REVIEW

Molecular pathways disrupted by gestational diabetes mellitus

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Abstract

Gestational diabetes mellitus (GDM) imposes serious short- and long-term health problems for mother and baby. An effective therapeutic that can reduce the incidence of GDM and improve long-term maternal and fetal outcomes is a major research priority, crucially important for public health. A lack of knowledge about the underlying pathophysiology of GDM has hampered the development of such therapeutics. What we do know, however, is that maternal insulin resistance, low-grade inflammation and endothelial cell dysfunction are three central features of pregnancies complicated by GDM. Indeed, data generated over the past decade have implicated a number of candidate regulators of insulin resistance, inflammation and endothelial cell dysfunction in placenta, maternal adipose tissue and skeletal muscle. These include nuclear factor- κ B (NF- κ B), peroxisome proliferator-activated receptors (PPARs), sirtuins (SIRTs), 5' AMP-activated protein kinase (AMPK), glycogen synthase kinase 3 (GSK3), PI3K/mTOR, inflammasome and endoplasmic reticulum (ER) stress. In this review, the identification of these as key modulators of GDM will be discussed. The biochemical pathways involved in the formation of these may represent potential sites for intervention that may translate to therapeutic interventions to prevent the development of GDM.

Key Words

- GDM
- pathophysiology
- inflammation
- placenta
- transcription factors

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Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance of variable severity with first recognition during pregnancy (HAPO Study Cooperative Research Group *et al.* 2008). GDM is the most common pregnancy complication, affecting up to 20% of all pregnancies (Xiong *et al.* 2001, Ferrara 2007, Reece *et al.* 2009, Sunton & Panichkul 2015). The prevalence is greater amongst indigenous Australians (Ishak & Petocz 2003) and in women whose native country is China or India

compared to those from a Caucasian background (Beischer *et al.* 1991).

GDM is a multifactorial disease. Risk factors include family history of type 2 diabetes and GDM (Solomon *et al.* 1997, Cedergren 2004, Setji *et al.* 2005), and having polycystic ovarian syndrome (PCOS) (Lo *et al.* 2006, Norman *et al.* 2007). Furthermore, the prevalence of GDM is growing with the obesity epidemic (Solomon *et al.* 1997, Kim *et al.* 2013b) and the older maternal age trend

that is characterised in developed countries (Solomon *et al.* 1997, Wolf *et al.* 2004). Both maternal pre-pregnancy weight (Chu *et al.* 2007) and maternal weight gain during pregnancy have a strong correlation with GDM (Hedderson *et al.* 2010, Gibson *et al.* 2012). Therefore, unsurprisingly, eating a high-fat or low-fibre and high-glycaemic index diet (Montonen *et al.* 2003, Zhang *et al.* 2006a) and having a sedentary lifestyle (Weissgerber *et al.* 2006) adds to the risk of GDM.

There are many short- and long-term complications associated to GDM for the mother and child (Dabelea *et al.* 2000). In the short term, GDM women are at a greater risk of hypertension induced by pregnancy, and even preeclampsia in severe cases (Xiong *et al.* 2001, HAPO Study Cooperative Research Group *et al.* 2008). Hyperglycaemia during late gestation is associated with macrosomia (or large-for-gestational age; greater than 90th percentile) (Langer *et al.* 2005). The Hyperglycemia Adverse Pregnancy Outcome (HAPO) Study indicated that even mild cases of hyperglycaemia increase the risk of macrosomia (HAPO Study Cooperative Research Group *et al.* 2008). Macrosomia is a surrogate risk for other complications, such as shoulder dystocia, induced birth and delivery by Caesarean section (Langer *et al.* 2005). Babies who are not macrosomic tend to have greater adiposity when adjusted for gestational age (Catalano *et al.* 2003b) which indicates metabolic dysfunction *in utero*. Other neonatal complications include hypoglycaemia, hyperbilirubinaemia, hypocalcaemia, and infant polycythaemia (Langer *et al.* 2005).

Long-term complications in both mother and baby include type 2 diabetes (Kim *et al.* 2002, Catalano *et al.* 2003a, Lee *et al.* 2007, Bellamy *et al.* 2009), obesity (Catalano *et al.* 2003a), cardiovascular disease (CVD) (Catalano *et al.* 2003a) and some cancers later in life (Perrin *et al.* 2007, 2008, Wu *et al.* 2012, Tong *et al.* 2014). Additionally, although fasting plasma glucose concentrations returns to the normal range (Ryan *et al.* 1985), the mother is more likely to experience recurrent GDM during the following pregnancy (Bottalico 2007). Depending on the population and the diagnostic criteria used, the risk of type 2 diabetes mellitus (T2DM) in women with GDM after 1 year can vary from 3 to 38% (Metzger *et al.* 1985, Lam *et al.* 1991, Lee *et al.* 2007). These health impacts of GDM present a significant challenge to finite healthcare resources. The current cost of caring for women with GDM is 34% greater than for healthy women during pregnancy or an estimated extra US\$1.3 billion per year (Gillespie *et al.* 2013). Exacerbating the problem are additional ongoing higher healthcare costs for mothers

who had GDM, which average 20% more 2–5 years after pregnancy (Gillespie *et al.* 2013). A better understanding of the pathophysiology of GDM is required in order to develop more effective intervention that can prevent GDM and thereby reduce its burden.

Pathophysiology of GDM

There are several distinct features of GDM pathophysiology including peripheral maternal insulin resistance, inflammation, and placental and endothelial dysfunction. These features will be discussed in depth in the following sections.

Maternal insulin resistance

During normal pregnancy, there is a progressive decline in insulin sensitivity, elevating the circulating levels of fatty acids and glucose to meet the increased energy demands of the developing fetus (Binder *et al.* 2015, Law & Zhang 2017). Progressive insulin resistance can occur at the start of the second trimester of pregnancy, and by the third trimester, glucose utilisation is demonstrated to decline 40–60% depending on BMI (Catalano *et al.* 1991, Sivan *et al.* 1997). Longitudinal studies have indicated that the maternal depots reduce fat accrual is insensitive to changes in circulating leptin levels (Highman *et al.* 1998), while lipid and glucose metabolism is hindered during late pregnancy (Herrera 2000). Pregnancy also causes hypertriglyceridaemia since plasma-free fatty acids become the maternal supplementary energy source, particularly during late pregnancy (Silliman *et al.* 1994, Sattar *et al.* 1997, Hubel *et al.* 1998, Brizzi *et al.* 1999). Nonetheless, euglycaemia is achieved as pancreatic β -cells compensate by hypertrophy and hyperplasia to produce more insulin (Assche *et al.* 1978, Ryan *et al.* 1985). The supply of glucose and amino acids are prioritised for the growing fetus (Barbour *et al.* 2004). However, in GDM, β -cell function is reduced by 30–70%, indicating that β -cells are unable to compensate for the increase in insulin resistance, resulting in the development of GDM (Xiang *et al.* 1999, Lain & Catalano 2007, Baeyens *et al.* 2016). Insulin resistance associated with GDM is further exacerbated by approximately 56% due to defective insulin signalling in adipose tissue and skeletal muscle (Catalano *et al.* 1991, Colomiere *et al.* 2009). GDM patients exhibit lower protein expression and tyrosine phosphorylation of the insulin receptor (IR)- β and insulin receptor substrate (IRS)-1 in adipose tissue and skeletal muscle compared to pregnant women with normal

