Role of Insulin in Placental Transport of Nutrients in Gestational Diabetes Mellitus

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Keywords

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Abstract

Background: Gestational diabetes mellitus (GDM) is associated with increased fetal adiposity, which may increase the risk of obesity in adulthood. The placenta has insulin receptors and maternal insulin can activate its signaling pathways, affecting the transport of nutrients to the fetus. However, the effects of diet or insulin treatment on the placental pathophysiology of GDM are unknown. **Summary:** There are very few studies on possible defects in the insulin signaling pathway in the GDM placenta. Such defects could influence the placental transport of nutrients to the fetus. In this review we discuss the state of insulin signaling pathways in placentas of women with GDM, as well as the role of exogenous insulin in placental nutrient transport to the fetus, and fetal adiposity.

Key Messages: Maternal insulin in the third trimester is correlated with fetal abdominal circumference at that time, suggesting the important role of insulin in this process. Since treatment with insulin at the end of pregnancy may activate placental nutrient transport to the fetus and promote placental fatty acid transfer, it would be interesting to improve maternal hyperlipidemia control in GDM subjects treated with this hormone. More research in this area with high number of subjects is necessary.

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Introduction

Gestational diabetes mellitus (GDM) is associated with perinatal complications, such as macrosomia in the offspring and increased fetal adiposity, which may increase the risk of obesity, diabetes type 2, and metabolic syndrome in adulthood [1]. Pregnant women diagnosed with gestational diabetes are treated through diet (and exercise) or with insulin in order to avoid hyperglycemia and its adverse effects on fetal development. However, during recent years, other therapeutic approaches such as oral antidiabetic drugs like metformin or glyburide have also been used [2], since insulin therapy has several disadvantages in GDM, including the lack of a clear definition of the dose, the need for multiple daily injections, and the risk of hypoglycemia and excessive maternal weight gain [3]. In addition, insulin therapy is potentially associated with a risk of developing type 2 diabetes mellitus in the offspring later in life [4]. A recent study has shown metformin to be more suitable than glyburide for use in the management of gestational diabetes, since glyburide has been associated with increased risk of neonatal hypoglycemia, high maternal weight gain, high neonatal birth weight, and macrosomia [5]. However, very few studies have analyzed the effects of diet or insulin treatment on the placental pathophysiology of this disease.

The classic mechanism to explain fetal macrosomia in GDM is based on the Pedersen hypothesis, according to

which maternal hyperglycemia leads to fetal hyperglycemia that would in turn establish fetal hyperinsulinemia to control fetal glucose levels [6]. This fetal insulin would activate fetal anabolism, thus promoting excessive fetal growth. In fact, the HAPO study showed a strong correlation between increased maternal glucose levels and fetal adiposity [7], even when glucose values were below the pathological limits. However, some studies have shown macrosomia even in pregnancies where maternal glucose is well controlled [8]. Thus, in addition to hyperglycemia, GDM-associated conditions, such as larger placentas, hyperleptinemia, hyperinsulinemia, and oxidative stress, may be involved in the pathophysiology of GDM and affect fetal development.

Gestational diabetes changes the placental structure, which may alter the transport of nutrients to the fetus [9–11]. Maternal insulin is not required for the placental transfer of glucose, since this occurs mainly via GLUT-1, which is a carrier that acts independently of insulin. However, the placenta has insulin receptors (IRs) and maternal insulin can activate its signaling pathways, affecting the placental metabolism [12] and, therefore, placental and fetal development. There are very few studies on possible defects in the insulin signaling pathway in the GDM placenta. Such defects would lead to insulin resistance in the placenta, which could influence the placental transport of nutrients to the fetus. Therefore, the study of the role of insulin in the placental transfer of nutrients in GDM is justified. It is very important to design strategies that prevent fetal adiposity and macrosomia in the offspring, avoiding fetal programming of obesity in later life.

In this review, we discuss the state of insulin signaling pathways in placentas of women with GDM, as well as the role of exogenous insulin on placental nutrient transport to the fetus and fetal adiposity. However, we are well aware that insulin is only one of the links in a complex network of metabolic pathways that could regulate the transfer of nutrients to the fetus and the placental metabolism [13, 14]. Insulin treatment could be considered as the “gold standard” for the treatment of gestational diabetes in many countries, and it is very important to analyze its mechanism of action in the placenta, especially in cases of GDM.

