Hypoxanthine–xanthine oxidase down-regulates GLUT1 transcription via SIRT1 resulting in decreased glucose uptake in human placenta

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Abstract

Appropriate foetal growth and development is dependent on adequate placental glucose uptake. Oxidative stress regulates glucose uptake in various tissues. The effect of oxidative stress on placental glucose transport is not known. Thus, the aim of this study was to determine the effect of oxidative stress on glucose uptake and glucose transporters (GLUTs) in human placenta. Human placenta was incubated in the absence or presence of 0.5 mM hypoxanthine + 15 mU/ml xanthine oxidase (HX/XO) for 24 h. Gene and protein expressions of the GLUTs were analysed by quantitative RT-PCR and western blotting respectively. Glucose uptake was measured using radio-labelled (¹⁴C) glucose. HX/XO significantly decreased

GLUT1 gene and protein expression and resultant glucose uptake. There was no effect of the antioxidants N-acetylcysteine, catalase and superoxide dismutase or the NF-κB inhibitor BAY 11-0782 on HX/XO-induced decrease in glucose uptake. However, HX/XO treatment significantly decreased both gene and protein expression of SIRT1. In the presence of the SIRT1 activator resveratrol, the decrease in GLUT1 expression and glucose uptake mediated by HX/XO was abolished. Collectively, the data presented here demonstrate that oxidative stress reduces placental glucose uptake and GLUT1 expression by a SIRT1-dependent mechanism.

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Introduction

Placental glucose uptake and transport is paramount for foetal growth and development (Hay 2006, Jansson & Powell 2007). In the absence of significant foetal gluconeogenesis (Kalhan & Parimi 2000), for appropriate growth and development the foetus entirely depends on glucose from maternal circulation. To accommodate this, maternal metabolism shifts into an insulin-resistant state, resulting in decreased glucose uptake by insulin target tissues of the mother. This increase in extracellular glucose levels enables placental uptake and transfer of glucose to the foetus, mediated by glucose transporters (GLUTs; Hauguel-de Mouzon *et al.* 1997, Xing *et al.* 1998, Illsley 2000, Colomiere *et al.* 2010). Glucose uptake and transport by the endothelial, epithelial and trophoblast cells of the placenta are mediated by GLUTs, with GLUT1 and -3 being the most abundant in human term placenta (Hauguel-de Mouzon *et al.* 1997, Xing *et al.* 1998, Illsley 2000, Colomiere *et al.* 2010). Their respective cellular localisations suggest that GLUT1 is responsible for supplying glucose for use as a placental fuel and that GLUT3 is important for glucose transfer to the foetus (Hauguel-de Mouzon *et al.* 1997,

Illsley 2000). Certainly, a number of significant pregnancy complications, including gestational diabetes mellitus (GDM) and pre-eclampsia (Hubel 1999, Myatt & Cui 2004, Siddiqui *et al.* 2010), are associated with altered placental glucose metabolism (Osmond *et al.* 2001, Jansson & Powell 2006). However, the molecular mechanisms of human placental glucose transport and its regulation remain to be fully elucidated.

The mechanisms that regulate placental glucose uptake are largely unknown, yet they are fundamental to understand how the placenta directly affects foetal growth and development. In non-gestational tissues, oxidative stress impairs glucose uptake, though the effects of oxidative stress on glucose uptake are not consistent. For example, reactive oxygen species (ROS) increases glucose transport in skeletal muscle (Kozlovsky *et al.* 1997a,b, Toyoda *et al.* 2004, Higaki *et al.* 2008) and M07 human leukaemic megakaryocytic cell lines (Fiorentini *et al.* 2001). On the other hand, in 3T3-L1 adipocytes, induction of oxidative stress inhibits insulin-stimulated glucose transport (Tirosh *et al.* 1999, Regazzetti *et al.* 2009).