glucose tolerance (NGT) (Shao *et al.* 2002, Colomiere *et al.* 2009). However, women with GDM also display a greater proportion of serine phosphorylated IRS-1 (Shao *et al.* 2002, Barbour *et al.* 2006). Phosphorylation of IRS-1 at the serine residue impairs its ability to bind to phosphoinositide 3 kinase (PI3K) (Cheatham *et al.* 1994, Aguirre *et al.* 2002) and inhibits the tyrosine kinase activity of IR- β (Aguirre *et al.* 2002). Transgenic mouse studies imply that IRS-1 serine phosphorylation may play a role in the development of insulin resistance (Morino *et al.* 2008). The exact mechanism responsible for insulin resistance in pregnancy is yet to be fully elucidated. Nonetheless, the temporal changes of pregnancy hormones and cytokines are thought to be related to the shift in metabolism. The increase in progesterone level was proposed to have an impact on maternal carbohydrate metabolism (Branisteanu & Mathieu 2003). Wada *et al.* have demonstrated that progesterone can inhibit glucose uptake in 3T3-L1 adipocytes by reducing the expression of IRS-1 which suppresses the PI-3K mediated pathway (Wada *et al.* 2010). Furthermore, knockout of the progesterone receptors in mice improved glucose tolerance as there was greater number of β -cells (Picard *et al.* 2002). Similarly, human placental growth hormone can also trigger insulin resistance in skeletal muscle (Barbour *et al.* 2004). However, Catalano *et al.* (2002) reported no differences in plasma progesterone levels in GDM women compared to NGT pregnant women. On the other hand, discrepancies exist on human placental lactogen profile in GDM pregnancies where the human placental lactogen level was reported to be normal (Beck *et al.* 1965, Catalano *et al.* 2002, Barbour 2003, Endo *et al.* 2006), higher (Saxena *et al.* 1969, Singer 1970, Selenkow *et al.* 1971) or lower (Spellacy *et al.* 1971) compared to normal pregnant controls. Emerging evidence, however, now implicates a more important role for pro-inflammatory cytokines released from placenta and maternal adipose tissue in inducing insulin resistance. The role of inflammation in GDM pathophysiology will be discussed further below.

Inflammation

Increasing 'omics'-based evidence suggest that GDM elicits major changes in the placental gene profile (Radaelli *et al.* 2003, 2009, Enquobahrie *et al.* 2009, Zhao *et al.* 2011b, Binder *et al.* 2015). Of note, these studies most commonly identify enrichment for inflammatory pathways (both markers and mediators of inflammation) in the GDM placenta. Results of previous studies indicate that moderate inflammation during pregnancy is correlated

with insulin resistance and GDM (Kirwan *et al.* 2002, Wolf *et al.* 2004). Several excellent reviews further detail the role of inflammation in GDM pathophysiology (Vrachnis *et al.* 2012, Abell *et al.* 2015, Lekva *et al.* 2016). Increased secretion of pro-inflammatory cytokines have been noted to occur in early pregnancy, followed by insulin resistance in the third trimester, hence suggesting that pro-inflammatory cytokines and chemokines may be involved in the development of insulin resistance (Abell *et al.* 2015). Elevated circulating levels of pro-inflammatory cytokines TNF- α and IL-6, and reduced levels of anti-inflammatory cytokines IL-10 and IL-4 have been identified in GDM, regardless of BMI (Ategbro *et al.* 2006). Specifically, TNF- α has been shown to be associated with insulin resistance in human pregnancy (Kirwan *et al.* 2002), while non-pregnant mouse studies have demonstrated that TNF- α is involved in the development of insulin resistance (Uysal *et al.* 1997, 1998, Li *et al.* 2009). Together, these findings suggest that elevated expression of pro-inflammatory cytokines such as TNF- α during gestation may be a cause of insulin resistance associated with GDM.

Notably, *in vitro* studies have shown that maternal adipose tissue, maternal skeletal muscle and placenta may contribute to a differential inflammatory profile in GDM compared with NGT pregnancies. Adipose tissue is a complex active endocrine tissue that is thought to play key roles in GDM pathophysiology. Increased depth of adipose tissue has been found significantly correlated with risk of GDM, where adipocytes isolated from GDM pregnancies displayed increased cell size when compared to NGT adipocytes (Rojas-Rodriguez *et al.* 2015, De Souza *et al.* 2016). This larger adipocyte size was significantly correlated with increased serum glucose (Rojas-Rodriguez *et al.* 2015). Adipose tissue from GDM pregnancies also displayed reduced capillary density, and increased expression of markers of endothelial dysfunction, which may contribute to the altered physiology of the adipose tissue (Lappas 2014d, Rojas-Rodriguez *et al.* 2015). Adipose tissue is also known to differentially regulate the expression of adipokines and inflammatory markers. For example, adiponectin mRNA expression is reduced in both omental and subcutaneous adipose tissue (Ott *et al.* 2018), but leptin mRNA expression and protein secretion are increased in both adipose tissues from GDM pregnancies compared with NGT pregnancies (Lappas *et al.* 2005a, Lappas 2014b, Tsiotra *et al.* 2018). The increase in leptin expression may also be contributing to the abnormal expression and regulation of fatty acid uptake and transport in GDM pregnancies (Lappas 2014b). Finally, adipose tissue from GDM pregnancies exhibit increased

gene expression of IL-6 and IL-8 in GDM pregnancies, compared to NGT pregnancies (Kleiblova *et al.* 2010, Kuzmicki *et al.* 2012, Bari *et al.* 2014).

Likewise, in skeletal muscle, TNF- α is known to promote expression and secretion of other cytokines and chemokines IL-6, IL-8 and MCP-1 in skeletal muscle (Nagaraju *et al.* 1998, Lappas *et al.* 2004). These pro-inflammatory mediators may also interfere with the insulin signalling pathway, and thus, induce insulin resistance in the skeletal muscle (del Aguila *et al.* 1999, Rieusset *et al.* 2004).

The placenta has been widely demonstrated to be a source of inflammation. Several studies have found increased concentrations of leptin and upregulation of TNF- α signalling genes, as well as upregulated IL-1 receptor and IL-8 receptor genes in GDM placentas compared to NGT placentas (Radaelli *et al.* 2003, Lappas *et al.* 2005a, Enquobahrie *et al.* 2009, Magee *et al.* 2014). In addition, GDM pregnancies exhibit increased serum macrophagic marker sCD163 in early pregnancy (Ueland *et al.* 2019), and increased placental expression of macrophagic markers CD14+ and CD68+ at term, accompanied by enhanced mRNA expression of TNF- α and IL-6 (Yu *et al.* 2013, Mrizak *et al.* 2014). Importantly, these pro-inflammatory cytokines are known to promote the expression and secretion of the chemokines IL-8 and MCP-1 in the placenta (Lappas *et al.* 2004, 2006).

