RESEARCH

Glucocorticoid regulation of amino acid transport in primary human trophoblast cells

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Abstract

Excess maternal glucocorticoids reduce placental amino acid transport and fetal growth, but whether these effects are mediated directly on the syncytiotrophoblast remains unknown. We hypothesised that glucocorticoids inhibit mechanistic target of rapamycin (mTOR) signaling and insulin-stimulated System A amino acid transport activity in primary human trophoblast (PHT) cells. Syncytialised PHTs, isolated from term placentas ($n = 15$), were treated with either cortisol (1 μ M) or dexamethasone (1 μ M), $\pm$ insulin (1 nM) for 24 h. Compared to vehicle, dexamethasone increased mRNA expression, but not protein abundance of the mTOR suppressor, regulated in development and DNA damage response 1 (REDD1). Dexamethasone enhanced insulin receptor abundance, activated mTOR complex 1 and 2 signaling and stimulated System A activity, measured by Na⁺-dependent ¹⁴C-methylaminoisobutyric acid uptake. Cortisol also activated mTORC1 without significantly altering insulin receptor or mTORC2 read-outs or System A activity. Both glucocorticoids downregulated expression of the glucocorticoid receptor and the System A transporter genes *SLC38A1*, *SLC38A2* and *SLC38A4*, without altering SNAT1 or SNAT4 protein abundance. Neither cortisol nor dexamethasone affected System L amino acid transport. Insulin further enhanced mTOR and System A activity, irrespective of glucocorticoid treatment and despite downregulating its own receptor. Contrary to our hypothesis, glucocorticoids do not inhibit mTOR signaling or cause insulin resistance in cultured PHT cells. We speculate that glucocorticoids stimulate System A activity in PHT cells by activating mTOR signaling, which regulates amino acid transporters post-translationally. We conclude that downregulation of placental nutrient transport *in vivo* following excess maternal glucocorticoids is not mediated by a direct effect on the placenta.

Key Words:

- ▶ placenta
- ▶ maternal–fetal exchange
- ▶ fetal development
- ▶ mechanistic target of rapamycin
- ▶ solute carrier family 38A members 1, 2 and 4

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Introduction

Exposure of the placenta and fetus to excess glucocorticoids is associated with reduced fetal growth and increased risk for cardiovascular and metabolic disease later in life. Despite beneficial effects on neonatal viability, repeated antenatal administration of synthetic glucocorticoids to

women delivering preterm reduces birthweight (Crowther *et al.* 2015), increases blood pressure and impairs glucose metabolism in their children (Doyle *et al.* 2000, Kelly *et al.* 2012). Elevated endogenous cortisol concentrations, as a result of maternal stress and hypothalamic-pituitary

adrenal axis activation, are also linked to reduced fetal growth in humans (Duthie & Reynolds 2013). Conversely, low maternal cortisol levels in obese women are associated with fetal overgrowth (Stirrat *et al.* 2014, 2017). Natural and synthetic glucocorticoid administration to pregnant animals similarly reduces birth weight and alters lifelong metabolic, cardiovascular and endocrine phenotype in the offspring (Fowden *et al.* 2016). Maternal glucocorticoids of both exogenous and endogenous origin therefore limit fetal growth. Since the presence of the inactivating enzyme 11 β -hydroxysteroid dehydrogenase type II (11 β HSD2) in the syncytiotrophoblast limits direct passage of maternal cortisol into the fetal circulation (Stirrat *et al.* 2018), the effects of maternal glucocorticoid excess on intrauterine development may be mediated indirectly, via effects on placental function.