In pregnancies complicated by diabetes (both pre-existing and GDM), there is an overproduction of free radicals,

placental oxidation reactions are accelerated and the radical scavenger function mechanisms are impaired (Coughlan *et al.* 2004, Lappas *et al.* 2010, 2011a). Similarly, in pre-eclampsia, the placenta also displays evidence of excessive oxidative stress (Hubel 1999, Myatt & Cui 2004, Siddiqui *et al.* 2010). GDM and pre-eclampsia are two complications of pregnancy that are associated with placental insulin resistance (Scioscia *et al.* 2006, Colomiere *et al.* 2010). It is not known what effects oxidative stress has on glucose transport in human placenta. Thus, the aim of this study was to determine the effect of oxidative stress on glucose transport in human placenta. Hypoxanthine (HX) and xanthine oxidase (XO) were used to induce ROS as we have previously described (Coughlan *et al.* 2004, Lappas *et al.* 2010). Using this system, we have previously demonstrated an increase in placental release of 8-isoprostane, a marker of oxidative stress. Of note, the level of 8-isoprostane produced by HX/XO was similar to the release of 8-isoprostane produced by the GDM placenta (Coughlan *et al.* 2004, Lappas *et al.* 2010).

Materials and Methods

Tissue collection

Approval for this study was obtained from the Mercy Hospital for Women's Research and Ethics Committee, and informed consent was obtained from all participating subjects. Human placenta was obtained from normal glucose-tolerant women at the time of term (37–40 weeks of gestation) Caesarean section before the onset of labour. Indications for Caesarean section included repeat Caesarean section or breech presentation. Women with any adverse underlying medical condition (i.e. asthma, diabetes, pre-eclampsia and intrauterine growth restriction) were excluded.

Placental explant incubation

Placental lobules (cotyledons) were obtained from various locations of the placenta; the basal plate and chorionic surface were removed from the cotyledon, and villous tissue was obtained from the middle cross-section. Placental tissues were bluntly dissected to remove visible connective tissue and calcium deposits. The dissected tissues were placed in DMEM (containing 5 mM glucose, 100 U/ml penicillin G and 100 µg/ml streptomycin) at 37 °C in a humidified atmosphere of 5% CO₂ and 8% O₂ for a pre-incubation period of 1 h. Explants were blotted dry on sterile filter paper and transferred to 24-well tissue culture plates (50 mg wet weight per well). The tissues were then incubated in 1 ml DMEM with and without treatments for 24 h as detailed below. Each treatment was performed in duplicate. Initial explants were incubated in the absence or presence of 0.5 mM HX and 15 mU/ml XO (HX/XO; *n*=7); 0.25, 0.5 and 1 mM H₂O₂ (*n*=4); and 0.5 mM HX (*n*=4). The concentration of HX/XO used in this study was based on our

previously published studies (Coughlan *et al.* 2004, Lappas *et al.* 2010). Additional explants were pre-treated with 300 U/ml polyethylene glycol (PEG)–superoxide dismutase (SOD), 1000 U/ml PEG–catalase, 5 mM *N*-acetylcysteine (NAC), 100 mM thiourea, 100 mM mannitol, 1% DMSO, 50 µM BAY 11-7082, 50 µM resveratrol and 10 µM SRT1720 for 30 min before incubation with HX/XO (*n*=3–6 per treatment). HX, XO, PEG–SOD, PEG–catalase, NAC, thiourea, mannitol, DMSO and resveratrol were purchased from Sigma. After the 24 h incubation, medium and tissue were collected separately and stored at –80 °C until assayed as detailed below.

Oxidative stress assays

For the measurement of hydrogen peroxide in the incubation buffer in the presence or absence of HX/XO, a colorimetric hydrogen peroxide assay kit was used (BioVision, Inc., Mountain View, CA, USA). The calculated coefficients of variation were all <10%.

