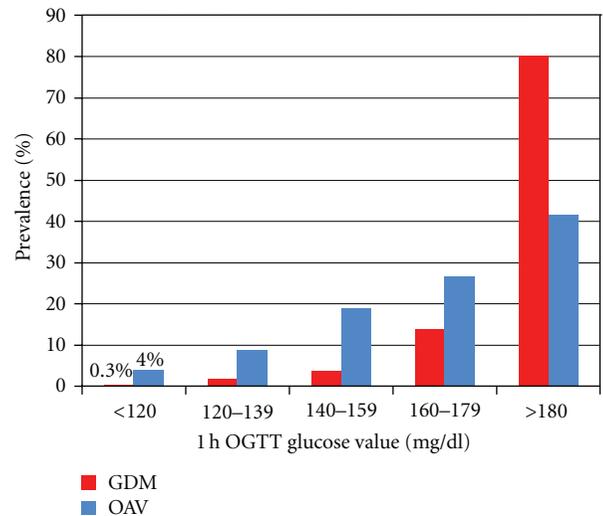


FIGURE 1: GDM and OAV prevalence according to 1-hour OGTT plasma glycaemia. GDM: gestational diabetes mellitus; OAV: women with one abnormal value during OGTT. The prevalence of GDM and OAV increases in the five groups of pregnant women (both $P < 0.0001$).

variables, we used the χ^2 test to compare observed frequency between groups. All statistical comparisons were considered significant at $P < 0.05$. Statistical analyses were performed using a statistical package (Statview SE; SAS Institute, Cary, NC) on a Macintosh computer (Apple, Cupertino, CA).

3. Results

The main clinical characteristics of the 5 groups of ascending 1-hour plasma glucose are shown in Table 1. These 5 groups were comparable for all parameters with the exception of BMI although absolute differences did not exceed 1.2 kg/m^2 . Of interest, no difference was apparent in body weight gain during pregnancy among the 5 groups. The prevalence of GDM (0.3, 1.8, 3.9, 13.7, 80.3%) and that of one abnormal value (including 1-hour, 2-hour, 3-hour OAV) progressively increased in the 5 groups of pregnant women (both $P < 0.0001$, Figure 1). Fasting glucose and insulin levels increased among the 5 groups as shown in Table 2 ($P < 0.0001$; $P < 0.01$, resp.). Accordingly, both AUC_{gluc} and AUC_{ins} progressively increased over the spectrum of 1-hour plasma glucose concentrations (AUC_{gluc} from 8894 ± 1295 mg/dL/min to 14493 ± 1841 mg/dL/min, AUC_{ins} from 6562 ± 3274 pmol/l/min to 9150 ± 5516 pmol/l/min; both $P < 0.0001$ for trend). Moreover, HOMA-R increased in a linear manner from the group with the lowest to the one with the highest 1-hour plasma glucose level ($P < 0.001$) (Figure 2). HOMA-B progressively declined ($P < 0.001$) over the entire spectrum (Figure 3(a)). AUC_{ins} / AUC_{gluc} decreased in a linear manner across the 5 groups (from 13.19 ± 6.59 to 11.24 ± 6.53 , $P < 0.001$, Figure 3(b)). When the analysis was restricted to groups with 1-hour plasma glucose < 180 mg/dL, the progressive nature of defects in insulin secretion (HOMA-B: -29.7%)

TABLE 1: Clinical features of pregnant women related to 1-hour glucose values during OGTT (data are means \pm SD).

1 h glycaemia (mg/dL)	<120	120–139	140–159	160–179	\geq 180	ANOVA (<i>P</i>)
<i>N</i>	661	710	912	885	886	
Age (years)	30.7 \pm 4	31.1 \pm 4	31.7 \pm 4	32.1 \pm 4	32.2 \pm 4	NS
Prepregnancy weight (kg)	64.9 \pm 10	63.5 \pm 9	64.3 \pm 11	65.1 \pm 12	66.2 \pm 12	<0.001
Prepregnancy BMI (kg/m ²)	24.4 \pm 3.8	23.7 \pm 3.6	24 \pm 4.2	24.3 \pm 4.6	24.9 \pm 4.4	<0.01
Weight gain (kg)	7.5 \pm 3.4	7.7 \pm 3.3	7.5 \pm 3.3	7.7 \pm 3.5	7.7 \pm 3.6	NS
Systolic BP (mmHg)	116.8 \pm 11	115.1 \pm 10	116 \pm 12	114.8 \pm 12	116.7 \pm 12	NS
Diastolic BP (mmHg)	71.9 \pm 8	71.4 \pm 8	71.2 \pm 8	71.1 \pm 8	71.7 \pm 8	NS
Total cholesterol (mg/dL)	261 \pm 45	258 \pm 46	257 \pm 44	254 \pm 39	263 \pm 50	NS
LDL cholesterol (mg/dL)	160 \pm 43	179 \pm 32	162 \pm 36	166 \pm 45	166 \pm 40	NS
HDL cholesterol (mg/dL)	54.3 \pm 18	58.3 \pm 18	59.2 \pm 20	61.1 \pm 18	62.2 \pm 19	NS
Triglycerides (mg/dL)	199 \pm 67	205 \pm 81	201 \pm 66	191 \pm 79	197 \pm 77	NS

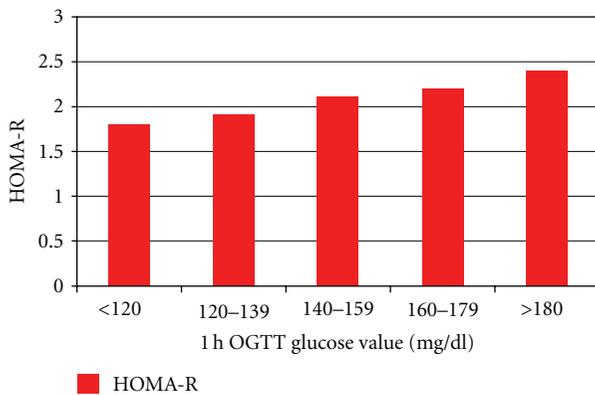
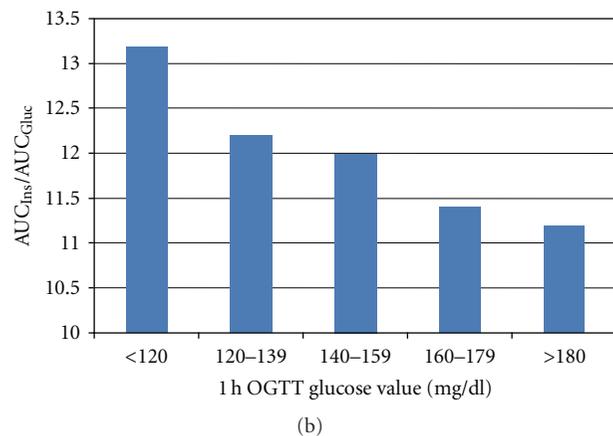
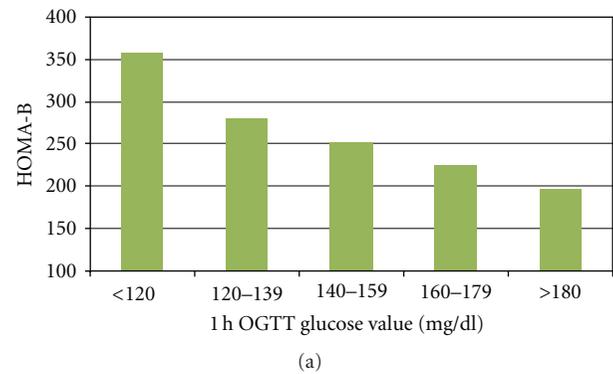


FIGURE 2: HOMA-R index according to 1-hour OGTT plasma glycaemia. HOMA-R index is estimated using the formulas proposed by Matthews et al. [7].

and insulin sensitivity (HOMA-R: +15%) were still apparent (all $P < 0.001$).

4. Discussion

Although gestational diabetes is a well-recognized condition that may affect maternal and foetus health state, evidence is mounting that nondiabetic abnormalities of glucose homeostasis should be looked at with some caution [8, 9]. Carr et al. [10] have shown that pregnant women with OAV have twofold greater risk for subsequent diabetes as compared to women with no abnormal values in response to an OGTT. More recently, the HAPO study (Hyperglycemia and Adverse Pregnancy Outcome) [5], a clinical trial conducted on a large cohort of pregnant women aimed at clarifying the risk of adverse outcomes associated with various nondiabetic degrees of maternal glucose intolerance, showed a strong, continuous association between maternal glucose levels and increased birth weight as well as poor pregnancy outcomes. Moreover, among pregnant women with OAV, the alteration of 1-hour plasma glucose seems to provide the best predictive effect in terms of both pathophysiologic involvement and clinical outcome as initially highlighted by Retnakaran and

FIGURE 3: HOMA-B index and AUC_{ins}/AUC_{gluc} according to 1-hour OGTT plasma glycaemia. HOMA-B index is estimated using the formulas proposed by Matthews et al. [7].

colleagues [11]. In their paper they showed that all measures of severity of glycemic control were highest in women with GDM group, followed by the 1-hour OAV, 2-hour or 3-hour OAV, and NGT groups. Consistent with this finding, insulin sensitivity was highest in the NGT group and worsened in a progressive manner through the 2-hour or 3-hour OAV, 1-h OAV, and GDM groups. In our previous study, we showed that pregnant women with 1-hour OAV also

TABLE 2: Fasting glucose and insulin levels related to 1-hour glucose values during OGTT (data are means \pm SD).

1 h glycaemia (mg/dL)	<120	120–139	140–159	160–179	\geq 180	ANOVA (<i>P</i>)
Fasting glucose (mg/dL)	72.4 \pm 10.4	80.8 \pm 9.14	82.11 \pm 9.16	84.1 \pm 10	87.5 \pm 11	<0.0001
Fasting insulin (pmol/L)	55.19 \pm 29.5	57.5 \pm 36.6	61.8 \pm 47	63.5 \pm 53	67.7 \pm 51	<0.01

had a greater impairment in their measures of β -cell function [6]. Moreover, pregnant women with 1-hour OAV, compared with those with 2-hour or 3-hour OAV, have a higher prevalence of adverse obstetric outcome, including caesarean delivery, macrosomia, and hypertensive disorder [12]. Finally, 3 months after delivery, women with 1-hour OAV tend to have persistent metabolic dysfunction, including higher plasma glucose levels, greater insulin resistance, and poorer β -cell function, very much alike women with prior GDM [13].

In summary, 1-hour OAV in pregnant women may be used for the stratification of the pregnancy risk. In order to gain further insights in the significance of 1-hour glucose, we have reanalyzed data from a large cohort of pregnant women showing that the progressive increase in this parameter is associated with a progressive loss of β -cell function as well as a decline in insulin sensitivity. Moreover the deterioration of the two main homeostatic parameters becomes apparent well within the so-called normal range of glucose tolerance in pregnancy. We would underline that in the same range (i.e., 1 h glucose values <120 mg/dL) GDM (0,3%) and OAV (4%) were also present and their prevalence progressively increases according to 1 h glucose values. These data suggest that even in categories with normal or very low 1-hour glucose values an alteration in glucose homeostasis is already apparent.

This also raises the question on the reason for 1-hour plasma glucose value to be so linked to degeneration of glucose homeostatic mechanisms. Although not directly assessed in the present study, it has already been reported that in nondiabetic individuals, the progressive increase of plasma glucose concentration at 30–60 min after an OGTT is dependent on β -cell function and insulin sensitivity in peripheral tissues [14, 15]. Therefore, it is plausible that development of insulin resistance in the liver and in the skeletal muscle and concomitant weakening of insulin secretion will result in progressive elevation of 1-hour glucose concentration, as suggested by our results. Moreover, in nondiabetic individuals it has been shown that 1-hour plasma glucose concentration has a stronger correlation with surrogates measures of hepatic and muscle insulin resistance and β -cell dysfunction compared with 2-hour plasma glucose levels [16].

The 1-hour plasma glucose value has been advocated as a risk marker not only in pregnant women. Abdul-Ghani et al. [17] have shown that the plasma glucose concentration at 1-hour after an OGTT is a powerful predictor of future risk for type 2 diabetes. He was also proposing a cutoff point of 155/mg/dL to stratify diabetes risk. Moreover, Succurro et al. [18] have recently shown that individuals with normal glucose tolerance but a 1-hour plasma glucose levels \geq 155 mg/dL have an atherogenic profile similar to IGT subjects. Obviously, we cannot extrapolate the continuous relationship between 1-hour plasma glucose levels and

alterations in insulin action and secretion to the general population, but the strong similarities existing between GDM and IGT/type 2 diabetes are worth being considered. Our data, indeed, strongly suggest that alterations in glucose homeostasis related to increasing *t* 1-hour plasma glucose value are continuous variables: for each 20 mg/dL increase in 1-hour plasma glucose concentration there is a concomitant impairment in insulin action and a reduction in insulin secretion.

A limitation of our study should be represented by the indexes we used to determine insulin secretion and action that involve only basal glucose and insulin concentration; anyway, these indexes decrease as 1 h glucose value increases.

In conclusion, this study underlines the concept that the spectrum of glucose tolerance in pregnancy identifies a continuum of disturbance of glucose homeostasis and that 1-hour glucose level after 100 g OGTT may be considered a relevant marker for improving risk stratification in pregnant women. Moreover, although new IADPSG criteria for GDM diagnosis [19] erase the difference between GDM and OAV, the importance of 1-hour glucose level impairment still remains and our data reinforce the concept that the new lower diagnostic criteria will be useful in reducing the risk for short- (obstetrical) and long-term (metabolic and cardiovascular) complications.

Abbreviations

OGTT:	Oral glucose tolerance test
1 h OGTT:	1-hour during OGTT
GDM:	Gestational diabetes
OAV:	One abnormal value
AUC _{gluc} :	Area under glucose curve
AUC _{ins} :	Area under insulin curve
HOMA-B:	Index of insulin secretion (homeostasis model assessment: β cell function)
HOMA R:	Index of insulin sensitivity (homeostasis model assessment: insulin resistance).

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Review Article

Role of Adipokines and Other Inflammatory Mediators in Gestational Diabetes Mellitus and Previous Gestational Diabetes Mellitus

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Previous Gestational Diabetes Mellitus (pGDM) is a common condition and has been associated with future development of Type 2 Diabetes Mellitus (T2DM) and Metabolic Syndrome (MS) in women affected. The pathogenesis and risk factors implicated in the development of these conditions later in the lives of women with pGDM are not as yet fully understood. Research has recently focused on a group of substances produced mainly by adipose tissue called adipokines, this group including, among others, adiponectin, leptin, Retinol-Binding Protein-4 (RBP-4), and resistin. These substances as well as other inflammatory mediators (CRP, IL-6, PAI-1, TNF- α) seem to play an important role in glucose tolerance and insulin sensitivity dysregulation in women with pGDM. We summarize the data available on the role of these molecules.

1. Introduction

Pregnancy is a progressively hyperglycemic period of life, accompanied by increasing insulin resistance as from mid-gestation, with the hyperglycemia serving a highly important role in the nutrition and development of the fetus by providing it with adequate levels of glucose [1].

Gestational Diabetes Mellitus is a common pathologic state that increases the incidence of complications in both the mother and the fetus [2]. Furthermore, GDM and gestational dysregulation of blood glucose levels expose the women affected to higher risk for subsequent development of type 2 diabetes mellitus and cardiovascular disease later in their lives [3–5], the risk being proportional to the degree of the dysregulation.

Glucose tolerance and metabolism as well as insulin resistance are altered in Type II Diabetes Mellitus (T2DM),

Gestational Diabetes Mellitus (GDM), and the postpartum period of a pregnancy complicated by pGDM. T2DM and pGDM have the same predisposing factors, namely, high body mass index before pregnancy, elevated levels of fasting glucose, and a degree of hyperglycemia in pregnancy, these leading to dysglycemia 1 to 4 months after delivery and recurrent gestational diabetes mellitus [6–26].

Although the pathophysiologic mechanisms responsible for these changes are not as yet completely understood, growing insight into the processes involved has been gained over the last few years. There are two main pathways leading to GDM, T2DM, and possibly pGDM: insulin resistance and chronic subclinical inflammation.

Insulin resistance is caused by the inability of tissues to respond to insulin and the deficient secretion of insulin by pancreatic beta cells [27–29]. The deficient secretion cannot compensate for the pregnancy-induced insulin resistance,

this resulting in GDM, a condition which sometimes persists after delivery [8–12, 30].

With regard to the contribution of inflammatory processes to the pathogenesis of dysglycemia conditions, it has been reported that long-term activation of the acute phase inflammatory response is a risk factor for T2DM and cardiovascular disease [31].

Furthermore, obesity has a role in the development of both T2DM and GDM through chronic subclinical inflammation, low-grade activation of the acute phase response, and dysregulation of adipokines [31–33]. Increased levels of inflammatory agents during and after pregnancy have been reported in patients with GDM, while increased body fat has been strongly associated with inflammation and adipocyte necrosis, hypoxia, and release of chemokines which cause macrophages to infiltrate adipose tissue. Macrophages secrete cytokines which activate the subsequent secretion of inflammation mediating agents, specifically interleukin-6 (IL-6) and C-reactive protein (CRP). Moreover, other molecules such as Plasminogen Activator Inhibitor 1 (PAI-1) and sialic acid lead to dysregulations of metabolism, hyperglycemia, insulin resistance, and, finally, overt T2DM [31, 34–46].