Glucocorticoids may limit fetal growth by decreasing placental nutrient delivery (Fowden *et al.* 2015, Vaughan *et al.* 2017). Maternal glucocorticoid administration suppresses placental transport of neutral amino acids, mediated by System A transporters (SNATs 1, 2 and 4) (Audette *et al.* 2011, 2014, Vaughan *et al.* 2012, 2013). These transporters actively accumulate amino acids in the syncytiotrophoblast, thereby establishing fetal circulating amino acid concentrations that are 2–3-fold higher than maternal concentrations. Indeed, placental System A amino acid transport may be critical for normal fetal growth (Cramer *et al.* 2002) and System A activity is often reduced in the placentas of severely growth-restricted infants (Mahendran *et al.* 1993, Glazier *et al.* 1997, Shibata *et al.* 2008, Mando *et al.* 2013). Insulin and insulin-like growth factor I (IGF-I) stimulate trophoblast System A activity (Karl *et al.* 1992, Karl 1995, Jansson *et al.* 2003, Roos *et al.* 2009a). System A is also regulated by mechanistic target of rapamycin (mTOR) signaling, which promotes SNAT2 translocation to the syncytiotrophoblast microvillous plasma membrane (Roos *et al.* 2009b, Rosario *et al.* 2013, 2016a). In skeletal muscle, glucocorticoids inhibit insulin signaling and impair mTOR activation by upregulating transcription of the mTOR repressor, *regulated in DNA damage and repair 1* (REDD1) (Wang *et al.* 2006, Shimizu *et al.* 2011). Glucocorticoids similarly enhance *Redd1* expression and suppress mTOR activation in the mouse placenta *in vivo* (Vaughan *et al.* 2015), but the role of REDD1 and mTOR in glucocorticoid-regulated System A transport in human trophoblast remains unknown. We hypothesized that glucocorticoids inhibit insulin-stimulated System A amino acid transport in syncytialised primary human trophoblast (PHT) cells by upregulating REDD1 expression and inhibiting mTORC1 signaling.

To test this hypothesis, PHT cells were treated with cortisol, at physiological concentrations in the maternal circulation in late pregnancy (Li *et al.* 2012) or dexamethasone, at concentrations achieved in maternal serum following treatment with synthetic glucocorticoids (Ballard & Ballard 1995). Cortisol acts on both the mineralocorticoid and glucocorticoid receptors, whereas dexamethasone is a more potent synthetic glucocorticoid that acts exclusively on the glucocorticoid receptor. Human trophoblast cells express functional mineralocorticoid and glucocorticoid receptors (Driver *et al.* 2003, Lee *et al.* 2005).

Methods

Human tissue collection

All procedures were conducted with informed consent and ethical approval from the University of Colorado Institutional Review Board. Placentae ($n=15$) were collected from vaginal deliveries or uncomplicated, non-labored Caesarean sections at term. Clinical characteristics of the study subjects are given in Table 1. Maternal smoking was an exclusion criterion for this study. None of the subjects received antenatal glucocorticoid treatment.

Isolation, culture and treatment of primary human trophoblast cells

Primary cytotrophoblasts were isolated from dissected villous tissue by trypsin digestion and discontinuous Percoll gradient separation, as described previously (Kliman *et al.* 1986, Roos *et al.* 2009b). Isolated cells were plated at 1.4×10^6 per well in six-well plates and cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM, 25 mM glucose) and Ham's F12 medium (10 mM glucose) with 10% fetal bovine serum, 2 mM glutamine, 50 μ g/mL gentamicin, 60 μ g/mL penicillin and 100 μ g/mL streptomycin. Cells were washed and the media was changed 18 h after isolation and daily thereafter. An aliquot of spent medium was collected each day for measurement

Table 1 Clinical characteristics of study subjects.

Characteristic	Value
n	15
Maternal body mass index (kg/m ²)	24.1 $\pm$ 1.2
Gestational age at delivery (wk)	39.6 $\pm$ 0.2
Delivery type (number vaginal)	7
Infant sex (number of females)	8
Birth weight (kg)	3.31 $\pm$ 0.06
Placenta weight (g)	612 $\pm$ 34

Continuous variables presented as mean $\pm$ s.e.m.

of human chorionic gonadotrophin (hCG) concentration by ELISA (IBL America, Minneapolis, MN, USA). At 66h in culture, either cortisol (1 μ M, H0888, Sigma-Aldrich), dexamethasone (1 μ M, D2915, Sigma-Aldrich), or vehicle (DMEM) was added to the culture medium for 24h. To determine the effect of the glucocorticoids on insulin signaling and insulin-stimulated amino acid transport, cells were concurrently treated with bovine insulin (1 nM, I0516, Sigma-Aldrich).