Functions of Insulin within the Placenta

Insulin is a peptide hormone that is essential for regulating both intracellular and plasma levels of glucose in different tissues, such as adipose tissue, skeletal muscle, or liver. This hormone induces a variety of cellular responses, such as glucose uptake via GLUT4 (in periph-

eral tissues but not in placenta), and glycogen, protein, and lipid synthesis; it may also decrease lipolysis in adipose tissue [15]. Insulin resistance is, by definition, a disorder in the signal transduction of this hormone [16].

Although insulin cannot cross the placenta or activate the placental glucose carrier GLUT-1, it can bind to its specific receptor (IR) present in the trophoblast membrane, activating the signaling pathways of this hormone [17], thus contributing to the placental metabolism of nutrients [12]. Insulin resistance in peripheral tissues in women with GDM is exacerbated, but few studies have examined the extent of insulin resistance in placenta in this disease and the ways in which this resistance could contribute to alter the placental transport of nutrients [18, 19]. Moreover, insulin, along with glucose, is involved in the regulation of angiogenesis and vasculogenesis in the fetus [20, 21], where they bind to fetal endothelial cells [22]. Thus, fetal hyperinsulinemia may also contribute to the hypervascular placenta typical of GDM [23]. Fetal insulin binds to the fetal endothelium IRs and alters the expression of several genes involved in signal transduction, metabolism, and transport [12]. In addition, fetal insulin stimulates endothelial glycogen synthesis [17]. Nevertheless, there are no specific studies on IR and lipid transfer in fetal endothelial cells.

Recent studies have shown the altered expression of IRs in the fetoplacental endothelium of GDM women, leading to abnormal fetal endothelium function [24], even when GDM is treated with diet containing normal levels of glucose. Nevertheless, insulin treatment was seen to restore IR expression in these patients leading to normal endothelial function and a normal newborn [25].

Placental sensitivity to maternal insulin during pregnancy is controversial. Some studies suggest that placenta is most sensitive to maternal insulin at the beginning of pregnancy, while by the end of the third trimester, it loses sensitivity, perhaps because of changes in the distribution of their receptors between the maternal and fetal compartments. In the first trimester of pregnancy, IRs are more abundant in the syncytiotrophoblast, while they are mainly found in fetal endothelial cells at the end of pregnancy [12, 26]. The response to insulin in early stages of pregnancy is strongly associated with placental weight at term and with placental volume in the mid-pregnancy stage, while this role is less prominent at the end of pregnancy [27]. We also found an association between maternal insulin levels at the beginning of the third trimester and the fetal abdominal circumference z-score, supporting the importance of the role of maternal insulin in the early stages of pregnancy for fetoplacental development [28].

Fig. 1. Main pathways of insulin signaling: ERK and PI3K-Akt-mTOR.

Nevertheless, maternal insulin may also affect placental function at the end of pregnancy. Some studies have reported the high expression of the IR in the microvillous membrane (MVM) of the syncytiotrophoblast at term, suggesting that maternal insulin affects the trophoblastic function *in vivo* at the end of pregnancy [29]. In addition, Friis et al. [30] found that maternal insulin at the end of pregnancy was strongly associated with the weight of the placenta, highlighting the effect of this hormone in the final stages of gestation. Nevertheless, several metabolic factors associated with maternal BMI were seen to be determinants of placental weight in this study.

Placental Insulin Signaling Cascade

In the placenta, insulin binds to the IR receptor in the trophoblast membrane and may activate 2 insulin signaling pathways: the Ras-extracellular-signal-regulated kinase (Ras-ERK) and the IRS (IR substrate)-PI3K-Akt-mTOR pathway [18, 31] (Fig. 1). These 2 signaling path-

ways are the main mechanisms through which cells control survival, differentiation, proliferation, and metabolism, all of which have been described in placenta [18, 28, 32]. Many studies point at a cross regulation of insulin pathways [33], which further hinders our understanding of the molecular mechanisms of this hormone. In addition, insulin is not the only molecule able to activate these signaling pathways, since cytokines, as well as other hormones and growth factors, play a very important role in their activation [9, 31, 33]. These molecules are also involved in cellular metabolism and may also affect the transport of nutrients.

The PI3K-Akt-mTOR pathway is mainly associated with nutrient metabolism, although it also affects apoptosis and cell proliferation. This pathway is the most studied in cases of insulin resistance in peripheral tissues and, to a lesser extent, in placenta. The activation of Akt (also known as Protein Kinase B) triggers the translocation of the glucose transporter GLUT-4 from the cytoplasm to the membrane in peripheral tissues, with the consequent uptake of glucose. However, in the syncytiotrophoblast,

GLUT-4 is not involved in glucose transfer, which is mediated by GLUT-1, so the activation of Akt would have a different role in the placenta.

