Insulin therapy and its consequences for the mother, foetus, and newborn in gestational diabetes mellitus

Subiabre, Mario; Silva, Luis; Toledo, Fernando; Paublo, Mario; Lopez, Marcia A.; Boric, Mauricio P.; Sobrevia, Luis

Published in:
Biochimica et biophysica acta-Molecular basis of disease

DOI:
10.1016/j.bbadis.2018.06.005

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

BBA - Molecular Basis of Disease 1864 (2018) 2949–2956

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Insulin therapy and its consequences for the mother, foetus, and newborn in gestational diabetes mellitus

Mario Subiabre[a], Luis Silva[a,b], Fernando Toledo[a,c], Mario Paublo[d], Marcia A. López[d], Mauricio P. Boric[e], Luis Sobrevia[a,f,g,*]

[a] Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile
[b] Immunoendocrinology, Division of Medical Biology, Department of Pathology and Medical Biology, University of Groningen, University Medical Centre Groningen (UMCG), Groningen 9700, RB, the Netherlands
[c] Department of Basic Sciences, Faculty of Sciences, Universidad del Bío-Bío, Chillán 3780000, Chile
[d] Gynaecology and Obstetrics Service, Hospital San Juan de Dios, Santiago 8330024, Chile
[e] Department of Physiology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile
[f] Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Seville E-41012, Spain
[g] University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, QLD 4029, Queensland, Australia

ARTICLE INFO

Keywords:
Diabetes
Insulin therapy
Diet
Human
Placenta

ABSTRACT

Gestational diabetes mellitus (GDM) is a disease characterised by glucose intolerance and first diagnosed in pregnancy. This condition relates to an anomalous placental environment and aberrant placental vascular function. GDM-associated hyperglycaemia changes the placenta structure leading to abnormal development and functionality of this vital organ. Aiming to avoid the GDM-hyperglycaemia and its deleterious consequences in the mother, the foetus and newborn, women with GDM are firstly treated with a controlled diet therapy; however, some of the women fail to reach the recommended glycaemia values and therefore they are passed to the second line of treatment, i.e., insulin therapy. The several protocols available in the literature regarding insulin therapy are variable and not a clear consensus is yet reached. Insulin therapy restores maternal glycaemia, but this beneficial effect is not reflected in the foetus and newborn metabolism, suggesting that other factors than D-glucose may be involved in the pathophysiology of GDM. Worryingly, insulin therapy may cause alterations in the placenta and umbilical vessels as well as the foetus and newborn additional to those seen in pregnant women with GDM treated with diet. In this review, we summarised the variable information regarding indications and protocols for administration of the insulin therapy and the possible outcomes on the function and structure of the foetoplacental unit and the neonate parameters from women with GDM.

1. Introduction

Gestational diabetes mellitus (GDM) corresponds to any degree of glucose intolerance developing or first recognised during pregnancy with a prevalence 6–20% of pregnant women worldwide [1]. GDM shows with defective insulin secretion, insulin resistance, and abnormal foetoplacental vascular function [2, 3]. Also, newborns of women with GDM present an adverse outcome and higher risk of developing obesity, impaired glucose tolerance, and type 2 diabetes mellitus (T2DM) later in adulthood [1, 4].

Clinical management of women with GDM begins with a controlled nutritional therapy, glucose monitoring, and moderate physical activity. Around 70–80% of these women achieve glycaemia suggested values, i.e., fasting: ≤95 mg/dL (5.3 mmol/L), 1 h postprandial: ≤140 mg/dL (7.8 mmol/L), 2 h postprandial: ≤120 mg/dL (6.7 mmol/L) [1, 4, 5]. However, when women under a nutritional therapy do not achieve the suggested glycaemia, insulin therapy or oral anti-diabetic pharmacological agents are used [1, 3, 4]. Interestingly, insulin therapy in pregnant women with GDM seems to be equally effective as diet [4]. However, not clear conclusions are presented for these protocols in GDM since not sufficient evidence is still available [4]. It is highlighted that several studies include women with pre-gestational diabetes mellitus and those with GDM, and, furthermore, do not explicitly differentiate between pregnant women with GDM on a diet from those

* Corresponding author at: Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, P.O. Box 114-D, Santiago 8330024, Chile.
E-mail address: lsobrevia@uc.cl (L. Sobrevia).

https://doi.org/10.1016/j.bbadis.2018.06.005
Received 23 March 2018; Received in revised form 26 May 2018; Accepted 6 June 2018
Available online 08 June 2018

Table 1
Outcome of insulin therapy versus other protocols in gestational diabetes mellitus.

Type of insulin	Other protocols	Outcome	References
[a]NPH and regular insulin	Diet	Lower newborn macrosomia	[16]
[b]Not specified	Diet	No differences in neonatal complications	[52]
[c]NPH and regular insulin	Diet	Lower macrosomia, birth weight, and ponderal index	[17]
[d]NPH and rapid-acting regular insulin	Diet	No differences in newborn macrosomia	[13]
[e]Long-acting insulin	Short-acting insulin	Higher macrosomia and birth weight	[24]
[f]Regular insulin and NPH insulin	Insulin analogues	Lower cranial-thoracic circumference ratio	[20]
[g]Insulin lispro or regular insulin	Insulin analogues	No differences in neonatal height, weight, and Apgar score	[21]
[h]Regular and intermediate acting insulin	Glyburide	No differences in neonatal complications	[12]
[i]Short- and long-acting insulin	Glyburide	Lower macrosomia	[25]
[j]NPH and regular insulin	Metformin	Higher arm and chest circumference, height, and birth weight	[56]
[k]NPH and regular insulin	Metformin	Higher jaundice, respiratory distress, neonatal intensive care unit admission, and preterm labour	[57]
[l]NPH insulin	Metformin	Higher neonatal hypoglycaemia	[22]
[m]Long-acting and rapid-acting insulin	Metformin	Lower incidence of vacuum extraction deliveries and caesarean sections	[18]
[n]NPH and regular insulin	Metformin	No differences in newborn BMI, glycaemia, umbilical cord C-peptide, and birthweight	[23]
[o]Not specified	Metformin	No differences in shoulder dystocia, hyperbilirubinemia, prenatal mortality, and need for intensive care treatment	[58]

NPH, Neutral protamine Hagedorn; BMI, body mass index. Protocols used in the different studies quoted in this table are the following: [a] 20 IU of NPH insulin and 10 IU of regular insulin 30 min before breakfast. [b] 8–12 IU per day of fast- or intermediate-acting insulin (time of the day not specified). [c] 20 IU of NPH insulin and 10 IU of regular insulin (time of the day not specified). [d] NPH insulin (dose not specified) before breakfast and at bedtime, plus three injections of rapid-acting regular insulin (time of the day not specified). [e] Long-acting human insulin (Protaphan®) was given one daily injection at an initial dose of 14 IU in the morning. Short-acting insulin (Actrapid®) was given in three daily injections at doses of 4, 6, and 4 IU before breakfast, lunch and dinner, respectively. [f] Regular human insulin (dose and time of the day not specified). NPH insulin (6–10 IU) was added at bedtime with fasting glucose > 5.2 mmol/L. [g] 0.7 IU per kg body weight per day with insulin therapy (Humalog® or regular human insulin (Humulin R) initiated by the 21 weeks of gestation, 0.8 IU per kg body weight per day whether initiated between the 21 and 26 weeks of gestation, or 0.9 IU per kg body weight per day whether initiated at ≥ 26 weeks of gestation or later. [h] 0.7 IU per kg body weight three times daily, i.e., regular and intermediate-acting insulin in the morning, regular insulin at dinner, and intermediate acting insulin at bedtime, and increased weekly as needed. [i] 0.8 IU per kg body weight in multiple daily doses (time of the day and specific dosage not specified) and increased up to twice weekly. [j] Initial dose of 0.2 IU of NPH insulin per kg body weight. NPH insulin is given before bedtime with hyperglycaemia at fasting. Regular insulin is given before meals with hyperglycaemia postprandial (1 IU for every 10 mg/dL glucose over the target value). Regular insulin is given (starting total dose of 0.7 IU per kg body weight) with hyperglycaemia at both fasting and postprandial. Insulin was divided into two thirds as NPH insulin (given in two thirds half-an-hour before breakfast and one third before bedtime) and either one third in two or three preprandial regular insulin injection. [k] 0.5 IU per kg body weight per day with two thirds of total NPH dose in the morning and one third of regular human insulin in the afternoon. [l] Initial dose of 0.4 IU per kg body weight per day, with half-dose before breakfast, one quarter of total dose before lunch, and one quarter at 22:00 h. [m] Long-acting insulin (Protaphan®) at fasting (dose not specified) and rapid-acting insulin (Humalog®) postprandial (dose and time of the day not specified). [n] Combination of regular and NPH insulin in a weight-based fashion. Total starting 0.7 IU per kg body weight per day was divided in two thirds given in the morning (two thirds NPH plus one third regular insulin) and one third in the evening (half NPH plus half regular insulin). [o] Initial dose of 0.2 IU per kg body weight per day (time of the day not specified) and titrated to meet glycaemia targets.

