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Interleukin-1β inhibits insulin signaling and prevents insulin-stimulated system A amino acid transport in primary human trophoblasts

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ABSTRACT

Interleukin-1β (IL-1β) promotes insulin resistance in tissues such as liver and skeletal muscle; however the influence of IL-1β on placental insulin signaling is unknown. We recently reported increased IL-1β protein expression in placentas of obese mothers, which could contribute to insulin resistance. In this study, we tested the hypothesis that IL-1β inhibits insulin signaling and prevents insulin-stimulated amino acid transport in cultured primary human trophoblast (PHT) cells. Cultured trophoblasts isolated from term placentas were treated with physiological concentrations of IL-1β (10 pg/ml) for 24 h. IL-1β increased the phosphorylation of insulin receptor substrate-1 (IRS-1) at Ser307 (inhibitory) and decreased total IRS-1 protein abundance but did not affect insulin receptor β expression. Furthermore, IL-1β inhibited insulin-stimulated phosphorylation of IRS-1 (Tyr612, activation site) and Akt (Thr308) and prevented insulin-stimulated increase in PI3K/p85 and Grb2 protein expression. IL-1β alone stimulated cRaf (Ser338), MEK (Ser221) and Erk1/2 (Thr202/Tyr204) phosphorylation. The inflammatory pathways nuclear factor kappa B and c-Jun N-terminal kinase, which are involved in insulin resistance, were also activated by IL-1β treatment. Moreover, IL-1β inhibited insulin-stimulated System A, but not System L amino acid uptake, indicating functional impairment of insulin signaling. In conclusion, IL-1β inhibited the insulin signaling pathway by inhibiting IRS-1 signaling and prevented insulin-stimulated System A transport, thereby promoting insulin resistance in cultured PHT cells. These findings indicate that conditions which lead to increased systemic maternal or placental IL-1β levels may attenuate the effects of maternal insulin on placental function and consequently fetal growth.

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1. Introduction

Chronic low-grade inflammation causes insulin resistance and is believed to contribute to the pathogenesis of obesity and diabetes. Normal pregnancy is associated with elevated systemic inflammation and decreased insulin sensitivity in the mother, as compared to the non-pregnant state. In pregnant women who are obese or have gestational diabetes mellitus (GDM), inflammation is increased further, as evidenced by the elevation of pro-inflammatory cytokines such as interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-alpha (TNF-α) in maternal serum (Basu et al., 2011b; Catalano et al., 2009; Challier et al., 2008; Madan et al., 2009; Roberts et al., 2011). Pregnant women with obesity and/or GDM are also more insulin resistant than normal pregnant women (Norman and Reynolds, 2011). In addition, recent studies indicate that the placentas of obese women exhibit increased expression of pro-inflammatory cytokines either through increased systemic inflammation in the mother, infiltration of maternal macrophages into the placenta, or via *de novo* activation of placental inflammatory pathways (Basu et al., 2011a; Challier et al., 2008, Roberts et al., 2011).

IL-1β is one of the major pro-inflammatory cytokines produced by both macrophages and the placenta. IL-1β binding to IL-1 type I receptor activates a number of inflammatory pathways including nuclear factor-kappa B (NF-κB) and c-jun N-terminal kinase (JNK), which cause insulin resistance by attenuating insulin receptor substrate-1 (IRS-1) activation (Maedler et al., 2009). IL-1β is linked to diabetes through defective insulin secretion in pancreatic islets (Maedler et al., 2002) and increased IL-1β production in adipose tissue of obese individuals decreases whole-body insulin sensitivity (Vandanmagsar et al., 2011). Recent clinical studies aimed at attenuating IL-1β activity in subjects with Type 2 diabetes or metabolic syndrome using human recombinant IL-1 receptor antagonist, have yielded promising results demonstrating improvements in glycaemia, insulin secretion, β-cell function and lower levels of systemic inflammation (Larsen et al., 2007; van Asseldonk et al., 2011).

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The insulin receptor consists of two extracellular α-subunits that bind growth factors and two transmembrane β-subunits mediating the intrinsic tyrosine kinase activity (Lee and Pilch, 1994). Activation of the insulin receptor leads to tyrosine phosphorylation of insulin receptor substrates (IRS-1 and 2). In addition to the insulin receptor, IRS proteins are also activated by insulin-like growth factor (IGF) receptors, thereby representing an integrated platform for insulin and IGF signaling. Tyrosine-phosphorylated sites on the IRS-1 protein create binding sites for various signal transducing molecules such as phosphoinositide 3-kinase (PI3K) and growth factor receptor-bound protein 2 (Grb2) (Van den Berghe, 2004). PI3K activation leads to Akt/protein kinase B signaling which is often referred to as the "metabolic pathway" of insulin signaling due to the downstream effects of Akt and mammalian target of rapamycin (mTOR) signaling on glucose, lipid and protein metabolism (Van den Berghe, 2004). On the other hand, Grb2 dependent activation of the Ras-Raf-MEK-ERK pathway is referred to as the insulin-mediated "growth pathway" signal based on its broad proliferative and anti-apoptotic effects (Van den Berghe, 2004).

The placenta represents the interface between maternal and fetal circulations and plays a crucial role in fetal development through a multitude of functions including nutrient transport, gas exchange and hormone production. Placental insulin signaling has been reported to be altered in pregnant women with obesity or diabetes, both conditions associated with fetal overgrowth. The placentas of these women exhibit decreased protein expression of IRS-1 and PI3K regulatory subunit p85α (Colomiere et al., 2009). Similarly, decreased expression of insulin signaling proteins has been identified in placentas of intrauterine growth restricted (IUGR) newborns (Laviola et al., 2005; Street et al., 2011). Collectively, these studies suggest that both excessive and restricted fetal growth are associated with altered placental insulin-signaling.