Gene expression and protein abundance

For analysis of gene expression ($n=9$ placentas; four male, five female), RNA was extracted from PHT cells lysed in guanidine isothiocyanate following 24h of hormone treatment by ethanol precipitation and spin column separation. One microgram of extracted RNA was reverse transcribed to cDNA and quantitative RT-PCR was performed to determine the relative expression of genes encoding REDD1 (*REDD1*, Taqman probe Hs01111686_g1), insulin receptor (*INSR*, Taqman probe Hs00961557_m1), glucocorticoid receptor (*NR3C1*, F: AAACCTTACTGCTTCTCTCTTCA, R: GTTAAGGAGATTTTCAACCACTTC; Gutling *et al.* 2016), SNAT1 (*SLC38A1*, F: GTGTATGCTTTACCCACCATTGC, R: GCACGTTGTCATAGAATGTCAAGT; Audette *et al.* 2014), SNAT2 (*SLC38A2*, Taqman probe Hs01089954_m1) and SNAT4 (*SLC38A4*, F: TTGCCGCCCTCTTTGGTTAC, R: GAGGACAATGGGCACAGTTAGT; Audette *et al.* 2014). Expression of the target transcripts was normalized to ribosomal protein S29 (*RPS29*, Taqman probe Hs03004310_g1) expression using the ddCt method. Average Ct values for RPS29 were not affected by cell treatment (mean $\pm$ s.e.m., control 25.3 ± 0.3 , cortisol 25.3 ± 0.4 , dexamethasone 25.0 ± 0.3 , insulin 25.2 ± 0.2 , cortisol+insulin 25.2 ± 0.3 , cortisol+dexamethasone 25.0 ± 0.3). Mean Ct values for the targets of interest were as follows: *REDD1* 28.2, *INSR* 26.0, *NR3C1* 25.5, *SLC38A1* 20.0, *SLC38A2* 27.4, *SLC38A4* 30.9.

Trophoblast abundance of glucocorticoid, insulin, mTOR signaling proteins, SNAT1 and SNAT4 was determined by Western blot following 24h of hormone treatment ($n=6$ placentas; three male, three female). Protein lysates, containing protease and phosphatase inhibitors, were resolved by SDS-PAGE under denaturing, reducing conditions and then transferred to PVDF membranes. Following blocking in 5% bovine-serum albumin, membranes were incubated with primary antibodies targeting glucocorticoid receptor (Cell Signaling Technologies), insulin receptor β (Santa Cruz Biotechnology), REDD1, phosphorylated (Ser235/236) and

total S6 ribosomal protein (S6), phosphorylated (Ser473) and total Akt, phosphorylated (Thr202/Tyr204) and total extracellular signal-regulated kinase 1/2 (ERK1/2, all Cell Signaling Technologies), SNAT1 (AV43810, Sigma-Aldrich) and SNAT4 (Eurogentec). Abundances of cytokeratin 7 and vimentin were also assessed relative to crude placental homogenate, as markers of trophoblast and mesenchymal cells (MA5-11986, Invitrogen and ab20346). Membranes were then washed and incubated with a horseradish-peroxidase-linked, species-specific secondary antibody and protein bands were visualized using enhanced chemiluminescence reagents and an automated image acquisition system (G:BOX, Syngene, Cambridge, UK). Protein abundance was quantified by band densitometry (total fluorescence, GeneTools, Syngene) and amido black staining was used as a loading control.

Amino acid uptake

System A and System L-mediated amino acid transport activity was also measured in syncytialised PHT cells ($n=9$ placentas; five male, four female) after 24h of hormone treatment as the rate of Na⁺-dependent ¹⁴C-methylaminoisobutyric acid (MeAIB) uptake and 4-amino-norbornane carboxylic acid (BCH)-inhibitable ³H-leucine uptake, as described previously (Roos *et al.* 2009b). Briefly, cells were washed and then incubated in Tyrode's buffer containing 20 μ M ¹⁴C-MeAIB and 12.5 nM ³H-leucine for 8 min. Non-mediated tracer uptake was determined in parallel by incubating cells with Na⁺-free Tyrode's buffer containing BCH. In both cases, uptake was terminated by washing with ice-cold, Na⁺-free Tyrode's buffer. Cells were lysed in distilled water to release intracellular tracer, which was quantified by liquid scintillation counting then corrected for protein content, determined by Lowry assay. The rate of transporter-mediated tracer uptake was determined as the difference between total uptake, in the presence of Na⁺, and non-mediated uptake. Preliminary experiments established that radiotracer uptake was linear with respect to time up to 10 min of incubation; therefore, the rate of uptake was quantified at 8 min in all treatment groups.