Crucially, hormones produced by the placenta [47] and increased maternal fat mass [48] have been reported to play a major role in GDM. In this context, the gaps in the mechanisms underlying glucose metabolism in pregnancy and nonpregnancy states have initiated research efforts to uncover other potential mediators of insulin resistance, namely, the adipokines. These are a group of substances, knowledge about which is continuously expanding, that are produced mainly in the adipose tissue [49]. The group includes leptin, adiponectin, tumor necrosis factor alpha (TNF- α), retinol-binding protein-4 (RBP-4), resistin, visfatin, and apelin. These molecules are involved in a wide range of physiological processes including lipid metabolism, atherosclerosis, blood pressure regulation, insulin sensitivity, and angiogenesis, while they also influence immunity and inflammation. Their levels in pathologic states appear increased, with the exception of adiponectin which shows decreased levels.

In this paper we summarize the data available concerning the role of these mediators in women with pGDM.

2. Adipokines in pGDM

Adipokines, whose significant role in the pathogenesis of numerous pathologic conditions has recently been recognized, are adipose tissue-derived substances mediating communication and endocrine function between this metabolically active tissue and other sites throughout the body. A summary of the levels of adipokines and other inflammatory mediators in cases of pGDM is shown in Table 1.

2.1. Adiponectin. Adiponectin, a well-studied protein, is secreted by adipose tissue. It has insulin-sensitizing action, stimulating glucose uptake in skeletal muscle, and reduces hepatic glucose production through AMP-activated protein

kinase [50], while it also possesses antiatherogenic and anti-inflammatory properties [51, 52]. The levels of adiponectin decrease as visceral fat increases [53–56] in such conditions as central obesity, insulin resistance, and diabetes mellitus; lower adiponectin levels have notably been associated with subclinical inflammation [43]. It has been shown that adiponectin levels begin to decrease early in the pathogenesis of diabetes, as adipose tissue increases in tandem with reduction in insulin sensitivity [57]. Hypoadiponectinemia has also been associated with beta cell dysfunction [58, 59], while it has additionally been linked to future development of insulin resistance [60] and type 2 diabetes mellitus [61–64], in the development of which adiponectin appears to have a causative role.

Adiponectin has also been studied in animal experiments in which it was demonstrated that it can reduce insulin resistance and enhance the action of insulin in liver, resulting in lowering of glucose blood levels [65–67]. In normal human pregnancy, adiponectin levels have been found to be unchanged or decreased [68–72] and negatively correlated with maternal BMI and insulin sensitivity [73].

In GDM pregnancies adiponectin appears to be decreased independently of maternal body mass index (BMI) or insulin sensitivity [68, 74–85]. Additionally, the fact that low circulating levels of adiponectin are found early in pregnancy has been associated with subsequent development of GDM [81]. Meanwhile, low levels of adiponectin in pregnancy, which have incidentally been associated with certain ethnic groups such as women of South Asian origin, have a significant impact on the development of GDM [86–88].

While hypoadiponectinemia is strongly associated with beta-cell dysfunction in pregnancy [89], the levels of adiponectin after delivery have been investigated in only a few studies. In one of them the investigators measured adiponectin levels in 89 women with pGDM at both 3 and 12 months postpartum and compared the values obtained with normal controls (women with normal pregnancies). They found that adiponectin levels were lower in women with pGDM at 3 months postpartum [43]. This registered decrease remains valid even after correction for body fat mass (BFM). The lower values of adiponectin are also associated with decreased insulin sensitivity and low HDL. It is of note that plasma adiponectin further decreased by 10% after 1 year in insulin-resistant women with pGDM.

Other investigators [90] studying 25 women with pGDM and comparing their adiponectin levels with those of 23 women with normal pregnancies at least 1 year after the index pregnancy (mean 4 years) found that the levels were significantly lower in women with pGDM compared to normal controls. The difference remained significant even after adjustment for BMI. This study also showed that adiponectin was negatively correlated to other inflammatory markers, namely, CRP, PAI-1, and IL-6, the which correlations remained unchanged even after adjustment for BMI.

In another study, 140 women with previous GDM and various states of glucose tolerance after delivery (8 with diabetes mellitus, 60 with impaired glucose tolerance and 72 with normal glucose tolerance) were studied and compared with 17 women with normal pregnancies [91].

TABLE 1: Adipokines and other inflammatory mediators in normal pregnancy, obesity/DM, GDM, and pGDM.

	Pregnancy	Obesity/DM	GDM	pGDM
Adiponectin [43, 50–92]	Decreased Unaltered	Decreased	Decreased	Decreased
Leptin [43, 91–105]	Increased	Increased	Increased Unaltered Decreased	Increased Unaltered
Resistin [68, 70, 73, 76, 106–121]	Increased	Increased	Increased Unaltered Decreased	Increased
RBP-4 [106, 122–138]	Increased Decreased	Increased Decreased	Increased Decreased Unaltered	Increased
Visfatin [139–154]	Increased	Increased	Increased	?
CRP [31, 34–36, 40–46, 90, 155–161]	Increased	Increased	Increased	Increased
PAI-1 [31, 34–36, 42, 43, 98, 162]	Increased	Increased	Increased	Increased
IL-6 [98, 163–167]	Increased	Increased	Increased	Increased
TNF- α [43, 84, 100, 103, 104, 155, 163–167]	Increased	Increased	Increased	Increased

The authors reported lower adiponectin levels in the women with pGDM 1.57 years postdelivery, while it is also of interest that the levels of adiponectin were progressively lower, the postpartum glucose tolerance values ranging from normality to impaired glucose tolerance and finally Diabetes Mellitus after GDM (P for linearity = 0.006). Plasma adiponectin was moreover negatively correlated with fasting glucose, fasting insulin, and RBP-4 levels.

Another recent study examined 60 women with GDM at 30 weeks of gestation and 6 weeks and 6 months postpartum and compared the results with normal pregnancies [92]. They did not find significantly different levels of adiponectin during and after pregnancy in the group of GDM women, but they did observe a significant difference in the group of non-GDM women (both at 6 weeks and 6 months postpartum, $P < 0.01$).

2.2. Leptin. Leptin is an adipokine that is produced by the ob (obese) gene in adipose tissue cells, especially in white adipose tissue, its action at the hypothalamus resulting in decreasing food intake and increasing energy consumption [93, 94]. It also regulates endocrine function, inflammation, immune response, and angiogenesis.

Its mechanism of action is to increase insulin sensitivity by influencing insulin secretion, glucose utilization, glycogen synthesis, and fatty acid metabolism [93–95]. Furthermore, it adjusts gonadotropin releasing-hormone secretion from the hypothalamus and activates the sympathetic nervous system.

In normal pregnancy, leptin concentration increases from early pregnancy onwards and decreases to normal pre-pregnancy levels before delivery [96–99]. This initial increase may be due to placental synthesis, since it occurs before the rise in maternal BMI and rapidly falls after delivery [99]. The function of increased maternal leptin is to enhance the mobilization of maternal fat stores thus enabling access of lipid substrates to the fetus [100].

In GDM, leptin has a more debatable role, since it appears to be elevated in women with GDM [101–103] but, after adjustment for BMI and insulin resistance [104, 105], it is shown to be decreased or even unaltered [102], while it has also been associated with insulin resistance in pregnancy [101, 103, 105].

There is to date an insufficient number of studies examining the role of leptin in the postpartum period following a GDM pregnancy. In one study, 89 women with pGDM were followed and found to have significantly increased plasma leptin at 3 months after delivery ($P < 0.003$) compared to controls [43]. Leptin levels were negatively associated with adiponectin but this association was not significant after the adjustment for BFM. Meanwhile, other authors have failed to find any difference in leptin levels between pGDM and normal pregnancies 18 months after delivery [91].

Leptin levels were studied [92] during pregnancy and 6 weeks and 6 months after delivery both in normal pregnancies and in pregnancies complicated by GDM. There were significantly higher levels of leptin in previous GDM pregnancies compared with normal pregnancies both at 6 weeks and 6 months postpartum.

2.3. Retinol-Binding Protein-4. RBP-4, which is an adipokine synthesized in hepatocytes and adipocytes, serves as a carrier for retinol in blood and is postulated to play a role in regulating glucose metabolism and insulin sensitivity [122, 123].

In pathologic glucose tolerance states (such as obesity, insulin resistance, polycystic ovary syndrome, and cardiovascular disease), RBP-4 concentration has been shown to be elevated [106, 124–128]. Other studies have reported low levels of the protein in humans with T2DM and have determined that RBP-4 concentration does not relate to insulin sensitivity in calorie restricted obese individuals [129–131]. It has moreover been demonstrated that overexpression of RBP-4 in normal mice increases insulin resistance, whereas

genetic disruption of this adipokine increases insulin sensitivity [123].

In normal pregnancy, RBP-4 increases significantly between early and late pregnancy with a parallel decrease in insulin sensitivity [132], although other authors have reported a decrease in the levels of this hormone after early gestation [133].

GDM has been associated with increased, decreased, or even stable levels of RBP-4 [134–138]. In pGDM it has been shown that RBP-4 was significantly higher more than 18 months postpartum in women with normal or impaired glucose tolerance or with diabetes mellitus in the postpartum period compared with women without GDM. A trend was also documented of increasing RBP-4 values from normality to DM in the pGDM group (P for linearity = 0.006). Furthermore, RBP-4 was positively correlated with fasting insulin, whereas the correlation to adiponectin was negative. Finally, RBP-4 concentration was significantly higher in women with metabolic syndrome than in those without [91].

Another study [92] that measured RBP-4 levels in 60 women with GDM during pregnancy as well as 6 weeks and 6 months after delivery found a positive correlation of RBP-4 with fasting insulin levels. Additionally, there was a significant reduction in RBP-4 in the control group between delivery and 6 weeks and 6 months postpartum, although there was no respective decrease in the GDM group. Between the two groups, a significant difference in the levels of RBP-4 ($P < 0.05$) was not observed until 6 weeks postpartum.

2.4. Resistin. Resistin, a hormone expressed by adipocytes as well as monocytes and macrophages [107], appears to have levels that parallel the mass of adipose tissue [107–109]. In obesity and insulin resistance, the role of resistin is as yet highly controversial [110, 111], although its function has been associated with impaired glucose tolerance [106, 111]. The results in animal experiments are indicative of insulin resistance induction in animals, but the same is not true in humans [107, 111–113].

In normal pregnancy it is expressed in human placenta [114], with plasma resistin levels in pregnant women being significantly higher as compared to normal controls. It increases in the third trimester [68, 76, 114–116] and may regulate energy metabolism during pregnancy.

In GDM its levels have been found either elevated or decreased [76, 117–120], with some studies having reported elevated maternal resistin in GDM [68, 118, 121, 147], while others found lower [73, 118] or unaltered values [70].

With regard to pGDM, one study [91] showed that resistin was significantly higher 18 months postpartum in women with normal or impaired glucose tolerance who had pGDM compared to a group of women who did not develop DM during pregnancy. This could be explained by a biphasic effect of insulin on the release of resistin, whereby low concentration of insulin increases the release of resistin, while this is reduced at higher insulin levels [76]. The authors also reported that plasma resistin levels correlated with BMI, fat quantity, and plasma insulin and that the presence of

metabolic syndrome was not significantly associated with plasma resistin levels in the postpartum period.

2.5. Visfatin. Visfatin is an adipocytokine produced mainly in visceral fat, as compared to subcutaneous fat, which exerts insulin mimetic action [139] and, additionally, plays a pro-inflammatory role [140].

It has higher concentrations in cases of obesity or insulin resistance, including T2DM and metabolic syndrome [139, 141, 142]. Conversely, other studies showed no relation of visfatin to insulin sensitivity or increased BMI and visceral fat mass [143, 144].

In pregnancy, although this hormone has been reported by some authors to maintain the same levels in the third trimester as in the non-pregnant state [145], other studies have demonstrated an increase [72, 146]. Visfatin levels peak between 19 and 26 weeks of gestation, while at between 27 and 34 weeks visfatin has the lowest serum concentrations [147].

Morgan et al. reported that visfatin may have a paracrine or autocrine action since it is locally increased in omental fat without increased plasma levels in pregnancy [148].

Some studies have shown elevated levels in women with GDM [149–151] with a further increase of these levels detected in the presence of high maternal blood glucose levels. In contrast, a number of other studies found that visfatin levels are lower in GDM [146, 152–154]. Figure 1 displays aspects of the aforementioned adipokines.

3. Other Mediators of Inflammation in pGDM

3.1. TNF- α and IL-6. TNF- α and IL-6 are inflammatory mediators produced by monocytes and macrophages in the adipose tissue. These cytokines are increased in obesity and have multiple effects on insulin sensitivity in muscles, liver, or beta cells of the pancreas, ultimately leading to insulin resistance [32, 168]. In pregnancy, TNF- α and IL-6 production occurs in placenta [163, 164], while it is considered that a chronic inflammatory process in the adipose tissue may contribute to pregnancy-induced insulin resistance [164–167, 169]. Placental production of TNF has been shown to be maximized late in pregnancy [163] and to decline rapidly after pregnancy, this being in accordance with placental production of TNF- α [155]. In early pregnancy TNF- α mRNA is present mainly in syncytiotrophoblast. Later in pregnancy and specifically near term, TNF- α mRNA also appears in villous stromal cells and TNF- α transcripts are present in maternal cells in the decidua adjacent to the extracellular membranes [164]. Most of the TNF- α produced by the placenta is delivered to maternal circulation and by comparison only a small amount to the fetal compartment [164].

Though in a GDM pregnancy IL-6 and TNF- α rarely increase [165–167], when this does occur, the increase is caused by the oxidative stress and the inflammation associated with the hyperglycemia [169]. Conversely, TNF- α possibly inhibits insulin secretion and insulin regulated glucose uptake in GDM [84, 104, 165, 166, 170]. An in vitro

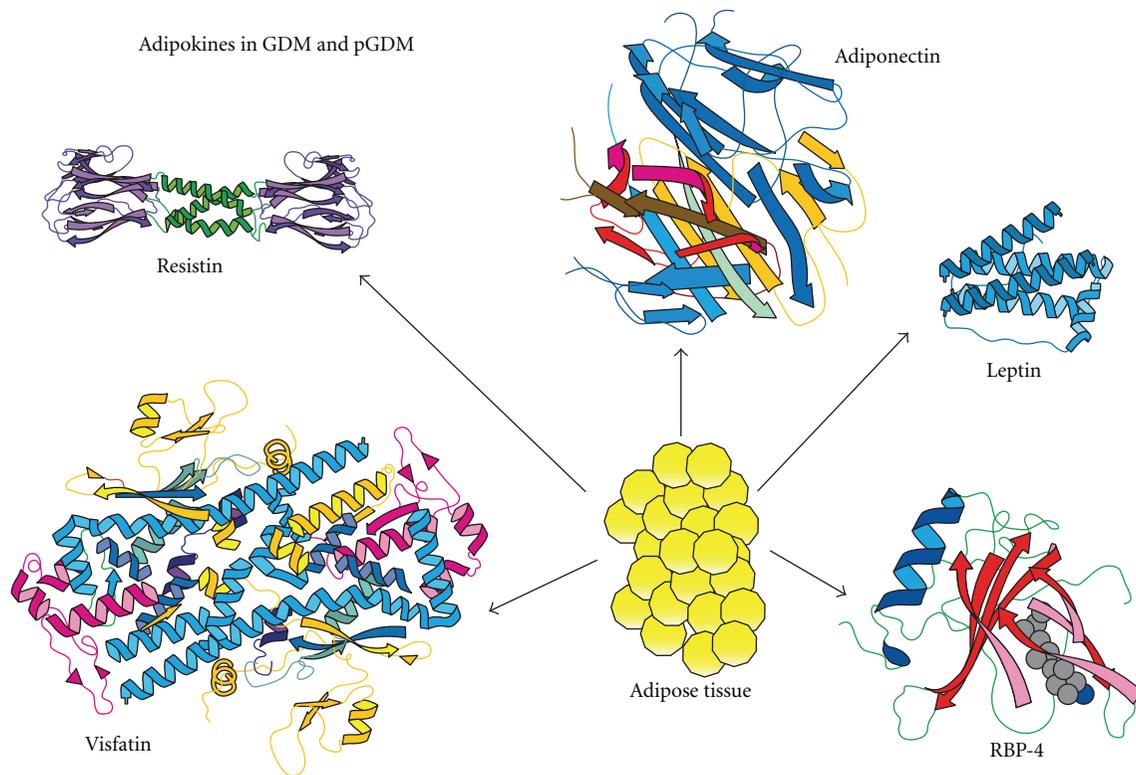


FIGURE 1: Molecular structure of the main adipokines produced by adipose tissue in GDM and pGDM. Adiponectin is a 244-amino-acid polypeptide that decreases as adipose tissue increases; it enhances insulin action and is decreased in pGDM and GDM. Leptin is a 167-amino-acid protein; it causes insulin action to increase and its levels show variability with respect to GDM and pGDM. Resistin (or adipose tissue-specific secretory factor) has 108 amino acids in the prepeptide form; its action on insulin has yet to be clarified. RBP-4 is a 183-amino-acid protein which reduces insulin action. Both resistin and RBP-4 levels appear increased, decreased, or unaltered in GDM but are uniformly increased in pGDM. The visfatin molecule consists of 491 amino acids; it facilitates insulin action and increases in GDM, while its actions in pGDM need further clarification.

experiment showed that placentas from women with GDM released more TNF- α in response to a glucose stimulus than placentas from women with normal glucose tolerance [156].

