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Triamcinolone up-regulates GLUT 1 and GLUT 3 expression in cultured human placental endothelial cells

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The placenta is a glucocorticoid target organ, and glucocorticoids (GCs) are essential for the development and maturation of fetal organs. They are widely used for treatment of a variety of diseases during pregnancy. In various tissues, GCs have regulated by glucose transport systems; however, their effects on glucose transporters in the human placental endothelial cells (HPECs) are unknown.

In the present study, HPECs were cultured 24 h in the presence or absence of 0·5, 5 and 50 $\mu mol \cdot l^{-1}$ of synthetic GC triamcinolone (TA). The glucose carrier proteins GLUT 1, GLUT 3 and GC receptor (GR) were detected in the HPECs.

We showed increased expression of GLUT 1 and GLUT 3 proteins and messenger RNA (mRNA) levels ($p < 0{\cdot}05$) after 24-h cell culture in the presence of 0·5, 5 and 50 $\mu mol \cdot l^{-1}$ of TA. In contrast, GR protein and mRNA expressions were down-regulated ($p < 0{\cdot}05$) with 0·5, 5 and 50 $\mu mol \cdot l^{-1}$ of TA 24-h cell culture.

The results demonstrate that GCs are potent regulators of placental GLUT 1 and GLUT 3 expression through GR.

Excessive exposure to GCs causes maternal and fetal hypoglycemia and diminished fetal growth. We speculate that to compensate for fetal hypoglycemia and diminished fetal growth, the expression of placental endothelial glucose transporters might be increased. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS—glucocorticoids; glucose transport; pregnancy; fetal growth

INTRODUCTION

The placenta receives and transmits endocrine signals between the mother and fetus and is the site of nutrient and waste exchange. Several aspects of placental function are critical for human fetal growth and development, including transport of nutrients such as glucose from mother to fetus. Glucose is the primary source of energy for metabolism and development of the fetus and placenta.[1,2] Because the fetus is not capable of producing appreciable amounts of glucose until late gestation, it is critically dependent of the net transfer of glucose across the placenta.[3] The fetal consumption of glucose increases rapidly towards term because of the almost 20-fold increase in fetal weight during the second half of pregnancy.[1] The high fetal demand for glucose, especially during the third trimester, necessitates the presence of rapid, high-volume system for maternal–fetal glucose transfer. On the other hand, down-regulation of placental glucose transport is associated with intrauterine growth restriction.[4]

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Glucose passes the placental barrier not only by simple diffusion but also by the action of facilitated diffusion transport machinery.[5] This process is brought about by facilitated diffusion along a concentration gradient rendering substrate entry about 10 000 times faster than calculated for diffusion across the lipid membrane layer. The transport facilitators are about 500 amino acids in length and belong to a growing superfamily of integral membrane glycoproteins with 12 membrane-spanning domains that presumably form a channel through which glucose can move in one or more association–dissociation steps.[6] Up to now, 14 functional mammalian-facilitated hexose carriers (GLUTs) have been characterized by molecular cloning. According to the sequence similarities, three classes of GLUTs have been defined. The isoforms GLUT 1, 3 and 4 are included in Class I and represent high-affinity transport facilitators. Because of their low Michaelis constant (Km), these transporters function at rates close to maximal velocity. Thus, their level of cell surface expression greatly influences the rate of glucose uptake into the cells.[7]

Uptake of glucose by the placenta is facilitated primarily by GLUT 1 and in part by GLUT 3 transporters. A possible major glucose transfer mechanism in the human placental villi may be depicted as follows. Glucose in the maternal

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bloodstream passes the apical microvillous plasma membrane of syncytiotrophoblast cells by means of GLUT 1. Glucose moves through the cytoplasm of the syncytiotrophoblast by simple diffusion and leaves the cytoplasm via GLUT 1 in the basal plasma membrane. GLUT 1 and GLUT 3 proteins contribute to the uptake of glucose by placental endothelial cells, as well as facilitate the transfer of glucose into and out of the fetal blood vessels in the villous core.[8–11] About 25% of glucose entering the placenta is metabolized within this tissue; the majority of glucose is passed to the fetus through placental endothelial cells.[12]