Western blotting

Assessment of tissue protein expression was analysed by western blotting as described previously (Colomiere *et al.* 2009, 2010). Rabbit polyclonal anti-IR-β (0.4 µg/ml), rabbit polyclonal anti-IRS1 (0.8 µg/ml), rabbit polyclonal anti-GLUT1 (0.5 µg/ml) and mouse monoclonal anti-GLUT3 (0.5 µg/ml) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Membranes were viewed and analysed using the Chemi-Doc system (Bio-Rad). Quantitative analysis of the relative density of the bands in western blots was performed using Quantity One 4.2.1 image analysis software (Bio-Rad). Data were corrected for background and then normalised to β-actin expression.

RNA extraction and RT-PCR

Total RNA was extracted from ~100 mg tissue using Tri Reagent according to the manufacturer's instructions (Sigma–Aldrich). RNA concentrations were quantified using a spectrophotometer (Smart Spec; Bio-Rad). RNA quality was determined via the A₂₆₀/A₂₈₀ ratio. RNA (1 µg) was converted to cDNA using the SuperScript VILO cDNA synthesis kit (Invitrogen) according to the manufacturer's instructions. The cDNA was diluted tenfold, and 2 µl cDNA was used to perform RT-PCR using Sensimix Plus SYBR green (Quantace, Alexandria, NSW, Australia) and 100 nM QuantiTect Primer Assays (Qiagen). Pre-validated primers for IR-β (QT00082810), IRS1 (QT00074144), PI3K p85α (QT01005984), GLUT1 (QT00068957), GLUT3 (QT00047124), GLUT4 (QT00097902) and SIRT1 (QT00051261) were purchased from Qiagen. The syncytin primer has previously been described (Paiva *et al.* 2011). Average gene C_T values were normalised to the average GAPDH values of the same cDNA sample. Of note, there was

no effect of experimental treatment on *GAPDH* gene expression. There was equal efficiency of PCR amplification of target and reference mRNA (average between 90 and 110%). The specificity of the product was assessed from melting curve analysis. RNA without reverse transcriptase during cDNA synthesis as well as PCR reactions using water instead of template showed no amplification. Gene expression levels were determined using the comparative threshold cycle (C_t) method.

Glucose uptake

Placental explants were performed as detailed above. After 24 h incubation with treatment, tissues were pre-incubated in the absence or presence of 20 μ M cytochalasin B (Sigma) in Krebs buffer for 5 min. 2-deoxy-D-glucose (2DG) uptake was measured by adding 3 μ Ci/ml [14 C]-2DG (Perkin Elmer) and 1 mM 2DG to Krebs buffer containing 0.1% BSA (fatty acid free). Glucose transport was measured at 37 °C for 20 min and was stopped by washing three times in ice-cold PBS. Tissues were solubilised with 1 M NaOH, neutralised with 1 M HCl and then centrifuged at 15 000 g for 5 min to pellet insoluble material. The supernatant was added to 3 ml liquid scintillation fluid, and 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 cytochalasin B. The rate of [14 C]-2DG transport was expressed in nanomoles per minute per milligram tissue.

Statistical analysis

Statistical analyses were performed using a commercially available statistical software package (Statgraphics Plus version 3.1; Statistical Graphics Corp., Rockville, MD, USA). Student's *t*-test was used to assess statistical significance between normally distributed data; otherwise, the non-parametric Mann–Whitney *U* (Wilcoxon) test was used. Statistical difference was indicated by a *P* value of <0.05. Data are expressed as mean $\pm$ S.E.M.

Results

Effect of HX/XO on GLUT expression in human placenta

GLUT1, *GLUT3* and *GLUT4* have all been localised in human placenta (Hauguel-de Mouzon *et al.* 1997, Xing *et al.* 1998, Illsley 2000, Colomiere *et al.* 2010) and thus may be responsible for glucose uptake in this tissue. Thus, our next aim was to determine the effect of HX/XO on GLUT expression. The effect of 24 h treatment of human placenta with HX/XO on GLUT mRNA and protein expression is shown in Fig. 1. HX/XO treatment significantly decreased GLUT1 mRNA and protein expression. On the other hand, there was no effect of HX/XO on GLUT3 and GLUT4 mRNA and protein expression.