Statistical analysis

Data are reported as mean $\pm$ s.e.m. The main effects of glucocorticoid and insulin treatment on trophoblast amino acid uptake, gene and protein expression were determined by two-way ANOVA with repeated-measures within each primary cell preparation (each placenta).

When there was an overall effect of glucocorticoid treatment, Holm–Sidak *post hoc* tests were performed for pairwise comparison of vehicle, cortisol and dexamethasone treatments. When there was an overall effect of insulin treatment, the simple effect of insulin within each level of glucocorticoid treatment (vehicle, cortisol, dexamethasone) was determined by Holm–Sidak test. When there was a significant interaction between glucocorticoid and insulin effects, Holm–Sidak post-tests were performed for both factors. The effect of dexamethasone on SNAT1 and SNAT4 abundance was determined by Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results

Characterisation of isolated primary human trophoblast cells

In the isolated placental cell preparations, mean daily hCG secretion rate into the medium increased over the course of the culture period, consistent with trophoblast syncytialisation by 66 h post isolation (Fig. 1A). Immunoblotting confirmed that the cultured cells expressed the trophoblast marker cytokeratin 7, but not the mesenchymal cell marker, vimentin (Fig. 1B).

Glucocorticoid and insulin receptor signaling

Cortisol and dexamethasone induced qualitatively similar changes in gene and protein expression in PHT cells, although the effects of dexamethasone were greater in magnitude. *REDD1* gene expression was greater in cortisol- (+27%) and dexamethasone-treated (+56%) cells compared to vehicle-treated controls (Fig. 2B) but neither glucocorticoid altered trophoblast *REDD1* protein abundance (Fig. 2C). Dexamethasone significantly increased IR β protein abundance but not *INSR* mRNA expression in the PHT cells, whereas cortisol did not affect either IR β nor *INSR* mRNA expression (Fig. 2D and E). Consistent with this insulin receptor upregulation, glucocorticoid treatment enhanced phosphorylation of the mTORC1 and mTORC2 downstream targets, S6 and Akt, without any change in total S6 or Akt abundance (Fig. 3A, B, C, D and E). Moreover, glucocorticoids did not impair insulin receptor signaling, as insulin independently increased S6 and Akt phosphorylation in vehicle- and glucocorticoid-treated cells (Fig. 3B and D). Insulin reduced IR β abundance in dexamethasone-treated cells and downregulated *INSR* expression irrespective of

Figure 1

Characterisation of primary human trophoblast cells. (A) Daily rate of hCG secretion into the culture medium by PHT cells at 18, 42, 66 and 90 h after isolation. Overall effect of time point determined by one-way ANOVA with Holm–Sidak *post hoc*. Differing superscripts a, b indicate significantly different time points. $n = 9$ placentas. Mean $\pm$ s.e.m. (B) Western blots of cytokeratin 7 (trophoblast marker) and vimentin (mesenchyme marker) in lysates collected from syncytialised PHT cells 90 h after isolation, compared to crude placental homogenate (Plac. Hom.).

glucocorticoid treatment but did not affect *REDD1*/*REDD1* gene expression or protein abundance (Fig. 2). There was no effect of either insulin or glucocorticoids on phosphorylated or total ERK1/2 abundance (Fig. 3F and G). Glucocorticoid receptor mRNA and protein abundance was lower in glucocorticoid-treated compared to vehicle-treated PHTs, but was not affected by insulin (Fig. 2F and G).