treated with insulin. Even when diet or insulin therapy are approaches that normalise maternal and newborn glycaemia the deleterious effect of GDM on the placenta function and the neonate metabolic state persist [3, 6]. In this review, we summarise the current evidence about the treatment with insulin (i.e., insulin therapy) in pregnant women with GDM and its effects on the foetoplacental tissue and impact on the newborn outcome.

2. Insulin therapy in gestational diabetes mellitus

The final expected outcome of treating GDM is avoiding the hyperglycaemia-associated maternal and foetal complications [3, 4]. The criteria for passing pregnant women with GDM on a diet (hereafter referred as GDM*d*) to insulin therapy (hereafter referred as GDM*i*) is broad and different approaches adjusted to the realities in different regions and populations are reported [4]. Individual studies report glycaemia values after 2 h postprandial over which insulin therapy is initiated varying between 5.2 and 5.6 mmol/L (94–101 mg/dL) at fasting and 6.6–7.9 mmol/L (119–142 mg/dL) determined either at the second or third trimester of pregnancy. Unfortunately, one of the leading conclusions recently reported for 7381 women with GDM is that there are not enough high-quality results to propose significant differences for health outcomes after using insulin compared with oral anti-diabetic pharmacological therapies, non-pharmacological interventions, or different insulin analogues or regimens in women with this disease [4]. Several reports show data that is analysed considering the local reality in terms of local guidelines for treating GDM, socio-economical differences, life style, ethnicity, age, among other factors. Despite the intrinsic value of treating patients with GDM with insulin as deeply described [4], it is highlighted the fact that local realities may be underestimated when defining the conditions determining the use of insulin in patients with GDM.

2.1. Different protocols of insulin therapy

The mainstay of insulin therapy in pregnancy has been the use of neutral protamine Hagedorn (NPH) insulin for two to four times daily. Continuous insulin injection of a rapid-acting insulin analogue, such as lispro and aspart, is used in women with GDM as well as in pregnant women with type I diabetes mellitus [7] whom frequently check their blood glucose level and use glucose monitoring devices [8]. However, several factors determine the decision of a protocol for treating the patients with insulin. These protocols are determinant when treating pregnant women with GDM. It is proposed that factors in addition to the clinical characteristics of the patients should be considered, including the high dependence of insulin treatment protocol on the characteristics of the studied population (v.g, ethnicity, socio-economic factors, physical activity, stress degree, feed quality), the use of a guideline based on the local sociocultural reality, the availability of proper technical facilities, and the actual benefits of different starting points for insulin treatment during the day, evening, or night [4, 5, 9]. In general, the existing perinatal guidelines indicate a low daily dose of insulin which also consider the characteristics of the woman and the frequency of self-monitoring [10]. It is emphasised that more than a general approach a specific insulin therapy planning dependent on factors as those mentioned above is applied worldwide [4, 10]. Interestingly, The American Diabetes Association [11] recently suggested that the sole measurement of glycated haemoglobin A1c (HbA_{1c}) level

may show a lower sensitivity compared with the classical oral glucose tolerance test (OGTT) approach in GDM. Also, other reports show no differences between the HbA_{1c} level in women with GDM [12] contrasting with another study where the HbA_{1c} level was suggested as the variable giving the best measure of glycaemia control when measured every two weeks in women with GDM [13].

2.2. Insulin therapy and the growing foetus and newborn

Normalization of maternal glycaemia with insulin therapy in GDM is expected to result in (*i*) reduced abnormal D-glucose handling by the maternal tissues, (*ii*) generation of maternal metabolites in a physiological range which are transferred through the placenta into the foetal circulation, and (*iii*) appropriate transfer of waste metabolites from the foetus to the maternal circulation. This is supported by findings showing that insulin therapy ends in normal glycaemia of the mother and the neonate at delivery [3, 4, 6, 14]. However, the adverse foetal clinical outcomes seem to be independent of the maternal therapy used, since no concluding differences were found when comparing pregnant women that were subjected to diet, oral anti-diabetic pharmacological treatment, or insulin therapy [3, 4].

Insulin therapy applied to women with GDM results in a better, unaltered, or adverse maternal and newborn outcome compared with women with GDM treated with diet, oral anti-diabetic drugs, or insulin analogues (Table 1). Beneficial effects of insulin therapy include lower incidence of macrosomia [12, 15, 16], lower cranial-thoracic circumference ratio compared with insulin analogues [17], or minor incidence of caesarean sections [18] (Fig. 1). Several other characteristics of the neonate following the use of insulin in GDM pregnancy are unaltered [13, 19–23]. However, the use of long-acting versus short-acting insulin increased the incidence of macrosomia [24], and the use of glyburide increases the neonate length and birth weight [25]. Interestingly, since the level of HbA_{1c} measured at first, second, and third trimester of pregnancy were similar in women with GDM treated with metformin versus those treated with insulin [23], and unaltered following treatment of women with GDM with long- versus short-acting insulin [24] it is likely that this parameter may not be determinant when deciding for insulin therapy or the use of pharmacological agents in GDM (see [9]). It is important to highlight that the even with the reduced number of reported studies comparing maternal insulin therapy versus diet in GDM it is suggested that insulin therapy could eventually be as good as diet improving the foetal and newborn alterations caused by GDM [4]. Noticeable, insulin therapy in GDM associated with excessive maternal gestational weight gain (GWG) and decreased infant weight between 12 and 18 months old compared with the use of metformin [18] (Fig. 1). Since excessive GWG is a key factor associated with dysfunction of the foetoplacental vasculature [26] insulin treatment of women with GDM may result in GWG-associated foetoplacental vascular alterations [3, 26]. Interestingly, diet or insulin therapy applied in pregnant women with GDM seems not to be enough to restore most of the parameters leading to deleterious consequences of the foetoplacental vasculature seen at birth [3, 6].

