ABSTRACT:
GESTATIONAL DIABETES MELLITUS (GDM) IS A COMPLICATION OF PREGNANCY CHARACTERIZED BY HYPERGLYCAEMIA WITH ONSET OR FIRST RECOGNITION IN PREGNANCY. PREVALENCE OF GDM IS INCREASING GLOBALLY. GDM IS ASSOCIATED WITH SHORT AND LONG-TERM MATERNOFETAL COMPLICATIONS AND IS CONSIDERED A PREDIABETES STATE BECAUSE IT HAS A HIGH RISK OF DEVELOPING TYPE 2 DIABETES FOR BOTH MOTHER AND CHILD. THE PATHOGENESIS OF GDM INCLUDES INSULIN RESISTANCE (IR) AND B-CELL DYSFUNCTION. PATIENTS WITH GDM ARE MORE INSULIN RESISTANT THAN PREGNANT NONDIABETIC WOMEN AND THEY ARE PREDISPOSED TO HAVE DEFECTIVE INSULIN SECRETION AND DEFECTIVE INSULIN ACTION. IN GDM, HYPERPROINSULINEMIA DEVELOPS IN PARALLEL WITH PROGRESSIVE B-CELL DYSFUNCTION. THUS, MEAN LEVELS OF PRO-INSULIN ARE SIGNIFICANTLY HIGHER DURING A GDM PREGNANCY AS COMPARED TO A NORMAL PREGNANCY. THE PURPOSE OF THIS REVIEW IS TO ANALYSE CHANGES OF SERUM LEVELS AND THE ROLE OF INSULIN AND PROINSULIN IN THE PATHOPHYSIOLOGY OF PREGNANCIES COMPLICATED WITH GESTATIONAL DIABETES. UNDERSTANDING THE PATHOPHYSIOLOGY OF GDM IS ESSENTIAL FOR A BETTER MANAGEMENT OF THIS DISEASE.

KEY WORDS: GESTATIONAL DIABETES, PATHOGENESIS, ADIPOKINES, INSULIN RESISTANCE, B-CELL DYSFUNCTION

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BACKGROUND

Gestational diabetes is a complex metabolic pregnancy complication, defined by glucose intolerance that is first diagnosed during pregnancy\(^8\). Due to the epidemic of overweight and obesity among women of reproductive age, the prevalence of GDM has increased and is currently estimated to be approximately 20\% of all pregnancies worldwide. The gold standard screening test recommended by the guidelines for the diagnosis of GDM is the 75g 2-hour Oral glucose tolerance test (OGTT)\(^9\). Gestational diabetes is diagnosed if one or more values are equal to or exceed the cut off values: FPG (fasting plasma glucose) of 5.1mmol/l (92 mg/dl), 1-h plasma glucose of 10 mmol/l (180 mg/dl) and 2-h plasma glucose of 8.5 mmol/l (153 mg/dl). There are various clinical factors that predispose women to develop GDM: an advanced maternal age, being overweight or obese, high parity, ethnicity, hypertension, family history of GDM, previous delivery of a macrosomic infant, personal history of GDM, type 2 diabetes or polycystic ovarian syndrome. All these factors are associated with impaired β-cell function and insulin resistance\(^10\). Studies reported that women with a personal history of GDM have a 7 times higher risk of developing type 2 diabetes than women with a normoglycemic pregnancy\(^11\). Understanding the pathophysiology of GDM is essential for a better management of this disease\(^12\). The pathogenesis includes insulin resistance (IR) and β-cell dysfunction.

NORMAL PREGNANCY - A STATE OF INSULIN RESISTANCE

Pregnancy is a physiological condition in which a series of metabolic changes facilitate the development and the growth of the fetus. One of these changes is a temporary, progressive increase of insulin resistance which becomes apparent during the second half of pregnancy. In pregnancy, maternal tissues progressively develop insulin insensitivity and insulin secretion increases by 200\%. Insulin sensitivity varies during gestation according to the requirements of pregnancy. Studies have shown that the decrease in insulin sensitivity in pregnancy could be imputed to postreceptor defects in the insulin signalling cascade. Insulin resistance has a peak in the third trimester when foetal energy demands rise\(^13\).