Placental dysfunction

The placenta is the key site of nutrient transfer from the mother to the fetus. Without significant fetal gluconeogenesis (Kalhan & Parimi 2000), the fetus relies on obtaining glucose from maternal circulation via the placenta. To accommodate this, the maternal state becomes insulin resistant and reduces glucose uptake by maternal insulin target tissues, instead allowing placental uptake and transfer of glucose to the fetus, mediated by glucose transporters (GLUTs). In the placenta, the GLUT-1 isoform is localised to the syncytiotrophoblast and is thought to be involved in glucose uptake from the maternal circulation (Illsley 2000). On the other hand, the GLUT-3 isoform is localised to placental endothelial cells and thought to be involved in glucose transfer to the fetus (Illsley 2000). GLUT-1 acts as the rate-limiting step for glucose transfer and the changes in the density of GLUT-1 can potentially affect the rate of transfer (Illsley 2000). The basal membrane (glucose delivery) expresses greater number of GLUT-1 compared to microvillous membrane (glucose uptake)

demonstrating the maternal to fetal transfer (Jansson *et al.* 1993). GDM is associated with altered placental glucose metabolism (Osmond *et al.* 2001, Jansson & Powell 2006) and altered amino acid transport (Jansson *et al.* 2002) and lipid (Segura *et al.* 2017) concentrations. Consequentially, GDM trophoblasts exhibit a two-fold increase in GLUT-1 expression and 40% increase in glucose uptake in the trophoblast basal membrane (Gaither *et al.* 1999). These observed changes in GLUT-1 expression may potentially increase glucose uptake from the maternal circulation into the basal membrane. On the other hand, the mRNA expression of GLUT-3 was increased in GDM placenta (Dekker Nitert *et al.* 2014). Given the higher affinity of GLUT-3 for glucose (Hay 2006) and its localisation in endothelial cells, the increased expression of GLUT-3 in GDM may play a role in the transmission of glucose to fetus after the trans-syncytial transport contributing for fetal overgrowth.

This observed increase in trophoblast glucose uptake may contribute to further inflammation in the placenta, as hyperglycaemic conditions have been noted to stimulate a pro-inflammatory response in human trophoblasts (Heim *et al.* 2018). Metainflammation which is triggered by metabolites causes development of systemic insulin resistance (Gregor & Hotamisligil 2011). In line with this, increased circulating levels of inflammatory molecules in GDM pregnancies is known (Hauguel-de Mouzon & Guerre-Millo 2006) and changes in circulating inflammatory profile can enhance insulin resistance in mothers which subsequently influences placental nutrient transport. Evidently, the overexpression of placental TNF- α is associated with increased fetal adiposity (Radaelli *et al.* 2003). Besides fetal adiposity, increased glucose transfer to the fetus causes fetal hyperglycaemia and fetal hyperinsulinaemia (Díaz *et al.* 2017). Since the basal membrane GLUT-1 expression is regulated by insulin-like growth factor 1 (IGF1) (Baumann *et al.* 2014), fetal hyperglycaemia may induce the secretion of fetal IGF1 which can regulate the expression of basal membrane GLUT-1 in a feedback loop manner. This will maintain the increase in transplacental glucose transport and consequently excessive fetal weight gain or macrosomia.

Insulin receptor expression is also present in the placenta, and its distribution varies depending on gestational age. While the insulin receptor can be found at the microvillous membrane in early pregnancy, term insulin receptor expression is primarily localised at the endothelium (Desoye *et al.* 1994, 1997). These spatio-temporal changes suggest a role for maternal and fetal

insulin in regulating placental function at different stage of pregnancies. The binding of insulin to insulin receptor activates the autophosphorylation on specific cytoplasmic tyrosine residues, which ensues downstream signalling (Pessin & Saltiel 2000). Although the insulin receptor is not implicated in placental glucose transport, placental insulin signalling may instead contribute to lipid metabolism (Ruiz-Palacios *et al.* 2017). Examination of GDM placenta displayed reduced expression of insulin receptor together with reduced phosphorylation of its downstream molecule, Akt (Li *et al.* 2016). However, limited data are available on the consequences of altered placental insulin signalling on fetal outcome.

Other biochemical changes in the placenta also contribute to the development of GDM placenta including inflammation (as discussed in the previous section), increased placental oxidative stress (Lappas *et al.* 2011a), mitochondrial damage (Muralimanoharan *et al.* 2016) and ER stress (Yung *et al.* 2016). Several anatomical changes to the placenta, including significantly lower fetal-to-placental weight ratios (Taricco *et al.* 2003) and abnormal placental vascularisation (Suranyi *et al.* 2013), are also associated with GDM pathophysiology.

Leptin has been found to be overexpressed along with its receptor in GDM placenta (Perez-Perez *et al.* 2013) and has been demonstrated to exert anti-apoptotic effects on human trophoblast cells (Magarinos *et al.* 2007, Perez-Perez *et al.* 2008). Thus, hyperleptinaemia in GDM may drive placental overgrowth by suppressing trophoblast apoptosis (Kautzky-Willer *et al.* 2001). Indeed, higher placental weight has been significantly associated with lower trophoblast apoptosis in GDM, thereby increasing the surface area available for nutrient transfer (Magee *et al.* 2014). Altogether, these trophoblast abnormalities may compromise placental function and contribute towards intrauterine fetal programming for metabolic disease later in life (Mele *et al.* 2014).

Endothelial cell dysfunction

Endothelial dysfunction is a state of imbalance between vasoconstriction and vasodilatation, predisposing patients to atherosclerosis and CVD – the principal complications of T2DM. Normal pregnancy is characterised by vasodilation, which reduces peripheral vascular resistance and enables an increase in uteroplacental blood flow. In GDM, however, endothelial function is impaired in the arteries that control blood flow to the placenta (uterine arteries), as well as those involved in controlling systemic vascular resistance (mesenteric arteries) (Knock *et al.* 1997,

Chirayath *et al.* 2010, Mrizak *et al.* 2013). Despite this, abnormal uterine blood flow has not been evidenced to affect glucose transfer to the fetus (Palacín *et al.* 1985). Instead, endothelial dysfunction during GDM pregnancy may be associated with impaired cardiometabolism postpartum (Göbl *et al.* 2014). The GDM placenta exhibits significant increases in key markers of endothelial dysfunction, such as endothelial-derived reactive oxygen and nitrative-derived species (Casanello *et al.* 2007, Westermeier *et al.* 2009). Markers of endothelial cell dysfunction, such as the cell adhesion molecules, vascular cell adhesion protein 1 (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, are increased in omental adipose tissue with maternal obesity and GDM (Lappas 2014d), while circulating VCAM-1 and ICAM-1 are increased with GDM (Mordwinkin *et al.* 2013). Notably, alterations in endothelial dysfunction are related to inflammatory status (Mrizak *et al.* 2013, Di Fulvio *et al.* 2014, Lappas 2014d).