3. The placenta in insulin therapy and GDM

3.1. Structural characteristics

Studies addressing the effect of insulin therapy in the foetoplacental tissue and cells in pregnancies with GDM are scarce (Table 2). It is reported that placenta from GDM*i* where the women were also controlling their diet show increased cell proliferation of villous cytotrophoblasts and cytotrophoblast hypertrophy but syncytiotrophoblast atrophy with multiple vacuoles and glycogen storage areas [27]. Trophoblast basal membrane was thicker with scarce microvilli compared with normal pregnancies. The villous nucleus showed congested capillaries, stromal macrophages, edematous spaces, glycogen storage areas, and fibrosis. Interestingly, even when the patients included in this study showed normoglycaemia from the 24–28 weeks of gestation until delivery, the authors suggest that hyperglycaemia plays a role in the aetiology of GDM leading to increased trophoblast vacuolation and altered transplacental metabolic substrates exchange perhaps due to hyperglycaemia-induced hypoxia and oxidative stress in the placenta [27]. However, the possibility that maternal hyperglycaemia was a factor it would be early in pregnancy, at least before the 24th week of gestation.

The vasculature of the human placenta from women with GDM*i* (insulin treatment from 28 weeks of pregnancy onwards) shows disruption in adherens junctions (VE-cadherin and β-catenin) and tight junctions (ZO-1 and occludin) compared with placentas from normal pregnancies [28]. Changes in the placenta vasculature were correlated with increased tyrosine phosphorylation at junctional zones and impaired placental barrier function. Another study shows that the placenta from women with GDM treated with diet plus insulin show lower number of microvilli with syncytial nodes, thick-walled vessels, edematous spaces, areas of fibrosis, and peri-villous fibrinoid degeneration compared with normal pregnancies [27, 29].

3.2. Functional characteristics

3.2.1. Insulin receptors and signalling

The human placenta shows differential expression of insulin receptors (IRs) required to maintain placenta function and foetus growth and development [6, 30]. The number of IRs is different in the trophoblast and placental endothelium along gestation in a normal pregnancy [30]. Interestingly, IRs expression in the placenta was higher in women with GDM*i* or GDM*d* compared with normal pregnancies, and trophoblast plasma membranes from GDM*i* pregnancies show higher IRs expression than in women with GDM*d* [30]. Since the blood glycated HbA_{1c} level at delivery was similar in women with GDM*d* and GDM*i* this phenomenon may not be due to altered D-glucose metabolism. Instead, GDM-increased expression of IRs could due to the state of insulin resistance in the foetus from a GDM pregnancy [31]. Recent results show that human umbilical vein endothelial cells (HUVECs) from GDM*i* pregnancies exhibit higher mRNA expression of the isoform A (IR-A) but an unaltered expression of the isoform B (IR-B) of IRs compared with cells from normal or GDM*d* pregnancies [6]. Thus, neither insulin therapy nor diet resolve the increased IR-A expression in this cell type caused by GDM.

Placentas from GDM*i* also show reduced insulin receptor substrate 1 (IRS-1) protein expression and increased IRS-2 mRNA and protein expression, without referring to their activity, compared with normal and GDM*d* pregnancies [32]. IRS-1 and IRS-2 may be differentially modulated in GDM*i* and GDM*d* thus contributing to the lower insulin response by the placenta tissue and foetal vessels. The reduced IRS-1 protein abundance in the placenta from GDM*i* was suggested to result in increased IRS-2 as a compensatory mechanism to counterbalance a defective insulin signalling via IRS-1 protein [32]. A similar mechanism was described in adipocytes from patients with T2DM highlighting the importance of this insulin-signalling protein in the response to insulin [33]. Since maternal insulin is apparently not a modulator of the expression of IRS-1 or IRS-2, these signalling proteins may be under the modulation of other maternal still undefined factors. Interestingly, since the umbilical vein blood at delivery from GDM*d* plus GDM*i* pregnancies contains higher levels of insulin (~8 μU/mL) compared with normal pregnancies (~5 μU/mL) [34, 35] it is likely that IRS-2 expression and perhaps its associated signalling may depend on foetal more than maternal insulin level. However, this is a possibility that has not been yet addressed for GDM*i* compared with GDM*d* and normal pregnancies.

A reduced protein and mRNA expression of the regulatory subunit of phosphatidylinositol 3-kinase (PI3-K p85α) in placenta homogenates from GDM*i* was seen when compared with GDM*d* and normal