The human placenta is of the hemochorial type, i.e. maternal blood delivered by the spiral arteries into the intervillous spaces comes in direct contact with the trophoblast villi containing the fetal capillaries. There are only two cell layers separating the fetal and maternal circulations in the term human placenta: the fetal capillary endothelium and the syncytiotrophoblast. In early pregnancy, cytotrophoblast cells are highly abundant, creating a continuous cell layer between the syncytium and the fetal capillary.[13] Therefore, glucose transport in term placenta is mainly dependent on microvilli of syncytiotrophoblast and endothelial cells. Moreover, cytotrophoblast cells are less abundant in late pregnancy. Consequently, in late pregnancy, placental glucose transport mechanism mainly depends on placental endothelial cells when compared with human term placental trophoblast cells. For these reasons, unlike Hahn *et al.*[14] we investigated the glucose transport mechanism in endothelial cells in which fetal glucose transport primarily takes place.

On the other hand, placenta is a glucocorticoid target organ,[15] and GCs are essential for the development and maturation of fetal organs. GCs could potentially affect a wide range of cellular functions within the embryo and the placenta, from trophoblast differentiation soon after implantation to hormone synthesis in the fully differentiated placenta.[16] In human pregnancy, GCs are now used mainly in two circumstances: the management of women at risk of preterm delivery and the antenatal treatment of fetuses at risk of congenital adrenal hyperplasia. Today, antenatal GCs are given to 7–10% of pregnant women in Europe and North America[17] during preterm labor, to mature the fetal lungs and reduce the risk of neonatal morbidity and mortality. Although GCs promote lung maturation, conversely, fetal exposure to excess GCs has been implicated as a causative factor in fetal growth retardation[15,18–20] linked with an increased risk of developing hypertension, cardiovascular disease and glucose intolerance in adult life.[21]

At cellular level, exposure to GCs *in utero* alters receptors, enzymes, ion channels and transporters in a wide range of different cell types during late gestation.[22] Today, the effect of glucocorticoids on glucose transport is not clear. On the one hand, GCs specifically inhibit glucose transport in skeletal muscle cells,[23] adipocytes[24] and human term placental trophoblast cells.[14] On the other hand, GCs stimulated glucose transporter protein expression in the rat placenta[25] and cardiac cells.[26] Moreover, in many species, placental 11β-hydoxysteroid dehydrogenase type 2 (11βHSD2) inactivates glucocorticoids and limits fetoplacental exposure to the higher maternal glucocorticoid concentrations. In humans, 11β-HSD2 gene mutations cause low birth weight and reduced placental 11β-HSD2 activity associated with intrauterine growth retardation.[27] In the placenta of prenatal stressed rats, placental expression and activity of the glucocorticoid "barrier" enzyme 11βHSD2 was strongly reduced. It means increase in glucocorticoids in animals that causes decreased expression of GLUT1, whereas slightly increased expression of GLUT3 and GLUT4.[28] According to these studies,[14,25,28] the effect of glucocorticoids on placental glucose transport is not clear. Additionally, the effects of GCs on glucose transporters in the human placental endothelial cells are unknown. Therefore, the present study addressed the question of whether GCs effect placental glucose transporter expression in human placental endothelial cells.

MATERIALS AND METHODS

Isolation and culture of placental endothelial cells

Isolation and culture of human term placental endothelial cells was performed as previously described by Lang *et al.*[29,30] In brief, human term placenta of normal pregnancies were obtained under informed consent after vaginal delivery and placed directly on ice until isolation. During the preparation steps, the placenta was gently warmed up to 37 °C in a bath of sterile phosphate-buffered saline (PBS). Approval of the ethical committee of the Medical University of Akdeniz was granted. The amnion was removed, and corresponding venous chorionic blood vessels at the apical surface of the chorionic plate were resected. Each vessel was washed with Ca^{2+}/Mg^{2+}-free Hank's balanced salt solution (HBSS; Gibco, Vienna, Austria) to remove residual blood. Subsequently, HPECs were isolated by separate perfusion of chorionic blood vessels with HBSS containing 0·1 U·ml^{-1} of collagenase, 0·8 U·ml^{-1} of dispase (Roche, Vienna, Austria) and antibiotics (Gibco), pre-warmed to 37 °C. The perfusion time was limited to 7 min to avoid contamination with non-endothelial cells. The obtained cell suspension was centrifuged (200 *g* for 5 min), and the pellet was resuspended with EBM basal medium (cc-3121, Lonza, Verviers, Belgium).