Amino acid transport and transporter expression

Overall, glucocorticoids significantly stimulated System A amino acid transport in PHT cells, such that the rate of ^{14}C -MeAIB uptake was higher in dexamethasone-treated trophoblasts than in cortisol-treated or control cells, irrespective of insulin treatment (Fig. 4A, B, C). Both cortisol and dexamethasone increased basal ^{14}C -MeAIB uptake,

Figure 2

Effects of glucocorticoids and insulin on REDD1 and insulin receptor abundance. Representative Western blots (A), gene expression (B, D and F) and protein abundance (C, E and G) of REDD1 and insulin receptor in syncytialised primary human trophoblast cells treated with vehicle (Con), cortisol (Cort, 1 μM) and dexamethasone (Dex, 1 μM), ± insulin (concentration, 1 nM). Effects of glucocorticoid (P_{GC}), insulin (P_{Ins}) and interaction (P_{GC*Ins}) were determined by two-way ANOVA with repeated measures and significances ($P < 0.05$) are provided in the figure. Differing superscripts a, b indicate significantly different glucocorticoid treatment groups, * indicates significant simple effect of insulin and † indicates significant simple effect of dexamethasone by Holm–Sidak *post hoc*. $n = 9$ placentas (gene expression), $n = 6$ placentas (protein). Mean $\pm$ S.E.M.

Figure 3

Effects of glucocorticoids and insulin on mTORC1, mTORC2 and ERK1/2 signaling. (A) Representative Western blots and (B, C, D, E, F and G) phosphorylated and total protein abundance for readouts of mTORC1, mTORC2 and ERK1/2 signaling in syncytialised primary human trophoblast cells treated with vehicle (Con), cortisol (Cort, 1 μM) and dexamethasone (Dex, 1 μM), ± insulin (concentration, 1 nM). Effects of glucocorticoid (P_{GC}), insulin (P_{Ins}) and interaction (P_{GC*Ins}) were determined by two-way ANOVA with repeated measures and significances ($P < 0.05$) are provided in the figure. Differing superscripts a, b indicate significantly different glucocorticoid treatment groups, * indicates significant simple effect of insulin by Holm–Sidak *post hoc*. $n = 6$ placentas. Mean $\pm$ S.E.M.

Figure 4

Effects of glucocorticoids and insulin on System A and System L amino acid transport. System A-mediated ^{14}C -methylaminoisobutyric acid uptake (A and C) and System L-mediated ^3H -leucine uptake (B and D) in syncytialised primary human trophoblast cells. (A and B) Time-course of total, non-mediated (+BCH, $-\text{Na}^+$) and transporter-mediated (total - non-mediated) uptake in untreated cells ($n = 3$ placentas). (C and D) Rate of uptake assessed after 8 min of tracer incubation in vehicle (Con), cortisol (Cort, $1\ \mu\text{M}$) and dexamethasone (Dex, $1\ \mu\text{M}$) treated cells, $\pm$ insulin (concentration, $1\ \text{nM}$). Effects of glucocorticoid (P_{GC}), insulin (P_{Ins}) and interaction ($P_{\text{GC} \times \text{Ins}}$) were determined by two-way ANOVA with repeated measures and significances ($P < 0.05$) are provided in the figure. Differing superscripts a, b indicate significantly different glucocorticoid treatment groups, * indicates significant simple effect of insulin by Holm-Sidak *post hoc*. $n = 9$ placentas. Mean $\pm$ S.E.M.

in the absence of insulin ($P < 0.05$ vs control without insulin, Holm-Sidak *post hoc*). Insulin further enhanced System A activity in vehicle- and dexamethasone-treated cells, but not in cortisol-treated cells (Fig. 4C). In contrast, both cortisol and dexamethasone reduced PHT cell expression of the amino acid transporters *SLC38A1*, *SLC38A2* and *SLC38A4*, compared to vehicle-treated controls, whereas insulin did not alter amino acid transporter gene expression (Fig. 5A, B and C). Dexamethasone did not affect PHT cell total SNAT1 or SNAT4 abundance (Fig. 5D and E). Neither glucocorticoids nor insulin affected System L-mediated ^3H -Leu uptake (Fig. 4D).