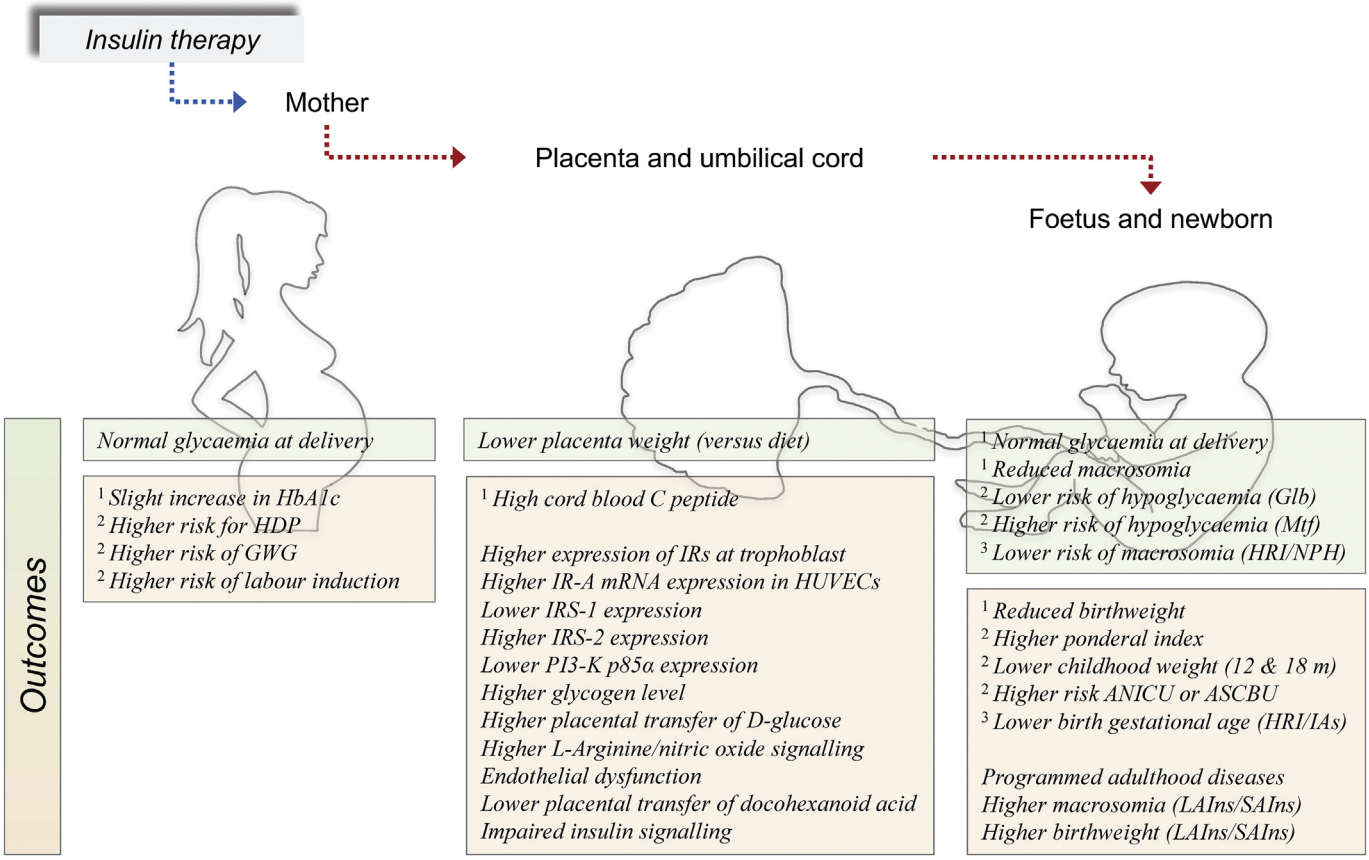

Fig. 1. Changes in the mother, the placenta and umbilical cord, and the foetus and newborn by insulin management of gestational diabetes mellitus. Insulin therapy is a protocol used in women with gestational diabetes mellitus (GDM) that were subjected to a controlled diet and did not reach the suggested normal glycaemia values in pregnancy. Normalising the maternal glycaemia after insulin therapy is likely to cause a variety of alterations that are beneficial (light green boxes) or negatively modified (light orange boxes) impacting several aspects of the physiology of the mother, the foetoplacental unit and the newborn. Thus, insulin therapy effects in the placenta and umbilical cord which may protect or having an adverse effect on the growing foetus and the newborn. Despite the well control of the maternal and foetal glycaemia by insulin therapy the detailed changes are not significantly different from women treated with diet or oral-pharmacological anti-glycaemiant drugs and are given as individual findings available in the literature. The numbers refer to comparisons between the insulin therapy and other approaches reported in Brown et al. [4] as follow: [1] insulin therapy compared with diet protocol, [2] insulin therapy compared with oral anti-diabetic pharmacological drugs (metformin, glibenclamide), and [3] insulin therapy compared with life style. *HbA1c*, haemoglobin A1c; *HDP*, hypertensive disorder of pregnancy; *GWG*, gestational weight gain; *IRs*, insulin receptors; *IR-A*, IR isoform A; *HUVECs*, human umbilical vein endothelial cells; *IRS-1*, insulin receptor substrate 1; *IRS-2*, insulin receptor substrate 2; *PI3-K p85α*, phosphatidyl-inositol-3-kinase regulatory (p85α) unit; *Glb*, glibenclamide; *Mtf*, metformin; *12 & 18 m*, 12 and 18 months; *HRI*, human regular insulin; *NHP*, Neutral protamine Hagedorn; *ANICU*, admission to neonatal intensive care unit; *ASCBU*, admission to special care baby unit; *IAs*, insulin aspart; *LAIns*, long-acting insulin; *SAIns*, short-acting insulin. Ratios *RHI/NPH*, *HRI/IAs*, *LAIns/SAIns* refers to the outcome using the first molecule versus the second molecule. Composed from references described in the text.

pregnancies [32]. Certainly, IRS-1 and IRS-2 act as major mediators of insulin signalling in the placenta vasculature and both proteins activate the protein kinase B/Akt via phosphatidylinositol 3 kinase (PI3K) [32, 36, 37]. This phenomenon results in activation of the endothelial nitric oxide synthase (eNOS). It is likely, unless still not documented, that a reduced expression of the PI3K signalling in GDM*i* may be compensated by an increase in the expression and signalling via IRS-2. However, GDM*d* and GDM*i* are conditions where the foetoplacental vasculature shows insulin resistance and several factors could result in an insulin-resistance–associated reduction in the NOS activity. This phenomenon may result from preferential activation of insulin receptors B (IR-B) form by insulin leading to at least IRS-2–mediated increase in the activity of the PI3K p85α, which will cause Akt inhibition thus reducing NO generation under insulin resistance [37]. Since activation of IRS-1, IRS-2, and PI3K-mediated signalling results in higher activity of the endothelial NOS (eNOS) in the placenta from normal pregnancies [37], a higher activity of these molecules, perhaps excluding IRS-1, may play a role in the increased NOS activity seen in the foetoplacental vasculature in GDM*i* and GDM*d* [6, 37].

3.2.2. *Transplacental transfer of substrates*

Placentas from GDM*d* also show less glycogen than placentas from GDM*i* or normal pregnancies [30]. GDM*i* also associated with a higher mean net uptake of D-glucose from the maternal perfusate and net D-glucose transfer to the foetal circulation in perfused placenta compared with GDM*d* [38]. Even when activation and increased expression of the D-glucose transporter 1 isoform (GLUT-1) at the basolateral membrane of the syncytiothrophoblast is described in a group of women with pregestational diabetes mellitus and GDM treated with insulin [39], few studies address the possibility that GLUT-1 in women with GDM*i* alone (i.e., not in mix with pregestational diabetes mellitus) may play a role in D-glucose transport compared with placentas from GDM*d* or normal pregnancies. GLUT-1 protein abundance was shown to be elevated in human placenta tissue from GDM*i* compared with placenta tissue from normal or GDM*d* pregnancies [32]. Thus, it is likely that D-glucose management by the placenta from GDM pregnancies is different depending on whether the mother received insulin treatment or not. Interestingly, GDM*i* also results in lower GLUT-4 mRNA expression and protein abundance [32], and increased expression and activity of the human cationic amino acid transporters (hCATs), preferentially hCAT-1

Table 2
Placental changes in gestational diabetes mellitus requiring insulin management.

Tissue or cell type	Effect	References
Placenta	Increased expression of insulin receptors	[30]
Placenta	Increased mother-*to*-foetus transfer of D-glucose	[30]
Placenta	Increased D-glucose transfer to the foetal circulation	[59]
Placenta	Decreased expression of IRS-1	[32]
Placenta	Increased expression of IRS-2	[32]
Placenta	Decreased expression of PI3-K p85α	[32]
Placenta	Increased expression of GLUT-1	[32]
Placenta	Decreased expression of GLUT-4	[32]
Placenta	Increased nitration of protein tyrosine residues	[60]
Placenta	Increased antioxidant genes expression	[43]
Placenta	Decreased expression of PPARγ, PPARα, and RXRα	[46]
Placenta	Decreased mother-*to*-foetus transfer of DHA	[50]
Placenta vasculature	Reduced VE-cadherin and β-catenin expression	[28]
Umbilical vein	Decreased vascular reactivity to insulin and CGRP	[6]
Trophoblast	Increased expression of insulin receptors	[30]
HUVECs	Increased expression and activity of hCAT-1	[6]
HUVECs	Increased expression and activity of eNOS	[6]
HUVECs	Increased expression of IR-A mRNA	[6]
HUVECs	Increased p44/42^{mapk} activation	[6]

IRS-1, insulin receptor substrate 1; IRS-2, I insulin receptor substrate 2; PI3-K p85α, phosphatidyl-inositol-3-kinase regulatory (p85α) unit; GLUT-1, glucose transporter 1; GLUT-4, glucose transporter 4; PPARγ, peroxisome proliferator-activated receptor gamma; PPARα, peroxisome proliferator-activated receptor alpha; RXRα, retinoid X receptor alpha; DHA, docosahexaenoic acid; CGRP, calcitonin gene-related peptide; HUVECs, human umbilical vein endothelial cells; hCAT-1, human cationic amino acid transporter 1; eNOS, endothelial nitric oxide synthase; IR-A, insulin receptor isoform A; p44/42^{mapk}, 44 and 42 kDa mitogen-activated protein kinases.

isoform, in the foetoplacental macrovasculature [6] compared with GDM*d* and normal pregnancies. L-Arginine is a semi-essential cationic amino acid that is metabolised by eNOS into L-citrulline forming NO as a coproduct. Membrane transport of L-arginine via hCAT-1 [35] and via the system y^+L activity [40, 41] is a phenomenon that seems crucial to maintain eNOS activity in foetoplacental endothelium. Thus, since NO generation is paralleled by an increase in the transport of L-arginine in GDM*d* and GDM*i* it is proposed as a mechanism involved in the endothelial dysfunction characteristic of these diseases of pregnancy. Unfortunately, insulin therapy in women with GDM*d* seems not to be enough to reverse this disease-increased L-arginine transport and NO synthesis in HUVECs [6]. Furthermore, the protein abundance and mRNA expression of hCAT-1 and eNOS, and mitogen-activated 44 and 42 kDa protein kinases (p44/42^{mapk}) activation were equally increased in GDM*d* and GDM*i* compared with cells from normal pregnancies. These alterations are proposed to play a role in the impaired insulin signalling pathway seen in this disease [37, 42]. In addition to the effect of maternal hyperglycaemia in GDM, foetal hyperglycaemia and hyperinsulinemia in this disease may be responsible for endothelial dysfunction, abnormal development of the placenta and foetal growth [42]. However, further studies are required to fully understand the potential involvement of insulin therapy in this phenomenon.