The cells were seeded into six-well culture plates that had been precoated with 1% (v/v) gelatin (Sigma Biosciences, Taufkirchen, Germany) in HBSS for 1 h at 37 °C. After endothelial cells became adherent, to discard the contaminating red blood cells, HPECs were washed with HBSS. In general, the percentage of endothelial cell marker-positive cells in the primary HPECs culture was about 95%. For the experiments, only pure endothelial cell cultures were used. The culture medium was changed after 24 h and, thereafter, every third day. Best results were achieved using complete medium (cc-3125, Lonza) containing 5% (v/v) dialysed fetal bovine serum, bovine brain extract (18 µg·ml^{-1}), epidermal growth factor (10 ng·ml^{-1}), hydrocortisone (1·0 µg·ml^{-1}), gentamycin (50 mg·ml^{-1}) and amphotericin B (50 ng·ml^{-1}).

Human placental endothelial cells were allowed to recover from the trypsinization for 24 h before starting the experiments. After this period (time zero), the cells were cultured in the presence or absence (controls) of 0·5, 5 and 50 $\mu mol \cdot l^{-1}$ of triamcinolone (TA; Volon A 40, Squibb-von Heiden, Munich, Germany) for another 24 h, as TA binding to placental cytosol peaks after 20 h of incubation *in vitro*.[31]

Cell characterization

Immunocytochemical characterization was performed on HPECs cultured up to passage 5 as described in detail elsewhere.[29,30] For each passage, the identity of the placental endothelial cells was tested with polyclonal von Willebrand factor antibody (vWf, immunoglobulin fraction, rabbit antihuman; Dako, Carpinteria, CA) and monoclonal CD31 [mouse immunoglobulin G (IgG) 1, antihuman endothelial cell; Dako]. Both are standard markers for endothelial cells. Placental endothelial cells were grown and stained on chamber slides (BD Biosciences, Bedford). At confluence, the chamber slides were washed in HBSS (Life Technologies Gibco, Wien, Austria) fixed in acetone for 5 min at room temperature and stained as previously described by Lang *et al*.[29] For the experiments, only pure endothelial cell cultures isolated from at least five different placentae were used.

Immunofluorescence staining

Human placental endothelial cells seeded on polylysine-coated glass chamber slides and cells were washed with PBS and fixed in ice-cold acetone for 5 min. The cells were then incubated with 5% bovine serum albumin for 10 min. Thereafter, cells were immunolabelled with von Willebrand factor (1:300 from Dako), CD31 (1:100 from Dako), GLUT 1 (1:500 from Chemicon, Temecula, CA), GLUT 3 (1:100 from Chemicon) and GR antibodies (1:50 from Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at room temperature for 60 min. All antibodies were diluted in antibody diluent (Dako). Normal rabbit immunoglobulin fraction and mouse IgG1 (Dako) served as controls. After three washings in PBS, the slides were incubated for 30 min with swine anti-rabbit IgG labelled with FITC (Dako) and for CD31, rhodamine conjugated goat anti-mouse IgG, (Chemicon) diluted 1:100 in antibody diluent. After three washings in PBS, the slides were counterstained with DAPI UltraCruz™ Mounting Medium (sc-24941; Santa Cruz Biotechnology) and examined using a fluorescence microscope (Olympus, BX61 Hamburg, Germany).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis and Western blotting

