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-kB (NF-κB), 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.

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, Suntorn & Panichkul 2015). The prevalence is greater amongst indigenous Australians (Ishak & Petocz 2003) and in women whose native country is China or India compared to whose 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 Hyperglycaemia 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 (Ategbo 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, Rieuisset 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. Consequently, 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 Nittert 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 microvillus 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 (Palacin et al. 1985). Instead, endothelial dysfunction during GDM pregnancy may be associated with impaired cardiometabolism post-partum (Göbl et al. 2014). The GDM placenta exhibits significant increases in key markers of endothelial dysfunction, such as endothelial-derived reactive oxygen and nitrate-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-κB signalling pathway**

The nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-κB) signalling pathway is one of the major and central pathways involved in gene expression for immune and inflammatory responses (Baldwin 1996). NF-κB exists in an inactive dimeric form in the cytoplasm, bound by the inhibitory protein IκB-α. Upon activation by stimuli such as pro-inflammatory cytokines and endotoxin, IκB kinases (IKKs) induce phosphorylation and degradation of IκB-α, thus allowing the NF-κB dimer to rapidly translocate to the nucleus. There, co-factor recruitment allows the NF-κB dimer to bind with cis-acting Rel-binding (κB) 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-κB pathway is implicated in diabetic pathophysiosologies. For example, high-dose salicylate administration is known to inhibit
the IKK-β/NF-κB 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-κB/IKK-β play in inducing insulin resistance (Hundal et al. 2002).

The importance of the NF-κB pathway extends to GDM – increased NF-κB 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-κB 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-κB pathway (Ma et al. 2015). Such evidence points to the theory that NF-κB 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-κB (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-κB-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, Puohit et al. 2013). We have recently shown that NOD1 (but not NOD2) expression in 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-κB, 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 (PPARα, PPARβ/δ, PPARγ) regulate the expression of genes involved in inflammation, adipogenesis, oxidative stress, insulin signalling and glucose metabolism (McCarthy et al. 2013, Wojcik et al. 2015). PPARγ 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 PPARγ agonist, 15-deoxy-delta12,14-prostaglandin J2 (15dPGJ2) are lower in women with GDM (Jawerbaum et al. 2004). In adipocytes, PPARγ signalling promotes blood glucose clearance by enhancing glucose uptake; thus, downregulated PPARγ expression in GDM may exacerbate glucose intolerance (Lendvai et al. 2016). Decreased expression of PPARα and RXRα have also been identified in placenta from women with GDM (Holdsworth-Carson et al. 2010). The roles of PPARα and RXRα 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, PPARγ 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 PPARγ 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-κB subunit RELA and inhibits NF-κB 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 placenta from pregnancies complicated with GDM (Lappas & Lioung 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-1β (Lappas et al. 2011b) and oxidative stress...
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 SIRTs in fetal cells may potentially link the development of long-term cardiovascular complications in offspring of GDM pregnancies.
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 (Laplante & 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 (Wullschleger et al. 2006). The transfer of nutrients is key in dictating normal birth weight, and pathological conditions.

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: GSK3α and GSK3β 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 GSK3α and ser9 in GSK3β, causing its inactivation (Woodgett 1990). Although GSK3α and GSK3β share many similar functions, they are not to be functionally redundant (Hoefflich et al. 2000). Subsequent studies revealed GSK3β 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, GSK3β inhibitor CHIR99021 improves insulin sensitivity and glucose metabolism (Cline et al. 2002, Ring et al. 2003). Interestingly, suppression of GSK3α/β is also associated with decreased inflammation in response to pro-inflammatory stimuli including TNF-α, IL-1β 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 GSK3β serum phosphorylation in their skeletal muscle and omental adipose tissue (Lappas 2014c). Given this increase in GSK3β 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-1β, IL-6, IL-8 and MCP-1) in adipose tissue and skeletal muscle stimulated with LPS or IL-1β (Lappas 2014c). Furthermore, GSK3 inhibition significantly decreased LPS or IL-1β 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-κB (Hoefflich et al. 2000, Martin et al. 2005). The gene transcription co-activator β-catenin plays an important role in GSK-mediated regulation of NF-κB gene transcription. GSK3 inactivation leads to the translocation of β-catenin from the cytoplasm to the nucleus, which in turn blocks NF-κB activity (Kim et al. 2014). Whether GSK3 regulates NF-κB 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-1β 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-1β-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-κB. For example, AICAR and phenformin block NF-κB signalling stimulated by LPS (Katerelos et al. 2010). It is thought that AMPK suppresses NF-κB signalling indirectly via its downstream inhibitory mediators such as SIRT1, Forkhead box O (FoxO) family, and peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) (Salminen et al. 2011).

**Inflammasome**

The inflammasome is a large multi-subunit protein complex that processes IL-1β 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-1β secretion via the inflammasome (Lappas 2014a). Of note, activation of the inflammasome significantly augments IL-1β 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-1β (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-κB activation via IkB degradation (Kaneko et al. 2003, Hu et al. 2006). Moreover, NF-κB-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-1β (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.
For example, either the ER stress inhibitor TUDCA or siRNA-induced gene silencing of ER stress markers GRP78, ATF6 or IRE1α significantly abolishes inflammation induced by toll-like receptors (TLR3, TLR4) or cytokines (IL-1β) in pregnant skeletal muscle (Liong & Lappas 2016). Further, mRNA expression of IRE1α, GRP78, XBP-1 and ATF6 was significantly increased in skeletal muscle stimulated with IL-1β, 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-1β 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 IRE1α 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
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 offsprings 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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