Discussion

Contrary to our original hypothesis, this study shows that glucocorticoids do not suppress insulin-stimulated System A amino acid transport or mTOR signaling in syncytialised primary human trophoblast. Instead, the study provides the first demonstration that glucocorticoids act additively with insulin to stimulate System A amino acid transport activity and mTOR signaling, in addition to enhancing insulin receptor abundance. Glucocorticoids downregulated System A amino acid transporter gene expression but did not alter total SNAT protein abundance.

Glucocorticoid stimulation of basal System A activity in primary human trophoblast cells in this study is in line with previous reports in choriocarcinoma cells and placental villous explants treated with cortisol or dexamethasone (Jones *et al.* 2006, Audette *et al.* 2010). The micromolar cortisol and dexamethasone concentrations used in culture in all these studies were

designed to reflect those in the circulation of pregnant women without treatment (Li *et al.* 2012) or following antenatal synthetic glucocorticoid administration (Ballard & Ballard 1995). Higher concentrations of dexamethasone have also been shown previously to stimulate System A activity in syncytialised primary human trophoblast (Karl *et al.* 1992). We extend these observations to show that this physiological effect is specific to System A activity, because there was no effect of either cortisol or dexamethasone on System L-mediated leucine uptake. Our study also demonstrates that the effects of cortisol and dexamethasone on basal System A activity are qualitatively similar, suggesting that they are mediated by the glucocorticoid receptor rather than the mineralocorticoid receptor, which is bound with lower affinity by dexamethasone than cortisol (Rogerson *et al.* 1999). The lesser effect of cortisol, compared to dexamethasone, may reflect its lower glucocorticoid receptor affinity and inactivation by 11BHSD2 in the trophoblast (Stirrat *et al.* 2018). Although the analyses in this study used similar numbers of placentas of male or female sex, and from vaginal or cesarean deliveries, we cannot exclude the possibility that glucocorticoid regulation of trophoblast amino acid transport differs by sex or delivery type. Labor inhibits mTOR signaling in the human placenta (Lager *et al.* 2014) and exogenous testosterone reduces placental SNAT1 expression in rats (Sathishkumar *et al.* 2011), but whether these factors modulate the response to glucocorticoids remains unstudied.

Increased *in vitro* System A activity was not mediated by a transcriptional or translational effect on

Figure 5

Effects of glucocorticoids and insulin on System A transporter gene and protein expression. (A) Representative Western blot, (B, D and E) mRNA expression and (C) protein abundance of System A transporters in syncytialised primary human trophoblast cells treated with vehicle (Con), cortisol (Cort, 1 μ M) and dexamethasone (Dex, 1 μ M), $\pm$ insulin (concentration, 1 nM). Effects of glucocorticoid (P_{GC}), insulin (P_{INS}) and interaction (P_{GC*INS}) were determined by two-way ANOVA with repeated measures and significances ($P < 0.05$) are provided in the figure. Differing superscripts a, b indicate significantly different glucocorticoid treatment groups, * indicates significant simple effect of insulin by Holm-Sidak *post hoc*. $n = 5$ placentas (gene expression), $n = 9$ placentas (protein). Mean $\pm$ S.E.M.

SLC38A/SNAT transporter gene expression or protein abundance. Indeed, glucocorticoid treatment downregulated *SLC38A1*, *SLC38A2* and *SLC38A4* expression, in contrast with previous studies in human placental villous explants showing that these genes are unaffected by dexamethasone (Audette *et al.* 2010). The discrepant RNA findings of these two studies most likely reflects the more complex cellular composition of placental villous explants, including trophoblast, mesenchymal and endothelial cells, and therefore, suggest that the suppressive effect of dexamethasone on transporter gene expression is specific to the syncytiotrophoblast. Nonetheless, dexamethasone had no effect on SNAT1 or SNAT4 protein abundance in either this or the previous study (Audette *et al.* 2010). We were unable to measure SNAT2 protein abundance in PHT cell lysates and therefore

cannot exclude the possibility of greater synthesis or stability of this transporter, but this is unlikely given the reduced *SLC38A2* mRNA expression. Instead, the data indicate that glucocorticoids may regulate amino acid transporters at the posttranslational level, a hypothesis supported by the demonstration that cortisol promotes trafficking of SNAT2 in choriocarcinoma cells (Jones *et al.* 2006). Certainly, both glucocorticoids resulted in a robust activation of mTORC1 and mTORC2 signaling pathways, which regulate trophoblast System A-mediated amino acid uptake by influencing the trafficking of specific transporter isoforms to the plasma membrane (Rosario *et al.* 2013). Our data therefore also imply that protein and gene expression of nutrient transporters in total cell lysates provides only limited information on transport activity given that trophoblast nutrient transporters are

often subjected to posttranslational regulation and these proteins mediate transport only when inserted in the plasma membrane (Rosario *et al.* 2013, 2016b).