3.2.3. Antioxidants

Placentas from GDM*i* show higher antioxidant gene expression and are less responsive to an oxidative environment than placentas from normal pregnancies [43]. Placentas for normal pregnancies react to hypoxanthine/xanthine oxidase (HX/XO) system by increasing the release of several cytokines and the mRNA expression of tumour necrosis factor α (*TNFα*), interleukin 6 (*IL6*) or 8 (*IL8*) genes and reducing catalase (*CAT*) and glutathione peroxidase (*GPX*) genes. In GDM*i*, activation of the HX/XO system increased the level of interleukin 1β (IL-1β), macrophage inflammatory protein 1β (MIP1β), and TNFα but did not alter the expression of antioxidant genes. Interestingly, a blunted response to oxidative stress in placenta tissue from women with GDM*i* is suggested to result from enhanced gene expression of antioxidant enzymes [43]. Thus, GDM*i* is a condition where the placenta shows a reduced capacity to respond to the oxidative stress compared with placentas from normal pregnancies. In another study in HUVECs from GDM pregnancies an impaired nuclear factor erythroid 2-related factor 2 (Nrf2)–mediated antioxidant gene expression was reported [44]. Thus, HUVECs from GDM would be less protected against oxidative stress. Unfortunately, the latter report included only five women with GDM and not specification addressing whether women were treated with diet, insulin, or both was indicated.

It is also reported that placentas from GDM*d* exhibit increased mRNA expression of the markers of macrophages infiltration cluster of differentiation 68 and 14, and mucin-like hormone receptor 1 in parallel with increased mRNA expression of the pro-inflammatory factors interleukin 6 (IL-6), Toll-like receptor 4, and transforming growth factor β (TGF-β) compared with normal pregnancies [45]. Thus, GDM*d* is a pathology that associates with an increase of inflammation in the placenta. Unfortunately, there are no studies addressing whether GDM*i* is a condition coursing with increased infiltration of inflammatory immune cells in the human placenta.

3.2.4. Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are integral regulators of placental development, trophoblast invasion, implantation, and decidualization and play a role in the pathophysiology of diabetes mellitus [46]. Placental tissue from GDM*i* was associated with downregulated expression of placental PPARα, PPARγ, and retinoid X receptor α (RXRα) [46, 47]. It is described that the activity of the transcription factor nuclear factor κB (NF-κB) is downregulated by PPARs, particularly PPARγ [48]. Thus, a deficiency of PPARs in placentas from women with GDM*i* [46, 47] could promote the development of this pathological phenotype likely due to impaired PPARγ-mediated trans-repression of NF-kB activity leading to increased gene transcription of proinflammatory mediators. In a study using placental tissue from GDM the NF-κB activity seems reduced [49]. Unfortunately, not a distinction between GDM*i* from GDM*d* was done. Also, there is no information addressing whether GDM*i* results in a similar phenomenon for NF-κB expression and activity and other factors involved in inflammation.

3.2.5. Lipids

Long-chain polyunsaturated fatty acids (PUFA-LC) and docosahexaenoic acid (DHA) are critical for the development of the foetal central nervous system [50, 51]. Some studies indicate that women with GDM*d* have a normal content of PUFA-LC in plasma lipids [51]. However, a lower PUFA-LC and higher DHA level in the plasma from mothers with GDM*d* is also reported [51]. Also, the plasma DHA concentration is lower in women with GDM*i* compared with normal pregnancies and lower in the umbilical vein blood in GDM*i* compared with GDM*d* [50]. One possible explanation for this phenomenon is that reduced DHA concentration in the umbilical blood may due to its lower maternal-*to*-foetal transfer in GDM, a phenomenon that may be emphasised with maternal insulin therapy. It is proposed that foetal insulin generation in response to severe foetal and maternal hyperglycaemia in GDM facilitates the accumulation of fatty acids in the foetal adipose tissue leading to reduced DHA content in the umbilical cord blood [50]. However, mechanisms other than a reduced placental transfer to the foetal circulation of DHA, which could also be independent of insulin, should be considered. Unfortunately, the mechanisms behind the effectiveness of maternal insulin therapy in reducing the plasma level of fatty acids in GDM*i* are not yet explicitly addressed.

4. The foetus and newborn from insulin therapy

It is reported that morphometric characteristics of the newborns (including birth weight, size, skinfold thickness triceps, skinfold thickness subscapular) from women with GDM*d* and GDM*i* are similar [19]. Since both the mothers and newborns in the latter study were with normal glycaemia at delivery, the possibility that an effective control of the maternal glycaemia may not protect the foetus from the potential GDM-associated harmful effects arises (see Table 1, Fig. 1). In a more recent report no differences in gestational age, birth weight, birth size, head/chest perimeter, Apgar scores for newborns, and women blood glycated HbA_{1c} level from GDM*i* and GDM*d* was shown [52]. These findings are reinforced by now several reports indicating that diet in women with GDM is comparable with other protocols such as insulin therapy or oral anti-diabetic pharmacological drugs [4]. The impact of the requirement of insulin in GDM is variable. To date, in a group of women with GDM from Australia, the newborns to GDM*i* show higher birthweight than those from GDM*d* but no differences in the need for caesareans between these two groups were seen [53]. Also, there were no differences in the Apgar score or the requirement for admission to neonatal intensive care unit between these groups. Another report in a group of women with GDM from Japan shows that the incidence of large for gestational age (LGA) newborns is reduced in GDM*i* compared with GDM*d* [54]. Thus, it is noticeable that beyond the vast beneficial effects of insulin management of GDM pregnancy it is an approach that could also results in altered newborn birth weight. Interestingly, insulin 'intensified treatment' of women with GDM (i.e., diet plus three preprandial doses of regular insulin and one dose of intermediate insulin at bedtime) compared with regular treatment (i.e., diet plus two doses of regular insulin per day) resulted in a better glycaemic control (HbA_{1c} ~5.5 versus 5.8%, respectively) and a significant reduction in the neonatal morbidity and hypoglycaemia risk [54]. However, no differences were observed in macrosomia, Apgar score, obstetrical trauma, caesarean rate, or IUGR. Thus, the consequences of insulin therapy on the foetus and the mother seems highly dependent on the protocols and type of insulin depending on the pregnant women physiological and immediate environment conditions or behaviour (v.g., diet, exercise, lifestyle) [4]. Also, it is conceivable that ethnicity may play a pivotal role in the response to the insulin treatment of women with GDM.

