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Hyperglycaemia-induced subcellular redistribution of GLUT1 glucose transporters in cultured human term placental trophoblast cells

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Abstract

Aims/hypothesis. We have recently shown that hyperglycaemia down-regulates the GLUT1 glucose transport system of term placental trophoblast. The reduction in GLUT1 protein alone was, however, not sufficient to explain the decrease in net glucose uptake, suggesting additional mechanisms. Therefore, we hypothesised that hyperglycaemia in vitro leads to a GLUT1 translocation from the trophoblast surface to intracellular sites.
Methods. This was tested in our study by determining the subcellular distribution of GLUT1 in human term placental trophoblast ($n = 5$ placentas) cultured for 48 h with 5 compared with 25 mmol/l D-glucose in vitro using immunogold labelling.
Results. Electron microscopic examination of cell profiles showed that 73 % of total GLUT1 molecules reside in the trophoblast plasma membrane under basal conditions. The reduced GLUT1 expression (–20 %; $p < 0.05$) after culture of the cells with 25 mmol/l glucose was accompanied by an internalisation of plasma membrane GLUT1, resulting in a loss of 40 % ($p < 0.05$) in cell surface transporter labelling. Western blotting identified a characteristically broad band between 55–65 kDa, confirming the specificity of the GLUT1 antiserum.
Conclusion/interpretation. We postulate that in addition to down-regulating human GLUT1 protein concentrations, glucose exerts its autoregulatory effect on hexose transport in term placental trophoblast by altering GLUT1 partitioning between the plasma membrane and intracellular sites in favour of the latter. [Diabetologia (2000) 43: 173–180]

Keywords GLUT1, translocation, glucose, transport, hyperglycaemia, diabetes, trophoblast, placenta, pregnancy, electron microscopy.

Adequate maternal-to-fetal transfer of glucose, the primary substrate for fetal oxidative metabolism, is crucial to sustain the normal development and survival of the fetus in utero, because its own glucose production is minimal [1]. Glucose is transferred across the placenta by stereospecific, sodium-independent facilitated diffusion along a concentration gradient [2]. This mechanism involves carriers that are about 500 amino acids in length and belong to a family of integral membrane proteins which render substrate entry about 10 000 times faster than calculated for diffusion across the lipid membrane layer [3]. In spite of the high sequence similarity these glucose transporters are encoded by six different genes designated *GLUT1 – GLUT6* [4], which are translated into protein with the exception of the pseudogene *GLUT6* [5]. The GLUT1 protein is a high-affinity isoform. Because of its low Michaelis constant (K_M), this transporter functions at rates close to maximum velocity. Thus its level of cell surface expression greatly influences the rate of glucose uptake into the cells. The highly tissue-specific expression of GLUT1 in

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Abbreviations: hCG, β-Human chorionic gonadotrophin; K_M, Michaelis constant; PBG, phosphate buffered saline containing 0.5 % (w/v) bovine serum albumin and 0.1 % (w/v) gelatine.

situ is restricted to epithelial blood-tissue barriers such as the placental trophoblast [6]. This narrow tissue layer separates the maternal and fetal circulations during pregnancy and thus represents the key regulator of transplacental glucose supply to the fetus [7].

In line with the general notion that placental glucose transporters are regulated by substrate availability and glucocorticoids, rather than by insulin changes [8–10], we have shown that hyperglycaemia (20 and 25 mmol/l D-glucose) down-regulates the GLUT1 glucose transport system of human term placental trophoblast [11]. This was recently confirmed by others with 20 mmol/l D-glucose [12]. We speculated that this down-regulation could represent a mechanism to protect fetal development in maternal diabetes. After hyperglycaemia there was, however, no kinetic evidence for a change in the intrinsic activity of the transporters neither in placental trophoblast [11] nor in trophoblast-derived choriocarcinoma cells [13]. The rather moderate extent of GLUT1 protein regulation alone was not sufficient to explain the considerable changes in net glucose uptake measured in these studies. We therefore hypothesised that hyperglycaemia changes the distribution pattern of GLUT1 between the plasma membrane and intracellular sites in human term placental trophoblast, which could have contributed to above apparent discrepancy.