In classical insulin sensitive tissues such as skeletal muscle and liver, glucose and fatty acid transport and metabolism are regulated by insulin. In the third trimester placenta, these processes are not insulin responsive (Challier et al., 1986; Magnusson-Olsson et al., 2007). Although insulin was able to stimulate glucose transport in first trimester placental villous explants (Ericsson et al., 2005b), previous reports have established that glucose transport in human term placental explants or trophoblast cell lines is not regulated by insulin (Boileau et al., 2001; Challier et al., 1986). This may be explained by the absence of the insulin-sensitive glucose transporter GLUT4 in the syncytiotrophoblast of the term placentas (Ericsson et al., 2005a), whereas the syncytium in early pregnancy expresses two insulin-sensitive glucose transporters, GLUT 4 and 12 (Ericsson et al., 2005a,b; Gude et al., 2003). However, insulin stimulates a number of other endocrine and metabolic activities in the term placenta. In response to insulin treatment, primary human trophoblast (PHT) cells from term placentas secrete human chorionic gonadotropin (hCG) and progesterone (Li and Zhuang, 1991). JAr placental choriocarcinoma cell line exhibits increased mitogenesis following insulin stimulation, an effect which was dependent on Erk activity (Boileau et al., 2001). Insulin is also believed to play a key role in placental vascular remodeling and function (Hiden et al., 2009, Leach, 2011) Furthermore, uptake of amino acids by placental explants and PHT cells has been shown to be stimulated by insulin (Ericsson et al., 2005a,b; Jansson et al., 2003; Jones et al., 2010).

Placental amino acid transport is positively correlated to fetal growth, with decreased transport activity in intrauterine growth restriction (IUGR) and increased placental amino acid transport activity in association with fetal overgrowth (Gaccioli et al., 2012; Lager and Powell, 2012). Over 20 amino acid transporters are expressed in the human placenta with System A and System L transport systems arguably the best characterized (Kudo and Boyd, 2002). System A mediates sodium-dependent uptake of small non-essential, neutral amino acids such as alanine, glycine and serine. Consequently, high intracellular concentrations of these non-essential amino acids such as glycine are then used in the exchange of essential amino acids such as leucine and phenylalanine via System L transporters.

While the downstream effects of IL-1β in non-gestational, insulin sensitive tissues such as the liver and skeletal muscle are well established, the role of IL-1β in activation of placental inflammatory pathways is largely unknown; and the effect of IL-1β, or indeed any other cytokines, on insulin signaling in the placenta has not been investigated. Thongsong et al. previously reported inhibition of System A amino acid transport in BeWo choriocarcinoma cell line following IL-1β treatment (Thongsong et al., 2005). Furthermore, injection of pregnant rats with IL-1β decreased System A activity in isolated rat placental brush-border membrane vesicles (Thongsong et al., 2005). However, it is currently unknown if IL-1β has similar effects in PHT cells and if the previously reported effects involve attenuation of insulin signaling. We recently reported increased IL-1β protein expression in placentas of obese mothers (Aye et al., 2013), which could contribute to insulin resistance. In this study, we tested the hypothesis that IL-1β inhibits insulin signaling and prevents insulin-stimulated amino acid transport in cultured PHT cells.

2. Materials and methods

2.1. Patient recruitment and tissue collection

Pregnant healthy women with normal term pregnancies who were scheduled for delivery by elective Cesarean section were recruited following written informed consent. Placental tissue was transported back to the laboratory within 15 min for cell isolation. Placentas were coded and de-identified relevant medical information was obtained. This study was approved by the Institutional Review Board UTHSCSA IRB (HSC20100262H). The early pregnancy BMI of the women included in this study ranged from 20.3 – 30.6 (mean ± sem; 24.9 ± 1.2). The maternal early pregnancy BMI did not influence the results of this study.

2.2. Primary human trophoblast cell culture

PHT cells were isolated from term placenta by trypsin digestion and Percoll purification as originally described (Kliman et al., 1986) with modifications (Roos et al., 2009). Briefly, approximately 30–40 g of villous tissue was dissected free of decidua and blood vessels, washed in saline and digested in trypsin (0.25%, Invitrogen, Carlsbad, CA) and DNAse I (Sigma–Aldrich, St. Louis, MO). Digests were then poured through 70 μm cell filters (BD Bioscience, San Jose, CA) and cytotrophoblast cells purified over a discontinuous 10–70% Percoll gradient. Cells which migrated between 35–55% Percoll layers were collected and cultured in 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich) and Ham's F-12 nutrient mixture (Invitrogen) containing 10% fetal bovine serum (FBS, Atlanta Biologicals, Lawrenceville, GA), 50 μg/ml gentamicin, 60 μg/ml benzyl penicillin and 100 μg/ml streptomycin (Sigma–Aldrich). Cells were plated in 6-well dishes at a density of 2 million per well for subsequent protein analyses or 1.2 million per well for uptake assays, and incubated in a 5% CO_2 humidified atmosphere at 37 °C. Following 18 h incubation, attached PHT cells were washed twice in warmed Dulbecco's PBS and culture media was changed daily. Trophoblast cell purity was verified by performing immunoblotting analyses to identify positive Cytokeratin-7 expression and negative Vimentin expression

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