5. Concluding remarks

The primary objective of passing women with GDM*d* to insulin therapy is to reach maternal normoglycaemia, thus avoiding potential risks of maternal disturbed D-glucose metabolism to the growing foetus [1, 9, 10]. Normalising the maternal glycaemia may result in a better outcome in its immediate perinatal period [4]. Surprisingly, even when glycaemia at delivery is normal in newborns to GDM*d* or GDM*i* several metabolic alterations at the foetoplacental function are present at this stage [3, 6, 42]. Beyond reaching maternal normoglycaemia in response to insulin therapy, this approach reduces the incidence of macrosomia and caesarean sections (Fig. 1). Insulin therapy in GDM is also an approach that shows associated modifications in the mother health or labour and placenta structure and functional properties. Endothelial dysfunction of the foetoplacental vasculature, as the most characterised phenomenon in GDM pregnancies, is not resolved by the insulin therapy [6]. However, further demonstration of a consequence to the newborn, childhood or adulthood is waiting to be addressed. Insulin therapy also normalises the glycaemia and reduces the risk of newborn hypoglycaemia. It is known that an altered intrauterine environment, as seen in GDM [42, 55], have consequences in adulthood where the most classical diseases including hypertension, obesity, insulin resistance, metabolic syndrome, are manifested. Based on the described studies summarised in this review, insulin therapy along with normalising the maternal and foetal glycaemia is an approach that is beneficial to reduce an adverse outcome in GDM; however, this approach has been shown to cause alterations to the foetoplacental unit that may affect the foetus wellbeing and newborn health. Despite the above-mentioned effects of insulin therapy, it is an approach that seems to be not detrimental for the mother and newborn health and similar to the use of diet in GDM as acutely reviewed in a recent series of Cochrane Database of Systematic Reviews [4]. We highlight the fact that insulin therapy is a beneficial approach for a wellbeing of the growing foetus and the newborn health, and possibly their adulthood and descendants. However, since the reported alterations in the foetoplacental structure and function following this approach compared with diet or with oral-pharmacological agents approaches, the possibility of potential adverse effects beyond the normalization of the maternal and foetal glycaemia in a GDM pregnancy should be considered. It is now clearer that local guidelines for treating GDM and other factors such as socio-economical differences between different populations and life style, among others, could be determinant in the outcome for the effects of insulin treatment of women with GDM. Despite the intrinsic value of treating patients with GDM with insulin as finely and critically described [4], it is highlighted the fact that local realities may be underestimated when defining the conditions determining the use of insulin in groups of patients with GDM. We suggest considering with caution the reported literature regarding the potential effects of insulin therapy in GDM since most of the studies make an unclear or definitively did not separate women treated with diet only from insulin treatment. Also, some studies did not separate the reported data on GDM from pre-gestational diabetes mellitus [4]. It seems clearer now that one of the main gaps in knowledge are insulin versus life style therapies, and insulin and other pharmacological interventions showed no differences regarding neonatal health [4], despite changes reported in placental vasculature due to GDM not being resolved [6]. Whether these potential adverse effects are of significance will have to wait for a better documentation in the literature with appropriate and significant population studies as for now the available evidence is not conclusive [4]. Finally, we must emphasize the possibility that that the lack of any long-term studies precludes for definitive conclusions that the alterations in the placenta and umbilical vessels could impact the health of mothers and their children in the long run.

Conflict of interest

The authors confirm that there are no conflicts of interest.

Sources of funding

This work was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) [grant number 1150377], Chile, and Servicio de Salud de Medicina Oriente, Hospital San Juan de Dios [grant number Res. 1938–2016], Chile. MS and LSi hold Comisión Nacional para la Investigación en Ciencia y Tecnología (CONICYT) and Vicerrectorate of Research and School of Medicine, PUC (Chile)-PhD fellowships. LSi holds PhD fellowship from The Abel Tasman Talent Program and University Medical Center Groningen (UMCG) (The Netherlands).

Disclosures

None.

Transparency document

The http://dx.doi.org/10.1016/j.bbadis.2018.06.005 associated with this article can be found, in online version.

References

[1] American Diabetes Association, Standards of medical care in diabetes–2017:

summary of revisions, Diabetes Care 40 (2017) 4–5, http://dx.doi.org/10.2337/dc17-S005.
[2] R. Wagner, L. Fritsche, M. Heni, E. Fehlert, N. Stefan, H. Staiger, H.U. Häring, A. Fritsche, A novel insulin sensitivity index particularly suitable to measure insulin sensitivity during gestation, Acta Diabetol. 53 (2016) 1037–1044, http://dx.doi.org/10.1007/s00592-016-0930-5.
[3] L. Sobrevia, R. Salsoso, T. Sáez, C. Sanhueza, F. Pardo, A. Leiva, Insulin therapy and fetoplacental vascular function in gestational diabetes mellitus, Exp. Physiol. 100 (2015) 231–238, http://dx.doi.org/10.1113/expphysiol.2014.082743.
[4] J. Brown, L. Grzeskowiak, K. Williamson, M.R. Downie, C.A. Crowther, Insulin for the treatment of women with gestational diabetes, Cochrane Database Syst. Rev. 2017 (2017) CD012037, , http://dx.doi.org/10.1002/14651858.CD012037.pub2.
[5] K. Mayo, N. Melamed, H. Vandenberghe, H. Berger, The impact of adoption of the International Association of Diabetes in pregnancy study group criteria for the screening and diagnosis of gestational diabetes, Am. J. Obstet. Gynecol. 212 (2015) 224 (e1-224.e9), https://doi.org/10.1016/j.ajog.2014.08.027.
[6] M. Subiabre, L. Silva, R. Villalobos-Labra, F. Toledo, M. Paublo, M.A. López, R. Salsoso, F. Pardo, A. Leiva, L. Sobrevia, Maternal insulin therapy does not restore foetoplacental endothelial dysfunction in gestational diabetes mellitus, Biochim. Biophys. Acta Mol. basis Dis. 1863 (2017) 2987–2998, http://dx.doi.org/10.1016/j.bbadis.2017.07.022.
[7] A. Bhattacharyya, S. Brown, S. Hughes, P.A. Vice, Insulin lispro and regular insulin in pregnancy, Q. J. Med. 94 (2001) 255–260, http://dx.doi.org/10.1093/qjmed/94.5.255.
[8] I.B. Hirsch, Insulin analogues, N. Engl. J. Med. 352 (2005) 174–183, http://dx.doi.org/10.1056/NEJMra040832.
[9] C. Kim, Gestational diabetes: risks, management, and treatment options, Int. J. Womens Health 2 (2010) 339–351, http://dx.doi.org/10.2147/IJWH.S13333.
[10] Committee on Practice Bulletins—Obstetrics, Practice bulletin no. 180, Obstet. Gynecol. 130 (2017) 17–37, http://dx.doi.org/10.1097/AOG.0000000000002159.
[11] American Diabetes Association, Classification and diagnosis of diabetes, Diabetes Care 40 (2017) S11–S24, http://dx.doi.org/10.2337/dc17-S005.
[12] O. Langer, D.L. Conway, M.D. Berkus, E.M.-J. Xenakis, O. Gonzales, A comparison of glyburide and insulin in women with gestational diabetes mellitus, N. Engl. J. Med. 343 (2000) 1134–1138, http://dx.doi.org/10.1056/NEJM200010193431601.
[13] P.R. Olmos, G.R. Borzone, R.I. Olmos, C.N. Valencia, F.A. Bravo, M.I. Hodgson, C.G. Belmar, J.A. Poblete, M.O. Escalona, B. Gómez, Gestational diabetes and pre-pregnancy overweight: possible factors involved in newborn macrosomia, J. Obstet. Gynaecol. Res. 38 (2012) 208–214, http://dx.doi.org/10.1111/j.1447-0756.2011.01681.x.
[14] O. Vérier-Mine, Outcomes in women with a history of gestational diabetes. Screening and prevention of type 2 diabetes. Literature review, Diabete Metab. 36 (2010) 595–616, http://dx.doi.org/10.1016/j.diabet.2010.11.011.
[15] T. Sáez, P. de Vos, L. Sobrevia, M.M. Faas, Is there a role for exosomes in foetoplacental endothelial dysfunction in gestational diabetes mellitus? Placenta 61 (2018) 48–54, http://dx.doi.org/10.1016/j.placenta.2017.11.007.
[16] D.R. Coustan, S.B. Lewis, L.S.B., Insulin therapy for gestational diabetes, Obstet. Gynecol. 51 (1978) 306–310, http://dx.doi.org/10.1097/00006250-197803000-00010.
[17] D.J. Thompson, K.B. Porter, D.J. Gunnells, P.C. Wagner, J.A. Spinnato, Prophylactic insulin in the management of gestational diabetes, Obstet. Gynecol. 75 (1990) 960–964, http://dx.doi.org/10.1016/0020-7292(91)90240-6.
[18] H. Ijäs, M. Vääräsmäki, L. Morin-Papunen, R. Keravuo, T. Ebeling, T. Saarela, T. Raudaskoski, Metformin should be considered in the treatment of gestational diabetes: a prospective randomised study, Br. J. Obstet. Gynaecol. 118 (2011) 880–885, http://dx.doi.org/10.1111/j.1471-0528.2010.02763.x.
[19] Y. Min, C. Lowy, K. Ghebremeskel, B. Thomas, D. Bitsanis, M.A. Crawford, Fetal erythrocyte membrane lipids modification: preliminary observation of an early sign of compromised insulin sensitivity in offspring of gestational diabetic women, Diabet. Med. 22 (2005) 914–920, http://dx.doi.org/10.1111/j.1464-5491.2005.01556.x.
[20] G. Di Cianni, L. Volpe, A. Ghio, C. Lencioni, I. Cuccuru, L. Benzi, S. Del Prato, Maternal metabolic control and perinatal outcome in women with gestational diabetes mellitus treated with lispro or aspart insulin: comparison with regular insulin, Diabetes Care 30 (2007) e11, http://dx.doi.org/10.2337/dc06-2586.
[21] L. Jovanovic, S. Ilic, D.J. Pettitt, K. Hugo, M. Gutierrez, R.R. Bowsher, E.J. Bastyr, Metabolic and immunologic effects of insulin lispro in gestational diabetes, Diabetes Care 22 (1999) 1422–1427, http://dx.doi.org/10.2337/diacare.22.9.1422.
[22] C.P. Spaulonci, L.S. Bernardes, T.C. Trindade, M. Zugaib, R.P.V. Francisco, Randomized trial of metformin vs insulin in the management of gestational diabetes, Am. J. Obstet. Gynecol. 209 (2013) 34.e1–34.e7, http://dx.doi.org/10.1016/j.ajog.2013.03.022.
[23] M.A. Hickman, R. McBride, K.A. Boggess, R. Strauss, Metformin compared with insulin in the treatment of pregnant women with overt diabetes: a randomized controlled trial, Am. J. Perinatol. 30 (2013) 483–489, http://dx.doi.org/10.1055/s-0032-1326994.
[24] M. Pöyhönen-Alho, K. Teramo, R. Kaaja, Treatment of gestational diabetes with short- or long-acting insulin and neonatal outcome: a pilot study, Acta Obstet. Gynecol. Scand. 81 (2002) 258–259, http://dx.doi.org/10.1034/j.1600-0412.2002.810312.x.
[25] K.Y. Lain, M.J. Garabedian, A. Daftary, A. Jeyabalan, Neonatal adiposity following maternal treatment of gestational diabetes with glyburide compared with insulin, Am. J. Obstet. Gynecol. 200 (2009) 501.e1–501.e6, http://dx.doi.org/10.1016/j.ajog.2009.02.038.
[26] F. Pardo, L. Silva, T. Sáez, R. Salsoso, J. Gutiérrez, C. Sanhueza, A. Leiva, L. Sobrevia, Human supraphysiological gestational weight gain and fetoplacental vascular dysfunction, Int. J. Obes. 39 (2015) 1264–1273, http://dx.doi.org/10.1038/ijo.2015.57.
[27] A. Hassan, T.M. Essa, Ultrastructure of the placenta in gestational diabetes mellitus, Anatomy 10 (2016) 159–169, http://dx.doi.org/10.2399/ana.16.019.
[28] M.O. Babawale, S. Lovat, T.M. Mayhew, M.J. Lammiman, D.K. James, L. Leach, Effects of gestational diabetes on junctional adhesion molecules in human term placental vasculature, Diabetologia 43 (2000) 1185–1196, http://dx.doi.org/10.1007/s001250051511.
[29] P. Jarmuzek, M. Wielgos, D. Bomba-Opon, Placental pathologic changes in gestational diabetes mellitus, Neuro. Endocrinol. Lett. 36 (2015) 101–105.
[30] G. Desoye, H.H. Hofmann, P.A.M. Weiss, Insulin binding to trophoblast plasma membranes and placental glycogen content in well-controlled gestational diabetic women treated with diet or insulin, in well-controlled overt diabetic patients and in healthy control subjects, Diabetologia 35 (1992) 45–55, http://dx.doi.org/10.1007/BF00400851.
[31] Q. Wang, R. Huang, B. Yu, F. Cao, H. Wang, M. Zhang, X. Wang, B. Zhang, H. Zhou, Z. Zhu, Higher fetal insulin resistance in Chinese pregnant women with gestational diabetes mellitus and correlation with maternal insulin resistance, PLoS One 8 (2013) e59845, , http://dx.doi.org/10.1371/journal.pone.0059845.
[32] M. Colomiere, M. Permezel, C. Riley, G. Desoye, M. Lappas, Defective insulin signaling in placenta from pregnancies complicated by gestational diabetes mellitus, Eur. J. Endocrinol. 160 (2009) 567–578, http://dx.doi.org/10.1530/EJE-09-0031.
[33] C.M. Rondinone, L.M. Wang, P. Lonnroth, C. Wesslau, J.H. Pierce, U. Smith, Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 4171–4175, http://dx.doi.org/10.1073/pnas.94.8.4171.
[34] F. Westermeier, C. Salomón, M. Farías, P. Arroyo, B. Fuenzalida, T. Sáez, R. Salsoso, C. Sanhueza, E. Guzmán-Gutiérrez, F. Pardo, A. Leiva, L. Sobrevia, Insulin requires normal expression and signaling of insulin receptor A to reverse gestational diabetes-reduced adenosine transport in human umbilical vein endothelium, FASEB J. 29 (2015) 37–49, http://dx.doi.org/10.1096/fj.14-254219.
[35] E. Guzmán-Gutiérrez, A. Armella, F. Toledo, F. Pardo, A. Leiva, L. Sobrevia, Insulin requires A_1 adenosine receptors expression to reverse gestational diabetes-increased L-arginine transport in human umbilical vein endothelium, Purinergic Signal 12 (2016) 175–190, http://dx.doi.org/10.1007/s11302-015-9491-2.
[36] S.H. Ong, Y.R. Hadari, N. Gotoh, G.R. Guy, J. Schlessinger, I. Lax, Stimulation of phosphatidylinositol 3-kinase by fibroblast growth factor receptors is mediated by coordinated recruitment of multiple docking proteins, Proc. Natl. Acad. Sci. U. S. A. (2001) 6074–6079, http://dx.doi.org/10.1073/pnas.111114298.
[37] R. Villalobos-Labra, L. Silva, M. Subiabre, J. Araos, R. Salsoso, B. Fuenzalida, T. Sáez, F. Toledo, M. González, C. Quezada, F. Pardo, D.I. Chiarello, A. Leiva, L. Sobrevia, Akt/mTOR role in human foetoplacental vascular insulin resistance in diseases of pregnancy, J. Diabetes. Res. 2017 (2017) 5947859, http://dx.doi.org/10.1155/2017/5947859.
[38] D.T. Osmond, R.G. King, S.P. Brennecke, N.M. Gude, Placental glucose transport and utilisation is altered at term in insulin-treated, gestational-diabetic patients, Diabetologia 44 (2001) 1133–1139, http://dx.doi.org/10.1007/s001250100609.
[39] K. Gaither, A.N. Quraishi, N.P. Illsley, Diabetes alters the expression and activity of the human placental GLUT1 glucose transporter, J. Clin. Endocrinol. Metab. 84 (1999) 695–701, http://dx.doi.org/10.1210/jcem.84.2.5438.
[40] Y. Arancibia-Garavilla, F. Toledo, P. Casanello, L. Sobrevia, Nitric oxide synthesis requires activity of the cationic and neutral amino acid transport system y+L in human umbilical vein endothelium, Exp. Physiol. 88 (2003) 699–710, http://dx.doi.org/10.1113/eph8802647.
[41] M.A. Ramírez, J. Morales, M. Cornejo, E.H. Blanco, E. Mancilla-Sierpe, F. Toledo, A.R. Beltrán, L. Sobrevia, Intracellular acidification reduces L-arginine transport via system y+L but not via system y+/CATs and nitric oxide synthase activity in human umbilical vein endothelial cells, Biochim. Biophys. Acta 1864 (2018) 1192–1202, http://dx.doi.org/10.1016/j.bbadis.2018.01.032.
[42] L. Silva, M. Subiabre, J. Araos, T. Sáez, R. Salsoso, F. Pardo, A. Leiva, R. San Martín, F. Toledo, L. Sobrevia, Insulin/adenosine axis linked signalling, Mol. Asp. Med. 55 (2017) 45–61, http://dx.doi.org/10.1016/j.mam.2016.11.002.
[43] M. Lappas, A. Mittion, M. Permezel, In response to oxidative stress, the expression of inflammatory cytokines and antioxidant enzymes are impaired in placenta, but not adipose tissue, of women with gestational diabetes, J. Endocrinol. 204 (2010) 75–84, http://dx.doi.org/10.1677/JOE-09-0321.
[44] X. Cheng, S.J. Chapple, B. Patel, W. Puszyk, D. Sugden, X. Yin, M. Mayr, R.C. Siow, G.E. Mann, Gestational diabetes mellitus impairs Nrf2-mediated adaptive antioxidant defenses and redox signaling in fetal endothelial cells in utero, Diabetes 62 (2013) 4088–4097, http://dx.doi.org/10.2337/db13-0169.
[45] I. Mrizak, O. Grissa, B. Henault, M. Fekih, A. Bouslema, I. Boumaiza, M. Zaouali, Z. Tabka, N.A. Khan, Placental infiltration of inflammatory markers in gestational diabetic women, Gen. Physiol. Biophys. 33 (2014) 169–176, http://dx.doi.org/10.4149/gpb_2013075.
[46] S.J. Holdsworth-Carson, R. Lim, A. Mitton, C. Whitehead, G.E. Rice, M. Permezel, M. Lappas, Peroxisome proliferator-activated receptors are altered in pathologies of the human placenta: gestational diabetes mellitus, intrauterine growth restriction and preeclampsia, Placenta 31 (2010) 222–229, http://dx.doi.org/10.1016/j.placenta.2009.12.009.
[47] M. Lappas, Effect of pre-existing maternal obesity, gestational diabetes and adipokines on the expression of genes involved in lipid metabolism in adipose tissue, Metabolism 63 (2014) 250–262, http://dx.doi.org/10.1016/j.metabol.2013.10.001.
[48] S.J. Bensinger, P. Tontonoz, Integration of metabolism and inflammation by lipid-activated nuclear receptors, Nature 454 (2008) 470–477, http://dx.doi.org/10.