We originally hypothesized that glucocorticoid treatment suppresses trophoblast mTOR signaling by upregulating the mTOR suppressor REDD1. Cortisol and dexamethasone both upregulated *REDD1* mRNA in PHT cells, in line with the effect of exogenous corticosterone administration on placental *Redd1* expression in mice (Vaughan *et al.* 2015). However, increased levels of the *REDD1* transcript in this study were not accompanied by a corresponding increase in the translated protein, suggesting that REDD1 may also be subject to post-transcriptional or post-translational control in trophoblast. Certainly, the REDD1 protein is highly labile *in vitro*, with a half-life of 5–7 min, and subject to rapid ubiquitination and proteasomal degradation (Katiyar *et al.* 2009, Tan & Hagen 2013).

In the absence of suppression by REDD1, the activation of mTORC1 and mTORC2 signaling and phosphorylation of S6 and Akt downstream may relate to increased insulin receptor abundance in the glucocorticoid treated PHT cells. Although glucocorticoids commonly impair peripheral post-receptor insulin signaling, they enhance insulin receptor abundance in lymphocytes (Fantus *et al.* 1982), fibroblasts (Knutson 1986) and in the sheep placenta *in vivo* (Vaughan *et al.* 2016). In agreement with our finding of IR β protein upregulation without increased *INSR* mRNA in dexamethasone-treated trophoblasts, the glucocorticoid-induced increase in total insulin receptor abundance in fibroblasts is coincident with slower degradation but no change in synthesis of the insulin receptor (Knutson 1986). Greater insulin receptor abundance at the trophoblast cell surface following dexamethasone treatment would be expected to increase intracellular insulin signaling in the presence of basal medium insulin concentrations, and therefore, further enhance signaling when exogenous insulin was added. We therefore speculate that the acute increase in System A uptake in glucocorticoid-treated primary human trophoblast cells is secondary to insulin receptor upregulation. This effect is additive with the mTOR-dependent effect of insulin to promote SNAT2 protein translation and translocation to the trophoblast plasma membrane (Rosario *et al.* 2013). The effect of insulin on System A was less marked in cortisol-treated cells, suggesting that cortisol binding to the mineralocorticoid receptor antagonizes the mTOR stimulatory glucocorticoid effect, for example by impinging elsewhere in the insulin receptor signaling cascade (Hitomi *et al.* 2007, Sherajee

et al. 2012). Moreover, glucocorticoids and insulin did not influence trophoblast ERK1/2 phosphorylation, which is normally positively regulated by insulin receptor signaling, suggesting that compensatory deactivating events also occurred in the growth promoting, mitogen-activated protein kinase arm of the cascade.

Taken together, the data demonstrate that glucocorticoids acutely stimulate System A amino acid transport and do not cause insulin resistance in cultured primary human trophoblast cells. These effects are opposite to the established suppressive action of *in vivo* glucocorticoid overexposure on placental System A transport capacity and fetal growth (Audette *et al.* 2011, 2014, Vaughan *et al.* 2012). Glucocorticoid suppression of trophoblast *SLC38A* gene transcription, demonstrated here after 24h, may ultimately outweigh the acute stimulatory effect to reduce steady state membrane transporter abundance and System A activity in the long term. Indeed, reduced placental System A activity in women given antenatal glucocorticoids is manifested more than two weeks after treatment and not within 24h of administration (Audette *et al.* 2014). Alternatively, our data are consistent with the model that downregulation of placental nutrient transport *in vivo* following excess maternal glucocorticoids is not mediated by a direct effect on the placenta.

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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