Cellular proteins from cultured human placental endothelial cells were solubilized in Laemmli sample buffer (Sigma Chemical Co.) supplemented with complete protease inhibitor cocktail (Boehringer Mannheim, Mannheim, Germany). Protein extraction and immunoblot analysis were performed as previously described by Lang *et al*.[30] Before electrophoresis, samples were boiled for 3 min at 100 °C. Equal amounts of protein, determined according to the method of Lowry *et al*.[32] were subjected to sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) on 8–18% gradient gels (ExcelGel, Pharmacia Biotech) using SDS buffer strips (ExcelGel, Pharmacia Biotech). Samples were run for 150 min at 600 volts, 50 mA and 30 watts. Proteins were transferred onto nitrocellulose membranes (Pharmacia Biotech) by semidry electroblotting in a buffer containing 0·2 $mol \cdot l^{-1}$ of glycine, 25 $\mu mol \cdot l^{-1}$ of Tris and 20% methanol for 45 min at 30 volts, 100 mA and 6 watts. Successful transfer was confirmed by Ponceau S (Sigma Chemical Co.) staining of the blots. The membranes were blocked for 1 h with 5% non-fat dry milk (Bio-Rad Laboratories, Inc., Hercules, CA) and 0.1% Tween-20 (Sigma Chemical Co.) in 0·14 $mol \cdot l^{-1}$ Tris-buffered saline, pH 7·2–7·4, at 4 °C. The same solution was used as diluents for the antibodies.

The blotted membranes were incubated for 2 h at room temperature with rabbit antisera against GLUT 1, GLUT 3 and GR (dilutions: GLUT 1, 1:3000; GLUT 3, 1:1000; GR, 1:1000) (GLUT 1 and GLUT 3 from Chemicon and GR from Santa Cruz Biotechnology, Inc.), respectively. After washing, the membranes were further incubated with horseradish peroxidase conjugated goat anti-rabbit IgG (Bio-Rad Laboratories, Inc.) diluted 1:5000 (GLUT 1), 1:3000 (GLUT 3) or 1:1000 (GR) for 2 h at room temperature. After three washings in Tris-buffered saline, pH 7·2–7·4, the immunolabelling was visualized using the chemiluminescence-based SuperSignal CL-HRP Substrate System (Pierce Chemical Co., Rockford, IL) according to the instructions of the manufacturer.

Membranes were exposed to Hyperfilm (Amersham Biosciences, Sweden), which was subsequently analysed using an Alpha Digi Doc 1000 gel documentation unit (Alpha Innotech Corporation, CA).

RNA isolation and complementary DNA synthesis

RNA isolation and complementary DNA (cDNA) synthesis were performed as previously described by Kipmen-Korgun *et al*.[33] Total RNA was isolated from HPECs using Trizol Reagent (Invitrogen, Rockville, MD) according to the manufacturer's instructions. RNA pellets were eluted in RNase-free water and stored at −70 °C until the time of the experiment. Each RNA sample was quantified using UV spectrophotometer.

Complementary DNA was synthesized from 1 μg of total RNA using the Promega Reverse Transcription System (Promega, Madison, WI). Briefly, 1 μg of total RNA was subjected to reverse transcription in a final reaction volume of 20 μl containing 5 $mmol \cdot l^{-1}$ of $MgCl_2$, 1 X reverse transcriptase (RT) buffer, 1 $mmol \cdot l^{-1}$ of each dNTP, 1 $U \cdot \mu l^{-1}$ of RNAsin, 20 U of AMV reverse transcriptase and 0·5 μg of random primers. Tubes with reaction mixtures were incubated for 10 min at room temperature, then at 42 °C for 15 min, with 5-min inactivation of enzyme at 95 °C, and chilled on ice for 5 min. Tubes were kept at −20 °C until use.

Semi-quantitative RT-polymerase chain reaction

Complementary DNA was amplified in a 50-µl reaction volume containing 0·2 mmol·l^{-1} of De oxynucleotide Triphosphates (dNTPs), 10 mmol·l^{-1} of specific primers, 1 X polymerase chain reaction (PCR) buffer and 1 U of Platinum® Taq DNA Polymerase (Invitrogen, Carlsbad, CA). After initial denaturation at 94 °C for 10 min, PCR was carried out for 40 cycles with denaturation for 30 s at 94 °C; annealing for 30 s at 56 °C for GLUT 1, GR and beta-actin and 54 °C for GLUT 3; and extension for 1 min at 72 °C, followed by a final extension of 10 min at 72 °C. The sequences of primers are shown in Table 1. The PCR products were visualized using electrophoresis with an ethidium bromide-stained 1·5% agarose gel, and the intensity of bands were quantified using a densitometer. All results were normalized to beta-actin.

