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Activation of inflammasomes in adipose tissue of women with gestational diabetes

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ABSTRACT

Gestational diabetes mellitus (GDM) is characterised by maternal peripheral insulin resistance, increased inflammation, and increasing levels of circulating free fatty acids (FFAs) and advanced glycation endproducts (AGEs). Caspase-1 is a key component of the inflammasome, which is activated upon cellular infection or stress to trigger the maturation IL-1β, a pro-inflammatory cytokine that mediated insulin resistance. The aim of this study was to determine whether the inflammasome is activated in adipose tissue from women with gestational diabetes mellitus (GDM) and if it interferes with the insulin signalling pathway leading to the insulin resistance that is evident in GDM. Protein expression of active caspase-1 and mature IL-1β secretion was increased in adipose tissue of women with GDM. Treatment of adipose tissue with IL-1β decreased insulin-stimulated phosphorylation of IRS-1, GLUT-4 expression and glucose uptake. Low-grade inflammation (induced by LPS), the FFA palmitate and AGE conjugated to BSA (AGE-BSA), induced IL-1β secretion via inflammasome activation. In conclusion, the present findings describe an important role for adipose tissue inflammasome activation in the development of insulin resistance associated in pregnancies complicated by GDM.

1. Introduction

Diabetes in pregnancy is a major health issue globally, affecting up to 14% of all pregnancies (Kim et al., 2007). Diabetes in pregnancy is defined as either pre-existing diabetes (type 1 and type 2) and gestational diabetes mellitus (GDM). GDM, characterised as any degree of glucose intolerance with first recognition during pregnancy, is the most common type of diabetes found in pregnancy. Of clinical importance, the rates of GDM are increasing worldwide, intensified with advancing maternal age, racial/ethnic disparities, and obesity (Ferrara, 2007). GDM is associated with substantial increased risks for both mother and infant (Dabelea et al., 2000; Sobngwi et al., 2003). Pregnancy complications include higher rates of macrosomia, shoulder dystocia, birth trauma, Caesarean birth and neonatal complications including jaundice and respiratory distress. The long term risks of exposure to GDM include increased risk of obesity, type 2 diabetes, insulin resistance, and other diseases. In the fetus, exposure to any form of diabetes results in a critically adverse fetal environment, enhancing susceptibility to a number of chronic diseases including obesity, diabetes, cardiovascular disease and certain cancers later in life.

Maternal adipose tissue plays an important role in fetal growth and development. The later part of normal human pregnancy is characterised by maternal hyperinsulinemia and insulin resistance, an adaptation that is required to meet the needs of the growing fetus (Buchanan and Xiang, 2005). In women with GDM however, peripheral insulin resistance is even more pronounced (Catalano et al., 2002; Colomiere et al., 2010), which results in greater substrate availability for fetal growth and development (Lain and Catalano, 2007). In addition, adipose tissue synthesises and secretes a number of cytokine hormones, which are dysregulated with GDM, that have been shown to correlate with fetal adiposity (Radaelli et al., 2006).

What causes insulin resistance is not known. Many factors can enhance insulin resistance, including genetics, a sedentary lifestyle, obesity, and other conditions, such as chronic inflammation or infection. Of note, GDM is characterised by low-grade inflammation (endotoxemia), elevated circulating free fatty acids (FFA) and advanced glycation endproducts (AGEs) (Catalano et al., 2002; Lappas et al., 2011; Winzer et al., 2004; Wolf et al., 2004). Bacterial endotoxin lipopolysaccharide (LPS), the saturated FFA palmitate and AGEs activate toll like receptors (TLRs) to promote the production of pro-inflammatory cytokines from adipose tissue and/or adipocytes (Ajuwon and Spurlock, 2005; Creely et al., 2007; Lappas et al., 2007; Lappas et al., 2005a). These pro-inflammatory cytokines have been shown to attenuate insulin signalling *in vitro* and *in vivo* (Kahn et al., 2006; Xu et al., 2003). IL-1β is one of the

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principal inflammatory cytokines implicated in this process (Jager et al., 2007). *In vitro*, the inflammasome, and in particular its generation of active caspase-1, is required to process pro IL-1β to an active, secreted molecule (Arend et al., 2008). It has been shown that in most systems, two signals are required for optimal IL-1β secretion (Bauernfeind et al., 2011). Signal 1 is induced by TLR stimulation, leading to the synthesis of pro IL-1β. Signal 2 is triggered by agents that can that can cause ionic perturbations (e.g. ATP) or pore forming toxins which induce the activation of caspase-1 followed by IL-1β processing and release.

Recent evidence has demonstrated an important role for caspase-1 mediated IL-1β in insulin resistance (Vandanmagsar et al., 2011). It is thus possible that in GDM, the activation of adipose tissue caspase-1 may enhance the release of IL-1β that underlies the development of insulin resistance evident in this disease. Therefore, in this study, the hypotheses to be tested are that GDM is associated with increased caspase-1 activation in maternal adipose tissue, and that LPS, palmitate and AGE activate caspase-1-dependent IL-1β secretion from adipose tissue which can interfere with the insulin signalling pathway leading to insulin resistance in adipose tissue.

Table 1
Characteristics of the study group.