Materials and methods

The study has been approved by the ethics committee of the University of Graz, Medical Faculty.

Cell culture. Mononucleated trophoblast was isolated from five term human placentas after uncomplicated pregnancy and vaginal delivery as was described in detail elsewhere [14]. Briefly, villous material was digested with a 0.125 % (v/v) trypsin solution (Gibco Life Technologies, Paisley, UK). The released cells were loaded on top of a Percoll (Pharmacia, Uppsala, Sweden) gradient ranging from 70 % (v/v) to 10 % (v/v). After centrifugation the band containing trophoblasts was removed. After extensive washings the trophoblasts were highly purified using immunomagnetic particles (Dynabeads M-280; Dynal, Hamburg, Germany), which had been conjugated with the monoclonal antibody W6/32 (Serotec, Kidlington, UK) against HLA-class I antigens. In the human placenta this antibody reacts only with stromal cells, macrophages, the endothelium and with the extravillous trophoblast. It does not identify villous trophoblast, which is devoid of HLA-class I antigens [15].

Cells were plated at a density of 500 cells/mm^2 into Transwell-COL culture chamber inserts (Costar, Cambridge, Mass., USA) and cultured in DMEM (Gibco) supplemented with 15 % defined fetal bovine serum (HyClone Laboratories, Logan, Utah, USA), 100 μg/ml streptomycin (Gibco), 100 IU/ml penicillin (Gibco) and 100 μg/ml amphotericin B (Gibco) at 37 °C in a humidified atmosphere of 5 % CO_2/air. Trophoblast cells were allowed to recover their microvillous surface after the trypsinisation for 24 h before starting the experiments. After this time (t = 0) the cells were further cultured for another 48 h in medium containing either 5.5 (normoglycaemia) or 25 mmol/l (hyperglycaemia) D-glucose (Sigma, Taufkirchen, Germany), or 19.5 mmol/l D-mannitol (Sigma) as osmotic control plus 5.5 mmol/l D-glucose, respectively. Osmolality of the culture media (means ± SD) was 326 ± 15 mosmol/kg in media containing 5.5 mmol/l D-glucose, 338 ± 12 mosmol/kg in media containing 25 mmol/l D-glucose and 340 ± 16 mosmol/kg in media containing 19.5 mmol/l D-mannitol plus 5.5 mmol/l D-glucose. Media were changed daily and stored at –40 °C for further analysis.

Purity and characterisation of cell preparations. Viability of the trophoblasts was assessed by 0.05 % (v/v) trypan blue (JRH Biosciences, Crawley Down, Sussex, UK) dye exclusion during a 2-min incubation and by measuring the concentration of β-human chorionic gonadotropin (OPUS sandwich immunoassay; Behring Diagnostics, Westwood, Mass., USA) secreted into the culture media.

Immunocytochemistry at the light microscopic level was carried out immediately after isolation and, in addition, after 72 h in culture. Cells were incubated with the following monoclonal antibodies: (1) anti-cytokeratin clone NCL5D3 (1:50; Monosan, Uden, Netherlands) for the identification of trophoblast cells [16], (2) W6/32 (1:10) and (3) anti-CD68 (1:50; Monosan) for monocyte and macrophage identification, and (4) Fluorescein-isothiocyanate-conjugated *Ulex europeaeus* lectin (UEA–I) (1:10; Sigma) was used for the identification of endothelial cells [17]. Immunoreactivity was visualised using a FITC-conjugated goat anti-mouse secondary antibody (1:20; Dianova, Hamburg, Germany).

Morphology was examined at the electron microscopic level (see below).