Statistical analysis

The data expressed as mean ± SD were analysed with standard methods for descriptive values and with one-way ANOVA to compare the size of the effect. Significant overall results were further examined with Tukey's highest significant difference (HSD) tests for all other pairwise comparisons. A significance level of 0·05 was used for all the tests. For all calculations, SPSS (SPSS, Chicago, IL) was used.

RESULTS

Characterization of cultured human placental endothelial cells and immunofluorescence staining

The viability of the cells was more than 90% by trypan blue exclusion. The immunocytochemical reaction pattern of the endothelial cell preparations revealed approximately 90% reactivity with the von Willebrand factor (Figure 1a) and CD31 antibodies (Figure 1b) immediately after isolation, and 90–95% were stained after 48 h in culture. HPECs expressed GLUT 1 (Figure 1c), GLUT 3 (Figure 1d) and GR (Figure 1e) proteins. Negative control sections in which the primary antibodies were replaced by their non-immune isotype controls showed no immunoreaction (Figure 1f for rabbit immunoglobulin, Figure 1g for mouse IgG1).

Effect of TA treatment on GLUTs protein and mRNA levels in HPECs

The measured expression levels relative to the controls are summarized in Table 2.

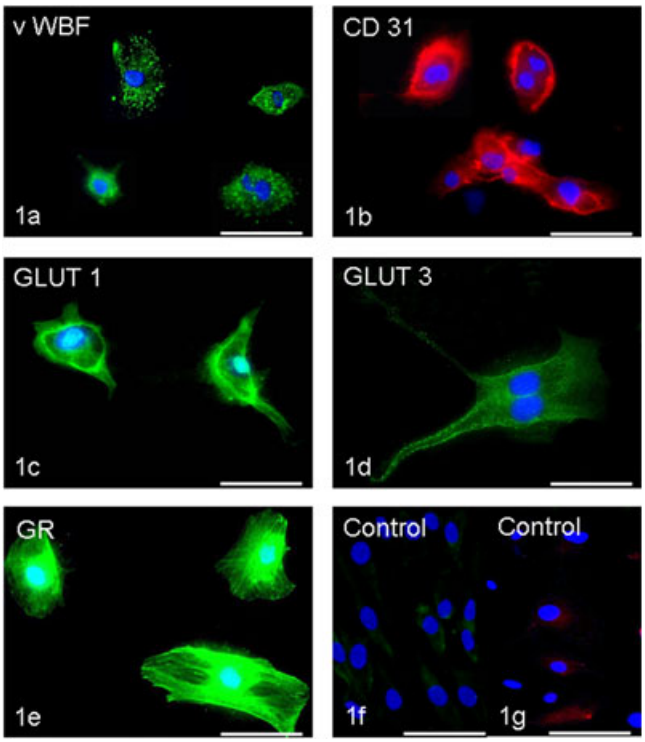

Figure 1. Immunocytochemical characterization of human placental endothelial cells with the cytoplasmatic protein von Willebrand factor antibody (a) and membrane protein CD31 (b). Immunofluorescence staining patterns of HPECs. Expression of GLUT 1 (c), GLUT 3 (d) and nuclear staining of GR (e). Control immunofluorescence staining for polyclonal antibody (f) and monoclonal antibody (g). Scale bars = 50 µm

Table 2. Expression levels relative to the controls (=100%) of GLUT 1, GLUT 3 and GR messenger RNA and protein of human placental endothelial cells cultured in the presence of 0·5, 5 and 50 µmol·l^{-1} of triamcinolone