Downstream of Akt is mTOR (mammalian target of rapamycin or mechanistic target of rapamycin), whose action is complex and involves a large number of mediators. The mTOR activation cascade regulates a wide range of intracellular processes, including survival, growth, metabolism, autophagy, and proliferation. The importance of the mTOR pathway lies in its recent discovery as a sensor of nutrients from the placenta [34]. Moreover, it is activated by growth factors other than insulin or by nutrients such as amino acids. One of the main functions of mTOR is to regulate the expression of amino acid (aa) carriers, thereby contributing to abnormal fetal growth in cases of GDM.

The MEK-ERK signaling pathway, which is mainly related to cell proliferation, has hardly been studied in cases of insulin resistance in the peripheral tissues, and even less so in human placenta. Recent studies have pointed to changes in the ERK pathway in the skeletal muscle of women with polycystic ovary syndrome, whilst the PI3K/Akt pathway was not altered in these subjects [35]. In addition, an alteration in the ERK pathway was also found in the skeletal muscle of obese women [36]. The consequences of ERK disruption for the insulin pathway have not been evaluated in the placenta and therefore merit further research.

Very few studies have examined changes in the insulin signaling pathways in cases of GDM, and those that have been made used a very small number of subjects. Lower levels of IR have been observed in GDM women treated with diet (perhaps due to insulin resistance) and higher levels of IR in those treated with insulin, compared with controls [17].

Nutrient excess in obesity and diabetes may inhibit Akt signaling through the mTORC1 complex, which would lead to a situation of insulin resistance in the placenta [37]. In addition to the changes that may occur at various levels of the insulin signaling pathway, there are proteins in the placenta that can interfere with the signaling pathway of this hormone, contributing to insulin resistance in this organ. A recent study has found overexpression in GDM placentas of insulin-related proteins such as Annexin 2 and 14-3-3 proteins. These proteins block the insulin pathway at various levels, thus contributing to insulin resistance in GDM [38]. In addition, in peripheral tissues, other molecules can inhibit the insulin signaling pathway, blocking some of its intermediates, for example, tumor necrosis factor- α (TNF α), which induces

the phosphorylation of serine residues of IR and IRS-1, thus inhibiting them [39]. Stress in the endoplasmic reticulum, which is characteristic of GDM, alters the insulin signaling pathway, also leading to insulin resistance [40].

Placental insulin resistance in GDM women treated with diet may occur as a result of increased levels of the PI3K subunit p85 α in these pregnant women [18, 19] or through a decrease in the levels of Akt [28]. This situation can be compensated by treatment with insulin, since the levels of this subunit were seen to decrease and the levels of p85 α /p110 dimer increase, which would involve activation of the Akt pathway in this group of women. However, in peripheral tissues, insulin therapy does not seem to be able to compensate insulin resistance, since p85 α subunit remained high with respect to the control in insulin-treated women with GDM [41]. In this regard, our results also support enhanced Akt and ERK in placenta after insulin treatment [28]. Thus, the insulin signaling pathway is less damaged in the placenta than in other peripheral tissues exposed to hyperglycemia at longer time, and thus, placental insulin resistance seems to be overcome by exogenous insulin treatment.

Effects of Insulin on Placental aa Transport in GDM

The placental transport of aa is complex, with more than 20 types of carriers involved. Some studies suggest that there is no alteration in some transporters, such as system L [42, 43], Y⁺, and TauT [44], while others have described an increase in the activity of system A in mothers with GDM. The above study also showed an increase in system L activity in the MVM of the trophoblast, but only in GDM mothers with large gestational age babies, which suggests a role for this transporter in fetal overgrowth [44]. However, there are no studies on the effect of GDM treatment with diet or insulin on aa carriers.

The mTOR signaling pathway is one of the main mechanisms related to protein levels in tissues, where it regulates numerous components involved in protein synthesis, including initiation and elongation factors, and the biogenesis of ribosomes themselves. In addition, aa themselves can modulate the levels of mTOR to regulate the expression of their membrane carriers [45] (Fig. 2). In cases of nutrient excess (obesity and GDM), mTOR might be activated, with the consequent activation of aa transporters accompanied by typical fetal overgrowth associated with these pathologies [34]. Park et al. [46] showed higher levels of some aa such as valine, tyrosine, and lysine in the serum of GDM mothers, where they are related with

Fig. 2. Signaling mechanisms of mTOR and link between maternal nutrient availability and placental growth.

insulin resistance. However, they did not study differences in the type of treatment. This would suggest a connection between the effect of maternal insulin and a greater contribution of aa to the fetus to promote macrosomia.