1038/nature07202.
[49] M.T. Coughlan, M. Permezel, H.M. Georgiou, G.E. Rice, Repression of oxidant-induced nuclear factor-κB activity mediates placental cytokine responses in gestational diabetes, J. Clin. Endocrinol. Metab. 89 (2004) 3585–3594, http://dx.doi.org/10.1210/jc.2003-031953.
[50] A. Pagán, M.T. Prieto-Sánchez, J.E. Blanco-Carnero, A. Gil-Sánchez, J.J. Parrilla, H. Demmelmair, B. Koletzko, E. Larqué, Materno-fetal transfer of docosahexaenoic acid is impaired by gestational diabetes mellitus, Am. J. Physiol. Endocrinol. Metab. 305 (2013) E826–E833, http://dx.doi.org/10.1152/ajpendo.00291.2013.
[51] V. Wijendran, R.B. Bendel, S.C. Couch, E.H. Philipson, K. Thomsen, X. Zhang, C.J. Lammi-Keefe, Maternal plasma phospholipid polyunsaturated fatty acids in pregnancy with and without gestational diabetes mellitus: relations with maternal factors, Am. J. Clin. Nutr. 70 (1999) 53–61, http://dx.doi.org/10.1093/ajcn/70.1.53.
[52] B. Persson, M. Stangenberg, U. Hansson, E. Nordlander, Gestational diabetes mellitus (GDM). Comparative evaluation of two treatment regimens, diet versus insulin and diet, Diabetes 34 (1985) 101–105, http://dx.doi.org/10.2337/diab.34.2.S101.
[53] T. Wong, G.P. Ross, B.B. Jalaludin, J.R. Flack, The clinical significance of overt diabetes in pregnancy, Diabet. Med. 30 (2013) 468–474, http://dx.doi.org/10.1111/dme.12110.
[54] Y. Ito, M. Shibuya, S. Hosokawa, Y. Motoki, R. Nagata, H. Konishi, T. Miyazaki, T. Matsunaga, Y. Nomura, T. Mihara, S. Ito, K. Sugiura, Y. Terauchi, Indicators of the need for insulin treatment and the effect of treatment for gestational diabetes on pregnancy outcomes in Japan, Endocr. J. 63 (2016) 231–237, http://dx.doi.org/10.1507/endocrj.EJ15-0427.
[55] B. Weisz, A. Shrim, C.J. Homko, E. Schiff, G.S. Epstein, E. Sivan, One hour versus two hours postprandial glucose measurement in gestational diabetes: a prospective study, J. Perinatol. 25 (2005) 241–244, http://dx.doi.org/10.1038/sj.jp.7211243.
[56] S. Niromanesh, A. Alavi, F.R. Sharbaf, N. Amjadi, S. Moosavi, S. Akbari, Metformin compared with insulin in the management of gestational diabetes mellitus: a randomized clinical trial, Diabetes Res. Clin. Pract. 98 (2012) 422–429, http://dx.doi.org/10.1016/j.diabres.2012.09.031.
[57] E. Mesdaghinia, M. Samimi, Z. Homaei, F. Saberi, S.G.A. Moosavi, M. Yaribakht, Comparison of newborn outcomes in women with gestational diabetes mellitus treated with metformin or insulin: a randomised blinded trial, Int, J. Prev. Med. 4 (2013) 327–333, http://dx.doi.org/10.1089/dia.2012.1221.
[58] S. Ruholamin, S. Eshaghian, Z. Allame, Neonatal outcomes in women with gestational diabetes mellitus treated with metformin in compare with insulin: a randomized clinical trial, J. Res. Med. Sci. 19 (2014) 970–975.
[59] D.T.D. Osmond, C.J. Nolan, R.G. King, S.P. Brennecke, N.M. Gude, Effects of gestational diabetes on human placental glucose uptake, transfer, and utilisation, Diabetologia 43 (2000) 576–582, http://dx.doi.org/10.1007/s001250051.
[60] E.M. Horváth, R. Magenheim, E. Kugler, G. Vácz, A. Szigethy, F. Lévárdi, M. Kollai, C. Szabo, Z. Lacza, C. Szabo, Nitrative stress and poly(ADP-ribose) polymerase activation in healthy and gestational diabetic pregnancies, Diabetologia 52 (2009) 1935–1943, http://dx.doi.org/10.1007/s00125-009-1435-3.