Molecular pathways disrupted by GDM

Extensive literature exists documenting the clinical consequences of GDM for the mother and fetus; however, much less is known in the way of the molecular basis of GDM pathogenesis. Several key pathways implicated in the development of GDM are shared with T2DM pathophysiology; however, these pathways will only be discussed in the context of GDM.

NF- κ B signalling pathway

The nuclear factor-kappa-light-chain-enhancer of activated B cells (NF- κ B) signalling pathway is one of the major and central pathways involved in gene expression for immune and inflammatory responses (Baldwin 1996). NF- κ B exists in an inactive dimeric form in the cytoplasm, bound by the inhibitory protein I κ B- α . Upon activation by stimuli such as pro-inflammatory cytokines and endotoxin, I κ B kinases (IKKs) induce phosphorylation and degradation of I κ B- α , thus allowing the NF- κ B dimer to rapidly translocate to the nucleus. There, co-factor recruitment allows the NF- κ B dimer to bind with *cis*-acting Rel-binding (κ B) sites promoter regions of several genes involved in inflammation and/or insulin resistance, thus initiating gene transcription.

Given that inflammation plays a key role in inducing insulin resistance, the activation of the NF- κ B pathway is implicated in diabetic pathophysiologies. For example, high-dose salicylate administration is known to inhibit

the IKK- β /NF- κ B pathway, and the related compound aspirin had been used in the late 1800s to treat diabetes and dramatically reduce hyperglycaemia (Hundal *et al.* 2002). At a molecular level, aspirin and salicylate administration was found to reduce insulin resistance by blocking IKK- β activity, allowing normalised activation of key insulin signalling molecules in skeletal muscle (Kim *et al.* 2001, Yuan *et al.* 2001). Indeed, patients with T2DM treated with high-dose aspirin reported improved glucose metabolism, highlighting the critical role that NF- κ B/IKK- β play in inducing insulin resistance (Hundal *et al.* 2002).

The importance of the NF- κ B pathway extends to GDM – increased NF- κ B mRNA has been reported in the GDM placenta (Feng *et al.* 2016). While its expression in adipose tissue or skeletal muscle of GDM pregnancies are yet to be assessed, NF- κ B remains a key factor for the regulation of the inflammation in these tissues obtained from pregnant women at the time of term Caesarean section (Lappas *et al.* 2005b). It is also worth noting that GDM pregnancies exhibit increased levels of chorionic gonadotrophin (CG), a pro-inflammatory compound that impairs insulin signalling in adipocytes through the NF- κ B pathway (Ma *et al.* 2015). Such evidence points to the theory that NF- κ B contributes to the development of GDM by promoting adipocyte inflammation and impairing insulin-related functions, such as glucose uptake.

Toll-like receptors (TLRs)

Toll-like receptors (TLRs) are key surface molecules responsible for recognising conserved components of pathogenic microorganisms known as pathogen-associated molecular patterns (PAMPs), thus playing an essential role in triggering an inflammatory innate immune response (Vasselon & Detmers 2002). Engagement of TLRs by PAMPs triggers intracellular signalling pathways that culminate in the activation of a number of pro-inflammatory transcription factors including NF- κ B (Barton & Medzhitov 2003). TLRs however lack catalytic domains and are connected to the cell-signalling machinery via intracellular adaptor molecules including the myeloid differentiation factor 88 (MyD88) and TNFR-associated factor 6 (TRAF6) (Medzhitov *et al.* 1998, Muzio *et al.* 1998).

To date, ten functional TLRs (TLR1-10) have been identified in humans; TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10 are found primarily on the cell surface, while TLR3, TLR7, TLR8, and TLR9 are expressed in intracellular endosomes. Human placenta expresses TLR1-10 (Nishimura & Naito 2005, Patni *et al.* 2009) with studies

showing that most TLRs are functionally active in human placenta (Tangeras *et al.* 2014). GDM is associated with increased expression of TLR4 mRNA (Mrizak *et al.* 2014, Feng *et al.* 2016) and MyD88 in the placenta (Feng *et al.* 2016). The cause of this increase in placenta is not known; however, saturated fatty acids (Yang *et al.* 2015) and oxidised cholesterol metabolites (oxysterols) (Aye *et al.* 2012) enhance TLR4-induced inflammation in human primary trophoblasts.

Interestingly, TLR2 mRNA expression is increased in PBMCs obtained from the pregnant women who were normoglycaemic at the time of sampling but later developed GDM (Kuzmicki *et al.* 2013). Further, elevated PBMC, TLR2 and TLR4 mRNA expression was evident in GDM in the second trimester; however, 4 weeks later, the difference was not significant, probably as a result of an increase in TLR expressions in the healthy pregnant women (Kuzmicki *et al.* 2013). On the other hand, another study reported that TLR4 mRNA expression was significantly higher in maternal monocytes of patients with GDM obtained after 37 weeks gestation (Xie *et al.* 2014). Additionally, there was a positive correlation between the TLR4 mRNA expression level in peripheral blood monocytes and serum TNF- α levels (Xie *et al.* 2014). It is not known what increases the expression of TLRs in PBMCs from women with GDM; however, high glucose (Dasu *et al.* 2008) and saturated fat (Ghanim *et al.* 2009, Deopurkar *et al.* 2010) have been shown to increase TLR expression in human monocytes.

Nucleotide-binding oligomerisation domains (NODs)

Nucleotide-binding oligomerisation domain-containing 1 and 2 (NOD1 and NOD2) are intracellular pattern recognition receptors involved in the sensing of numerous microbes or microbial components which have gained access to the cell's cytoplasm (Franchi *et al.* 2009). The activation of NOD1 or NOD2 promotes the activation of NF- κ B-mediated pro-inflammatory gene expression (Fritz *et al.* 2006, Strober *et al.* 2006). NOD1 and NOD2 have been shown to play an important role in inflammation and insulin resistance (Winzer *et al.* 2004, Zhao *et al.* 2011a, Yi-Jun *et al.* 2012, Zhou *et al.* 2012, Purohit *et al.* 2013). We have recently shown that NOD1 (but not NOD2) expression is increased in both subcutaneous and omental adipose tissue obtained from women with GDM when compared to BMI-matched NGT women (Lappas 2014b). Furthermore, treatment of subcutaneous and omental adipose tissue from NGT and GDM pregnant women with the NOD1 ligand iE-DAP

significantly increased the expression of a number of inflammatory markers. Specifically, there was an increase in the expression and secretion of the pro-inflammatory cytokine IL-6, the pro-inflammatory chemokine IL-8, COX-2 and subsequent prostaglandin production, the ECM remodelling/degrading enzyme MMP-9, and the gene expression and secretion of the adhesion molecules ICAM-1 and VCAM-1. These effects of NOD1 appear to be mediated via the pro-inflammatory transcription factor NF- κ B, as BAY 11-7082 ameliorated iE-DAP-induced inflammatory proteins. This study demonstrates that the NOD1 ligand iE-DAP significantly inhibits the insulin signalling pathway in omental adipose tissue from pregnant women, as evidenced by decreased phosphorylated IRS-1, GLUT-4 expression and glucose uptake. Together, these results indicate that NOD1 plays an important role in adipose tissue inflammation and insulin resistance that is evident in women with GDM.

Peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that heterodimerise with retinoid X receptor (RXR) and bind to peroxisome proliferator response elements (PPRE) in promoters of target genes (Ganss 2017). PPARs (PPAR α , PPAR β/δ , PPAR γ) regulate the expression of genes involved in inflammation, adipogenesis, oxidative stress, insulin signalling and glucose metabolism (McCarthy *et al.* 2013, Wójcik *et al.* 2015). PPAR γ expression is lower in maternal adipose tissue, placenta and placental cells (i.e. syncytiotrophoblasts and extravillous trophoblasts) from women with GDM (Catalano *et al.* 2002, Arck *et al.* 2010, Holdsworth-Carson *et al.* 2010, Knabl *et al.* 2014). Further, placental levels of the natural anti-inflammatory PPAR γ agonist, 15-deoxy-delta12,14-prostaglandin J₂ (15dPGJ₂) are lower in women with GDM (Jawerbaum *et al.* 2004). In adipocytes, PPAR γ signalling promotes blood glucose clearance by enhancing glucose uptake; thus, downregulated PPAR γ expression in GDM may exacerbate glucose intolerance (Lendvai *et al.* 2016). Decreased expression of PPAR α and RXR α have also been identified in placentas from women with GDM (Holdsworth-Carson *et al.* 2010). The roles of PPAR α and RXR α in GDM are not known.

Although it is unclear if dysregulated PPAR expression is a cause or a consequence of GDM, PPARs may be potential therapeutic targets for the prevention or treatment of GDM. Indeed, PPAR γ ligands such as thiazolidinediones (TZDs), which are synthetic agonists, are used in patients with T2DM to improve insulin

sensitivity and glucose tolerance (Knabl *et al.* 2014, Ganss 2017). Likewise, resveratrol, a natural PPAR γ ligand, decreases inflammation in human placenta and adipose tissue obtained from pregnant women and improves skeletal muscle insulin resistance *in vitro* (Tran *et al.* 2017). Moreover, animal studies have shown that resveratrol has beneficial effects on maternal glucose metabolism and insulin sensitivity (Roberts *et al.* 2014).

Sirtuins

In mammals, there are seven sirtuins (SIRT1-7) that differ in tissue distribution, subcellular localisation and substrate specificity (Michishita *et al.* 2005). They are classified accordingly to their amino acid sequences: class I (SIRT1-3), class II (SIRT4), class III (SIRT5) and class IV (SIRT6 and 7). SIRTs possess either NAD⁺-dependent histone deacetylase (SIRT1, 2, 3, and 5) or mono-ribosyltransferase (SIRT4 and 6) activity that contribute the transcription of certain genes in response stress stimuli or ageing. For the purpose of this review we will discuss the role of SIRT1, 3 and 6 in regulating inflammation and metabolic dysfunction.

SIRT1

SIRT1 is involved in dampening the inflammatory response, regulating the ageing process, cell death/survival, metabolism, stress resistance and endocrine signalling (Michan & Sinclair 2007). SIRT1 has been shown to directly interact with the p65 NF- κ B subunit RELA and inhibits NF- κ B transcriptional activity by the deacetylation of RELA at lysine residue Lys310 (Chen *et al.* 2005, Rajendrasozhan *et al.* 2008). Other studies have shown SIRT1 to regulate other transcription factors such as inhibiting the pro-inflammatory FOXO protein (Motta *et al.* 2004) and upregulates the activity of the anti-inflammatory transcription factor PPAR (Purushotham *et al.* 2009).

There are, however, limited studies on the role of SIRT1 and GDM. Nevertheless, the available data suggest that SIRT1 may play a role in regulating inflammation and glucose metabolism in human placenta. Our preliminary data show that SIRT1 is expressed in human placenta and is significantly downregulated in placentas from pregnancies complicated with GDM (Lappas & Liong unpublished observation). It is not known what causes the decrease in placental SIRT1 expression; however, bacterial endotoxin LPS (Lappas *et al.* 2011b), the pro-inflammatory cytokines TNF- α and IL-1 β (Lappas *et al.* 2011b) and oxidative stress

(Lappas *et al.* 2012) have all been shown to decrease SIRT1 expression in human placenta. Activation of SIRT1 by resveratrol, a phytochemical compound present in plants, red grapes, peanuts, vegetables, berries, beverages, and herbal medicines (Sanders *et al.* 2000, Burns *et al.* 2002), and the synthetic SIRT1 activator SRT1720, significantly suppressed LPS-induced release of pro-inflammatory cytokines and prostaglandins in human placenta (Lappas *et al.* 2011b). Furthermore, SIRT1 is involved in placental glucose uptake by regulating the transcription of GLUT-1 (Lappas *et al.* 2012).

Interestingly, women with GDM have elevated leukocyte SIRT1 mRNA compared to women with normal glucose tolerance (Anna Turek *et al.* 2014, Wojcik *et al.* 2014). Given the anti-inflammatory function of SIRT1, this increase in SIRT1 expression may be the result of a compensatory mechanism in response to the heightened inflammatory state associated with GDM. On the other hand, reduced SIRT1 expression and activity in fetal endothelial colony-forming cells (ECFCs) and human umbilical vein endothelial cells (HUVECs) were found in pregnancies complicated by GDM (Lappas 2012, Gui *et al.* 2016). This reduction of SIRT1s in fetal cells may potentially link the development of long-term cardiovascular complications in offspring of GDM pregnancies.

Whether specific SIRT1 activators can prevent the development of GDM *in vivo* is unknown. However, of promise, a recent study found resveratrol to alleviate insulin resistance and improve glucose metabolism and fetal outcomes complicated by GDM in a mouse model (Yao *et al.* 2015). GDM mice treated with resveratrol had increased activation of the adenosine monophosphate (AMP)-activated protein kinase (AMPK) in maternal liver as well as in their offspring. There is an extensive body of evidence that supports the involvement of AMPK in GDM by regulating skeletal muscle glucose metabolism and inflammation, which will be further discussed in the review.