Electron microscopy. During processing the trophoblast cells isolated from $n = 5$ placentas remained adherent to the Transwell-COL membrane (Costar) on which they were grown in culture. After washing with PBS the cells were fixed and permeabilised for 10 min in cold (–20 °C) methanol. The fixative was replaced after 10 s. The cells were washed three times for 5 min with PBS and then transferred to PBS containing 0.5 % (w/v) BSA and 0.1 % (w/v) gelatine (Merck, Darmstadt, Germany) (PBG) supplemented with 20 % (w/v) normal goat serum for blocking. Blocking solution was replaced by a polyclonal GLUT1 antiserum (East Acres, Southbridge, Mass., USA), diluted 1:200 in PBS and, for the controls, by serum of non-immunised rabbits or by antibody diluent. Incubation was carried out for 1 h at room temperature, followed by three washings for 5 min each in PBG. Subsequently, samples were incubated with the goat anti-rabbit secondary antibody coated with 5 nm colloidal gold particles (AuroProbe; Amersham, Buckinghamshire, UK) diluted 1:10 in PBG for 1 h at room temperature, followed by four washings in PBG and one in pure PBS for 10 min each. The cells were postfixed with 2.5 % (v/v) glutaraldehyde (Fluka Chemie, Buchs, Switzerland) in PBS for 30 min at room temperature and then washed in ultrapure water. The labelling was silver-enhanced for 9 min at room temperature using the intenSE kit (Amersham). After three washings in ultrapure water the samples were treated with 2 % (w/v) osmium tetroxide in cacodylate buffer at room temperature for 20 min. The cells were washed with cacodylate buffer (three times for 10 min) and subsequently with distilled water for 10 min. Samples were dehydrated in 70 % (v/v) ethanol that was replaced three times after each 15 min. Afterwards they were contrasted with 0.5 % (w/v) uranyl acetate in 1 % (w/v) phosphorotungsten acid and 70 % (v/v) ethanol for 30 min and further dehydrated in 80 %, 90 % and twice 100 % (v/v) ethanol for each 15 min. The samples were embedded in resin (TAAB Laboratories Equipment, Aldermaston, UK) after preinfiltration with a terpineol/resin mixture (1:1 and 1:3,

Table 1. Accumulated β-hCG values in the media of human term placental trophoblast cells

Time after plating	β-hCG (mIU/10^6 cells)		
	Normo-glycaemia	Hyper-glycaemia	Osmotic control
24 h	19 (11–64)	–	–
48 h	81 (32–288)	74 (24–502)	70 (25–294)
72 h	51 (14–172)	56 (33–145)	59 (42–113)

Cells were cultured under normoglycaemic conditions for 24 h and further in the presence of either 5.5 mmol/l glucose (normoglycaemia) or 25 mmol/l glucose (hyperglycaemia) or 25 mmol/l D-mannitol (osmotic control), respectively, for another 48 h. Data are medians and (range)

respectively, for each 15 min). Ultrathin (50 nm) sections were examined with a Zeiss 902 electron microscope at an accelerating voltage of 50 kV. Photographs were taken on Kodak electron microscope film SO 163 at 2 s exposure time.

Specificity of the GLUT1 antiserum. The antiserum was raised against the human erythrocyte glucose transporter and identified a recombinant lambda gt11 bacteriophage in a cDNA library prepared from immunoselected polysomal RNA from adult rat brain. This cDNA predicts a 492-amino acid protein that has 97.6 % identity to the human hepatoma hexose carrier [18]. The amino acid residues 480–492 of the carboxyl terminus of the deduced sequence of the glucose transporter are bound by the antiserum and it specifically immunoblots the 55 kDa glucose transporter in erythrocyte membranes and the purified erythrocyte transporter [19]. This binding is saturable and competitively inhibited by peptide 480–492 [19]. The antiserum cross-reacts with the HepG2 glucose transporter [20] as well as with GLUT1 in Caco-2 cells [21].

Quantification of GLUT1 distribution. The GLUT1 distribution was quantified by counting immunogold particles in ultrathin sections according to a standard procedure [22]. Three grids among 15–20 from each trophoblast preparation ($n = 5$) were selected on the basis of good morphology at a magnification of x 700. Within each grid, cells that showed the nucleus were randomly chosen and used for counting over the entire cross-sectional profile of a cell. Grid squares were used to outline cytoplasmic fields in which gold particles were counted one after the other. The numbers of gold particles were counted at $\times 30\,000$ magnification. Higher magnification (up to $\times 85\,000$) was chosen if the gold grains were accumulated in clusters. For each experiment ten profiles per grid were scored which were examined in strict sequence, ensuring that no trophoblast cell was scored twice. The average number of gold particles was calculated by subtracting the labelling of a control profile from that of the experimental specimen.