Figure 1 Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO for 24 h ($n=7$). (A) GLUT gene expression is expressed as mean $\pm$ S.E.M. (B) Protein expression is expressed as mean (normalised to β -actin) $\pm$ S.E.M. A representative GLUT1 and GLUT3 western blot is also shown. * $P<0.05$ vs basal GLUT1 expression.

Effect of HX/XO on glucose uptake in human placenta

To investigate the effects of oxidative stress on glucose uptake, an *ex situ* tissue explant incubation model was used. Human placenta was incubated in the absence (basal) and presence of HX/XO for 24 h ($n=7$). When compared with basal uptake, glucose uptake was significantly decreased with HX/XO treatment in placenta (Fig. 2A). As placenta is a source of XO (Biri *et al.* 2006), control experiments were also performed in the presence of HX alone. There was, however, no effect of HX on placental glucose uptake (Fig. 2B). The insulin signalling pathway regulates glucose uptake; however, in this study, there was no effect of HX/XO on IR- β , IRS1 and PI3K mRNA and protein expression (data not shown).

Figure 2 Placenta was incubated in the absence or presence of (A) 0.5 mM HX + 15 mU/ml XO ($n=7$) for 24 h, (B) 0.5 mM HX ($n=4$) for 24 h or (C) 0.5 mM HX + 15 mU/ml XO ($n=4$) for 4 h. Data are expressed as mean 2DG uptake (nmol/min per mg protein) $\pm$ S.E.M. * $P<0.05$ vs basal 2DG uptake. (D) Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO for 24 h ($n=7$). Syncytin gene expression is expressed as mean $\pm$ S.E.M.

HX/XO treatment is not associated with syncytial damage

In this study, all treatments were performed for 24 h. Thus, it is possible that the effects of HX/XO on 2DG uptake may be due to damage and loss of the syncytium. Thus, we also determined the effect of HX/XO on 2DG uptake after 4 h. As shown in Fig. 2C, HX/XO significantly decreased placental glucose uptake after 4 h (Fig. 2C). In addition, there was no effect of HX/XO on syncytin gene expression (Fig. 2D).

Superoxide and hydrogen peroxide do not regulate glucose uptake in human placenta

Catalytic oxidation of HX by XO produces H_2O_2 and superoxide anions. To determine whether this occurs in our system, we measured H_2O_2 concentration in the incubation buffer containing HX/XO. As shown in Fig. 3A, there was a significant increase in H_2O_2 concentration in the presence of HX/XO. We then determined whether the increase in H_2O_2 and/or superoxide anions was responsible for the decrease in glucose uptake. Experiments were carried out by pre-incubating placental tissue explants with the superoxide scavenger SOD (300 U/ml) or the H_2O_2 scavenger catalase (1000 U/ml) for 30 min, before incubation with HX/XO for 24 h. Figure 3B shows that catalase and SOD treatment did not restore the decrease in glucose uptake induced by HX/XO. Further, there was no effect of 0.25, 0.5 and 1 mM H_2O_2 on placental glucose uptake (Fig. 3C). Collectively, these data show that superoxide and H_2O_2 are not involved in the regulation of glucose uptake by HX/XO in this system.

Hydroxyl radical scavengers do not restore the decrease in glucose uptake induced by HX/XO

H_2O_2 can readily pass through cell membranes and interact with intracellular iron to form highly reactive oxidants such as the hydroxyl radical ($\cdot OH$). Thus, we assessed the effect of $\cdot OH$ scavengers on placental glucose uptake in the presence

Figure 3 (A) Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO for 24 h ($n=7$). Data are expressed as mean H_2O_2 release (μM /mg protein) $\pm$ S.E.M. * $P<0.05$ vs basal H_2O_2 release. (B) Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO with or without 1000 U/ml catalase-PEG ($n=4$), 300 U/ml SOD-PEG ($n=4$), 100 mM mannitol ($n=3$), 1% DMSO ($n=3$), 5 mM NAC ($n=3$) and 50 μM BAY 11-7082 ($n=4$) for 24 h. Data are expressed as mean 2DG uptake (nmol/min per mg protein) $\pm$ S.E.M. * $P<0.05$ vs basal 2DG uptake. (C) Placenta was incubated in the absence or presence of 0.25, 0.5 and 1 mM H_2O_2 for 24 h ($n=4$). Data are expressed as mean 2DG uptake (nmol/min per mg protein) $\pm$ S.E.M.