As for the insulin levels, the initial phase of gestation has increased insulin values, but as pregnancy evolves, there is a reduction by 50-60\%. To obtain an euglycemic status, the whole organism reduces glucose disposal by 50\%.\(^14\) Increased levels of hPL (human

\(^8\) Cristiane de Freitas Paganoti, Rafaela Alkmin da Costa, Ana Maria da Silva Sousa Oliveira, Mara Sandra Hoshidac, Rossana Pulcineli Vieira Francisco, Adiponectin does not improve the prediction of insulin need in pregnancy, in J. E. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care 2001; 14: 1316

\(^9\) Management of Diabetes in Pregnancy: Standards of Medical Care in Diabetes—2022, American Diabetes Association Professional Practice Committee, Diabetes Care 2022;45(Supplement_1):S232–S243


\(^12\) Flavius-Cristian Mărçiău, Sorin Purec, George Niculescu, „Study on the refusal of vaccination against Covid-19 in Romania” in Vaccines 2022, 10, 261.10.3390/vaccines10020261


placental lactogen), estrogen, progesterone, cortisol and prolactin are involved in this process\textsuperscript{15}. The elevated levels of placental hormones increase insulin resistance, leading to hyperglycemia\textsuperscript{16}.

Insulin resistance stimulates hepatic gluconeogenesis, increases lipolysis in the adipose tissue, reduces glucose uptake in the skeletal muscle and adipose tissue\textsuperscript{17}. All these processes assure the energy requirements for the mother and the glucose directed through the placenta maintains the development of the fetus\textsuperscript{18}.

**INSULIN LEVELS IN GESTATIONAL DIABETES**

Gestational diabetes develops when there is an inadequate insulin secretion in pregnancy. In pregnant women without pre-existing diabetes, if pancreatic β cells can secrete sufficient insulin to fight off the insulin resistance state (physiologic in pregnancy), normoglycaemia is maintained; when the secretory response is inadequate, hyperglycaemia occurs, leading to a diagnosis of GDM.

The physiology of gestational diabetes involves changes in the tissues’ sensitivity to insulin. Ryan et al. reported a 40% decrease in insulin sensitivity in women with gestational diabetes as compared to normal pregnancies\textsuperscript{19}.

Insulin resistance in GDM has two components: 1) on the one hand, the physiologic pregnancy associated increase in insulin resistance that we evoked earlier, which is completely reversible postpartum; 2) on the other hand, there is a component of insulin resistance present since before pregnancy (and which is partially inherited and partially acquired)\textsuperscript{20}. If the insulin resistance cannot be resolved, hyperglycaemia develops and by consequence GDM.

Studies on women with a history of gestational diabetes showed that the abnormalities in the insulin secretory response and the insulin sensitivity were identical with those depicted in type 2 diabetes. Thus, in women with gestational diabetes, the metabolic changes inherent to the pregnancy may reveal a genetic susceptibility to type 2 diabetes. Patients with GDM are more insulin resistant than pregnant nondiabetic women\textsuperscript{21} and they are predisposed to have defective insulin secretion and defective insulin action.

\textsuperscript{17} Wilcox G. Insulin and Insulin Resistance. Clin. Biochem. Rev. 2005
Data on insulin secretion in GDM is however contradictory. Some studies have shown a low secretion of insulin after orally or iv administered glucose, whereas in others there are reports that insulin responses in GDM patients were either comparable to or higher than in nondiabetic pregnant controls22.