Sodium dodecyl sulphate-PAGE and western blotting. Trophoblast proteins obtained from samples ($n = 5$) after 48 h in hyperglycaemic culture and respective controls were rendered soluble in Laemmli sample buffer (Sigma) supplemented with Complete protease inhibitor cocktail (Boehringer, Mannheim, Germany). Insoluble material was removed by centrifugation at $100\,000 \times g$ for 1 h at 4 °C. Before electrophoresis, samples were boiled for 3 min at 100 °C.

Equal amounts of protein, determined as described previously [23], were subjected to SDS-PAGE on 8 %–18 % gradient gels (ExcelGel, Pharmacia) using SDS buffer strips (ExcelGel, Pharmacia). Samples were run for 150 min at a constant 600 V/50 mA/30 W. Proteins were transferred onto nitrocellulose membranes (Pharmacia) by semi-dry electroblotting in a buffer containing 0.2 mol/l glycine, 25 mmol/l TRIS and 20 % (v/v) methanol for 45 min at 30 V/100 mA/6 W. Successful transfer was confirmed by Ponceau S (Sigma) staining of the blots.

The membranes were blocked for 12 h with 5 % (w/v) nonfat dry milk (BioRad, Hercules, Calif., USA) and 0.1 % (v/v) Tween-20 (Sigma) in 0.14 mol/l TRIS-buffered saline pH 7.2–7.4 at 4 °C. The same solution was used for subsequent washings and as diluent for the antibodies. The blotting membranes were incubated for 1 h at room temperature with a rabbit antiserum against GLUT1 (East Acres), diluted 1:10000. After washing they were further incubated with goat anti-rabbit IgG horseradish peroxidase conjugate (BioRad) diluted 1:5000 for 1 h at room temperature. After three washings in 0.14 mol/l TRIS-buffered saline, pH 7.2–7.4, the immunolabelling was made visible using the SuperSignal Ultra chemiluminescence substrate (Pierce, Rockford, Ill., USA) according to the instructions of the manufacturer. Membranes were exposed to Hyperfilm (Amersham).

Control blots were incubated with serum of non-immunised rabbits or the primary antibody was omitted.

Statistics. Experimental data were not normally distributed (Kolmogoroff-Smirnov test) and thus presented as median with range. Statistical analysis was done by one-way non-parametric ANOVA (Kruskal-Wallis procedure) followed by the Mann-Whitney U test. A level of less than 0.05 was chosen to identify significant differences.

Results

Characterisation of cells. Viability of the cells was greater than 90 % by trypan blue exclusion. The cytokeratin antibody stained approximately 85 % of the cells immediately after isolation and 90–95 % after 72 h in culture. Of the freshly prepared cells 10 % reacted with W6/32 and this level was reduced to 6–2 % after 72 h in culture. Anti-CD68 for monocyte and macrophage labelled 4–5 % of the cells after seeding and less than 3 % after 72 h. About 1 % of the cells were stained with the endothelial cell marker *Ulex europeaeus* lectin.

The range of β-human chorionic gonadotropin released into the media was not significantly different between trophoblast cultured in the presence of 5.5 and 25 mmol/l glucose or 19.5 mmol/l D-mannitol plus 5.5 mmol/l D-glucose ($p = 0.31$ and $p = 0.24$, respectively; Table 1).

Electron microscopic examination showed a pale cytoplasm of the cells and pleomorphic nuclei with diffuse chromatin pattern. Common cytologic features included sparse stacks of rough endoplasmic reticulum, perinuclear Golgi apparatus, few mitochondria and a microvillous membrane on the media-facing surface (Fig. 1). Trophoblasts were rich in coated pits and multiple coated vesicles containing electron dense material. Scattered endocytotic vesicles (multivesicular bodies) and lipid droplets were occasionally seen. Cells usually possessed bundles of