% of control (=100%)	0·5 µmol·l^{-1}	5 µmol·l^{-1}	50 µmol·l^{-1}
GLUT 1			
Protein	120 ± 12·5*	207 ± 15·3*	221 ± 15·1*
mRNA	132 ± 13·1*	165 ± 12·3*	189 ± 16·7*
GLUT 3			
Protein	178 ± 13·3*	228 ± 15·2*	314 ± 17·2*
mRNA	154 ± 12·4*	220 ± 18·3*	231 ± 13·6*
GR			
Protein	80 ± 9·3*	52 ± 7·7*	54 ± 9·3*
mRNA	60 ± 7·2*	64 ± 9·1*	72 ± 11·4*

Data (mean ± SD) were tested statistically by the one-way ANOVA test. *$p < 0·05$ versus control.

Table 1. Oligonucleotide sequences used for semiquantitative reverse transcriptase-polymerase chain reaction

Genes	Forward primers	Reverse primers	Product (bp)
GLUT 1	**5′-GGAGAAGAAGGTCACCATCC-3′**	**5′CCACGATGCTCAGATAGGAC-3′**	**353**
GLUT 3	**5′-GCATATGATAGGCCTTGGAG-3′**	**5′-CATTGGTGGTGGTCTCCTTA-3′**	**485**
GR	**5′-GGAATAGGTGCCAAGGATCT-3′**	**5′-GTGGTAACGTTGCAGGAACT-3′**	**489**
Beta-actin	**5′-CCTTCTACAATGAGCTGCGT-3′**	**5′-TCGGTGAGGATCTTCATGAG-3′**	**319**

Both GLUT 1 and GLUT 3 protein expression were detectable by Western blotting in the HPECs (Figure 2). GLUT 1 protein expression of HPECs was analysed using Western blotting at 24 h cell culture and presented in Figure 2. Direct comparison of HPECs between control and TA-treated endothelial cells revealed that TA treatment of 0·5, 5 and 50 $\mu mol \cdot l^{-1}$ led to a dose-dependent increase in placental GLUT 1 relative protein abundance at 1·2-fold ($p < 0{\cdot}05$), 2·0-fold ($p < 0{\cdot}05$), and 2·2-fold ($p < 0{\cdot}05$), respectively (Table 2).

GLUT 3 protein levels were increased by TA in a dose-dependent manner similar to GLUT 1. Treatment of HPECs with the synthetic glucocorticoid TA increased GLUT 3 activity approximately 1·7-fold (0·5 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$), 2·2-fold (5 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$) and 3·1-fold (50 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$) after 24-h culture (Table 2).

GLUT 1 and GLUT 3 mRNA levels were up-regulated in cultured human placental endothelial cells after TA administration (Figure 3). In cultured endothelial cells, this effect was clearly dose dependent. TA treatment increased GLUT 1 mRNA levels to 1·3-fold (0·5 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$), 1·6-fold (5 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$) and 1·9-fold (50 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$) during 24-h culture (Table 2). Densitometric scanning analysis of GLUT 3 mRNA levels showed that TA increased GLUT 3 activity approximately 1·5-fold (0·5 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$), 2·2-fold (5 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$) and 2·3-fold (50 $\mu mol \cdot l^{-1}$ of TA; $p < 0{\cdot}05$) during 24-h culture (Table 2).

Effect of TA treatment on levels of GR protein and mRNA in HPECs

We used Western blot and RT-PCR analyses to determine the effects of GC treatment on levels of GR protein and mRNA in HPECs (Figures 2 and 3). We observed that treatment of HPECs with 0·5, 5 and 50 $\mu mol \cdot l^{-1}$ of TA for 24 h reduced GR protein ($p < 0{\cdot}05$) and mRNA expression ($p < 0{\cdot}05$) significantly (Table 2) when normalized to beta-actin protein and mRNA levels (Figures 2 and 3). These data suggest that TA treatment reduced GR protein and mRNA expressions in HPECs (Table 2). The expression of beta-actin was a control for equal protein and mRNA loading for GLUT 1, GLUT 3 and GR (Figures 2 and 3).