Figure 4 Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO with or without 50 μ M resveratrol for 24 h ($n=6$). (A) *SIRT1* gene expression is expressed as mean $\pm$ S.E.M. * $P<0.05$ vs HX/XO *SIRT1* mRNA expression. (B) *SIRT1* protein expression is expressed as mean (normalised to β -actin) $\pm$ S.E.M. * $P<0.05$ vs HX/XO *SIRT1* protein expression. Two representative western blots are shown.

of HX/XO. As shown in Fig. 3B, there was no effect of thiourea, mannitol, DMSO or NAC on glucose uptake in the presence of HX/XO.

Inhibition of NF- κ B does not restore the decrease in glucose uptake induced by HX/XO

There are multiple mechanisms that regulate glucose uptake, and in recent years NF- κ B has been suggested to be a key signalling protein regulating insulin-independent glucose uptake. Previous studies from our laboratory have shown that in response to HX/XO treatment, placenta increases NF- κ B DNA-binding activity (Coughlan *et al.* 2004). Thus, in order to elucidate whether NF- κ B is involved in the HX/XO regulation of glucose uptake, placenta was incubated with the NF- κ B inhibitor BAY 11-7082 for 24 h, in the presence of HX/XO. There was, however, no effect of BAY

11-7082 on placental glucose uptake in the presence of HX/XO (Fig. 3B).

Resveratrol restores the decrease in glucose uptake induced by HX/XO

In non-gestational tissues, recent studies have shown that SIRT1 regulates glucose uptake (Yoshizaki *et al.* 2009). The natural photochemical, resveratrol, is an activator of SIRT1 (Bai *et al.* 2008, Csiszar *et al.* 2008, Backesjo *et al.* 2009, Kennedy *et al.* 2009). Similarly, we have previously shown that, in human placenta, resveratrol inhibits lipopolysaccharide (LPS)-induced inflammation via SIRT1 (Lappas *et al.* 2011b). Thus, we sought to investigate whether resveratrol can restore the decrease in glucose uptake induced by HX/XO. First, we determined the effect of HX/XO on SIRT1 gene and protein expression. As shown in Fig. 4, *SIRT1* mRNA gene and protein expression was significantly decreased in the presence of HX/XO. Co-incubation with resveratrol was associated with an increase in SIRT1 gene and protein expression.

Then, we determined the effect of resveratrol on glucose uptake. Placenta was incubated with HX/XO in the absence or presence of 50 μ M resveratrol for 24 h. The effect of resveratrol on glucose uptake is shown in Fig. 5A. Resveratrol significantly attenuated the decrease in placental glucose uptake induced by HX/XO. In addition, co-incubation with resveratrol significantly attenuated the HX/XO-induced decrease in *GLUT1* mRNA (Fig. 5B) and protein expression (Fig. 5C).

Discussion

In this study, we have demonstrated that HX/XO-induced oxidative stress decreases placental *GLUT1* expression and glucose uptake via SIRT1. Specifically, we have shown that oxidative stress decreases SIRT1 gene and protein expression in human placenta, and the SIRT1 activator resveratrol attenuates the decrease in *GLUT1* expression and glucose uptake mediated by HX/XO.