IL-6 levels are significantly higher in women with pGDM at least 1 year postdelivery (mean 4 years postpartum) compared with normal controls [98], this difference becoming nonsignificant when it was adjusted for BMI. It is interesting to note that when nonobese women with pGDM were examined, the difference remained significant for IL-6 (BMI played no role) and that IL-6 was positively related to CRP, this persisting even after adjustment for BMI.

TNF- α was not significantly different in pGDM 3 months after delivery compared to women with normal glucose tolerance during pregnancy [43]. By contrast, IL-6 was significantly higher before adjusting for BFM but after adjustment for BFM adiponectin did not correlate to IL-6. Hauguel-de Mouzon et al. reported in a recent study that TNF- α rose significantly 6 weeks postpartum both in women with normal pregnancies and in GDM pregnancies when compared to antepartum values [100]. The increase for TNF- α was not significant for both groups 6 months postpartum.

In another study, 18 women with pGDM at least 12 months after delivery were compared with normal controls and women with polycystic ovary syndrome (PCOS).

They found no significant difference in TNF- α between the pGDM cases and controls, although the difference between PCOS and controls was significant [157].

3.2. C-Reactive Protein. CRP, an inflammatory agent common in numerous pathologic conditions, has been associated with metabolic abnormal states such as insulin resistance, hyperglycemia, and T2DM [31, 34–36], while it also appears to be associated with central obesity [44, 46].

In the first trimester of pregnancy the levels of CRP are increased and have been related to higher risk for GDM development [156, 157], this association also being valid with measurement of CRP later in the course of pregnancy [160, 170]. Furthermore, CRP is increased in maternal obesity, insulin resistance, and maternal dysglycemia [158, 161, 162]. The pathophysiologic role of inflammatory proteins and adiponectin seem to be the gradual impairment of beta cell function and increasing insulin resistance, which results in ineffective plasma glucose regulation and subsequent dysglycemia in the months and years that follow pregnancy.

As concerns pGDM, there is some evidence that women with a history of prior GDM have postpartum increased CRP that manifests chronic subclinical inflammation [40–46]. Increased CRP levels in women with pGDM, which have

also been related to metabolic syndrome [45], have been reported in several studies [43–46].

The postpartum period which is complicated by gestational diabetes is a period of chronic subclinical inflammation. Some investigators have shown significantly increased levels of CRP 3 months after delivery in women with pGDM compared with controls [43]. Other authors also found a negative correlation between adiponectin levels and CRP, but this correlation became nonsignificant after adjustment for BFM, the latter being explained by the finding that CRP is also related to central obesity [44, 46]. The NHANES III study showed that adjustment of CRP values for waist circumference attenuated differences in women with pGDM and normal women [171].

Another study [172] recruiting 46 women with pGDM 3 years postpartum reported that women with pGDM had significantly elevated high-sensitivity CRP (hs-CRP) compared with controls.

Heitritter et al. [90] studied 25 women with pGDM and found that in a mean period of 4 years postpartum they had significantly higher CRP levels compared to controls. The difference remained significant after adjustment for BMI. CRP was negatively related to adiponectin and positively related to IL-6 and these associations remained unchanged after adjustment for BMI.

In another study, 18 women with pGDM at least 12 months after index delivery were found to have no significant difference in hs-CRP compared with normal controls [157].

On the other hand, other authors [46] studied 70 women with pGDM 6 years after their pregnancy and found significantly higher CRP levels in women with pGDM in the presence of abdominal obesity; they also found abnormal glucose tolerance compared with the women without a history of GDM. This was further confirmed in another study where significantly elevated CRP levels and fibrinogen were detected in 26 women with pGDM as compared with controls [44].

3.3. Plasminogen Activator Inhibitor 1. Plasminogen Activator Inhibitor 1 (PAI-1) is a protein that in humans is encoded by the SERPINE1 gene and is mainly produced by the endothelial cells, though it is also secreted by other tissue types, such as adipose tissue. Its main function is to inhibit tissue plasminogen activator (tPA) and urokinase (uPA), the activators of plasminogen, and hence fibrinolysis. PAI-1 is increased in various disease states, such as obesity, MS, insulin resistance, and T2DM [31, 34–36].

PAI-1 is increased in women with pGDM compared with normal women 3 months after delivery [43]. In this study PAI-1 remained higher after adjustment for BFM, while the authors also found a negative correlation between adiponectin levels and PAI-1.

Another study [172] reported that women with pGDM had significantly elevated PAI-1 compared with controls 3 years after delivery.

Other authors studying 74 women with pGDM 3 months after delivery found them to exhibit increased PAI-1 levels when they had impaired insulin sensitivity postpartum,

while tPA was also observed to be elevated [42]. In another study, 25 women with pGDM demonstrated significantly higher PAI-1 levels compared to controls in a mean period of 4 years postpartum [98], although the difference lost significance after adjustment for BMI. Meanwhile, adiponectin levels correlated to PAI-1 levels before and after adjustment for BMI.

4. Conclusions

Women with pGDM are characterized by chronic subclinical inflammation which is associated with insulin resistance and abnormality in glucose metabolism. Approximately 30% of these women have metabolic syndrome and many of them will develop T2DM within 5 years of diagnosis [26, 31, 173]. The conversion rates from GDM to T2DM range from 2.6% to 70% over a period of 6 weeks to 28 years postpartum [174]. The problem of gestational diabetes is common and its incidence is exhibiting an increasing prevalence. Early recognition and management of women predisposed to develop T2DM later in their lives is thus crucial in the development of primary health care strategies, modification of lifestyle, and dietary habits significantly enabling prevention or delay of appearance of glucose intolerance states in predisposed women.

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Clinical Study

Pilot Study of an Individualised Early Postpartum Intervention to Increase Physical Activity in Women with Previous Gestational Diabetes

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Optimal strategies to prevent progression towards overt diabetes in women with recent gestational diabetes remain ill defined. We report a pilot study of a convenient, home based exercise program with telephone support, suited to the early post-partum period. Twenty eight women with recent gestational diabetes were enrolled at six weeks post-partum into a 12 week randomised controlled trial of Usual Care ($n = 13$) versus Supported Care (individualised exercise program with regular telephone support; $n = 15$). Baseline characteristics (Mean \pm SD) were: Age 33 ± 4 years; Weight 80 ± 20 kg and Body Mass Index (BMI) 30.0 ± 9.7 kg/m². The primary outcome, planned physical activity {Median (Range)}, increased by 60 (0–540) mins/week in the SC group versus 0 (0–580) mins/week in the UC group ($P = 0.234$). Walking was the predominant physical activity. Body weight, BMI, waist circumference, % body fat, fasting glucose and insulin did not change significantly over time in either group. This intervention designed to increase physical activity in post-partum women with previous gestational diabetes proved feasible. However, no measurable improvement in metabolic or biometric parameters was observed over a three month period.

1. Background

Strategies to prevent the progression from impaired glucose tolerance to overt (principally type 2) diabetes in middle-aged and older adults have been developed by a number of groups worldwide, drawing on the results of major randomised controlled trials [1–3]. Women with previous gestational diabetes (GDM) are known to be at high risk of progression to type 2 diabetes [4]. However, strategies for diabetes prevention for women with previous GDM in the period immediately following pregnancy are less well defined. The TRIPOD [5] and PIPOD studies [6] demonstrated that thiazolidinedione (TZD) therapy could delay progression to diabetes in a high risk group of women. Some benefits have also been suggested for metformin by Ratner and colleagues [7] in women with previous GDM

(mean age at study entry 43 years) who participated in the Diabetes Prevention Program (DPP). In women with previous GDM, metformin led to a 50% reduction of the risk of progression from impaired glucose tolerance to overt diabetes, whereas lifestyle intervention was associated with a 53% risk reduction. However, medication-based strategies may not be appropriate for women of child-bearing age and are unlikely to be feasible or desirable on a broader scale.

Anecdotally, the pressures of caring for a new baby tend to dominate the early postpartum period, with Australian women potentially experiencing difficulty focusing on their own long-term health, and specifically their exercise, in this context. This belief is supported by a recent qualitative study conducted in the USA that found that having young children/child was a major barrier to an active lifestyle in the first 12 months postpartum [8]. Our recent work [9] has

demonstrated that women with previous GDM frequently have ongoing deficits in health promoting physical activity. By contrast, the recent findings of Retnakaran et al. [10] were more positive, suggesting some improvement in physical activity following a GDM pregnancy.

Changes in lifestyle patterns at this time might potentially prove to be valuable in preventing longer term progression towards diabetes, as well as influencing the woman's entire family towards adopting health promoting behaviours. However, Cheung et al. have reported little success with a group intervention that used patient-centred counselling [11] or more recently with a pedometer-linked programme [12]. In contrast, several intervention studies based on the Social Cognitive Theory [13] have demonstrated success in increasing and even maintaining physical activity among individuals with type 2 diabetes [14]. This pilot study sought to evaluate the feasibility and efficacy of an individualised programme, based on the social cognitive theory, to assist women to be more physically active in the early post natal period.

2. Research Design and Methods

The protocol was approved by Hospital and University Human Research Ethics Committees. Participants consented in writing after appropriate verbal and written explanation of the study. The study was registered with the Australian and New Zealand Clinical Trials Registry: ACTRN 12608000280303.

Seventy-two women were approached to join the trial prior to six weeks postpartum. Forty-three women refused participation and one was excluded due to detection of overt diabetes on the entry oral glucose tolerance test (OGTT), leaving 28 randomised participants. At six weeks after delivery of the index pregnancy complicated by GDM, participants underwent baseline assessment. Parameters assessed included a 75 g OGTT, fasting insulin, body weight and height using standardised instruments, and body composition using bioimpedance methodology. Insulin resistance was estimated using the HOMA-IR equation [15] ($\text{HOMA-IR} = \text{Fasting insulin } (\mu\text{U/mL}) \times \text{Fasting glucose (mmol/L)} / 22.5$). Physical Activity was assessed using the validated Australian Women's Activity Survey [16].

Women were then randomly assigned to one of two groups. The Usual Care group ("UC", $n = 13$) received brief printed materials outlining the importance of diet and exercise for the prevention of future diabetes. The Supported Care ("SC", $n = 15$) group underwent an initial face-to-face consultation with an exercise physiologist where specific, individualised goals for initiating and maintaining regular health-enhancing physical activity were developed. Consistent with current physical activity guidelines a physical activity target of 150 mins/wk was set, to be achieved gradually over the 12 weeks intervention through activities acceptable to the individual. The exercise physiologist contacted each woman in the SC group weekly by telephone for the next four weeks and then every 2 weeks thereafter to assess progress, promote accountability, and to provide tailored

expert support for recognising and overcoming experienced constraints to physical activity behaviour change.

Twelve weeks following baseline assessment (total 18 weeks postpartum), both groups underwent repeat examinations as noted above, except that samples for fasting glucose and insulin alone were taken without a repeat OGTT. The primary outcome measure was change in self-reported physical activity. Secondary outcomes were change in insulin resistance (HOMA-IR), change in weight, and changes in body composition.

Statistical analyses were performed using data from those women who completed both assessments $n = 11$ "UC" and $n = 14$ "SC" women. All comparisons between the UC and SC groups consider differences between these groups in the change or "Delta" ($\text{Delta} = \text{Value}_{18 \text{ weeks post partum}} - \text{Value}_{6 \text{ weeks post partum}}$) in each variable between six and 18 weeks postpartum. Statistical comparisons have been performed using unpaired *t*-tests for normally distributed variables and Mann Whitney *U* tests for nonnormally distributed variables. Categorical variables were analysed using Fisher's exact test due to small cell sizes. Significance was accepted at the 5% level for two-tailed analysis for all variables.

3. Results

Typical of an Australian GDM cohort, the women were generally in their early thirties and their mean body mass index (BMI) at six weeks postpartum was in the obese range. Importantly there were no significant differences between the study groups demographic, physical activity or insulin resistance at baseline (see Table 1). Two UC and one SC participant dropped out of the study prior to the follow-up assessment.

Consistent with previous studies, the physical activity data were nonnormally distributed (see Table 2). Median (range) planned physical activity increased by 60 (0–540) mins/wk in the SC group versus 0 (0–580) mins/wk in the UC group, but this change was not statistically significant ($P = 0.234$, Mann-Whitney *U* test). The change observed in the SC group's physical activity comprised mostly increased planned walking. A pre defined categorical analysis examined differences between SC and UC groups in the proportion of women increasing planned physical activity by >60 mins/wk; 67% of women who received SC achieved this criterion compared to 31% of women who received UC. Despite this, most women regardless of group allocation failed to reach the recommended physical activity level of 150 mins/wk (see Table 2).

Metabolic assessments revealed no changes in weight or insulin resistance in either group (see Table 2). Body composition (% lean mass, % fat mass) was also unchanged. Breast feeding status (full/partial/nil) was also noted at six and 18 weeks postpartum. Weight loss and other metabolic parameters did not differ between breastfeeding groups.

Open-ended feedback regarding the intervention programme was obtained from the SC group. Whilst most women responded positively to the programme, many commented that the starting point of six weeks postpartum was

TABLE 1: Prestudy characteristics of women, at the baseline visit (six weeks postpartum), divided by treatment group.

Parameter	Usual Care group (<i>n</i> = 13)	Supported Care group (<i>n</i> = 15)
	Mean ± SD	Mean ± SD
Age—years	31.5 ± 3.9	34.6 ± 4.4
Weight—kg	80.3 ± 17.4	79.3 ± 20.7
BMI—kg/m ²	30.3 ± 7.4	30.6 ± 8.5
Waist circumference (cm)	96.0 ± 11.0	97.6 ± 15.2
% Body fat	32.7 ± 8.1	33.5 ± 8.3
Fasting glucose—mmol/L;	4.7 ± 0.7	4.6 ± 0.7
Fasting insulin—μU/mL;	8.4 ± 7.5	8.4 ± 7.5
HOMA—IR	1.9 ± 2.0	1.8 ± 1.8
	<i>n</i> (%)	<i>n</i> (%)
Parity > 1	9 (69%)	9 (60%)
Education > high school	8 (62%)	9 (60%)
	Median (range)	Median (range)
Planned physical activity (mins/wk)	0 (0–420)	0 (0–300)

TABLE 2: Changes in physical activity, weight, and insulin resistance of women between baseline (six weeks postpartum) and followup (18 weeks postpartum) by treatment group. All changes calculated as (Value_{18 weeks post partum} – Value_{6 weeks post partum}).

Parameter	Usual Care group (<i>n</i> = 11 at end of study)	Supported Care group (<i>n</i> = 14 at end of study)
	Median (range)	Median (range)
Change in planned physical activity (mins/wk)	0 (0–580)	60 (0–540)
Change in planned walking (mins/wk)	0 (0–360)	60(0–345)
	%	%
Change in planned physical activity > 60 mins/wk	31%	67%
	Never: 54%	Never 53%
Meets physical activity goal of 150 mins/wk	18 weeks only 31% 6 & 18 weeks 15%	18 weeks only 40% 6 & 18 weeks 7%
	Mean ± SD	Mean ± SD
Change in Weight (kg)	0.22 ± 4.2	0.97 ± 3.7
Change in Waist circumference (cm)	–3.6 ± 7.3	–0.35 ± 3.8
Change in % Body Fat	–1.2 ± 3.3	1.0 ± 4.4
Change in fasting glucose (mmol/L)	0.12 ± 0.42	0.25 ± 0.56
Change in fasting insulin (μU/mL)	0.06 ± 3.89	1.49 ± 4.23
Change in HOMA IR	–0.08 ± 1.02	0.43 ± 1.28

too early for maximum benefit, as they were still adapting to life with a new baby and found it difficult to focus on personal lifestyle changes such as increasing physical activity at that time.

4. Discussion

This pilot study was designed to evaluate and refine a potential early postpartum intervention designed to increase physical activity in women with previous gestational diabetes for future dissemination and evaluation. Our findings suggest that a postpartum programme designed to encourage

and assist women with prior GDM to be more physically active is feasible.

Specific strengths of our study included the randomized design and good overall retention of participants. Weaknesses included relatively poor recruitment rates, anecdotally contributed to by the predominance of “baby-related” concerns in early postpartum period, short duration of followup, and small total study cohort.

As noted in Tables 1 and 2, there was great variability in physical activity both at baseline and at followup, with many women reporting essentially zero planned physical activity. The variance in all biophysical study measures was large,

in particular for HOMA-IR where the standard deviations approached or exceeded the mean values. In designing future studies, it may be worthwhile to stratify women according to BMI at entry, as this is likely to be a major factor influencing the degree of insulin resistance.

Notwithstanding the timing of commencement, the intervention was well received. Anecdotally, women were happy that their potential health problems were being addressed in an organised programme. Although changes in physical activity between groups did not reach statistical significance, the proportion of women increasing their physical activity by >60 mins/wk in the SC group was twice that of women in the UC group. If confirmed in a larger study sample and maintained over a longer period of time, this would provide significant health benefits [17].

Commencement of programmes designed to increase physical activity in the early postpartum period has some potential advantages in terms of capitalizing on the increased motivation often seen in pregnancy. However, the focus of attention frequently shifts to the baby at this stage, making alterations in ingrained maternal behaviours potentially difficult to achieve. The emotional stress of adapting to a new baby and the fear of receiving a diagnosis of diabetes are key barriers to follow-up care for GDM [18].