Using explants and primary cultures of human trophoblasts, it has been shown that mTORC1 is a positive regulator of the aa transporter systems A and L, which are critical for aa transport to the fetus [47, 48]. In addition, in obese women (non-GDM), insulin and IGF1 were seen to activate mTORC1 and molecules of the mTORC1 pathway, and both were correlated with birth weight and aa transport in large babies [32]. In an animal model, several mediators of the insulin pathway and mTOR were activated in placentas of obese mice with macrosomic offspring, indicating a role for these pathways in fetal overgrowth [49]. Therefore, insulin, aa, other growth factors, and cytokines could act on the mTOR pathway, although the mechanism is very complex and may follow other pathways of the cellular metabolism.

The Effects of Insulin on Placental Lipids Transport in GDM

GDM women have heavier and larger placentas than healthy women even when they have good glycemic control [50, 51]. The placental weight provides an indirect measurement of the area available for nutrient exchange, and is directly involved in the transport of nutrients to the fetus [52]. Numerous studies pointed to a close correlation between placental weight and birth weight [52–54].

Moreover, placentomegaly has been correlated not only with macrosomia but also with fetal adiposity [51, 55]. Therefore, study of the possible effects of insulin treatment on placental fatty acid carriers is of great interest. There are very few studies along these lines and those that exist involve in vitro assays. The state of insulin resistance within the placenta may help unravel the controversy concerning the results on placental fatty acid transport.

Placental transfer of fatty acids is a complex process that involves the release of fatty acids from maternal lipoproteins by several lipases such as lipoprotein lipase (LPL) and endothelial lipase (EL), followed by the uptake of these free fatty acids by membrane-binding proteins (Fig. 3). Numerous membrane-bound proteins are expressed in the trophoblast, including fatty acid binding protein (FABP) plasma membrane, FAT/CD36 (fatty acid translocase), and FATP-1 to -6 (fatty acid transport proteins) [56]. Recently, the presence of the major facilitator superfamily domain 2A (MFSD2A) transporter was reported in the placenta [57], which may enhance the transfer of lysophospholipids across the cell membrane [58]. Lysophospholipids could be an additional source of fatty acids for the placenta. This is supported by reports that show how lysophosphatidylcholine may represent a preferred physiological carrier of docosahexaenoic acid (DHA) compared with NEFA for its incorporation into brain [59, 60]. Similarly, lysophosphatidylcholines seem to deliver ¹³C-DHA to erythrocytes more efficiently than NEFA [61]. Once in the cytosol, fatty acids are bound to cytosolic FABPs to interact with subcellular organelles, including the endoplasmic reticulum, mitochondria, lipid droplets, and peroxisomes. The incorporation of fatty acids in placental lipid droplets may also interfere in their rate of fetal transfer.

With regard to placental lipases, Radaelli et al. [62] found an increase in EL expression in insulin-treated women with GDM accompanied by a decrease in the expression of LPL, which is consistent with results published by our group [28]. A reduction in LPL may help to counteract the excess of maternal–fetal fatty acid transfer across the placenta. However, other authors did not report any change in LPL activity in GDM patients [63–65], although neither diet nor insulin treatment was considered in these studies. LPL seems to be modulated by insulin, and trophoblast cells exposed to insulin for 24 h show reduced LPL expression, but not when the exposure time was 3 h [66]. Taken together, these findings suggest that insulin regulates these lipases in some way.

In terms of lipid-binding carriers, no change or an increase in both FAT and FATP-1 expression was observed

Fig. 3. Theoretical model of fatty acid transport in a trophoblast cell, adapted from Hanebutt et al. [81]. EL, endothelial lipase; Lyso-PL, lysophospholipids; LP, lipoprotein; LPL, lipoprotein lipase; NEFA, non-esterified fatty acids; FATP, fatty acid transport protein; FAT, fatty acid translocase; FABPpm, plasma membrane fat-

ty acid binding protein; L-FABP, liver-fatty acid binding protein; H-FABP, heart-fatty acid binding protein; MFSD2A, major superfamily domain 2A; PPAR, peroxisome proliferator activated receptor; RXR, retinoid X receptor. FATP show acyl-coA activity and would produce FA-acyl coA.

in placental membranes, as well as in A-FABP in the cytosol of placentas from GDM women treated with diet compared with GDM women treated with insulin [28, 62, 67]. A similar trend was also reported in placentas of obese women [68]. In peripheral tissues, insulin modulated the expression and translocation to the membrane of FAT via the Akt pathway, [69, 70]. We also found an association between placental FAT and A-FABP, which may enhance fatty acid translocation from the maternal side to the cytosol and placental fatty acids uptake [28] (Fig. 4). In addition, insulin was seen to regulate the expression and translocation to the cytoplasm of FATP-1 in adipocytes and skeletal muscle of rats, although without activating the Akt pathway [71].

