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Endoplasmic reticulum stress regulates inflammation and insulin resistance in skeletal muscle from pregnant women

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A B S T R A C T

Sterile inflammation and infection are key mediators of inflammation and peripheral insulin resistance associated with gestational diabetes mellitus (GDM). Studies have shown endoplasmic reticulum (ER) stress to induce inflammation and insulin resistance associated with obesity and type 2 diabetes, however is paucity of studies investigating the effects of ER stress in skeletal muscle on inflammation and insulin resistance associated with GDM. ER stress proteins IRE1α, GRP78 and XBP-1s were upregulated in skeletal muscle of obese pregnant women, whereas IRE1α was increased in GDM women. Suppression of ER stress, using ER stress inhibitor tauroursodeoxycholic acid (TUDCA) or siRNA knockdown of IRE1α and GRP78, significantly downregulated LPS-, poly(I:C)- or IL-1β-induced production of IL-6, IL-8, IL-1β and MCP-1. Furthermore, LPS-, poly(I:C)- or TNF-α-induced insulin resistance was improved following suppression of ER stress, by increasing insulin-stimulated phosphorylation of IR-β, IRS-1, GLUT-4 expression and glucose uptake. In summary, our inducible obesity and GDM-like models suggests that the development of GDM may be involved in activating ER stress-induced inflammation and insulin resistance in human skeletal muscle.

1. Introduction

Gestational diabetes mellitus (GDM), defined as glucose intolerance with first onset during pregnancy, affects up to 18% of all pregnant women (Coustan et al., 2010). Advancing maternal age, ethnicity and obesity are known risk factors for GDM (Ferrara, 2007). GDM is associated with pregnancy complications and long-term adverse health problems for both mother and infant (Perkins et al., 2007; Al-Khalifah et al., 2012; Mitanchez, 2010). Pregnancy complications associated with GDM include fetal macrosomia, shoulder dystocia and higher rates of Caesarean delivery. Neonatal complications include jaundice, respiratory distress and hypoglycaemia. The long-term health risks for mothers and their infants include the development of cardiovascular diseases, obesity, type 2 diabetes and certain cancers later in life (Dabelea et al., 2000). With the growing rates of maternal obesity and GDM in developed countries, they are of significant public health concern with enormous long-term financial costs to the health care system and the community (Hossain et al., 2007).

Peripheral insulin resistance (Cani et al., 2007; Friedman et al., 2008; Borst, 2004; Catalano et al., 2003; Friedman et al., 1999; Kuhl, 1991) is a central feature of pregnancies complicated by maternal obesity and/or GDM. Insulin sensitivity is about 56% less in GDM pregnancies (Kuhl, 1991) and 47% less in obese normal glucose tolerant (NGT) pregnant women when compared to lean pregnant NGT women (Catalano et al., 1999). This decrease in insulin sensitivity is caused by multiple defects in the insulin signalling pathway in skeletal muscle of GDM and obese women (Friedman et al., 1999; Colomiere et al., 2009; Shao et al., 2002; Barbour et al., 2006). This pronounced peripheral insulin resistance increases nutrient availability to the fetus, which in turn increases the risk of fetal adiposity (Lain and Catalano, 2007) and also the development of childhood obesity and other metabolic dysfunctions later on in life (Dabelea et al., 2000).

Inflammation and endotoxemia are also key features of GDM and obese pregnancies (Lappas, 2014a,b,c,d; Radaelli et al., 2003; Basu et al., 2011; Sobel et al., 1992; Dhurandhar et al., 1997). We have previously shown that sterile inflammation and infections by bacteria or viruses can induce inflammation and insulin resistance in tissues from pregnant women (Lappas, 2014a,b,c,d, 2015; Liong

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and Lappas, 2015a,b). There is, however, a paucity of studies that have investigated the mechanisms involved in peripheral insulin resistance and inflammation in skeletal muscle.

There is now increasing evidence that the inositol requiring enzyme 1α (IRE1α) arm of the endoplasmic reticulum (ER) stress response plays a key role in regulating inflammation and insulin resistance. Various endogenous and exogenous cellular insults such as viral infection, inflammation and environmental toxins are known to trigger the ER stress response (Shen et al., 2004) resulting in the induction of pro-inflammatory cytokines, chemokines and other mediators of the immune response in gestational and non-gestational tissues (Liong and Lappas, 2015a,b; Kim et al., 2014; Gargalovic et al., 2006; Martinon et al., 2010; Liong and Lappas, 2014). ER stress has also been implicated to be responsible for the development of peripheral insulin resistance, obesity and type 2 diabetes (Ozcan et al., 2004, 2006. Indeed, we have previously described the IRE1α arm of the ER stress response is increased in maternal adipose tissue from pregnancies complicated by GDM and obesity (Liong and Lappas, 2015a,b).