As noted previously, other studies of interventions in the postpartum period [10–12] have met with limited and variable success and the optimal timing and content of postpartum programmes remains undefined. Group-based programmes may help increase motivation [11, 12] but achieving “buy in” and maintaining participation appears challenging. Despite the theoretical advantage of commencing diabetes prevention at an earlier stage of pathophysiology, practical barriers may make women more resistant to change at this stage of the life cycle.

Despite some evidence of increased physical activity, measures of glucose metabolism were not altered by this intervention over a three-month period. This was not unexpected given the small sample size and short duration of the study, but we noted absolutely no trends in favour of metabolic improvement. Weight and body composition were also unchanged. Although early postpartum breast feeding status did not appear to influence our findings, the potential importance of breast feeding in longer term diabetes prevention has also been noted in a recent review [18].

Alternatively, one could argue in favour of pharmacologic prevention of progression towards diabetes following GDM, citing the results of the TRIPOD [5], PIPOD [6], and DPP [7] studies. However, thiazolidinediones are rapidly disappearing from the pharmacotherapy of type 2 diabetes due to an unfavourable risk/benefit profile and their potential use in diabetes prevention appears severely limited. Metformin was reported to be equally efficacious as an intensive diet/lifestyle programme in women with previous GDM who participated in the DPP [7], but this finding relates to much older women (mean age 46 years at study entry), rather than those in the early postpartum period. For large scale intervention, lifestyle measures appear intrinsically more

attractive, though metformin may still deserve consideration in those struggling to make effective lifestyle changes.

Further research is warranted to improve the physical activity levels and general health of women with previous GDM. We suggest that studies combining physical activity and dietary interventions may potentially offer greater benefits and we are currently planning such studies, using the pilot data reported in this paper. We hope that our findings will also assist other researchers in determining in the design and conduct of more definitive studies, in particular by allowing *pre hoc* power calculations to be performed in a more robust fashion.

Abbreviations

BMI:	Body mass index
GDM:	Gestational diabetes mellitus
HOMA:	Homeostasis model assessment
IR:	Insulin resistance
OGTT:	Oral glucose tolerance test
SC:	Supported care
UC:	Usual care.

Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contribution

All authors made substantial contributions to conception and design, analysis, and interpretation of data. All authors were involved in drafting the paper and revised it critically for important intellectual content and have read and approved the final paper.

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Clinical Study

Fetuin-A Characteristics during and after Pregnancy: Result from a Case Control Pilot Study

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Objective. Fetuin-A has been associated with gestational diabetes mellitus (GDM). We investigated fetuin-A levels during and after pregnancy in women with GDM. Fetuin-A measurements were performed in 10 women with GDM and 10 age and body mass index (BMI) matched healthy pregnant women. All women underwent an oral glucose tolerance test (OGTT) in and 3 months after gestation. **Results.** Fasting fetuin-A correlated with BMI in women with former GDM ($r = 0.90$, $P < 0.0001$) but showed no association with parameters of glucose tolerance in women with GDM or post-GDM. GDM featured significantly lower insulin sensitivity and higher insulin and C-peptide secretion profiles compared to NGT during pregnancy ($P < 0.05$). Fasting and postprandial fetuin-A did not differ between groups, neither during nor after pregnancy. **Conclusion.** Fetuin-A is not influenced by glucose tolerance during or after pregnancy or acute glucose elevations following glucose ingestion in young women, but closely relates to BMI early postpartum.

1. Introduction

Fetuin-A is a potent systemic calcification inhibitor [1]. Beside this function fetuin-A has been shown to interact with insulin receptor tyrosine kinase, thereby inducing insulin resistance in rodents [2, 3]. In cross-sectional human studies, fetuin-A has been linked to insulin resistance and metabolic syndrome [4]. Furthermore fetuin-A has been linked to incident diabetes mellitus in the Health ABC study [5]. Stefan et al. were able to demonstrate a relation between fetuin-A and liver fat accumulation in insulin-resistant subjects [6]. In pregnant women fetuin-A levels were shown to increase with gestational age [7]. Furthermore women with

gestational diabetes (GDM) display higher levels of fetuin-A compared to normal glucose tolerant counterparts [7]. However, GDM being regarded as pre-type 2 diabetes may comprise a very heterogeneous group of women of different ethnicity and with varying degree of obesity. Therefore, investigating fetuin-A behavior in women with GDM may provide a possibility to further elucidate the role of fetuin-A in early stages of diabetes mellitus. Therefore we aimed to investigate the role of acute glucose ingestion during a standardized oral glucose tolerance test (OGTT) on plasma fetuin-A levels in a homogeneous group of European young normal-weight to moderately overweight women with and without GDM during as well as after partum.

2. Methods

The study was performed as a case-control study. Women with GDM were recruited consecutively from the Department of Obstetrics and Gynecology of the Medical University of Vienna. Those who gave informed consent underwent a 2 h oral glucose tolerance test (OGTT). Data from 10 women with GDM and 10 women with NGT during pregnancy (28th week of gestation), matched for age and BMI in a 1:1 ratio, were analyzed. A second OGTT was performed 10–12 weeks after delivery. Women with previous ketoacidosis and/or beta-cell antibodies (GAD, ICA, IA2), severe chronic disease, kidney or liver disease, and those on chronic medication of drugs known to influence carbohydrate metabolism were excluded.

2.1. OGTT. GDM was diagnosed according to the criteria of the 4th International. Workshop Conference on GDM [8]. After an overnight fast for at least 12 h, blood samples for the measurement of glucose, insulin, and C-peptide were taken at baseline as well as at 30, 60, 90, and 120 min after ingestion of 75 g glucose. Fetuin-A levels were measured at fasting as well as 30, 60, and 120 minutes after glucose load.

2.2. Plasma Metabolites. Plasma levels of fetuin-A were measured by an enzyme-linked radioimmunoassay (ELISA) (Biovendor laboratory medicine, Modreci, Czech republic). Intra-assay coefficients of variation were 3.5% and interassay coefficient of variation 5.4%. Glucose, insulin, C-peptide, glutamate oxalacetat transaminase (GOT), glutamat-pyruvat-transaminase (GPT), bilirubin, cholinesterase (CHE), cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglyceride (TG), and HbA1c were measured using standard kits available in our central laboratory.

2.3. Data Analysis. The kinetics of glucose, insulin, and C-peptide during OGTT were analyzed by quantitative methods to obtain metabolic parameters, such as insulin sensitivity through oral glucose insulin sensitivity index (OGIS), which describes glucose clearance per unit change of insulin concentration [9]. Total insulin secretion (TIS) from C-peptide, its suprabaal component (dynamic TIS), and hepatic insulin extraction (HIE) were obtained with a mathematical model of insulin/C-peptide interactions [10, 11]. β -Cell function was described as the ability of the β -cell to adapt insulin secretion to the prevailing insulin resistance and was quantified by the products: OGIS_dynamic AUC insulin (termed disposition index) and OGIS_dynamic TIS (termed adaptation index), where AUC is the area under the insulin concentration curve during the whole test.

2.4. Statistical Analysis. Comparisons of quantitative variables among groups were performed using Student's *t*-test. Associations between continuous variables are described by the Spearman correlation coefficient. Levels of statistical significance were set at $P < 0.05$.

3. Results

Women with and without GDM were comparable in terms of their medical history. There were also no differences in baseline as well as 3 months postpartum laboratory parameters of lipoproteins and liver enzymes (Table 1). During gestation women with GDM displayed significant higher fasting insulin, C-peptide, and AUC of both parameters during OGTT. OGIS was significantly lower in women with GDM compared to those with NGT (Table 1) and Figure 1(a).

Three months after delivery, 3 women from the GDM group had a pathologic glucose tolerance status and the remaining 7 returned to normal glucose values. Insulin and C-peptide secretion profiles were still higher in women from the former GDM compared to NGT group but without reaching statistical significance (Table 1). Also OGIS were still lower in the former GDM group compared to women with NGT without statistical significance (Table 1) and Figure 1(b).

Fasting and postprandial fetuin-A plasma levels did not differ between women with GDM and NGT during as well as 3 months after pregnancy (Table 1). During pregnancy there were no correlations between fetuin-A plasma levels and parameters of insulin resistance and secretion. Three months after gestation there was significant correlation between fasting fetuin-A and BMI ($r = 0.90$, $P < 0.0001$) in women with former GDM (Figure 2). There were no correlations between fetuin-A plasma concentrations and GOT, GPT, or bilirubin.

4. Discussion

In the present study we found that fetuin-A plasma levels did not differ between women with and without GDM even when measured during as well as after pregnancy. Additionally we found that fetuin-A was not affected by acute glucose ingestion both during as well as after gestation. Interestingly we found that fetuin-A did not correlate with parameters of obesity and insulin resistance during pregnancy. However three months after gestation there was a strong correlation between fasting fetuin-A and BMI in those from the former GDM group. One study investigated the relation between fetuin-A levels and parameters of insulin resistance during normal pregnancy and in women with GDM [7]. In that study fetuin-A levels increased during the course of uncomplicated gestation [7]. Additionally women with GDM had significantly higher fetuin-A levels compared to healthy pregnant women [7]. Furthermore they found a significant correlation between fetuin-A levels and parameters of insulin resistance such as fasting C-peptide and C-peptide to glucose ratio [7]. However the differences between those results and our findings could be explained by study design and methods. GDM women in the study of Kabaly et al. were more obese than subjects in our investigation. Furthermore we used more sophisticated methods to measure insulin resistance. Under experimental conditions high glucose levels were able to activate fetuin-A gene promoter [12]. This activation was dose dependent and occurred after at least 3

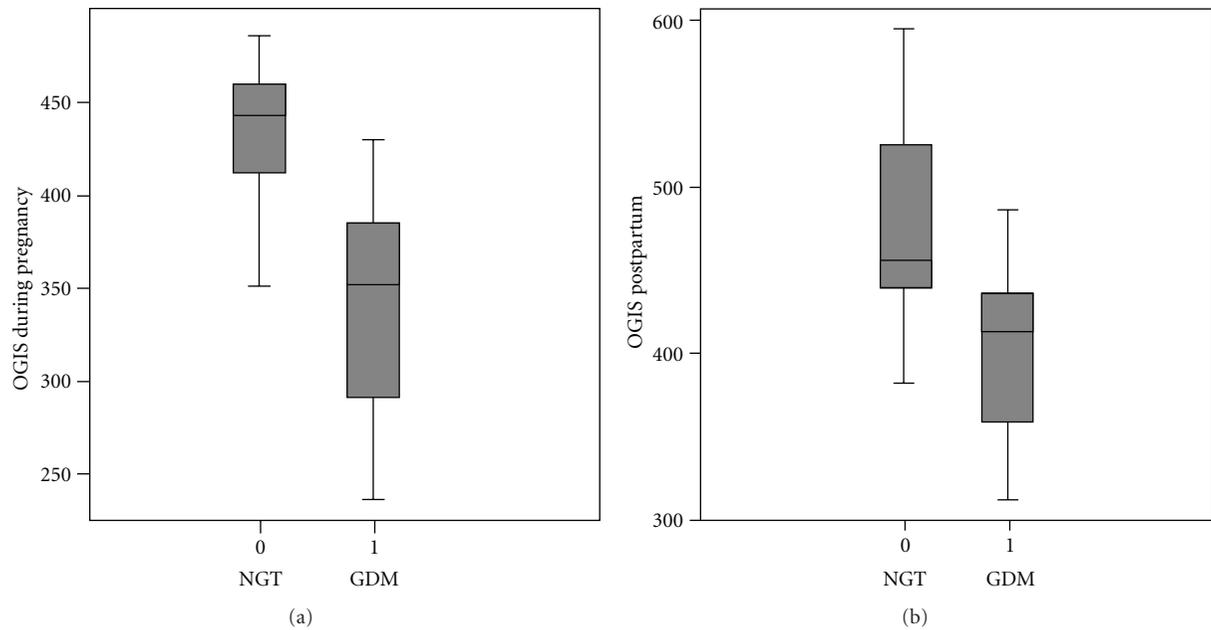


FIGURE 1: (a) Boxplot of OGIS level ($\text{mL min}^{-1} \text{m}^{-2}$) in women with and without gestational diabetes mellitus during pregnancy ($P = 0.001$). (b) Boxplot of OGIS level ($\text{mL min}^{-1} \text{m}^{-2}$) in women with and without gestational diabetes mellitus 3 months after delivery ($P = 0.06$).

TABLE 1: Patient's characteristics and laboratory parameters.

Variable	Third trimester of gestation		<i>P</i> -Value	Three months postpartum		<i>P</i> Value
	NGT (<i>n</i> = 10)	GDM (<i>n</i> = 10)		NGT (<i>n</i> = 10)	GDM (<i>n</i> = 10)	
BMI (Kg/m^2)	27.3 ± 1.3	28.9 ± 1.31	0.4	26.5 ± 1.58	27.9 ± 1.30	0.5
Age (years)	30.4 ± 1.7	32 ± 2.3	0.5	—	—	—
Parity	1.1 ± 0.4	2 ± 1	0.4	—	—	—
Systolic blood pressure (mmHg)	116.5 ± 4.2	110 ± 4.1	0.2	117.6 ± 2.9	106.4 ± 4.4	0.05
Diastolic blood pressure (mmHg)	71.5 ± 2.5	67.7 ± 2	0.2	73.1 ± 2.4	65 ± 2.6	0.04
Total cholesterol (mg/dL)	277.1 ± 16.4	227.2 ± 11.6	0.02	204.5 ± 15.2	203 ± 10.2	0.9
LDL cholesterol (mg/dL)	159 ± 14.8	115 ± 9.1	0.02	129.5 ± 13	118.4 ± 6.8	0.4
HDL cholesterol (mg/dL)	75.6 ± 21.3	69.9 ± 3.7	0.4	64.7 ± 6	61.6 ± 4.5	0.6
Triglycerides (mg/dL)	197.1 ± 20.2	207 ± 24.9	0.7	81 ± 7.5	116.3 ± 18.7	0.09
HbA1c (%)	4.9 ± 0.11	4.9 ± 0.14	0.9	5.2 ± 0.1	5.3 ± 0.08	0.5
Cholinesterase ()	5.31 ± 0.71	5.23 ± 1.39	0.6	7.07 ± 0.7	7.76 ± 1.48	0.1
Bilirubin ()	0.33 ± 0.13	0.37 ± 0.14	0.5	0.7 ± 0.42	0.48 ± 0.16	0.1
GOT (U/L)	21.6 ± 5.1	20.1 ± 3.9	0.8	26.3 ± 7.5	26.3 ± 8.9	0.2
GPT (U/L)	15.8 ± 6.1	17.1 ± 7.4	0.4	27 ± 13.7	40.1 ± 23.3	0.9
hs-CRP (mg/dL)	0.43 ± 0.05	2.15 ± 1.75	0.3	0.29 ± 0.07	0.21 ± 0.07	0.5
Fasting glucose (mg/dL)	80.3 ± 2.3	88.1 ± 2.6	0.039	82.4 ± 2.9	92.4 ± 3.6	0.04
1-hour postload glucose (mg/dL)	135.6 ± 7.2	186.1 ± 11.5	0.002	105.3 ± 8.6	114.1 ± 13.4	0.6
Fasting insulin secretion (pmol/L)	12.2 ± 1.6	16.0 ± 1.81	0.1	7.8 ± 1.4	11.3 ± 2.3	0.2
OGIS	434.05 ± 12	341.51 ± 19.35	0.001	476.79 ± 30.23	408.19 ± 18.72	0.06
Disposition index (nmol/m^3)	3.87 ± 0.44	5.15 ± 0.50	0.07	2.49 ± 0.43	3.57 ± 0.31	0.059
AUC-glucose (mmol/L/min.)	14.21 ± 0.54	19.28 ± 0.73	0.0001	14.17 ± 0.89	17.26 ± 1.26	0.1
AUC-insulin (nmol/L/min.)	8.93 ± 0.98	15.7 ± 2.01	0.007	5.41 ± 1.08	9.11 ± 1.15	0.46
AUC-C-peptide (nmol/L/min.)	1016.7 ± 44.9	1441.3 ± 137.9	0.009	777.5 ± 98.9	1131.4 ± 156.3	0.1
Fasting fetuin-A (ng/mL)	497.5 ± 64.3	580 ± 46.4	0.8	560.38 ± 69.3	571.4 ± 80.4	0.8
30-min. after load fetuin-A (ng/mL)	453.9 ± 29.8	549.3 ± 50	0.5	569.4 ± 86.1	571.3 ± 87.2	0.5
60-min. after load fetuin-A (ng/mL)	581 ± 43.2	513 ± 42.1	0.7	577.5 ± 114.1	542.7 ± 43.8	0.7
120-min. after load fetuin-A (ng/mL)	524.5 ± 39.9	539.6 ± 33	0.1	414.8 ± 48.6	552 ± 47.6	0.1

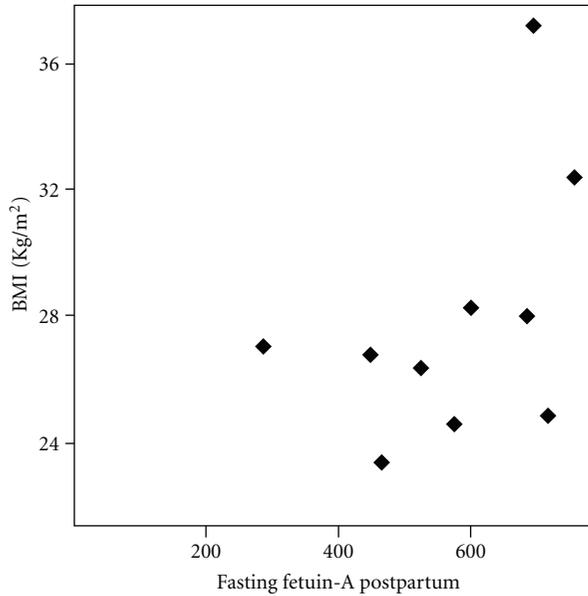


FIGURE 2: Scatter diagram of the correlation between fasting postpartum fetuin-A (ng/mL) and body mass index (BMI) (Kg/m^2) in women with history of gestational diabetes mellitus ($r = 0.90$, $P < 0.0001$).

hours of incubation [12]. In our study during OGTT fetuin-A plasma levels remained unchanged. We could not exclude a late elevation of fetuin-A after OGTT. Further studies with longer fetuin-A collection time points during OGTT or intravenous glucose tolerance test are needed to further investigate the influence of glucose per se on fetuin-A plasma concentrations.