HX/XO treatment significantly reduced *GLUT1* expression that was associated with a concomitant decrease in glucose uptake. *GLUT1* is located at both the maternal-facing microvillous trophoblast membrane and the foetal-facing basal trophoblast membrane, thereby allowing bidirectional transport across the placenta (Illsley 2000). In this study, we found that there was no effect of HX/XO on *GLUT3* and *GLUT4* expression. *GLUT3* is mainly localised to the vascular endothelium (Hauguel-de Mouzon *et al.* 1997, Illsley 2000) and therefore cannot be involved in placental glucose uptake from the maternal circulation; it may act specifically in the transport of glucose to the foetus. *GLUT4* also has been found in the human placenta (Xing *et al.* 1998), and insulin mediates glucose uptake in first-trimester chorionic villous explants (Kniss *et al.* 1994), but its role in term placenta is controversial (Challier *et al.* 1986).

Figure 5 (A) Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO with or without 50 μ M resveratrol for 24 h ($n=6$). Data are expressed as mean 2DG uptake (nmol/min per mg protein) $\pm$ S.E.M. * $P<0.05$ vs HX/XO 2DG uptake. (B) Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO with or without 50 μ M resveratrol for 24 h ($n=6$). *GLUT1* gene expression is expressed as mean $\pm$ S.E.M. * $P<0.05$ vs HX/XO *GLUT1* mRNA expression. (C) Placenta was incubated in the absence or presence of 0.5 mM HX + 15 mU/ml XO with or without 50 μ M resveratrol for 24 h ($n=6$). *GLUT1* protein expression is expressed as mean (normalised to β -actin) $\pm$ S.E.M. * $P<0.05$ vs HX/XO *GLUT1* protein expression. A representative western blot image is shown.

In this study, though we could detect low levels of *GLUT4* at the mRNA level, we were unable to detect *GLUT4* protein expression in the placenta. There was also no effect of HX/XO on total expression levels of proteins of the insulin signalling pathways, namely IR- β , IRS1 and PI3K, though activation of this pathway was not examined.

The molecular mechanism responsible for this decrease is not known but is not attributable to ROS. In this study, we used various antioxidants, including NAC, SOD and catalase, that quench the production of superoxide anion and hydrogen peroxide produced by the HX and XO reaction; however, there was no effect of these antioxidants on the reduction of placental glucose uptake-induced HX/XO. It is possible that the concentration of antioxidants used in this study was not optimal or that a combination of various antioxidants is required to see an effect. A previous study has, however, reported that the overexpression of antioxidant thioredoxin increases placental *GLUT1* in mice (Umekawa *et al.* 2008). Previous studies from our laboratory have shown that cytokine response to HX/XO may be mediated by the oxidant-responsive transcription factor NF- κ B (Coughlan *et al.* 2004). However, in this study, the NF- κ B inhibitor was unable to restore the decrease in placental glucose uptake in the presence of oxidative stress.

HX/XO reduced *SIRT1* gene and protein expression in human placenta. Likewise, oxidative stress decreases *SIRT1* expression in the hippocampus and cerebral cortex (Wu *et al.* 2006), and human umbilical vein endothelial cells exposed to cigarette smoke extract and hydrogen peroxide showed decreased *SIRT1* levels and activity (Arunachalam *et al.* 2010). More recently, it has been shown that direct modification of *SIRT1* by ROS or alkylating agents can mediate its degradation (Caito *et al.* 2010). Given that HX/XO decreased *SIRT1* expression, we sought to determine the effect of the *SIRT1* activator, resveratrol (Bai *et al.* 2008, Csiszar *et al.* 2008, Backesjo *et al.* 2009, Kennedy *et al.* 2009). Treatment with resveratrol ameliorated the HX/XO-induced impairment in glucose uptake and this coincided with significant increases in the expression of *GLUT1*. This suggests that *SIRT1* is responsible for the regulation of glucose uptake mediated by HX/XO in human placenta. This is in keeping with our previous studies in human placenta, whereby resveratrol inhibits inflammation by a *SIRT1*-dependent mechanism (Lappas *et al.* 2011b). Of note, these studies showed that resveratrol had no effect on NF- κ B and MAPK pathways in human placenta. Our data is in keeping with other studies; in muscle cells (Breen *et al.* 2008) and adipocytes (Fischer-Posovszky *et al.* 2010), resveratrol elevates glucose uptake via *SIRT1* and improves insulin sensitivity in adipocytes (Yoshizaki *et al.* 2009).