In diet-treated GDM, we found slight placental insulin resistance in the form of a reduction in phosphorylated AKT (p-AKT), that was reversed in GDM patients treated with insulin during the last trimester of pregnancy [28]. Even in these situations, placental p-Akt and p-ERK were correlated with some placental fatty acid carriers like EL, A-FABP, and FAT, which lends weight to the idea that the state of placental insulin resistance is a key issue in modulating lipid transporters via p-Akt and p-ERK activation. These results were also confirmed in an in vitro study in which a choriocarcinoma cell line (BeWo cells) was stimulated with insulin and treated with Akt inhibitors or ERK inhibitors [28]. Thus, maternal insulin could contribute to adiposity in the fetuses of GDM especially if a

Fig. 4. In both peripheral tissues and placenta, insulin modulates the expression and translocation to the membrane of FAT, via Akt pathway. FAT, fatty acid translocase; Akt, protein kinase B; A-FABP, adipose fatty acid binding protein; IR, insulin receptor.

Fig. 5. Proposed mechanisms of disturbed nutrient transport to the fetus associated with GDM and obesity. Z-CA, Z-score of ultrasound fetal abdominal circumference; p-Akt, phosphorylated protein kinase B; p-ERK phosphorylated extracellular signal-regulated kinase; TG, triglyceride.

hyperlipidemic status is present at that time in their mothers.

Insulin plus fatty acids also stimulate the expression of adipophilin and A-FABP, in primary trophoblasts via Akt, promoting the accumulation of lipids in placental lipid droplets [72]. In this sense, placentas of GDM mothers could modulate fatty acids transfer to the fetus [67]. Other molecules, such as IGF-1, hormones such as leptin [66, 73], and some cytokines such as interleukin-6 and TNF α [11, 63], in addition to PPAR γ [74, 75] could also regulate the expression of fatty acid transporters. All of them are part of a much more complex regulatory mechanism involving other cytokines, oxidative stress, hypoxia, and growth factors, as well as fatty acids themselves.

It is important to mention that although higher fetal adiposity is associated with GDM, the levels of n-3 polyunsaturated fatty acids such as DHA are lower in the cord

of GDM babies [76, 77]. Unlike other fatty acid transporters, our group found lower levels of FATP-4 and MFSD2A expression in GDM placentas [57]. Moreover, Soygur et al. [78] confirmed a reduction in MFSD2A in GDM placentas. These transporters have previously been associated with the selective placental transport of DHA [58, 79].

Summary

Figure 5 summarizes the possible mechanisms involved in altered nutrient transfer to the fetus in GDM, which would contribute to the fetal programming of obesity in the offspring (Fig. 5). At the beginning of the third trimester, a maternal environment of hyperlipidemia and hyperinsulinemia is common in GDM, which would lead

to a disturbed placental weight and thickness and activation of the main insulin pathways as p-ERK and p-Akt. Such activation of the insulin cascade is even greater after GDM treatment with exogenous insulin. The activation of insulin signaling pathways increases some placental lipid carriers, which might increase fetal lipid transport and storage and even lower TG levels in cord blood in the offspring. Therefore, insulin resistance from the early stages of pregnancy alter both the placental structure and insulin signaling pathways in this tissue, resulting in fetal adiposity and contributing to the fetal programming of obesity.

In addition, low levels of DHA in the cord blood of GDM babies could be due to the decrease in placental expression of MFSD2A, which has been linked to the selective transport of DHA and other long chain polyunsaturated fatty acids. Given the importance of DHA in the

correct neurodevelopment of fetuses, it is essential to understand the mechanisms that regulate this carrier in detail before specific drugs can be designed to improve its expression. In this context, the efficiency of DHA supplementation in GDM pregnancies could be poor, since DHA cannot cross the placenta properly [80].

Finally, since treatment with insulin at the end of pregnancy may overcome placental insulin resistance and promote placental fatty acid transfer, it would be interesting to improve maternal hyperlipidemia in GDM subjects.

Disclosure Statement

There are no conflicts of interest, financial or otherwise, declared by any of the authors.

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