However, the fact that even chronic hyperglycemia linked to GDM did not alter fetuin-A levels argues against a prominent effect of glucose fluctuations/elevation on fetuin-A release. Fetuin-A has been linked to obesity in a study performed in patients with morbid obesity [13]. In that study, fetuin-A levels decreased after weight loss due to gastric bypass surgery [13]. Another study investigated the relation between fetuin-A with parameters of insulin resistance and fatty liver [14]. In that study obese children with and without nonalcoholic fatty liver (NAFL) were compared. The authors found a relation between fetuin-A and NAFL as well as a reduction in fetuin-A after weight loss [14]. In our study we did not investigate the presence of NAFL by ultrasound as recommended. Despite this there were no differences in hepatic transaminases as well as cholinesterase between women with and without GDM neither in nor after pregnancy. Additionally we found no correlation of fetuin-A with those liver enzymes (data not presented). Women in our study were only of moderate obesity as compared to children from the investigation of Reinher and Roth [14]. Based on our results and in light of previous findings, it appears that fetuin-A could be the link between fatty liver disease and metabolic syndrome. Further investigations in subjects with obesity with and without NAFL should be performed to finally evidence a link between fatty liver disease, fetuin-A, and insulin resistance.

4.1. Study Limitations. First, as we examined a small cohort it is difficult to translate our findings to the general GDM population. Second the subjects investigated were young females, which were besides having GDM healthy. Also the mean BMI of our GDM patients was lower than that of the subjects investigated by Kalabay et al. [7]. Third we did not investigate the presence of NAFL by ultrasound examination.

5. Conclusion

Our preliminary study suggests that fetuin-A does not relate to parameters of insulin sensitivity or metabolic control and does not seem to play a role in the pathogenesis of GDM. Furthermore acute glucose changes during an OGTT do not affect fetuin-A plasma concentrations both during and after pregnancy. However preexisting overweight, which may be masked by pregnancy, related changes in body weight or weight retention after pregnancy may have a strong impact on fetuin-A release.

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Clinical Study

How Can We Increase Postpartum Glucose Screening in Women at High Risk for Gestational Diabetes Mellitus?

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Women with a history of gestational diabetes mellitus (GDM) are at increased risk for diabetes mellitus but postpartum followup is problematic for frequent nonattendance. Our aim was to increase coverage of postpartum oral glucose tolerance tests (ppOGTTs) and examine associated factors. This was a prospective observational study of altogether 266 high-risk women for GDM from 2005 to 2008 in four Finnish municipalities. The groups were as follows: women ($n = 54$) who had previously participated in early pregnancy lifestyle intervention study and high-risk women ($n = 102$) from the same municipalities studied within one-year after delivery. Furthermore, in two neighboring municipalities nurses were reminded to perform a ppOGTT on high-risk women ($n = 110$). The primary outcome was the prevalence of ppOGTT performed and associated factors. Overall the ppOGTT was performed in 35.7% of women. Only 14.7% of women returned for testing to health care centers, 30.9% after a reminder in municipalities, and 82.5% to the central hospital, respectively. The most important explaining factor was a special call or reminder from the central hospital (OR 13.4 (4.6–38.1), $P < 0.001$). Thus, additional reminders improved communication between primary care and secondary care and more attention to postpartum oral glucose testing in primary care are of great importance.

1. Introduction

Gestational diabetes mellitus (GDM) implies a substantial risk of later diabetes. Pregnancy seems to identify women who are at risk of developing diabetes later in life. In our study the prevalence of GDM in South Ostrobothnia was 13.0% [1]. About 10% of Finnish women with GDM will develop diabetes over the next 6 years; nearly half of them develop type 1 diabetes and the other half type 2 diabetes [2]. In the meta-analysis of 675,455 women with 10,859 cases of type 2 diabetes Bellamy found that women who have had gestational diabetes have at least a sevenfold increased risk of

developing type 2 diabetes mellitus in the future compared with those who have had a normoglycaemic pregnancy. The strength of the association between gestational diabetes and type 2 diabetes and the knowledge that many of the risk factors (family history of diabetes, raised body-mass index, increased age, and Asian and black origin) are the same, suggest that the two disorders might have an overlapping cause [3].

Recommendations from the International Workshop Conference of GDM suggest screening at 6 weeks postpartum using the 75 gram, 2 hour oral glucose tolerance test (OGTT), which should then be repeated at one-year

postpartum and then at least every 3 years thereafter [4]. The postpartum period allows for the identification of women at high risk for diabetes and provides an important opportunity for intervention. Type 2 diabetes has been prevented or delayed by lifestyle intervention not only in randomized controlled studies [5], but also in the primary health care setting [6].

Despite the elevated risk for diabetes and recommendations for close followup, the opportunities for postpartum screening and intervention are missed. The variation of screening rate is from 14% in usual care to 60% in a randomized control trial [7]. In a retrospective study a reminder system of automated orders to physicians and telephone and e-mail reminder messages to patients improves the coverage of tested women 50% [8].

GDM strongly predisposes to type 2 diabetes. GDM and type 2 diabetes also share many of the same risk factors, suggesting that the two disorders have an overlapping pathogenesis [3]. Excessive gestational weight gain is a risk factor for short-term postpartum weight retention and thus overweight in women. In a meta-analysis of >65,000 women Nehring showed that women with a gestational weight gain above recommendations retained 3 kg more weight 3 years postpartum than did those who gained weight within the recommendations [9]. Therefore, women at a high risk for GDM are a high-risk group for diabetes, especially if they have postpartum weight retention.

Our aim in this study was to explore the optimal strategies to increase the amount of postpartum oral glucose tolerance test (ppOGTT). The primary outcome was the prevalence of women at high risk for GDM who underwent an OGTT in the postpartum period in four rural municipalities. In two municipalities we had our previous early pregnancy intervention study group women for preventing GDM [1] and other high-risk women for GDM with usual care, and in two neighboring municipalities high-risk women who received a special telephone call for postpartum glucose testing.

2. Methods

This study was a prospective observational multicenter study of postpartum glucose screening in 266 women at high risk for GDM in South Ostrobothnia, Finland. The risk of GDM was estimated during index pregnancy from Apr 2005 to May 2006. We analyzed data from Apr 2005 to Jan 2008 from Seinäjoki Central Hospital and four rural municipalities Kauhajoki, Lapua, Jalasjärvi, and Kurikka.

A database contained clinical, glycemic, and delivery data of all women from four municipalities in the study period. The inclusion criteria for this study were at least one of the follows: (1) BMI >25 kg/m², (2) birth of child >4.5 kg, (3) age over 40 years, (4) family history of diabetes and (5) glucosuria. The risk factors for GDM were reasked in connection with the ppOGTT at the central hospital and in the neighboring municipalities. In the usual care municipalities the information of risk factors was extracted from the self reported questionnaire in the beginning of index pregnancy.

We included also our previous early pregnancy lifestyle intervention study group to the current examination. The protocol was approved by the ethics committee of South Ostrobothnia Hospital District in Seinäjoki, Finland. It was in accordance with Helsinki Declaration. All women participating in ppOGTT gave written informed consent.

2.1. Diabetes Care during Pregnancy. According to guidelines in Finland at the time of study, GDM testing was focused on women with risk factors. Diagnosis of glucose intolerance was established by 2-h 75-g OGTT performed between 24 and 28 weeks of gestation. In women with a high risk for diabetes, testing was done in early pregnancy. OGTT testing was also offered if women had polyhydramnion, macrosomia or glucosuria later during pregnancy [10]. The GDM criteria were modified from the World Health Organization as a fasting plasma glucose ≥ 5.6 mmol/L or 2 h plasma glucose ≥ 7.8 mmol/L [1]. From 1998 onward, the WHO classified any glucose levels above normal as indicative of gestational diabetes [11].

The health care nurses gave women counseling about healthy lifestyle in the beginning of pregnancy. The dietary and exercise advice were provided both verbally and writing. Women were advised to stop alcohol intake and smoking. The nurse in the health care centers had on average 13 appointments with the women during pregnancy.

In the study group a clinical nutritionist gave dietary advice tailored to each subject individually six times and a physiotherapist gave exercise advice six times. In the control group the women were given general information on diet and physical activity in a single session to decrease the risk of GDM during pregnancy group [1].

After a diagnosis of GDM women were given instructions and advised to perform blood glucose monitoring. Insulin therapy was initiated when fasting capillary plasma glucose exceeded 5.8 mmol/L and postprandial capillary plasma glucose was >8.5 mmol/L in the study period.

All the women were followed in maternal health care in municipalities and all the women gave birth at the Central Hospital of Seinäjoki. During pregnancy the women were seen at the central hospital only if fasting capillary plasma glucose exceeded 5.8 mmol/L or postprandial capillary plasma glucose exceeded 8.5 mmol/L or in the case of macrosomia, polyhydramnion, lack of compliance, BMI >30 kg/m², or some complicating illness.

2.2. Postpartum Testing. Women with GDM requiring insulin were asked to continue glucose testing at home after discharge. The women with a diagnosis of GDM were offered an OGTT after one year after delivery based on local instructions.

The high-risk women who underwent a lifestyle intervention during pregnancy were offered an OGTT at the central hospital irrespective of whether they developed GDM. We generated a list of women at high risk for GDM in two neighboring municipalities, took it to the health care nurses and advised them to call the women by telephone for glucose testing in the primary care.

TABLE 1: Characteristics of the women at high risk for gestational diabetes mellitus during the index pregnancy.

Characteristics	No postpartum glucose screening (<i>n</i> = 171)	Postpartum glucose screening (<i>n</i> = 95)	<i>P</i> ¹
Age (years), mean ± SD	30.1 ± 5.7	30.2 ± 5.7	0.968
<25 (%)	19.3 (33/171)	18.9 (18/95)	
25–35 (%)	63.2 (108/171)	61.1 (58/95)	
≥36 (%)	17.5 (30/171)	20.0 (19/95)	
Educational status			0.044
Higher education (%)	8.8 (15/171)	26.3 (25/95)	
Other education (%)	91.2 (156/171)	73.7 (70/95)	
Parity			0.001
Nulliparous (%)	25.1 (43/171)	45.3 (44/95)	
Multiparous (%)	74.9 (128/171)	53.7 (51/95)	
Body mass index (kg/m ²), mean ± SD	28.2 ± 5.4	26.7 ± 4.7	0.024
≤25.0 (%)	26.3 (45/171)	38.9 (37/95)	
25.1–30.0 (%)	45.0 (77/171)	45.3 (43/95)	
30.1–35.0 (%)	19.9 (35/171)	9.5 (9/95)	
>35.0 (%)	8.2 (14/171)	6.3 (6/95)	
Weight gain (kg), mean ± SD	11.5 ± 6.5	12.0 ± 5.8	0.453
≤11.5 (%)	50.9 (87/171)	46.3 (44/95)	
11.6–16.0 (%)	26.9 (47/171)	28.4 (27/95)	
>16.0 (%)	21.1 (37/171)	24.2 (23/95)	
GDM diagnosed (%)	19.9 (34/171)	24.2 (23/95)	0.385
Insulin therapy during pregnancy (%)	6.4 (11/171)	10.5 (10/95)	0.235
Blood sample of newborn for glucose (%)	36.3 (62/171)	48.4 (46/95)	0.049
Risk factors for GDM			
BMI >25 (kg/m ²) (%)	78.9 (135/171)	67.4 (64/95)	0.037
Previous birth of child >4.5 kg (%)	5.3 (9/171)	1.1 (1/95)	0.102
Age >40 years (%)	3.5 (6/171)	3.2 (3/95)	1.000
Previous history of GDM (%)	18.1 (31/171)	14.7 (14/95)	0.480
Family history of diabetes (%)	18.1 (31/171)	52.6 (50/95)	<0.001
Special call for OGTT (%)	49.1 (84/171)	83.2 (79/95)	<0.001

¹ *P* values (two sided): χ^2 test or Mann-Whitney *U*-test.

A 75 g 2 h oral glucose tolerance test after overnight fasting for 12 hour was performed one year post partum. Diabetes was diagnosed by either fasting venous plasma glucose ≥ 7.0 mmol/L or 2 h value ≥ 11.1 mmol/L, impaired fasting glucose (IFG) tolerance by fasting glucose ≥ 6.1 mmol/L and impaired glucose tolerance (IGT) by 2-h glucose ≥ 7.8 mmol/L and < 11.1 mmol/L.

2.3. Data Collection and Statistical Analysis. Antenatal maternal clinical, glycemic, delivery, neonatal, and ppOGTT data were derived from the database of the central hospital and the health care centers. Variables considered as potentially predictive for a participation in the ppOGTT were analyzed with the statistical package SPSS 19.0 (SPSS, Chicago, IL, USA). Data are presented as numbers and proportion for categorical variables or as means \pm SD for continuous variables, respectively. A χ^2 test or two-sided Mann-Whitney *U*-test was used to test for differences between women who participated in the ppOGTT and those who did not. Differences with *P* < 0.05 were regarded as statistically significant. A multivariable logistic regression model was used

to evaluate the association between participation in the ppOGTT and demographic factors, anthropometric and clinical risk factors and special call for ppOGTT. Results for each risk factor are presented as odds ratios (ORs) with 95% CI.

3. Results

The baseline characteristics of the 266 women at high risk for GDM are seen in Table 1. The women in the lifestyle intervention study group (*n* = 54) were thinner (*P* = 0.040), they were more often nulliparous (*P* = 0.001) and more frequently had a family history of diabetes (*P* = 0.005) than the other women at a high risk for GDM (*n* = 212). During pregnancy GDM was diagnosed in fewer women (*P* = 0.005) in the lifestyle intervention group (*P* = 0.010) than in the other high-risk women (data not shown). Otherwise there were no differences on tested baseline variables between the lifestyle intervention group and other risk women for GDM in four municipalities.

Women who underwent ppOGTT were more likely to have received a telephone call from the central hospital to

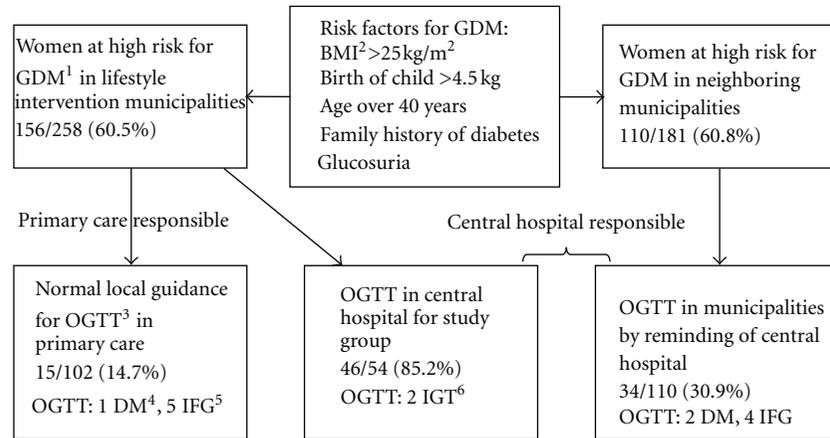


FIGURE 1: The flowchart of postprandial glucose screening in women at high risk for gestational diabetes mellitus.¹GDM: gestational diabetes mellitus, ²BMI: body mass index, ³OGTT: oral glucose tolerance test, ⁴DM: diabetes mellitus, ⁵IFG: impaired fasting glucose, ⁶IGT: impaired glucose tolerance.

remind about the OGTT than women who did not undergo testing (83.2% (79/95) and 49.1% (84/171), respectively, $P < 0.001$). The women in the lifestyle intervention study group returned for testing significantly more often than the other women at high risk for GDM, adjusted OR 2.5 (5.4–29.5), $P < 0.001$. The overall return rate for postpartum testing in women was 35.7% (95/266). In the usual care in the two municipalities in which no reminder call was made the return rate was lowest 14.5% (15/102), 30.9% (34/110) for those receiving the reminder call and in the lifestyle intervention study group 85.2% (46/54) (Figure 1). All of the women who participated in postpartum testing also had OGTT during pregnancy. In the lifestyle intervention study group, 6 women were pregnant again, one had moved away, and one was not willing to undergo testing. In the reminder group 7 women were pregnant, and 68 women refused testing for professional or child care reasons or most because of unknown problems. In the usual care group the reasons could not be assessed.