Our previous studies demonstrate that the pro-inflammatory cytokines tumour necrosis factor α (TNF α) and interleukin 1 β (IL1 β) decrease *SIRT1* expression in human placenta (Lappas *et al.* 2011b). Further, in recent studies in our laboratory, we showed that both TNF α and IL1 β down-regulate glucose uptake and *GLUT1* expression (R Lim and M Lappas 2010, unpublished observations). Whether these cytokines are also regulating glucose transport via *SIRT1* is not known at this stage; however, it is tempting to speculate that TNF α and IL1 β may also decrease placental glucose uptake via *SIRT1*-dependent mechanisms.

It is known that incubation of placental tissue causes significant syncytial damage and loss after ~6 h of incubation (Palmer *et al.* 1997). In this study, all treatments were performed for 24 h. Thus, it is possible that the effects of HX/XO on 2DG uptake may be due to damage and loss of the syncytium. However, we also determined the effect of HX/XO on 2DG uptake after 4 h. HX/XO was able to significantly decrease placental glucose uptake. In addition, we also assessed the general morphology of the placental tissue after incubation using hematoxylin and eosin (H&E). No difference was observed between the basal and the HX/XO-treated tissues (data not shown). There was also no effect of HX/XO on PI3K and syncytin—proteins that are specifically localised to the syncytiotrophoblast of the placenta. We have also previously reported no effect of HX/XO on placental release of lactate dehydrogenase, a marker of tissue viability (Coughlan *et al.* 2004, Lappas *et al.* 2010). Collectively, this suggests that the effects of HX/XO on glucose transport are not due to damage or loss of the syncytium.

Our original hypothesis was that ROS, generated by the HX/XO system, would regulate glucose uptake in human placenta. However, the data presented in this paper suggest that this is not the case; the antioxidants, including SOD, catalase and NAC, had no effect on HX/XO-induced decreases in glucose uptake and *GLUT1* expression. However, we did find that resveratrol, a SIRT1 activator, was able to restore the effects of HX/XO on glucose uptake and *GLUT1* expression. Though the underlying mechanism/pathway that elicited these changes is not known, it is possible that HX/XO, via its effects on SIRT1, is inducing chromatin modifications. SIRT1s possess NAD⁺-dependent histone deacetylase (HDAC) activity (North & Verdin 2004). Though there are no studies that have investigated the effect of HX/XO on histone acetylation, *GLUT1* expression and glucose uptake, at least in part, are regulated by changes in deacetylation. For example, in myeloma cells, a number of different HDAC inhibitors induced a decrease in glucose uptake and *GLUT1* expression (Wardell *et al.* 2009). Likewise, glucose uptake was inhibited in colorectal adenocarcinomas cells (HT29) treated with HDAC inhibitors (Boren *et al.* 2003). Future studies are required to determine whether HX/XO regulates glucose uptake by interfering with or promoting histone acetylation in human placenta.

There are much data showing that the placenta, by transferring nutrients to the foetus, plays a major role in the regulation of foetal metabolism and growth (Fowden *et al.* 2009). Alterations in the amounts of proteins, carbohydrates and fat that are supplied to the foetus may have long-term consequences (Jansson & Powell 2007). Changes in glucose metabolism (Osmond *et al.* 2001) and increased oxidative stress are characteristics of pregnancies complicated with diabetes (Lappas *et al.* 2011a). Excess uptake and transfer of maternal glucose across the placenta would lead to foetal hyperglycaemia with subsequent hyperinsulinaemia and its associated detrimental sequelae (Nold & Georgieff 2004). Thus, in order to circumvent and/or control the effects of

aberrant nutrient transfer and thus harmful effects on foetal development, it is of clinical significance to identify modulators of nutrient transport within the placenta. In this study, we provide evidence that the reduction in placental glucose uptake and *GLUT1* expression induced by HX/XO is mediated by SIRT1.

Declaration of interest

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

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