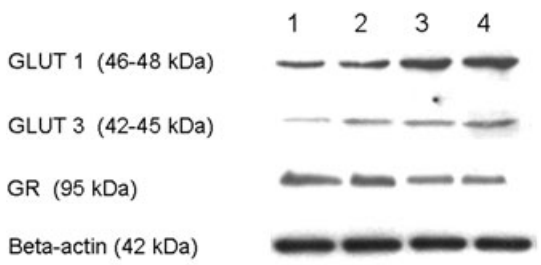

Figure 2. Representative Western blot of GLUT 1, GLUT 3 and GR protein, respectively. The expression of beta-actin is shown as a control for equal protein loading for GLUT 1, GLUT 3 and GR. Lanes show protein from the following samples: 1, control; 2, placental endothelial cells cultured in the presence of 0·5 $\mu mol \cdot l^{-1}$ of TA; 3, placental endothelial cells cultured in the presence of 5 $\mu mol \cdot l^{-1}$ of TA; and 4, placental endothelial cells cultured in the presence of 50 $\mu mol \cdot l^{-1}$ of TA

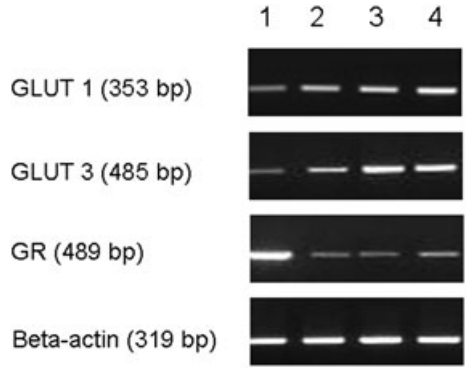

Figure 3. Representative reverse transcriptase-polymerase chain reaction of GLUT 1 messenger RNA (mRNA), GLUT 3 mRNA and GR mRNA, respectively. Beta-actin was used as a control to confirm mRNA integrity and equal loading for GLUT 1 mRNA, GLUT 3 mRNA and GR mRNA. Lanes show mRNA from the following samples: 1, control; 2, placental endothelial cells cultured in the presence of 0·5 $\mu mol \cdot l^{-1}$ of TA; 3, placental endothelial cells cultured in the presence of 5 $\mu mol \cdot l^{-1}$ of TA; and 4, placental endothelial cells cultured in the presence of 50 $\mu mol \cdot l^{-1}$ of TA

DISCUSSION

The effect of GCs on placental transport of glucose to the fetus *in vitro* has been poorly studied. GCs have been shown to strongly regulate maternal and fetal glucose metabolism, but direct effects on placental glucose transport are unclear.

In the present study, the high affinity glucose transporter isoforms GLUT 1 and GLUT 3 were expressed in the HPECs, which are cellular placental barriers fronting to the fetal circulation. Because of their locations, endothelial cell populations play a pivotal role in transplacental glucose transport in the hemochorial-type human placenta.

To the best of our knowledge, this is the first report of a GC effect on GLUT 1 and GLUT 3 expressions in HPECs. Our initial results using immunofluorescence methods showed that HPECs expressed GLUT 1, GLUT 3 and GR. The finding that HPECs are targets of GC action is not surprising because GC-regulated genes, including the VEGF proteins, are synthesized by placental HPECs.[34] Our Western blot results showed that GC overexposure significantly increased placental GLUT 1 and GLUT 3 protein levels in all experimental groups of HPECs. RT-PCR analysis of placental GLUT expressions indicated that both GLUT 1 and GLUT 3 mRNA levels were affected by the GC induction. It was supposed that GCs caused the increase in placental GLUT proteins and mRNA expression. This assumption is consistent with a previous study showing that glucocorticoid administration up-regulated GLUT 1 and GLUT 3 protein expression in rat term placenta.[25]

Maternal GCs circulate at five to ten times higher concentrations than those in the fetus,[35] and the fetus is not capable of synthesizing its own GCs until relatively late in

gestation.[36,37] Physiological cortisol levels in maternal plasma during human pregnancy are in the range of 1 μmol·l^{-1} [38]. We examined the effect of exposure to elevated levels of the synthetic glucocorticoid TA. Three doses were used. The lower (0·5 μmol·l^{-1}) dose is a concentration in the lower range of doses generally used in previous cell culture studies[14] and considered comparable to the doses used to promote lung maturation in rats.[39] Other doses (5 and 50 μmol·l^{-1}) were used to investigate the potentially detrimental effects of glucocorticoid excess. The highest TA dose administered to the endothelial cell cultures in the present study corresponds to the TA concentration in blood resulting after intravenous injection of a dose recommended by the manufacturers for therapy in humans.