SIRT3

Insulin resistance in skeletal muscle is associated with decreased mitochondrial metabolism and increased skeletal muscle oxidative stress in non-pregnant individuals (Koves *et al.* 2008, Anderson *et al.* 2009, Rains & Jain 2011) and in pregnancies complicated by obesity and/or GDM (Boyle *et al.* 2013). In obese animal models, SIRT3 expression and activity are significantly reduced (Hirschey *et al.* 2011, Kendrick *et al.* 2011). Indeed, obese pregnant women have significantly increased levels of

oxidative stress and reduced mitochondrial metabolic activity in skeletal muscle compared with non-obese pregnant women (Boyle *et al.* 2013). This increase in oxidative stress and altered mitochondrial activity corroborates with other studies in non-pregnant obese and insulin-resistant humans and animals (Koves *et al.* 2008, Anderson *et al.* 2009). GDM and obese pregnant women also have impaired antioxidant defence as determined by decreased MnSOD activity (Boyle *et al.* 2013). SIRT3 is the primary mitochondrial deacetylase in human skeletal muscle (Lombard *et al.* 2007) that is responsible for the activation of MnSOD enzymatic activity (Qiu *et al.* 2010, Tao *et al.* 2010, Finley *et al.* 2011). Acetylation is a reversible post-translational modification that is important for controlling enzyme activity involved in mitochondrial metabolism, including the citric acid cycle, fatty acid oxidation, electron transport system, antioxidant protection (Qiu *et al.* 2010, Tao *et al.* 2010, Finley *et al.* 2011). Although short-term high-fat diets increase SIRT3 gene expression, prolonged metabolic stress caused by chronic exposure to high-fat diets and the development of obesity are shown to downregulate SIRT3 (Hirschey *et al.* 2011). Notably, SIRT3-knockout mice share similar metabolic alternations that are common features of obesity, including signs of impaired skeletal muscle insulin signalling, increased oxidative stress/lipid peroxidation and reduced hepatic mitochondrial respiration (Ahn *et al.* 2008, Hirschey *et al.* 2011, Jing *et al.* 2011). Notably, obese normal glucose-tolerant women and obese GDM women had significantly reduced mitochondrial SIRT3 mRNA content and activity in their skeletal muscle compared to non-obese pregnant women (Boyle *et al.* 2013). In addition to adverse maternal health risks, GDM and obesity have long-term consequences for the offspring, such as increased risk of obesity and type 2 diabetes (Heerwagen *et al.* 2010, Strakovsky & Pan 2012).

Recent studies also found reduced SIRT3 expression and activity in ECFCs and HUVECs from pregnancies complicated by GDM (Lappas 2012, Gui *et al.* 2016). This reduction of SIRT3s may potentially link the development of long-term cardiovascular complications in offspring of GDM pregnancies.

To date, there are limited studies on the role of SIRT3s in regulating inflammation and insulin resistance associated with GDM. However, the studies discussed in this review have demonstrated SIRT3s to exert protective effects against inflammation, endothelial cell dysfunction, oxidative stress and insulin resistance. Thus, SIRT3s may be a potential therapeutic target in alleviating

maternal and fetal complications that are commonly associated with GDM.

PI3K/mTOR signalling

The PI3K pathway is one of many mechanisms required for survival in environments with variable nutrient availability. Within the PI3K kinase family, there exists two protein complexes, namely mechanistic target of rapamycin complex (mTORC)1 and mTORC2, each with distinct sensitivity to upstream and downstream regulation (Lapante & Sabatini 2012). The mTOR pathway is a primary responder to environmental cues for energy availability, making it a crucial regulator of high energy-consuming processes, including cell proliferation and growth. A compromised mTOR signalling mechanism is thought to at least partially underlie various pathologies, including cancer and diabetes (Populo *et al.* 2012, Blagosklonny 2013).

Given these essential roles in cell metabolism, the mTOR pathway is a key regulator of maternal-fetal nutrient transport across the placenta (Wullschlegel *et al.* 2006). The transfer of nutrients is key in dictating normal or abnormal growth of the fetus within the placenta and occurs across the microvillous and basal plasma membranes that form the syncytiotrophoblast through to the endothelium of fetal capillaries (Philipps *et al.* 1978). Amino acid transfer occurs from maternal blood to fetal blood through a process of active transport and key amino acid transporters. In cases of fetal macrosomia, it has been observed that the system A transporter for alanine, serine and glutamine has been upregulated in the microvillous membrane (Jansson *et al.* 2002). Similarly, increased activity within this membrane has also been observed in cases of diabetic pregnancies. Contrastingly, activity of this transporter is reduced in cases of intrauterine growth restriction (Jansson *et al.* 1998). Together, these data lead to the conclusion that the extent of growth within the fetus is largely dependent on the transport of nutrients across the placental barriers (Roos *et al.* 2009). It follows that pathways that underpin the transport of nutrients, such as the mTOR pathway, are essential in understanding how nutrient transfer affects fetal growth in both physiological and pathological conditions.

In an earlier study in cultured trophoblast cells, mTOR protein was found to regulate placental amino acid transfer in the placental epithelium, particularly through the L-amino acid transporter (Roos *et al.* 2007). Similarly, a study by Gaccioli *et al.* explored the role of the mTOR pathway in conjunction with the eukaryotic

initiation factor (EIF)-2 in the regulation of protein synthesis (Gaccioli *et al.* 2013). Based on the notion that mTOR upregulates the uptake of nutrients in the placenta, researchers hypothesised that when the mother was overweight due to a high consumption of saturated fats, mTOR activity and subsequent nutrient transfer would be upregulated leading to overgrowth of the fetus. Moreover, high saturated fats in the diet leading to overweight would result in an inhibition of EIF-2 and inflammation of the placenta. These hypotheses were tested on a population of female pregnant rats fed a high-fat diet. The rats exhibited significantly higher triglyceride, insulin and leptin levels in both the mother and fetus, accompanied by increased weight. Interestingly, there was increased activation of mTORC1, despite no change in placental nutrient transporter activation and no evidence of increased placental inflammation. Thus, the predominant change was exerted through increased mTORC1 activity and additionally, decreased eIF2 alpha phosphorylation. Maternal hormones, including insulin, leptin and IGF-1 (Karl *et al.* 1992, Karl 1995, Jansson *et al.* 2003) are the mediators of placental nutrient transporters (Jones *et al.* 2007). Accordingly, obese NGT pregnancies are associated with elevated level of fasting insulin and leptin in late pregnancy (Ramsay *et al.* 2002) and second trimester insulin concentrations which are positively correlated with pre-pregnancy BMI (Clausen *et al.* 2005). Maternal hormones, including insulin, leptin and IGF-1 (Karl *et al.* 1992, Karl 1995, Jansson *et al.* 2003), have been described as regulators of placental nutrient transporters (Jones *et al.* 2007). Of interest, obese NGT pregnancies are associated with elevated level of fasting insulin and leptin in late pregnancy (Ramsay *et al.* 2002) and second trimester insulin concentrations which are positively correlated with pre-pregnancy BMI (Clausen *et al.* 2005). The increased circulation of hormones can mediate the upregulation of placental nutrient transport linking obese pregnancies and fetal overgrowth. Furthering this, Jansson *et al.*'s prospective study found that mTOR-dependent nutrient transport was implicated in fetal overgrowth by obese NGT mothers (Jansson *et al.* 2013). Of interest, high birth weight was positively correlated with the mTOR signalling pathway as well as the placental insulin and insulin-like growth factor pathways, while also being inversely correlated with AMPK phosphorylation. A positive correlation was also observed with the amino acid transporter system A within the microvillous plasma membrane. This led to the conclusion that fetal overgrowth with obese mothers may occur as a result of upregulated activity of amino acid transporters.