We tested age, educational status, parity, BMI, weight gain during pregnancy, GDM diagnosed during pregnancy, blood sample of newborn for glucose and risk factors for GDM (BMI >25 kg/m², previous birth of child >4.5 kg, age >40 years, previous history of GDM and family history of diabetes), and special call for ppOGTT. In univariable analysis unadjusted nulliparous women, women with normal weight and higher education returned more often for glucose testing (Table 1).

The most important explanatory factor was the reminder telephone call for testing either at the central hospital or reminding from the central hospital to the care providers in the health care centers (adjusted OR 13.4 (4.6–38.1), $P < 0.001$). Another statistically significant risk factor was family history of diabetes, (adjusted OR 5.1 (2.1–12.2), $P < 0.001$) (Table 2).

Fourteen women (8.2%) had abnormal glucose tolerance: 3 had DM-, 4 had IGT- and 7 had IFG. Two women out of 4 with IGT diagnosis and one woman out of 7 with

IFG-diagnosis had normal glucose tolerance during pregnancy. One woman was obese, BMI 40 kg/m² and she had 2 kg of weight retention postpartum, the other had 8.5 kg of weight retention and the third 6 kg.

4. Discussion

GDM is a significant risk factor for the development of diabetes. Early identification of women at high risk for diabetes is critical to prevent or delay onset of diabetes. However, in the real world the opportunities for postpartum screening and intervention are frequently missed. In the present study the inclusion criteria were BMI >25 kg/m², birth of child >4.5 kg, age over 40 years, family history of diabetes and glucosuria. In the usual care in the health care centers, the testing rate was only 14.5%. The return rate was doubled (30.9%) in municipalities in which health professionals from the central hospital reminded the nurses in health care centers to call the high-risk women for glucose testing. The return rate for postpartum testing of women at high risk for GDM was highest (85.2%) in those who participated in the lifestyle intervention study group. Thus a simple reminder from the central hospital was the most important factor explaining better compliance with ppOGTT. To our knowledge there are no earlier reports of postpartum testing women with risk factors for GDM without GDM diagnosis.

The overall return rate for postpartum testing was 35.7% (14.5%, the usual care; 30.9%, after the reminder call; 82.5%, the study group) in the current study. The frequency of follow-up with an OGTT was 33.7% in a retrospective cohort study of women with previous GDM ($n = 745$) in New York [12]. The overall return rate was 51.1% (79.6%, Kiel; 65.0%, Bonn; 23.4%, Berlin) in a German prospective multicenter study of 605 women with GDM [13]. In the Canadian study the postpartum reminding system doubled screening rate from 14% to 28% in the usual care [7]. This result is in accordance with our study. All the Canadian women had

TABLE 2: Multivariable logistic regression model predicting postpartum glucose screening among women at high risk for gestational diabetes mellitus.

	Odds ratio (95% CI) ¹	P
Age (years)		
<25	1.00	
25–35	0.73 (0.25–2.15)	0.571
>35	0.75 (0.19–2.98)	0.684
Educational status		
Other education	1.00	
Higher education	0.65 (0.25–1.73)	0.393
Parity		
Nulliparous	1.00	
Multiparous	0.51 (0.20–1.27)	0.149
Body mass index (kg/m ²)		
≤25.0	1.00	
25.1–30.0	1.07 (0.18–6.36)	0.941
30.1–35.0	1.79 (0.25–12.86)	0.564
>35.0	1.67 (0.19–14.83)	0.646
Weight gain during pregnancy		
≤11.5	1.00	
11.6–16.0	0.71 (0.27–1.89)	0.491
>16.1	0.67 (0.23–1.92)	0.454
GDM diagnosed during index pregnancy	2.23 (0.54–9.26)	0.269
Insulin therapy during index pregnancy	1.31 (0.19–9.12)	0.782
Blood sample of newborn for glucose	1.30 (0.57–2.97)	0.536
Risk factors for GDM		
BMI >25 (kg/m ²)	0.99 (0.29–3.36)	0.989
Previous birth of child >4.5 kg	0.21 (0.01–3.09)	0.256
Age >40 years	0.95 (0.13–7.06)	0.961
Previous history of GDM	1.63 (0.48–5.52)	0.435
Family history of diabetes	5.09 (2.13–12.12)	<0.001
Special call for OGTT	13.4 (4.64–38.1)	<0.001

¹ Adjusted odds ratios (ORs), and their 95% confidence intervals (CIs) and P values.

GDM, whereas in our study 21.4% (57/266) had GDM, and the rest were at high risk for GDM. However, 42% (11/26) of those women who had GDM returned for glucose testing in usual care. All (4/4) of the women with GDM who had undergone in the lifestyle intervention and 33% (9/27) of the women with GDM who were given a reminder telephone call took a postpartum OGTT. It is worth noting that 2 women with IGT and one woman with IFG had normal glucose tolerance during pregnancy. One woman was morbidly obese and the rest had excessive weight retention one year after pregnancy. Gestational diabetes and type 2 diabetes have the same risk factors, and overweight and weight retention

after index pregnancy also seems to be important predictive factors for impaired postpartum glucose tolerance.

Hunt has identified in a prospective cohort study of 707 women several factors associated with postpartum screening: fewer children, lower fasting blood glucose levels at GDM diagnosis, and no insulin treatment in pregnancy [14]. Thus the women who returned for postpartum glucose screening had less severe GDM than women who failed to return. The same tendency was observed also in the current study: over half (58% 33/57) of women with GDM and (57% 12/21) of women with insulin therapy did not attend for testing. In an observational German study Schaefer-Graf examined the association between an abnormal postpartum OGTT and four risk factors: body mass index ≥ 30 kg/m², gestational age at diagnosis <24 weeks, 1 h antenatal glucose value >11.1 mmol/L and insulin therapy. Women with two or more risk factors had a high risk for an abnormal ppOGTT, and 86% of postpartum diabetes was diagnosed within this group [13].

Even though clinicians are aware that women with GDM are at high risk of developing type 2 diabetes they do not routinely screen patients. Providers identified poor communication between primary care providers and obstetric and gynecology care providers as a major barrier to screening [15]. The current study and the other reminding system studies [7, 16, 17] shows that we have other barriers as well. Feelings of emotional stress due to adjusting to a baby and the fear of receiving a diagnosis of diabetes at the visit were identified as key barriers in a small interview study of 22 women [16]. Child care availability and desire for checkup were among the key facilitators to screening. The fear of receiving a diagnosis of diabetes may have explained in the current study why overweight, multiparous and less educated women frequently did not attend. This may also be a reflection of a “healthy cohort” effect, in which individuals who are more health-conscious are more likely to seek treatment or followup. On the other hand guidance during pregnancy in the intervention group seems to increase the likelihood for postpartum testing.

One of the strengths of this study is that it was performed in a community-based setting in a rural area. This is the first study where the women with risk factors for GDM were called for postpartum glucose testing. The limitation of this study is the small number of women. The Electrical Medical Record (EMR) covers now the study area and improves the possibilities for expanding the system of reminding by telephone. Timing the testing six months after delivery when breastfeeding is finished and child care organized may remove barriers.

5. Conclusion

We should improve the communication between primary care providers and obstetrics and gynecology care providers and endocrinologists and develop a reminding system for primary care. A lifestyle intervention during pregnancy and the knowledge that diabetes can be prevented may encourage the women to participate in postpartum testing.

Abbreviations

BMI:	Body mass index
DM:	Diabetes mellitus
EMR:	Electrical Medical Record
GDM:	Gestational diabetes mellitus
IFG:	Impaired fasting glucose
IGT:	Impaired glucose tolerance
OGTT:	Oral glucose tolerance test
ppOGTT:	Postpartum oral glucose tolerance test
OR:	Odds ratios.

Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contribution

E. Korpi-Hyövähti participated in the design of the study, acquisition of the data, performed the statistical analysis, and drafted the paper. D. E. Laaksonen, L. Niskanen, U. Schwab and S. Heinonen had substantial contributions to conception and design of the study and they helped to draft the paper. All authors read and approved the final paper.

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Clinical Study

Changes in Maternal Glucose Metabolism after the Administration of Dexamethasone for Fetal Lung Development

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Aims. Antenatal dexamethasone administration for fetal lung development may impair maternal glucose tolerance. In this study, we investigated whether glucose and insulin levels differed among singleton and twin pregnancies and pregnancies with impaired glucose tolerance (IGT) after treatment with dexamethasone. **Methods.** Singleton pregnancies, twin pregnancies, and pregnancies with IGT between 28 and 33 weeks of gestation whose mothers were treated with dexamethasone were enrolled in this study. Exclusion criteria included gestational hypertension, diabetes, renal disorders, and infectious diseases. The fasting plasma glucose and insulin levels were checked before administration and 24 h, 48 h, and 72 h after treatment was completed. **Results.** Mean glucose levels were significantly higher in the twin pregnancy and IGT groups at 24 h and 48 h after the administration of dexamethasone than those in the singleton pregnancy group ($P < 0.05$). Although there was no significant difference in glucose levels before administration and 72 h after dexamethasone administration among the three groups, insulin levels in the IGT group were significantly higher ($P < 0.05$). Insulin levels in the singleton pregnancy group at 24 h and 48 h after treatment were significantly lower than in the twin and IGT groups. **Conclusion.** The effects on maternal fasting blood glucose and insulin levels of dexamethasone administered to promote fetal lung maturation correlated with embryo number and the presence of IGT.

1. Introduction

The most common cause of deaths among preterm babies is respiratory distress syndrome (RDS); antenatal corticosteroid (ACS) treatment for pregnant women at risk of preterm birth is an established intervention for the prevention of RDS. Liggins and Howie first described this indication in 1972, when they demonstrated that ACS could reduce the risk of neonatal RDS from 25.8% to 9.0%, and the rate of neonatal mortality dropped from 15.0% to 3.2% [1, 2]. In 1994, a National Institutes of Health (NIH) consensus conference recommended that women at risk of preterm birth before 34 weeks of gestation, who delivered within 7 days, should routinely be given a course of ACS [3]. Since then, the incidence and mortality rates of RDS, intraventricular hemorrhage, and necrotizing enterocolitis in preterm infants have been significantly reduced.

However, corticosteroid therapy has some adverse maternal effects, including adrenal suppression and altered glucose tolerance [4]. Mathiesen [5] reported that diabetic

mothers receiving glucocorticoid treatment for fetal lung maturation may have poor glycemic control and need to adjust insulin usage. To what extent short-term use of corticosteroids for fetal lung maturation affects fasting blood glucose and insulin levels in normal singleton pregnancies, normal twin pregnancies, and IGT pregnancies who do not require insulin treatment is still uncertain. In this study, we investigated whether glucose and insulin levels differ after the administration of dexamethasone among singleton and twin pregnancies and in pregnancies with impaired glucose tolerance (IGT) [6].

2. Materials and Methods

Fifty-six women aged between 25 and 35 years old who received dexamethasone therapy for fetal lung maturation between 28 and 33 weeks of gestation at the Women's Hospital School of the Medicine Zhejiang University from Jan 2009 to Jan 2011 were recruited into this study. All of

TABLE 1: Demographic characteristics ($x \pm s$).

Group	Number of people	Age (years)	Gestational age (weeks)	BMI
I	22	27.1 \pm 4.2	30.4 \pm 3.1	22.4 \pm 3.2
II	18	29.3 \pm 2.8	31.2 \pm 2.4	21.7 \pm 2.9
III	16	27.2 \pm 3.4	32.8 \pm 2.7	23.4 \pm 2.8

them had received oral glucose tolerance test (OGTT) [6] between 24 and 28 weeks to screen for gestational diabetes. Exclusion criteria included gestational hypertension, diabetes, renal disorders, and infectious diseases. Patients were divided into three groups: group I (singleton pregnancies) 22 people, group II (twin pregnancies) 18 people, and group III (singleton pregnancies with IGT) 16 people. All of the women gave their informed consent prior to inclusion into the study, which was approved by the Ethics Committee of Zhejiang University.

Dexamethasone usage was according to the Guideline by Obstetrics group of Chinese Medical Association [7]; the women with singleton pregnancies received four doses of 5 mg dexamethasone intramuscularly every 12 h; women with twin pregnancies received six doses of 5 mg dexamethasone intramuscularly every 8 h. Fasting blood samples were collected before the dexamethasone therapy was commenced in the morning and 24 h, 48 h, and 72 h after treatment was completed. Plasma glucose and insulin levels were then detected.

SPSS 11.5 statistical software was used for statistical analysis. All data were expressed as mean \pm standard deviation. The F-test was used to determine statistically significant differences between the groups. A P value of <0.05 was considered to be statistically significant.

3. Results

There was no significant difference in terms of age, gestational weeks, and prepregnancy body mass index (BMI; Table 1). The blood glucose level in group I was significantly lower than that in groups II and III 24 h and 48 h after dexamethasone therapy was started ($P < 0.01$). However, there was no significant difference before treatment was started and 72 h after treatment ($P > 0.05$; Table 2). The insulin level in group III was significantly higher than that in groups I and II before treatment was started and 72 h after treatment ($P < 0.05$); 24 h and 48 h after dexamethasone therapy, the insulin levels in group I were significantly lower than those in groups II and III ($P < 0.01$). There was no difference in the insulin level between groups II and III ($P > 0.05$; Table 3).

4. Discussion

In this study, we found that the fasting blood glucose levels 24 h and 48 h after the first injection of dexamethasone in groups II and III were significantly higher than those in group I ($P < 0.01$), but the difference of blood glucose

TABLE 2: Blood glucose levels before and after dexamethasone therapy ($x \pm s$ mmol/L).

Group	Before treatment	24 h	48 h	72 h
I	4.1 \pm 1.9	5.3 \pm 2.2*	5.4 \pm 1.6*	4.9 \pm 2.1
II	3.8 \pm 2.2	6.9 \pm 2.5	7.3 \pm 1.9	4.7 \pm 1.9
III	4.2 \pm .4	7.3 \pm 2.4	8.1 \pm 3.1	5.2 \pm 2.1

*Group I compared with group II and group III, * $P < 0.01$.

TABLE 3: Blood insulin levels before and after dexamethasone therapy ($x \pm s$ mmol/L).

Group	Before treatment	24 h	48 h	72 h
I	8.4 \pm 2.1	45.3 \pm 17.2**	56.2 \pm 21.4**	6.1 \pm 2.8
II	9.0 \pm 3.2	68.8 \pm 21.5	98.3 \pm 31.9	8.2 \pm 3.0
III	19.2 \pm 3.4*	76.9 \pm 22.4	106.1 \pm 33.1	20.2 \pm 4.4*

*Group III compared with group I and group II, * $P < 0.05$; **group I compared with group II and group III, ** $P < 0.01$.

levels among all three groups either before treatment or 72 h afterwards was not significant. Since the biologic half-life of dexamethasone is approximately 36 h to 54 h [8], this indicates that blood glucose levels elevation in groups II and III were consistent with elevated concentration of dexamethasone after injection.

In twin pregnancies, perhaps due to the enlarged placenta, more insulin antagonistic hormones are secreted, such as glucocorticoids, placental lactogen, progesterone, estrogen, and others. In IGT women, exceed insulins were secreted to maintain normal glucose levels although the baseline blood glucose level in groups II and III in our study was within the normal reference range before dexamethasone administration. In these groups, insulin secretion by the pancreatic β -cells was in a “full load” state due to the increased levels of insulin antagonistic hormones. After dexamethasone was given to promote fetal lung maturation, insulin resistance increased, which meant that β -cell secretion was not sufficient to offset the insulin antagonistic hormones. This was the most likely mechanism that led to the resultant hyperglycemia.

Determination of insulin levels before and after dexamethasone injection showed that the insulin levels before and 72 h after the injections in group III were significantly greater than those of groups I and II, which indicated an increased absolute insulin value and an insulin resistance state in women with IGT. The blood glucose and the insulin levels 24 h and 48 h after dexamethasone administration in groups II and III were both synchronized with the corresponding values in group I. Foglia et al. [9] have suggested that dexamethasone has different effect on maternal glucose levels in twin and singleton pregnancies. Within 24 h of the first corticosteroid injection, twin pregnancies had a higher average maternal glucose levels than singleton pregnancies. This may be due to a higher rate of abnormal glucose tolerance in mothers with twin pregnancies than singleton pregnancies. In addition, Yang et al. [10] found that

the use of dexamethasone in the final trimester of pregnancy could increase insulin resistance. Pregnant women with normal glucose tolerance have normal pancreatic islet β -cell function. Insulin secretion is increased through enhancement of pancreatic islet β -cell function. Consequently, the antagonistic effect of dexamethasone is ameliorated, so that blood glucose levels remain within a normal narrow range. However, the insulin secretion of pregnant women with IGT is significantly increased under normal circumstances. After the administration of dexamethasone, these women are unable to compensate for the action of corticosteroids by enhancing islet β -cell function, which results in the increased blood glucose levels.

In conclusion, promoting fetal lung maturation with dexamethasone affects maternal glucose metabolism. The degree of modification of the maternal fasting plasma glucose and insulin levels is correlated with the basic maternal glucose metabolic situation and may correlated with twin pregnancy status. Consequently, blood glucose levels of twin pregnancies and those with impaired glucose metabolism should be closely monitored during the use of dexamethasone for fetal lung maturation.