Triamcinolone administration at 0·5-, 5- and 50-μmol·l^{-1} doses led to a significant up-regulation of the placental expressions of both GLUT 1 and GLUT 3 mRNA and protein. That is compatible with a possible role of GCs in mediating or facilitating the increase in placental endothelial glucose transport.

Non-reciprocal changes in placental GLUT 1 and GLUT 3 protein expression in the present experiments indicated that the total placental glucose transporter capacity increased. This suggests that an alteration in placental glucose transport may contribute to the process of altered materno–placental–fetal transport of glucose. It is known that glucocorticoid exposure causes maternal[14] and fetal hypoglycemia.[25] The decrease of glucose concentration in the maternal circulation during late pregnancy seems to be caused by excess glucose requirements of overgrown fetuses. Fetal hypoglycemia in Intrauterine growth restriction (IUGR) is not caused by a decrease in placental glucose transporter density.[4,40] As a result of reduced glucose in the maternal and fetal circulation, to have sufficient glucose transport to the fetus, increase in GLUT expression in fetal endothelial cells can be to compensate for reduced glucose. However, increase of GLUTs may not provide enough glucose transport to the fetus, and as a result of this, IUGR may occur. The mechanisms underlying fetal hypoglycemia in IUGR remains to be fully established but may be related to increased fetal or placental glucose consumption. Because of the facilitated nature of transplacental glucose transport, net transfer will be dependent on the concentration gradient across the placental barrier, which has been shown to be increased in the compromised IUGR fetus.[41] Therefore, fetal hypoglycemia in IUGR may represent an adaptation to, e.g. reduced placental surface area to maintain a sufficient glucose delivery[41] or increased placental endothelial glucose transporter expression.

The results of GC induction of human placental cells (trophoblasts and endothelial cells) showed that the effects of glucocorticoids in glucose transport might be cell specific. Because one side of the placental barrier in trophoblasts is GLUT proteins decreased,[14] on the other side of placental barrier in endothelial cells is GLUT proteins increased. Perhaps, endothelial cells might try to compensate for decreased glucose transport in trophoblasts cells. It seems intrinsically that an increase in placental endothelial glucose transport capacity may contribute an immediate attempt to increase fetal glucose supply to the fetus.

The human placenta is a GC responsive organ consisting of multiple cell types including endothelial cells, fibroblasts and trophoblasts that demonstrate changes in gene expression after hormone treatment. However, little is known about the relative expression or activity of the GR among the various placental cell types. Previous studies have documented that placental endothelial cells expressed GR. [42] but the regulation of glucose transport have not been studied. We found that GR mRNA and protein expression is down-regulated after 24-h cell culture of HPECs. Our results suggest that GC-mediated down-regulation of GR levels occurs through changes in protein and mRNA stability in HPECs after TA treatment. The data from the cell culture further strengthen the hypothesis that increased GC levels specifically modulate GLUT expression via the GR.

Collectively, we conclude that the synthetic GC TA is a potent regulator of HPECs GLUT 1 and GLUT 3 expression. This effect is mediated by GR. We speculate that GC-induced up-regulation of the placental glucose transporter systems contributes to the retarded fetal and placental growth observed with GC treatment. The results taken together clearly demonstrate that dosages of GC exposure are likely to be important in determining the response of the placental glucose transporters to elevated glucocorticoid levels. The mechanism by which GC treatment leads to concomitant up-regulation of placental GLUT 1 and GLUT 3 protein expressions in our experiments is not yet elucidated. Further studies will be needed to elucidate how GC and GR interact with GLUT 1 and GLUT 3 in the HPECs to determine their role in placental glucose transport.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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