Sati *et al.* recently compared the expression of mTOR pathway in placentas from normal term babies and GDM babies (Sati *et al.* 2016). In NGT term placentas, the syncytiotrophoblast and vascular walls of villi exhibited immunoreactive mTOR within the cytoplasm, as well as p-mTOR. However, there was increased expression of the ribosomal protein p-p70S6K in stromal cells of GDM placentas. As a downstream constituent of the mTOR signalling network, p-p70S6K overexpression indicates that mTOR plays a role in the observed pathology exhibited by the placenta of GDM births (Sati *et al.* 2016). Further functional analyses continue to be performed to further understand the distinct molecular pathways involved in these pathologies. However, the literature so far suggests placental GDM pathology, marked by overgrowth, is underpinned by an over activation of the mTOR pathway.

Glycogen synthase kinase 3

Glycogen synthase kinase 3 (GSK3) is a serine/threonine protein kinases that was originally found to play a role in the storage of glucose into glycogen (Woodgett 1990). In mammals, GSK3 exists as two isoforms: GSK3 α and GSK3 β which although structurally similar are encoded by distinct genes and have different molecular weights of 51 and 47 kD, respectively (Woodgett 1990). GSK3 activity is regulated by the phosphorylation at one of its N-terminal serine (Ser) residues: ser21 in GSK3 α and ser9 in GSK3 β , causing its inactivation (Woodgett 1990). Although GSK3 α and GSK3 β share many similar functions, they are not to be functionally redundant (Hoeflich *et al.* 2000). Subsequent studies revealed GSK3 β is essential in a number of crucial cellular functions including cell cycle control, apoptosis, embryonic development, cell differentiation and adhesion (Frame & Cohen 2001, Grimes & Jope 2001, Doble & Woodgett 2003, Jope & Johnson 2004, Wang *et al.* 2011).

Overexpression of GSK3 in skeletal muscle of obese type 2 diabetic individuals and animal models of obesity is associated with insulin resistance. For example, in type 2 diabetes, the early development of insulin resistance as indicated by impaired glycogen synthesis is associated with increased GSK3 activity *in vivo* (Eldar-Finkelman *et al.* 1999). Furthermore, using type 2 diabetes animal models, GSK3 β inhibitor CHIR99021 improves insulin sensitivity and glucose metabolism (Cline *et al.* 2002, Ring *et al.* 2003). Interestingly, suppression of GSK3 α/β is also associated with decreased inflammation in response to pro-inflammatory stimuli including TNF- α , IL-1 β and LPS (Martin *et al.* 2005). More recently, increased GSK3

activity has been linked to a number of inflammatory diseases, including diabetes (Jope *et al.* 2007, Rayasam *et al.* 2009). GSK3 activity is significantly increased in non-pregnant obese and diabetic adipose tissue and skeletal muscle (Eldar-Finkelman *et al.* 1999, Nikoulina *et al.* 2000). Likewise, women with GDM have significantly reduced GSK3 β serine phosphorylation in their skeletal muscle and omental adipose tissue (Lappas 2014c). Given this increase in GSK3 β activity with GDM, the role of GSK3 in regulating GDM associated inflammation has been explored. The GSK3 inhibitor CHIR99021 significantly reduced the gene expression and secretion of pro-inflammatory mediators (TNF- α , IL-1 β , IL-6, IL-8 and MCP-1) in adipose tissue and skeletal muscle stimulated with LPS or IL-1 β (Lappas 2014c). Furthermore, GSK3 inhibition significantly decreased LPS or IL-1 β induced expression and secretion of the cell adhesion molecules VCAM-1 and ICAM-1 (Lappas 2014c).

The precise mechanism that GSK3 regulates inflammation in pregnant adipose tissue and skeletal muscle is not known. However, GSK3 is necessary for the full transcriptional activity of NF- κ B (Hoeflich *et al.* 2000, Martin *et al.* 2005). The gene transcription co-activator β -catenin plays an important role in GSK-mediated regulation of NF- κ B gene transcription. GSK3 inactivation leads to the translocation of β -catenin from the cytoplasm to the nucleus, which in turn blocks NF- κ B activity (Kim *et al.* 2014). Whether GSK3 regulates NF- κ B activity in pregnant adipose tissue and skeletal muscle is not known.

Adenosine monophosphate (AMP)-activated protein kinase (AMPK)

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a serine/threonine protein kinase that is formed from three heterogenic subunits, a catalytic (α) subunit and two regulatory subunits (β and γ). There is considerable evidence that AMPK regulates skeletal muscle glucose metabolism and inflammation (Hayashi *et al.* 1998, Lihn *et al.* 2008). Of note, in non-pregnant obese and diabetic individuals, AMPK activity in skeletal muscle and adipose tissue is diminished or impaired compared to normal healthy individuals (Sriwijitkamol *et al.* 2007, Xu *et al.* 2012). Recent data have also revealed that AMPK activity is significantly reduced in skeletal muscle and adipose tissue of women with GDM (Boyle *et al.* 2014, Liong & Lappas 2015a).

AMPK activators diminish inflammation in response to LPS and IL-1 β stimuli (Giri *et al.* 2004, Dasu *et al.* 2008). Studies in skeletal muscle and adipose tissue from

pregnant women have shown AMPK activators AICAR and phenformin to significantly reduce LPS or IL-1 β -stimulated production of pro-inflammatory cytokines IL-6, IL-8 and MCP-1 (Liong & Lappas 2015a). The precise mechanism(s) by which AMPK regulates inflammation and insulin sensitivity in skeletal muscle remains known. In skeletal muscle myoblasts, AMPK activates the histone/protein deacetylase and anti-inflammatory molecule sirtuin 1 (SIRT1) (Lappas *et al.* 2011b). It has been proposed that AMPK activators can upregulate SIRT1 activity resulting in the decreased production of bacterially induced pro-inflammatory mediators in human gestational tissues (Lappas *et al.* 2011b). Studies have also described AMPK to suppress the activity of the pro-inflammatory transcription NF- κ B. For example, AICAR and phenformin block NF- κ B signalling stimulated by LPS (Katerelos *et al.* 2010). It is thought that AMPK suppresses NF- κ B signalling indirectly via its downstream inhibitory mediators such as SIRT1, Forkhead box O (FoxO) family, and peroxisome proliferator-activated receptor γ co-activator 1 α (PGC-1 α) (Salminen *et al.* 2011).

Inflammasome

The inflammasome is a large multi-subunit protein complex that processes IL-1 β from its precursor form to its secreted active form. In pregnant adipose tissue, TLRs and pro-inflammatory cytokine signalling pathways play a crucial role in IL-1 β secretion via the inflammasome (Lappas 2014a). Of note, activation of the inflammasome significantly augments IL-1 β production while simultaneously decreasing AMPK activity in pregnant skeletal muscle and adipose tissue (Liong & Lappas 2015a). As a result, insulin-mediated glucose uptake and activation of the insulin signalling pathway (i.e. IRS-1, IR- β and GLUT-4) were significantly impaired compared to basal conditions (Lappas 2014a, Liong & Lappas 2015a). Therefore, the finding that women with GDM exhibit increased inflammasome activation in their adipose tissue is of great concern (Lappas 2014a).