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Review Article

Previous Gestational Diabetes Mellitus and Markers of Cardiovascular Risk

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The prevalence of gestational diabetes mellitus (GDM) in the developed world has increased at an alarming rate over the last few decades. GDM has been shown to be associated with postpartum diabetes, insulin resistance, hypertension, and dyslipidemia. A history of previous GDM (pGDM), associated or not with any of these metabolic abnormalities, can increase the risk of developing not only type 2 diabetes mellitus but also cardiovascular disease (CVD) independent of a diagnosis of type 2 diabetes later in life. In this paper we discuss the relationship among inflammatory markers, metabolic abnormalities, and vascular dysfunction in women with pGDM. We also review the current knowledge on metabolic modifications occurring in normal pregnancy and the link between alterations of a normal metabolic state with the long-term maternal complications that may result in increased CVD risk. Our review of studies on pGDM prompts us to recommend that these women be considered a population at risk for later CVD events, which however could be avoided via the use of specially designed follow-up programs in the future.

1. Introduction

Gestational diabetes mellitus (GDM) is any degree of glucose intolerance with onset or first recognition during pregnancy [1, 2]. In early gestation fasting blood glucose is lower and insulin sensitivity decreases slightly. This is followed by progressively increasing insulin resistance in the second and third trimesters with a borderline increase of insulin production or hyperinsulinemia. Furthermore, insulin resistance occurs as a result of placental hormones that antagonize insulin, estrogen, progesterone, human placental lactogen (HPL), human placental growth hormone, cortisol, prolactin, and tumor necrosis factor- α (TNF- α) [3]. The above different pathophysiologic mechanisms accompanying pregnancy result in metabolic changes that allow for higher postprandial maternal glucose. Pregnancy is a hyperinsulinemic state which may develop into impaired glucose tolerance if insulin secretion is unable to compensate for pregnancy-associated insulin resistance [3–5].

The condition of GDM is a state of chronic low-grade subclinical inflammation characterized by abnormal production of cytokine and mediators and activation of a network of inflammatory signaling pathways. Although the characteristic of GDM is insulin resistance, the exact mechanism involved in this process is still unknown. The increased insulin resistance during pregnancy has been, as just described, attributed to cortisol and gestational hormones, but more recent data have shown that cytokines may also be involved in this process [6]. The most significant maternal risk is that of development of metabolic syndrome characterized by central obesity, dyslipidemia, and insulin resistance, which predispose to increased risk for coronary artery disease, stroke, and type 2 diabetes later in life [7–11].

The incidence of type 2 diabetes in women with previous GDM (pGDM) who were examined six weeks to 28 years postpartum was estimated to range from 2.6% to 70% [12, 13]. Other researchers found that women with pGDM have a 18–50% risk of developing type 2 diabetes mellitus

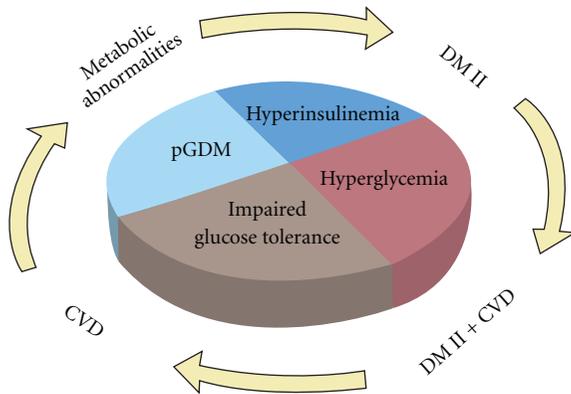


FIGURE 1: Metabolic status during pregnancy and pGDM may result in outcomes later in life like metabolic abnormalities, DMII, CVD, and DMII + CVD as it is shown following the arrows.

within 5 years following pregnancy [14–17], and diabetes is an established risk factor for CVD [18, 19]. In addition, women with a history of GDM are at increased risk of other cardiovascular risk factors, such as obesity, hypertension, dyslipidemia, and subclinical atherosclerosis [20–22]. It is unclear whether women with a history of GDM who do not subsequently develop type 2 diabetes mellitus are also at an increased CVD risk in the future. The metabolic abnormalities which accompany GDM preceding type 2 diabetes and which remain in effect during the natural course of the disease place women at high risk for CVD [23].

In this paper we review the interrelationship among inflammatory markers, metabolic abnormalities, and endothelium dysfunction in pGDM and discuss whether these women could be considered at risk for cardiovascular disease later in life. Based on the small amount of existing literature, we discuss the inflammatory and metabolic abnormalities underlying the status of pGDM and the potential that endothelial dysfunction is a marker of future CVD risk. To our knowledge, this is the first paper presented in the literature dealing with markers of CVD risk in women with a history of gestational diabetes.

2. Surrogate Markers of Increased Cardiovascular Risk

Although the majority of women with GDM return to normal glucose tolerance after delivery, they remain, as a group, at substantially increased risk of developing type 2 diabetes in later life, a known condition that leads to an increased risk for CVD [24].

Inflammation may contribute to atherosclerosis by a variety of mechanisms depending on the stage of the disease. Circulating markers of systemic inflammation have been shown to predict future CVD [25]. These markers include C-reactive protein (CRP), proinflammatory cytokines such as interleukin-6 (IL-6), and soluble adhesion molecules. Most attention has been focused on CRP which, along with IL-6,

has been revealed in large prospective studies to be a consistent predictor of future cardiovascular events [26, 27].

Epidemiological and experimental studies have established the association of markers of subclinical inflammation with CVD, type 2 diabetes, and metabolic syndrome (Figure 1). Pregnancy is a hyperinsulinemic state in which the increased insulin resistance during pregnancy may be attributed not only to gestational hormones but also possibly to cytokines, which, as mentioned in Section 1, may play a role [28–30]. Increased levels of inflammatory markers such as CRP, plasminogen activator inhibitor-1 (PAI-1), and IL-6 are predictors of future establishment of type 2 diabetes and CVD [31–34]. Adiponectin, a peptide with anti-inflammatory properties, has in some studies been associated with a decreased risk of type 2 diabetes and CVD [35, 36].

Markers of endothelial dysfunction, like circulating levels of E-selectin, vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), as well as inflammatory parameters like CRP and IL-6, have been reported to be associated with CVD in several studies [37–42]. Furthermore, the adhesion molecules E-selectin, VCAM-1, and ICAM-1 are thought to play a major role in the pathogenesis of vascular disease [6]. These molecules are markers of endothelial dysfunction and are expressed on the endothelial wall in response to inflammatory mediators. They contribute to the formation of atherosclerotic plaques and can be detected in soluble form in the circulation [43]. More reliable, and thus of great interest, are the measurements of carotid intima media thickness (IMT) and flow-mediated vasodilatation (FMD). Both indexes have been used in epidemiological studies as surrogate markers of early atherosclerosis. Carotid IMT increases with age, is correlated with cardiovascular risk factors, and identifies subjects at increased risk of severe coronary artery disease and cardiovascular morbidity [37]. Intima media thickness (IMT) is an ultrasound marker of CVD risk. Heiss et al. in their study found positive relation of this marker to cardiovascular risk factors and CVD risk [44].

Mediators of inflammation may exert pathologic action by inducing vascular dysfunction, thus leading to many of the diverse effects of the insulin resistance condition, like hypertension, dyslipidemia, and impaired fibrinolysis [62]. Insulin resistance has been associated with impaired endothelial function, which interacts with coagulation and hypofibrinolysis [63, 64], while hypofibrinolysis and procoagulant activity are linked with increased risk for cardiovascular events (Table 1). It is of note that raised levels of circulating inhibitors of the fibrinolytic system have been observed in patients with insulin resistance [64]. Plasminogen activator inhibitor-1 (PAI-1) is elevated in a variety of clinical situations that are associated with insulin resistance and cardiovascular disorders [65].

Osteoprotegerin (OPG) is a glycoprotein, a soluble member of the tumor necrosis factor (TNF) receptor superfamily, which inhibits receptor activator of nuclear-factor- κ B-ligand (RANKL-) mediated osteoclastic bone resorption [66]. It has been reported to be expressed in the arterial wall [67]. Elevated serum OPG levels have been found to be associated with atherosclerosis [68].

TABLE 1: Markers of increased CVD risk in normoglycemic women with GDM or pGDM.

Authors	Number of subjects	Months/years postpartum	Markers of CVD risk
Kousta et al., 2003 [45]	78	3 years	↑ Insulin resistance ↑ lipidemia
Anastasiou et al., 1998 [46]	68	3–6 months	↓ FMD
Hu et al., 1998 [47]	37	2–4 years	↓ Acetylcholine induced vasodilatation
Knock et al., 1997 [48]	32	During cesarean section	↑ Vascular pathology, vessel myography
Paradisi et al., 2002 [49]	38	During GDM pregnancy	↓ FMD
Heitritter et al., 2005 [50]	48	1 year	↓ Adiponectin
Winzer et al., 2004 [51]	108	3 months	↓ Adiponectin ↑ E-selectin ↑ ICAM-1 ↑ IMT
Bo et al., 2007 [52]	195	6-7 years	↑ E-selectin ↑ VCAM-1
Thomaseth et al., 1997 [53]	10	1 year	↑ VCAM-1
Lawrence et al., 2002 [54]	265	recent GDM	≈ E-selectin
Kautzky-Willer et al., 1997 [55]	41	3 months	↑ E-selectin ↑ VCAM-1
Shah et al., 2008 [56]	89.500	p GDM	↑ CVD events
Akinci et al., 2008 [57]	76	p GDM	↑ OPG ↑ IMT
Akinci et al., 2011 [58]	195	3 years	↑ OPG
Banerjee et al., 2011 [59]	29	2 years	↑ Vascular pathology, vessel myography
Farhan et al., 2006 [60]	70	recent GDM	↑ PAI-1
Madarász et al., 2009 [61]	107	4 years	↑ CVD risk factors, disturbed carbohydrate metabolism

Vessel stiffness measured by arterial tonometry is associated with endothelial dysfunction and increased CVD risk [69, 70].

3. Studies in pGDM Women for Identification of the Risk of Cardiovascular Complications

The study of women in the pGDM state serves as a model for the detection of early metabolic abnormalities. Normoglycemic women with pGDM have increased insulin resistance and decreased endothelium-dependent vasodilatation when compared with women who had uncomplicated pregnancies [45, 46]. During the first 3–6 months postpartum, women with pGDM had impaired endothelial function assessed by FMD, this tending to confirm the assumption that glucose metabolism derangement is closely related to vascular dysfunction [46]. A cross-sectional study showed that 2–4 years after the postpartum period, pGDM had impaired acetylcholine-induced skin vasodilatation in hand and foot, as assessed by laser Doppler flow, when compared with normal controls [47]. Two cohort studies have reported signs of vascular endothelial dysfunction in vitro and in vivo during pregnancies complicated by GDM. The first study evaluated vascular endothelial function in

small subcutaneous arteries dissected from biopsies obtained at cesarean section using vessel myograph and the second during pregnancy with impaired glucose tolerance and gestational diabetes mellitus assessing brachial artery FMD [48, 49].

Heitritter et al. compared biochemical and hemodynamic surrogate markers of CVD in nondiabetic women with and without a history of GDM who were at least one year postpartum and concluded that nondiabetic women with pGDM have evidence of subclinical inflammation, hypoadiponectinemia, and early vascular dysfunction and may be at increased risk of developing CVD [50]. Lower plasma adiponectin concentrations characterize women with pGDM by contrast to controls, independently of the prevailing insulin sensitivity or the degree of obesity and are associated with subclinical inflammation and atherogenic parameters [51]. Bo et al. showed in their study that pGDM women had higher values of markers of endothelial dysfunction and IMT than controls and an increased future CVD risk; however, few data are available concerning the association between pGDM and inflammation markers of endothelial dysfunction [52]. E-selectin and VCAM-1 concentrations were found to be elevated in a cohort study of women with pGDM one year after delivery [53]. A larger study many months postpartum failed to display the same results

[54]. Kautzky-Willer et al. demonstrated that pGDM was characterized by persistently raised levels of E-selectin and VCAM-1 12 weeks after delivery [55]. In a large population-based study, women who had GDM in pregnancy compared with controls were at higher risk of CVD events [56].

Akinci et al. observed that OPG serum levels tended to be elevated in pGDM and moreover reported an association with carotid IMT, thus showing that osteoprotegerin may play a role in the pathogenesis of endothelial dysfunction in these women [57]. Furthermore, a very recent study conducted by the same group concluded that OPG was related to CVD risk factors and metabolic syndrome and may be involved in the development of CVD disorders in pGDM [58]. Farhan et al. recorded elevated PAI-1 levels in pGDM [60].

Another study examined the relationship between glycemia during pregnancy and small artery function 2 years postpartum. In this study subcutaneous arteries from gluteal fat biopsy were examined as to structure, stiffness, and vasoconstrictor response using myography. The results showed that vascular pathology is detectable very early in women at risk of type 2 diabetes [59]. Studying the prevalence of abnormal glucose tolerance and metabolic syndrome in a cohort of pGDM, the results demonstrated disturbed carbohydrate metabolism and a clustering of CVD factors in these women [61, 71].

Surrogate markers of increased cardiovascular risk in population-based studies are commonly used in routine practice. However, though in studies of pGDM markers are used that link this condition with future CVD risk [72], the evidence is as yet inadequate for the markers to be applied in the routine follow-up of these women. Nevertheless, the aforementioned studies are promising, as several of these biochemical and hemodynamic markers may in future prove to be of great value in follow-up programs, contributing to reducing the risk in pGDM for cardiovascular morbidities later in life.

4. Conclusions

It has been shown that women with pGDM are more insulin resistant than women with normal carbohydrate tolerance during their pregnancies. Diabetic complications may be in progress during the phase of insulin resistance in pregnancy even in the absence of hyperglycemia, while furthermore there is evidence that pGDM is associated with postpartum diabetes, insulin resistance, hypertension, and dyslipidemia. A history of pGDM can increase the risk of developing not only type 2 diabetes mellitus, which is a major risk factor for the development of cardiovascular disorders, but also CVD independent of the presence of type 2 diabetes. Also mentioned in this paper is the fact that a number of studies have reported pGDM to be additionally associated with the increased prevalence of metabolic syndrome, an important factor of cardiovascular disorders.

Having reviewed the current literature concerning the relationship between inflammatory markers, metabolic abnormalities, and vascular dysfunction in pGDM, we proceeded to evaluate, for the first time to our knowledge, the sum total

of this information for the purpose of seeking to identify women at future risk. We additionally reviewed the current knowledge on normal metabolic modifications that occur in pregnancy and the link between these normal modifications and the ensuing long-term complications in this group of women. Based on the evidence related to pGDM, we suggest that these women be considered at an increased risk for subsequent cardiovascular morbidity. Identifying women at increased risk for developing cardiovascular morbidities and, at a later date, placing them in follow-up programs that will include the use of established selected markers, has the potential to substantially hold back their CVD risk in terms of both, lower incidence and reduced severity of cardiovascular events later in life.

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Clinical Study

A Pilot Study on Dietary Approaches in Multiethnicity: Two Methods Compared

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Background. Medical nutritional therapy is the most important method for normalizing glucose levels in pregnancy. In this setting, there is a new problem to consider relating to migrants, their personal food preferences, and ethnic, cultural, and religious aspects of their diet. We compared maternal and fetal outcomes between two multiethnic groups of pregnant women, one adopting a food plan that included dishes typical of the foreign women's original countries (the "ethnic meal plan" group), while the other group adopted a standard meal plan. *Findings.* To develop the meal plan, each dish chosen by the women was broken down into its principal ingredients. The quantity of each food was given in tablespoons, teaspoons, slices, and cups, and there were photographs of the complete dish. The group treated with the ethnic meal plan achieved a better metabolic control at the end of the pregnancy and a lower weight gain (though the difference was not statistically significant). As for fetal outcome, the group on the ethnic meal plan had babies with a lower birth weight and there were no cases of macrosomia or LGA babies. *Conclusions.* This preliminary study indicates the positive effect of an ethnic approach to diet on the outcome of pregnancy.

1. Introduction

Gestational diabetes (GDM), that is, carbohydrate intolerance first recognized and/or diagnosed during pregnancy [1], is the most common metabolic complication of gestation, with a prevalence estimated at around 14% [2, 3]. Obesity, family history of diabetes, and belonging to certain ethnic groups increase the risk of GDM [4–11]. If the disorder is not properly monitored and treated, it can cause severe complications in both mother (including preeclampsia, cesarean delivery, glucose intolerance, or type 2 diabetes after delivery) and child (macrosomia, hypoglycemia, hyperbilirubinemia, adolescent obesity, glucose intolerance, and diabetes) [2, 4, 12, 13].

Immigration rates have recently increased in Italy, and immigrants now account for about 4% of the resident population; 48% of the immigrant population consists of women, and 65% of them are of reproductive age [14]. Some studies have demonstrated a tendency for adverse outcomes

of pregnancy among immigrant women from countries with high rates of diabetes [5–8].

The first step in treating GDM is nutrition therapy [1, 15]. Immigrants differ in their cultural background and eating habits, so we aimed to assess such women's compliance with dietary restrictions and the possible benefit, in terms of maternal and fetal outcome, of adopting a nonstandard, ethnic-based approach to their diet.

2. Materials and Methods

For this pilot study, twenty pregnant immigrant women with GDM followed up at the Metabolic Disease and Diabetology Unit of Padua University between January and June 2008 were enrolled. The study protocol complied with the Helsinki Declaration and was approved by the local Ethics Committee, and written informed consent was obtained from all participants.

TABLE 1: Example of two diets on 1800 kilocalories.