Xiang et al. showed that in late gestational phases, the hepatic production of glucose increases in women with gestational diabetes as compared to a normoglycemic group23. In the longitudinal studies of Catalano et al. no difference was observed in either fasting glucose concentrations or hepatic glucose production24.

Another phenomenon witnessed in GDM is an increase in fasting insulin concentrations, associated with a lower suppression of the liver glucose production during insulin infusion. This occurrence suggests that, in GDM, hepatic insulin sensitivity decreases. Xiang et al. showed that there is a significant correlation between fasting free fatty acids levels and the hepatic glucose production. This suggests that free fatty acids contribute to the hepatic insulin resistance. The insulin resistance stimulates the pancreas to secrete insulin. Consequently, insulin stimulates the breakdown of fat stores, leading to a rise in blood glucose levels and free fatty acid (FFA) concentrations25.

Catalano et al. compared a group of lean women with gestational diabetes with a weight-matched control group and they concluded that in late gestation in GDM, there is a progressive decrease in the first-phase insulin response. In contrast, in obese women with gestational diabetes, the first-phase insulin response did not change, but the second phase of insulin secretion significantly increased as response to an intravenous glucose challenge26.

Friedman et al. revealed several defects in the insulin signalling cascade that also appear in late pregnancy27. Pregnant women have a decreased expression of insulin receptor substrate-1. Women with gestational diabetes have a lower capacity of the insulin receptor beta subunit to undergo tyrosine phosphorylation. This defect in the insulin signalling cascade leads to a 25% lower glucose transport activity in muscles compared with that of nondiabetic pregnant women.

Homko et al. showed that insulin resistance is higher in women with GDM and these women have a major β-cell defect with impossibility to compensate for their increased level of insulin resistance inherent in late pregnancy28. Pro-insulin levels and insulin concentrations increase in the context of insulin resistance29. Due to a disproportionate release of pro-insulin

from β-cells, pro-insulin to insulin ratio (PIR) rises and is considered an early marker of islet dysfunction.

**PROINSULIN LEVELS IN GESTATIONAL DIABETES**

Proinsulin is a precursor molecule for insulin and is produced by the pancreatic beta cells. Proinsulin is an 86 amino acid peptide, incorporating the A and B chains of insulin, linked together by the C peptide between amino acid residues 31 and 65. Normally, all proinsulin is cleaved at residues 32–33 and 65–66 to produce C peptide and insulin. A small amount of intact proinsulin may also be released into the circulation along with the 31–32 split proinsulin and 32–33 split proinsulin. In conditions of insulin resistance, the pancreatic beta-cell function is affected and secretion of proinsulin (both intact and split) is higher than that of insulin (a similar situation happens in type 2 diabetes). A β-cell dysfunction affects the process of pro-insulin secretion and determines a high raport of pro-insulin/insulin. This situation is defined by high levels of plasma pro-insulin (PI) and lower levels of plasma insulin.

All diabetes phenotypes, including GDM, are characterized by increased plasma proinsulin levels. Rentnakaran et al. demonstrated that PI secretion in late pregnancy is associated with insulin resistance and a positive PIR (proinsulin to insulin ratio). Women with a history of GDM can develop hyperproinsulinemia in parallel with progressive β-cell dysfunction. All these changes lead to the development of type 2 diabetes. Mean levels of pro-insulin are significantly higher and PIR are lower in GDM pregnancies as compared to normal pregnancies. The PIR can be a marker of pancreatic β-cells dysfunction in the early period after birth. An increase in the proinsulin levels appears in early pregnancy even before a change in glycaemic control could be identified. If we measure proinsulin level in high-risk pregnant women, we can identify those that will develop GDM at an earlier stage in their pregnancy and therefore have an earlier therapeutic intervention.

Data about pro-insulin levels in GDM is however inconsistent. Dornhorst et al. showed higher fasting proinsulin levels in GDM; Kühl et al. found contrasting results, showing elevated proinsulin levels in normal pregnant women as compared to women with GDM; Swinn et al. showed similar levels of fasting proinsulin in GDM compared to normal pregnancies; Kautzky-Willer et al. showed that in GDM there is an increase in the level of fasting proinsulin (versus normal pregnancies and versus non-pregnant control subjects).