	NGT (n = 9)	GDM (n = 16)	P value
Maternal age (years)	31.0 ± 1.7	34.9 ± 1.1	NS
Maternal BMI at ~12 weeks (kg/m^2)	35.7 ± 1.7	34.9 ± 1.7	NS
Maternal BMI at delivery (kg/m^2)	38.5 ± 1.8	35.8 ± 1.7	NS
Gestational age at birth (weeks)	38.5 ± 0.2	38.8 ± 0.2	NS
Fetal birth weight (g)	3865 ± 138	3525 ± 95	NS
Fetal gender	5 Female; 4 Male	7 Female; 9 Male	
Gravida	3.0 ± 0.6	2.9 ± 0.3	NS
Parity	2.1 ± 0.3	2.3 ± 0.2	NS
Maternal OGTT at ~28 weeks gestation			
Fasting plasma OGTT (mmol/l)	4.7 ± 0.1	5.6 ± 0.3	$P < 0.05$
1 h plasma OGTT (mmol/l)	7.5 ± 0.7	10.7 ± 0.5	$P < 0.05$
2 h plasma OGTT (mmol/l)	5.5 ± 0.4	8.8 ± 0.4	$P < 0.05$
Maternal OGTT at ~6 weeks postpartum			
Fasting plasma OGTT (mmol/l)	ND	5.0 ± 0.4	
1 h plasma OGTT (mmol/l)	ND	6.6 ± 0.5	
2 h plasma OGTT (mmol/l)	ND	5.7 ± 0.7	

Values represent mean ± SEM (Student's t-test).
NS, not significant; ND, not done; OGTT, oral glucose tolerance test.

2. Materials and methods

2.1. Tissue collection and preparation

Human omental adipose tissue was obtained (with the Research Ethics Committee of Mercy Health approval) from consenting women who delivered healthy, singleton infants at term (>37 weeks gestation). Indications for Caesarean section were breech presentation and/or previous Caesarean section. Tissues were obtained within 15 min of delivery.

Omental adipose tissue was obtained from normal glucose tolerant (NGT) women and women with GDM. Women with any underlying medical conditions such as pre-existing diabetes, asthma, polycystic ovarian syndrome, preeclampsia and macrovascular complications were excluded. Women with GDM were diagnosed according to the criteria of the Australasian Diabetes in Pregnancy Society (ADIPS) by either a fasting venous plasma glucose concentrations of ⩾5.5 mmol/l glucose, and/or ⩾8.0 mmol/l glucose 2 h after a 75 g oral glucose load at approximately 28 weeks gestation. Women with GDM were managed by diet alone (n = 8 patients) or insulin in addition to diet (n = 8 patients). Women were controlled by diet if their fasting glucose readings were maintained below 5.5 mmol/l over a 2 week period post diagnosis. Women with fasting glucose readings greater than 5.5 mmol/l were placed on insulin for optimal glucose control. All pregnant women were screened for GDM, and women participating in the normal group had a negative screen. The baseline characteristics for all patients used in this study are outlined in Table 1. Adipose tissue was obtained within 10 min of delivery, thoroughly washed in ice-cold PBS to remove and blood. Dissected fragments were stored at −80 °C until assayed as detailed below. The clinical details of these patients are presented in Table 1.

2.2. Tissue explant culture

For these studies, adipose tissue was obtained from NGT pregnant women, and tissue explants were performed as previously described (Barker et al., 2012; Lappas et al., 2010; Lappas et al., 2005b; Lappas et al., 2004). Briefly, adipose tissues was finely diced and placed in DMEM at 37 °C in a humidified atmosphere of 21% O_2 and 5% CO_2 for 1 h. Tissues were blotted dry on sterile filter paper and transferred to 24-well tissue culture plates (100 mg wet weight/well). The explants were incubated in 1 ml DMEM containing 100 U/ml penicillin G and 100 μg/ml streptomycin. Tissues were incubated in the absence or presence of 1 μg/ml LPS, 250 μM palmitate (conjugated to BSA; PA-BSA) or 250 μM glycated BSA (AGE-BSA) for 20 h, followed by incubation with 5 mM ATP for 2 h. For the palmitate and , the control (basal) experiments were performed in DMEM containing BSA. Additional experiments were also performed whereby adipose tissue was pre-treated with 10 μM of the caspase-1 inhibitor Ac-YVAD-CHO or the 10 μM of the P2X7 receptor antagonist KN-62 prior to the addition of LPS, palmitate or AGE-BSA. After the final incubation, medium was collected and assessment of cytokine concentrations was performed by ELISA. Each treatment was performed from at least four patients.

2.3. Glucose uptake

Adipose tissue explants were performed as detailed above and glucose uptake in adipose tissue was performed as previously described (Lappas et al., 2012). Briefly, after final incubation with treatment, tissues were pre-incubated in the absence or presence of 20 μM cytochalasin B in Krebs buffer for 5 min. 2-Deoxy-D-glucose (2DG) uptake was measured by adding 3 μCi/ml [^{14}C]-2DG (Perkin Elmer) and 1 mM 2DG to Krebs buffer containing 0.1% BSA (fatty acid free) and 0.1 μM insulin for 20 min. Tissues were then collected and washed in ice-cold PBS and solubilised for 4 h in 0.5 ml 1 M NaOH at 60 °C. Tissues were neutralised with 0.5 ml 1 M HCl and then centrifuged at 15,000g for 5 min to pellet insoluble material. The supernatant was transferred to a vial containing 3 ml of liquid scintillation fluid. All samples were counted for radioactivity in a liquid scintillation counter. GLUT-specific glucose uptake was measured by subtracting values for [^{14}C]-2DG uptake in the presence of 20 μM cytochalasin B. The rate of [^{14}C]-2DG transport was expressed in nanomoles per minute per milligram protein. Fold change was calculated relative to basal, which was set at 1.

2.4. RNA extraction and quantitative RT-PCR (qRT-PCR)

Total RNA was extracted from tissues using TRIsure™ according to manufacturer's instructions (Bioline). RNA concentration and purity were measured using a NanoDrop ND1000 spectrophotometer (Thermo Scientific, Pittsburgh, PA). RNA was converted to cDNA using the SuperScript® VILO™ cDNA synthesis kit (Invitrogen, Carlsbad, California, USA) according to the manufacturer's