	Standard meal plan		Ethnic meal plan	
	Meal	Porzion size	Meal	Porzion size
Breakfast	Milk, skimmed	1 cup	Yogurt, skimmed	1
	White bread	1 slice	Crackers	4 slices
Snack	Apple	1	Mango	1
	Pasta	1 cup	Cous cous	1 cup
Lunch	Vegetables	1/2 cup	Vegetables	1/2 cup
	Meat	100 g	Poultry	3/4
			Bread without yeast	1 slices
	Olive oil	2 T	Sunflower oil	2 T
Snack	Yogurt, skim	1	Banana	1
	White bread	1 slice	Yogurt, skim	1
Dinner	Soup with:		Soup with:	
	Pasta	1/2 cup	Beans	1/2 cup
	Parmesan	1 T	Potatoes	One small
			Lentils	1/2 cup
	White bread	2 slice	Plantain	one
	Vegetables	1/2 cup	Vegetables	1/2 cup
	Mozzarella	100 g		
	Olive oil	2 T	Olive oil	2 T
Snack at bedtime	Milk, skimmed	1 cup	Yogurt, skimmed	1
	White bread	1 slice	Crackers	2 slices

T: tablespoon = 10 g; 1 cup liquid = 200 mL; 1 cup solid = 80 g; one slice = 30 g; Plantain = 100 g; 1 Fruit = 200 g.

Screening for GDM was done with a glucose challenge test (GCT) between weeks 24 and 28 of gestation, and the diagnosis was confirmed with a 100 g oral glucose tolerance test (OGTT) as recommended by the 4th International Workshop Conference on GDM [1].

The women enrolled were randomly assigned to two groups: one adopted a standard meal plan (SMP) prepared according to the ADA guidelines [15], the other an ethnic meal plan (EMP) (Table 1).

All the women were monitored to achieve a good metabolic control, that is, fasting plasma glucose (FPG) <5.3 mmol/L and 1 h postprandial plasma glucose (1 h PPPG) <7.2 mmol/L, and nurses taught them to monitor their own blood glucose levels [15]. The pregnant women on diet treatment performed 2 measurements per day, measuring fasting and 1 h postprandial glucose on alternate meals over the course of a week. The women on insulin therapy performed self-glucose monitoring four times a day (fasting and 1 h after breakfast, lunch, and dinner). They saw a specialist every two weeks. Insulin treatment was started when FPG and/or 1-hour PPPG exceeded the above level in more than one measurement [15]. All GDM women were followed up for metabolic and obstetric purposes until delivery.

For maternal characteristics and outcome, we considered age, prepregnancy body mass index (BMI, kg/m²), time of GDM diagnosis, HbA1c (when GDM was diagnosed and at delivery), percentage of patients on insulin, weight gain, timing and mode of delivery, and hypertensive disorders.

For fetal outcome, we considered birth weight, infants large or small for gestational age (LGA, SGA), and fetal composite outcome (hypoglycemia, neonatal asphyxia, respiratory distress syndrome, and hyperbilirubinemia, hypocalcemia) and fetal malformations. Babies were LGA if their birth weight was above the 90th percentile and SGA if it was below the 10th percentile according to population-specific standard growth tables [16]. Macrosomia was diagnosed for a fetus weighing more than 4000 g.

A dietary assessment was conducted to determine whether a woman's intake of essential nutrients was adequate and whether she was eating excessively and to identify foods she avoided, as well as food intolerances or allergies. A meal plan was then developed, and patient and dietician prepared a sample menu. Food models, using measures in cups, glasses, and bowls, proved helpful props when teaching appropriate serving sizes.

The two groups received different meal plans: group 1 adopted the SMP for GDM according to the ADA guidelines [15]; group 2 adopted the EMP, which included typical foods of the women's home countries, identified using a photographic atlas (Dietmeter and Photographic Atlas, Scotti Bassani) [17–19].

The EMP included foods commonly consumed by patients according to their ethnicity. Dishes were broken down into the various ingredients, shown raw and cooked. Due to difficulties in using kitchen scales, measures such as cups, or spoonfuls handfulls or pinches, were preferred (Figure 1). Furthermore, the food pyramids of the specific country of origin were used.

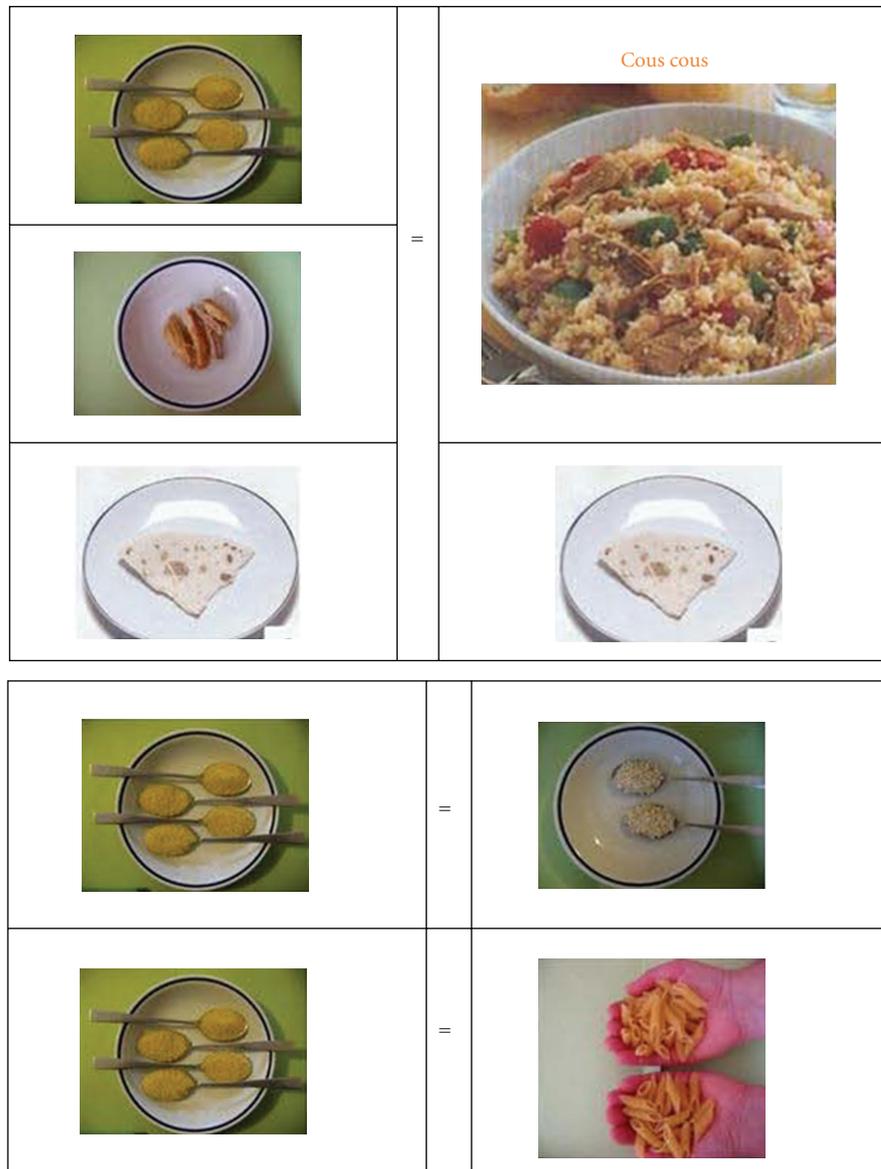


FIGURE 1: Example of ethnic meal plan.

The two meal plans had the same nutrient composition (SMP: CHO 53%, L 28%, P 18% fiber 26 g; EMP: CHO 55%, L 28%, P 17%, fiber 21 g), and energy intake was from 1800 to 2200 Kcal, depending on prepregnancy BMI.

Adherence to the diet was measured using a 24-hour food intake recall method and scored as 0 for an intake more than 20% higher than prescribed, 1 if the intake was 10–20% higher; 2 if it was consistent with the plan or up to 10% lower. The intake was calculated in individual tables, based on the INRAN nutritional tables, 2000 version [20].

Plasma glucose was evaluated using the glucose-oxidase method [21]. HbA1c was measured using standard HPLC; the normal range assumed for healthy pregnant women was 4.0–5.5% (20–37 mmol/mol) [22].

2.1. Statistical Evaluation. Data are given as means \pm standard deviations and were compared using Student's *t*-test for unpaired data or for paired data when comparisons were drawn at different times in the same sample. The groups were compared for categorical data or frequency of an event using the χ^2 test with Yates' correction. A *P* value of less than 0.05 was considered significant. The data were processed using the SPSS18 for Windows program.

3. Results

3.1. Clinical and Metabolic Features. The ethnic distribution in EMP group was Chinese 1; Filipino 1; Moroccan 1; Nigerian 3; Romanian 4; Bangladeshi 1. In SMP group, was Chinese 1; Moroccan 1; Nigerian 1; Romanian 4; Sudanese 1,

TABLE 2: Clinical and metabolic characteristics and pregnancy outcome of the subjects under study.

	SMP group <i>n</i> = 10	EMP group <i>n</i> = 10	<i>P</i>
Age (yrs)	30.2 ± 4.7	28.9 ± 3.3	0,622
Prepregnancy BMI (kg/m ²)	24.1 ± 4.7	25.7 ± 3.6	0,784
Time of GDM diagnosis (gw)	27.1 ± 5.9	21.3 ± 6.8	0,316
Weight gain during pregnancy (kg)	14.3 ± 6.9	12.1 ± 4.3	0,869
Insulin therapy (<i>n</i>)	1	2	0,509
Gestational hypertension (<i>n</i>)	1	0	0,330
Delivery (gw)	38.4 ± 1.1	38.0 ± 0.5	0,409
Cesarean section (<i>n</i> .)	5	6	1,000
Birth weight (g)	3434 ± 649	3064 ± 626	0,164
LGA babies (<i>n</i>)	3	0	0,001
SGA babies (<i>n</i>)	1	0	0,330
Macrosomia (<i>n</i>)	2	0	0,186
Fetal composite outcome (<i>n</i>)	0	0	—
Fetal malformations (<i>n</i>)	0	0	—

SMP: standard meal plan, EMP: ethnic meal plan, BMI: body mass index, LGA: large for gestational age, SGA: small for gestational age, gw: weeks of gestation.

Bangladeshi 1, Hungarian 1, with no difference between the two meal plan groups.

Table 2 shows the women's clinical and metabolic characteristics, and maternal and fetal outcomes. Mean age, prepregnancy BMI, and time of GDM diagnosis were comparable for the two groups. The EMP group had better FPG, 1hPPPG, and HbA1c values than the SMP group. Weight gain was lower, though not significantly, in the EMP group (Figure 2).

3.2. Pregnancy Outcome. No differences in maternal outcome emerged between the two groups. The newborns' birth weight was slightly higher in the SMP group, which also included more LGA (3 versus 0, $P < 0.001$) and macrosomic babies (2 versus 0) than the EMP group. There were no cases of SGA babies. No fetal complications or congenital malformations were seen in either group (Table 2).

3.3. Adherence to Dietary Recommendations. Adherence to the meal plans was better in the EMP group, with 7 women scoring 1 or 2 (good adherence), as opposed to 2 women in the SMP group. This difference was statistically significant ($\chi^2 = 0.025$).

4. Discussion

Immigration flows have increased in Italy, meaning rising numbers of pregnant immigrant women [14]. Italian data [14] on pregnancy indicate a relatively poor outcome of such pregnancies due to difficulties in accessing care and following medical recommendations for cultural and social reasons. Some immigrant populations are also at higher risk of type 2 diabetes and gestational diabetes [2, 8, 10].

Better standards of living in host countries often have negative effects on the health of immigrant populations, partly because foreign citizens acquire eating habits and

lifestyles unsuited to their genetic profile. Regarding GDM, the problem is exacerbated by a genetic predisposition of African, Afro-American, and Hispanic races to develop diabetes [4, 8, 9].

Nutritional therapy for immigrant women should take the social, cultural, and religious value attached to food by the various ethnic groups into account.

In our study, the main problems the women had with their prescribed diet were related to difficulties in changing their eating habits; problems with managing a meal plan and weighing the foods; doubts concerning which foods to choose; difficulties in achieving the right nutritional balance.

On the other hand, many studies have confirmed the important influence of dietary treatment on the outcome of GDM [12].

The real difference between the two meal plans we used lies in how the diet and examples of typical dishes are presented [23]. The meal plan containing new elements adapted to different ethnic and cultural needs, with photos and practical domestic units of measure to illustrate the quantities of each food, plus lists of alternative foods and photos showing the foods raw and cooked proved useful and effective for our immigrant women with GDM, since adherence was better among the patients adopting the EMP.

Better adherence coincided with better glycemic control, a more normal weight gain [24], and a better pregnancy outcome, that is, the birth weights were lower, and there were no LGA or SGA babies in the EMP group.

The weakness of our study lies in the small numbers of patients considered but, to the best of our knowledge, this is the first study on the feasibility and efficacy of customized dietary treatment for immigrant GDM patients.

5. Conclusion

This pilot study indicates the positive effect of an ethnic approach to diet on the outcome of pregnancy. The new

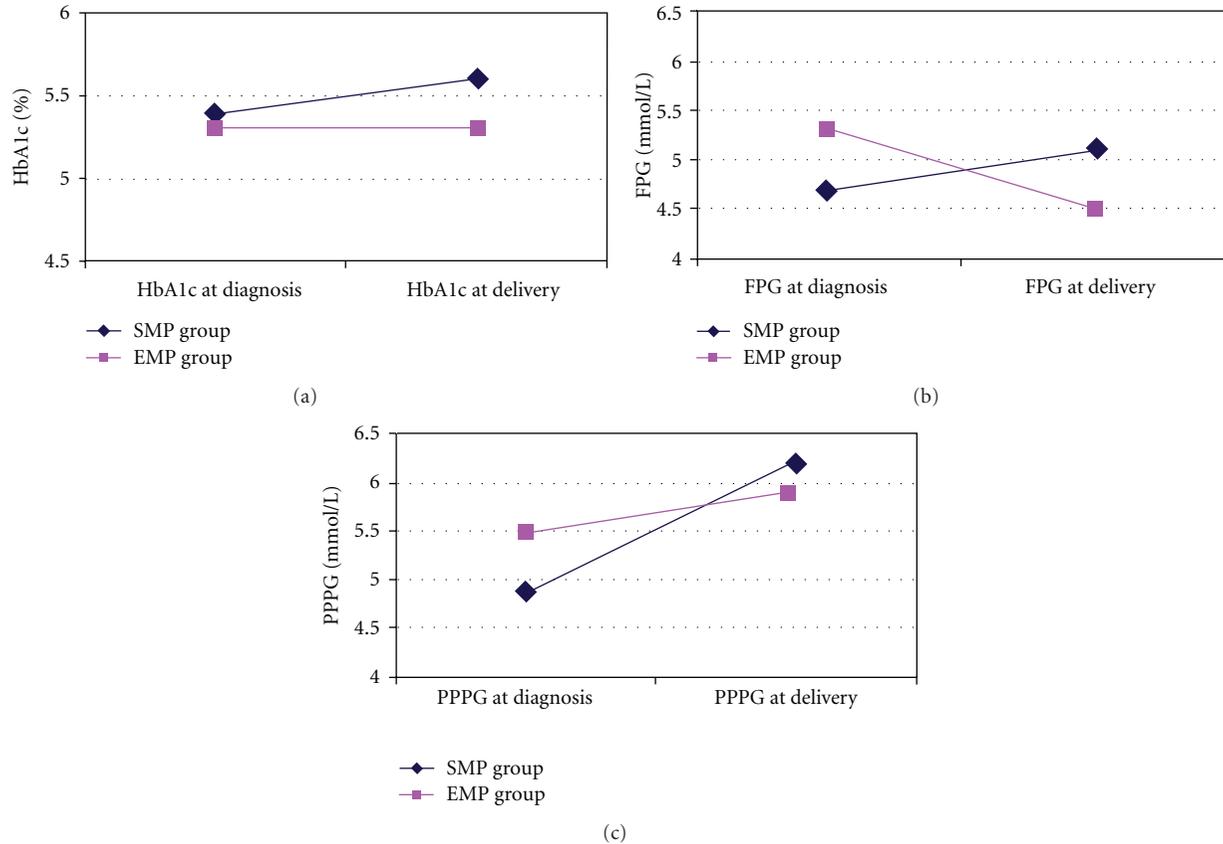


FIGURE 2: Metabolic parameters ((a) HbA1c; (b) FPG; (c) PPPG) evaluated in the subjects under study, AMP group: standard meal plan; EMP group: ethnic meal plan; FPG: fasting plasma glucose; PPPG: 1-hour postprandial plasma glucose.

methods introduced in our study could be considered a valid approach to the nutritional management of immigrant pregnant women with gestational diabetes mellitus. It points out that, when prescribing a diet for immigrant women, their different traditional eating habits should be borne in mind. A study with an adequate number of women chosen in accordance with the power calculation is necessary and useful to confirm these preliminary data.

Conflict of Interests

The authors declare nonfinancial and no competing interest.

Authors' Contribution

R. Valentini conceived and designed the study, participated in the coordination of the study, and wrote the paper. M. G. Dalfrà followed the patients, performed the statistical analysis, and participated to the preparation of the paper. M. Masin followed the patients. A. Barison followed the patients. M. Marialisa recorded the data, participated in the statistical analysis. E. Pegoraro followed the patients and recorded the data. A. Lapolla coordinated the study and participated in the preparation of the paper. All the authors read and approved the paper.

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