Interestingly, AMPK activation by the AICAR activator rescued insulin resistance induced by IL-1 β (Liong & Lappas 2015a). Other *in vivo* studies have found AMPK activation resulted in improved insulin resistance, glucose metabolism and fetal outcomes in GDM mice (Yao *et al.* 2015). In the liver, AMPK regulates glucose production, by inhibiting histone acetyltransferase (HDACs) enzymes, resulting in the downregulation of glucose 6-phosphatase (G6Pase) expression and activity (Mihaylova *et al.* 2011). Given that AMPK has been shown to be an important

regulator of inflammation and glucose metabolism, further studies are warranted to assess its role as a therapeutic target for GDM.

ER stress

ER stress is characterised by the accumulation of misfolded proteins in the ER lumen (Zhang *et al.* 2009, Hotamisligil 2010, McGuckin *et al.* 2010, Garg *et al.* 2012, Verfaillie *et al.* 2013) and is associated with a number of metabolic disease, including obesity and diabetes (Ozcan *et al.* 2004, 2006). It is thought that various endogenous and exogenous cellular insults, including environmental toxins, inflammation and viral infection, disturb the protein folding environment, thus activating the unfolded protein response (UPR). The UPR is mediated by three distinct arms, each activated by either the inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6) or pancreatic endoplasmic reticulum kinase (PERK). Through these signalling proteins, the UPR alleviates ER stress by inhibiting protein translation, removing misfolded proteins and increasing the ER protein folding capacity. However, if the UPR fails to alleviate ER stress, the cells will subsequently undergo caspase-mediated apoptosis (Nakagawa *et al.* 2000).

Recent evidence has implicated the IRE1 pathway of the UPR to regulate inflammation. Activation of the IRE1 pathway induces the production of pro-inflammatory cytokines such IL-8, IL-6, and TNF- α (Gargalovic *et al.* 2006, Martinon *et al.* 2010), and NF- κ B activation via I κ B degradation (Kaneko *et al.* 2003, Hu *et al.* 2006). Moreover, NF- κ B-mediated production of pro-inflammatory cytokines activate ER stress and thus augment the inflammatory state (Zhang *et al.* 2006b). More recently, studies have identified the IRE1 pathway to activate the inflammasome complex and secretion of IL-1 β (Kim *et al.* 2013a).

There is mounting evidence to show ER stress plays a central role in peripheral insulin resistance, obesity and type 2 diabetes (Ozcan *et al.* 2004, 2006). Studies in obese and diabetic mice have demonstrated a significant reduction in weight gain and improved insulin sensitivity in the liver, adipose tissue and muscle when administered with ER stress inhibitor 4-phenylbutyric acid (4-PBA) (Ozcan *et al.* 2006, Basseri *et al.* 2009). In pregnant women, ER stress is significantly increased in adipose tissue and skeletal muscle of women with GDM and with maternal obesity (Liong & Lappas 2015b, 2016). Recent studies have described the IRE1 arm of the ER stress signalling pathway in propagating inflammation associated with GDM pregnancies.

Table 1 Summary of changes in the expression or activity of pathways in adipose tissue, placenta and skeletal muscle of GDM pregnancies compared to normal pregnancies.

Pathways	Expression/activity in GDM compared to NGT pregnancies		
	Adipose tissue	Placenta	Skeletal muscle
NF- κ B	–	↑	–
TLRs	–	↑ TLR4	–
NODs	↑ NOD1	–	–
PPARs	↓ PPAR γ	↓ PPAR α , PPAR γ	–
SIRT	–	↓ SIRT1, SIRT3	↓ SIRT3
PI3K	↑	↓	↓
mTOR	–	↑	–
GSK3	↑	–	↑
AMPK	↓	↓	↓
Inflammasome	↑	–	–
ER stress	↑	↑	↑

↑, increased in expression/activity; ↓, decreased in expression/activity; –, no findings reported.

For example, either the ER stress inhibitor TUDCA or siRNA-induced gene silencing of ER stress markers GRP78, ATF6 or IRE1 α significantly abolishes inflammation induced by toll-like receptors (TLR3, TLR4) or cytokines (IL-1 β) in pregnant skeletal muscle (Liong & Lappas 2016). Further, mRNA expression of IRE1 α , GRP78, XBP-1 and ATF6 was significantly increased in skeletal muscle stimulated with

IL-1 β , LPS (TLR3 ligand) and poly(I:C) (TLR4 ligand) and was subsequently suppressed following TUDCA treatment. Studies in adipose tissue of pregnant women also found ER stress to be a key activator of the inflammasome complex and that the secretion of IL-1 β is regulated by ER stress (Liong & Lappas 2015b). *In vivo* studies have also shown ER stress inhibitors attenuate inflammasome activation in diabetic mice (Fang *et al.* 2013). Inhibition of ER stress by GRP78, ATF6 or IRE1 α siRNA gene silencing or with TUDCA treatment also restored the insulin signalling pathway and insulin-mediated glucose uptake in skeletal muscle impaired by LPS, poly(I:C) and TNF- α to basal levels (Liong & Lappas 2016).

In summary, ER stress may contribute to inflammation and insulin resistance that is characteristic of GDM and obese pregnancies. Of promise is the safe use of TUDCA in humans, which is currently used as a treatment for cholestatic liver diseases (Beuers 2006), and thus could have therapeutic applications in suppressing inflammation and improving peripheral insulin resistance associated with GDM.

Concluding remarks

The lifelong and severe health complications directly resulting for GDM for both mother and child cannot be

Figure 1

Model of GDM. Sterile inflammatory insults dysregulate the expression and/or activity of various signalling pathways in placenta, adipose tissue and skeletal muscle leading to altered function in these tissues which can contribute to adverse offspring outcomes associated with GDM.

ignored. GDM not only contributes heavily to obstetric and perinatal morbidity, but leaves a legacy of future long-term health risks that places an extraordinary economic burden on our healthcare system. A better understanding of the regulatory pathways involved in GDM may lead to the identification of molecular targets for therapy. It is important that any potential pharmaceutical use during pregnancy also needs to consider the impact on the developing fetus. Such therapies would significantly reduce public health costs and improve the lives of women and babies for generations to come.

As described earlier, a number of signalling pathways may play a role in the pathophysiology of GDM; see Table 1 for a summary of the data. Though this review discusses the key pathways of GDM development in a non-hierarchical manner, we are able to glean from T2DM studies that these key pathways often interact with and alongside each other. Unfortunately, there is no evidence in GDM to indicate that these pathways act in a hierarchical manner to contribute to GDM development. We postulate that sterile inflammatory insults dysregulate the expression and or activity of various signalling pathways in placenta, adipose tissue and skeletal muscle leading to altered function in these tissues which can contribute to adverse offspring outcomes associated with GDM (Fig. 1). These signalling pathways represent potential targets for improving both short- and long-term health outcomes of offspring complicated by GDM pregnancies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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