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as well as higher proinsulin/insulin ratios (versus nonpregnant control subjects)\textsuperscript{37}. Phelps et al. showed that high proinsulin levels in women with fasting hyperglycemia indicate more severe GDM\textsuperscript{38}. This lack of consistency in data about proinsulin levels in pregnancy could be imputed to different factors: differences in the size of the study populations; differences in inclusion criteria, gestational age, and definition of GDM; differences in the "severity" of GDM; differences in body weight among study populations.

Increased insulin resistance is significantly associated with decreased proinsulin–to–C-peptide ratio\textsuperscript{39}. This demonstrates that the efficiency of the processing of proinsulin is increased as response to the pregnancy-associated insulin resistance, independently of the glucose tolerance status. Proinsulin secretion in late pregnancy reflects insulin resistance rather than β-cell dysfunction.

In the Women’s Health Study, an increased proinsulin-to-insulin ratio in healthy women was most strongly associated with an imminent diagnosis of type 2 diabetes (within 2 years, as compared to 3 or 4 years)\textsuperscript{40}. β-cell dysfunction in women with GDM may not yet have advanced to the point of abnormal proinsulin processing. A follow-up of proinsulin–to–C-peptide ratio in women with previous GDM showed that hyperproinsulinemia developed over time, as β-cell function worsened\textsuperscript{41}.

There is an inverse relationship between maternal obesity/insulin resistance and the proinsulin–to–C-peptide ratio in pregnancy\textsuperscript{42}. Obesity and insulin resistance are associated with decreased proinsulin-to-insulin ratios in normoglycemic individuals\textsuperscript{43}. The use of the proinsulin–to–C-peptide ratio in this context supports the idea that increased proinsulin (rather than decreased hepatic insulin extraction) appears. Despite the β-cell defect, the efficiency of proinsulin processing can be increased in GDM, just as in women with normal β-cell function. Studies suggest that this ability to compensate for insulin resistance by increasing the efficiency of proinsulin secretion may decrease over time in these patients.

**CONCLUSIONS**

Gestational diabetes is defined by anomalies in pancreatic β-cell secretion, important for both aetiologies of gestational diabetes and non-insulin dependent diabetes. Changes in the insulin signalling pathway appear to be responsible for the metabolic changes that


\textsuperscript{39} Polonsky KS, Rubenstein AH: C-peptide as a measure of the secretion and hepatic extraction of insulin. Diabetes 33:486–494, 1984

\textsuperscript{40} Cuulin Zhang, Sjurdur F Olsen, Stefanie N Hinkle, Robert E Gore-Langton, Allan Vaag, Louise Groth Grunnet, Edwina H Yeung, Diabetes & Women’s Health (DWH) Study: an observational study of long-term health consequences of gestational diabetes, their determinants and underlying mechanisms in the USA and Denmark, BMJ, 2019

\textsuperscript{41} Polonsky KS, Rubenstein AH: C-peptide as a measure of the secretion and hepatic extraction of insulin. Diabetes 33:486–494, 1984


develop during pregnancy. Individual genetic background and environmental factors, in particular obesity, determine the ability of a pregnant women to balance these changes and avoid the development of GDM. In conclusion, patients with GDM are more insulin resistant than pregnant nondiabetic women and are predisposed to have defective insulin secretion and defective insulin action. Gestational diabetes is associated with abnormalities in insulin-sensitivity and insulin secretory response that are typical in type 2 diabetes. Pregnant women with GDM have elevated levels of pro-insulin and lower proinsulin/insulin raport which could serve as earlier markers for this condition.

CONFLICT OF INTERESTS
The authors declare that they have no conflict of interests.

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