To our knowledge there have been no studies on the role of the IRE1α pathway of the ER stress response in skeletal muscle in the context of obesity in pregnancy and/or GDM. We hypothesise that (i) the IRE1α arm of the ER stress response is increased in skeletal muscle in pregnancies complicated by maternal obesity and/or GDM; (ii) inflammation and infection activate the IRE1α arm of the ER stress response in skeletal muscle; and (iii) inhibition of inflammation- and infection-induced ER stress decreases inflammation, activates the insulin signalling pathway and increases glucose uptake. Inflammation and insulin resistance, tissues were obtained from normal glucose tolerant (NGT) women and stimulated with either bacterial lipopolysaccharide (LPS; a toll-like receptor (TLR)4 ligand), polyinosinic-polycytidylic acid (poly(I:C); a TLR3 ligand), or pro-inflammatory cytokines (i.e. IL-1β, TNF-α) to generate models of obese and GDM-like state. To inhibit ER stress, we used (i) the ER stress inhibitor tauroursodeoxycholic acid (TUDCA), which is an endogenous bile acid derivative that acts as a potent chemical chaperone that inhibits the ER stress response (Ozcan et al., 2006; Miller et al., 2007); and (ii) siRNA specific knockdown of ER stress components IRE1α and GRP78 in primary human skeletal muscle cells.

2. Materials and methods

2.1. Tissue collection and preparation

Human skeletal muscle tissue (pyramidalis) 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. Women with any underlying medical conditions such as pre-existing diabetes, asthma, polycystic ovarian syndrome, preeclampsia and macrovascular complications were excluded from this study. Tissues were collected and processed within 15 min of delivery. Tissue samples were snap-frozen and stored at −80 °C until assessed for ER stress markers by Western blotting, or immediately processed for tissue and cell culture studies as detailed below.

To examine the effect of GDM and/or maternal obesity on the expression of ER stress markers, skeletal muscle samples were obtained from the following groups: (i) normal glucose tolerant (*NGT*) women who entered pregnancy *lean* (BMI between 18 and <25 kg/m^2; n = 6 patients); (ii) *NGT* women who entered pregnancy *obese* (BMI ≥ 30 kg/m^2; n = 6 patients); (iii) women with *GDM* who were managed by *diet* alone (n = 6 lean and n = 6 obese); and (iv) women with *GDM* who were managed by *insulin* in addition to diet (n = 6 lean and n = 6 obese). The relevant clinical details of the subjects are detailed in Table 1.

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 5.1–6.9 mmol/l glucose, and/or 8.5–11 mmol/l glucose 2 h after a 75 g oral glucose load at approximately 28 weeks gestation. Women were controlled by diet if their fasting glucose readings were maintained below 5.1 mmol/l over a 2 week period post diagnosis. Women with fasting glucose readings greater than 5.1 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.

2.2. Skeletal muscle explant culture

Fresh, non-frozen tissue explants were performed to determine the effect of ER stress inhibitor TUDCA on inflammation and insulin signalling in pregnant skeletal muscle. For these experiments, fresh skeletal muscle was obtained from non-obese NGT (n = 6 patients per experiment) and GDM (n = 3 patients) pregnant women, and tissue explants were performed immediately as previously described (Lappas, 2015). Briefly, skeletal muscle tissue 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 (50 mg/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 2 mM TUDCA (AdooQ BioScience; Sapphire Bioscience, NSW, Australia) for 60 min before the addition of 10 μg/ml LPS (derived from *Escherichia coli* 026:B6; Sigma–Aldrich; St. Louis, MO, USA), 20 μg/ml poly(I:C) (Sigma–Aldrich; St. Louis, MO, USA) or 1 ng/ml IL-1β (PeproTech; Rocky Hill, NJ, USA) for 20 h. After final incubation, tissue and media were collected separately and stored at −80 °C for further analysis as detailed below. The concentration of TUDCA was based on previous studies in gestational (Liong and Lappas, 2014a,b,c,d) and non-gestational tissues (Giri et al., 2004; Zhao et al., 2008).

To assess the effect of ER stress inhibitor TUDCA on the insulin signalling pathway and glucose uptake, fresh skeletal muscle was obtained from NGT women and explants were performed as detailed above, however, after 20 h incubation, tissues were incubated with 0.1 μM insulin for 30 min. After final incubation, tissue was collected and assessment of glucose uptake and expression of the insulin signalling proteins by Western blot are detailed below. Each treatment was performed on tissues obtained from six patients for both the glucose uptake assays and Western blotting.

2.3. Gene knockdown of GRP78 and IRE1α with siRNA in primary skeletal muscle cells

Primary skeletal muscle cells were used to investigate the effect of siRNA-mediated gene silencing of GRP78 and IRE1α inflammation and glucose uptake. Skeletal muscle cells were isolated from fresh skeletal muscle obtained from lean NGT women who delivered healthy, singleton infants at term (>37 weeks' gestation) undergoing elective Caesarean section. Fresh skeletal muscle tissue was washed in PBS, finely dissected and digested for 1 h in Dulbecco's Modified Eagle's Medium (DMEM) with 0.25%(w/v) of trypsin (Gibco Laboratories, Grand Island, NY, USA) and 1 mM EDTA. Cells were centrifuged at 500 × *g* for 10 min and then cultured in a petri dish at 21% O_2, 5% CO_2 at 37 °C for 30 min to eliminate adherent fibroblasts. Non-adherent cells were then transferred and grown in a 25-cm^2 flask coated with 0.2% gelatin in DMEM containing 10% heat inactivated fetal calf serum (FCS),