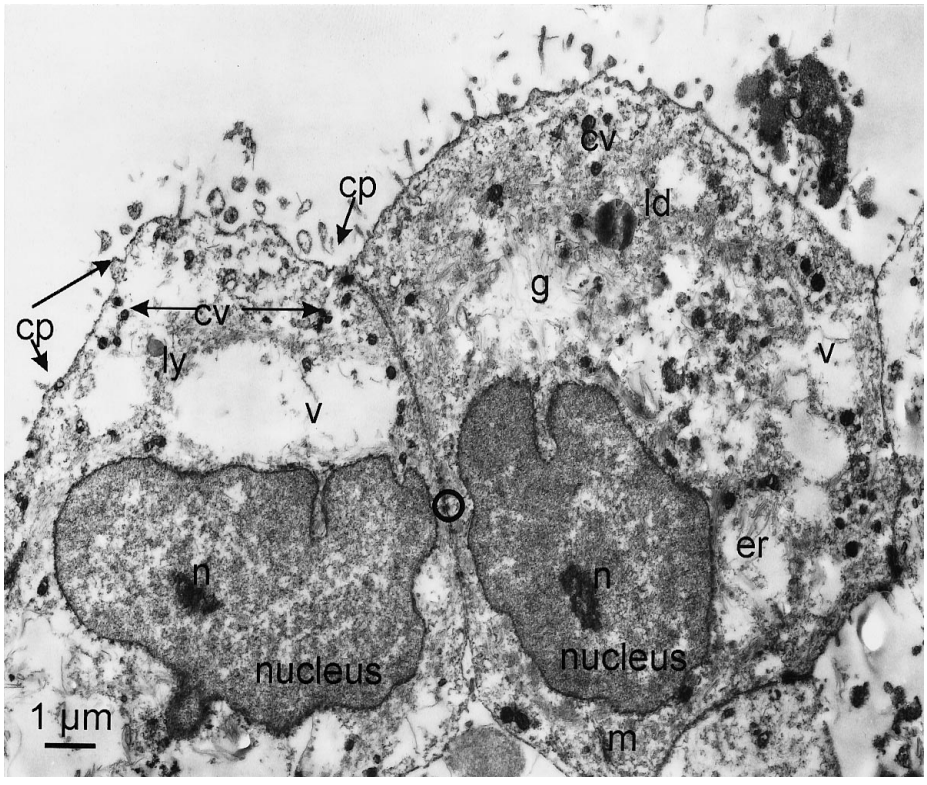

Fig. 1. Routine transmission electron microscopy of cultured human term placental trophoblast. Evident are pleomorphic nuclei with distinct nucleolus (n), segments of endoplasmic reticulum (er), Golgi apparatus (g), lysosomes (ly), mitochondrium (m), lipid droplets (ld), coated pits (cp), coated vesicles (cv), prominent cytoplasmic vacuoles (v) and intercellular desmosomal connections (encircled). Magnification: × 6500

cytoplasmic intermediate filaments which are typical of epithelium including trophoblasts. Some had prominent cytoplasmic vacuoles. Small glycogen granula and larger aggregates were randomly distributed throughout the cytoplasm. Desmosomal connections between adjacent cells were frequently observed (Fig. 1). Trophoblasts did not fuse to multinucleated syncytia even after 72 h regardless of the culture media.

GLUT1 distribution. Cultured trophoblast cells stained abundantly for GLUT1. Pre-embedding immunogold labelling revealed that 73% of total GLUT1 molecules reside in the trophoblast plasma membrane under basal conditions (Fig. 2). The majority of these cell surface transporters were located in the apical microvillous membrane, only 31% were visible in the basolateral membranes. Coated pits were generally devoid of label. Gold grains in the cells interior were found in clusters or singularily dispersed through the cytoplasm, being not preferably associated with glygogen aggregates.

Concomitantly with a reduced total GLUT1 expression (–20%; $p < 0.05$) after culture of the cells in media containing 25 mmol/l glucose for 48 h, the transporters were significantly redistributed resulting in a loss of 40% ($p < 0.05$) in plasma membrane transporter labelling (Fig. 3). The disappearance of cell surface transporters was more pronounced in the microvillous membrane (–45%; $p < 0.05$) than in the basolateral membranes (–23%), where this effect did not reach the level of statistical significance ($p = 0.06$; Table 2). Preliminary experiments showed similar effects on GLUT1 distribution also after 24 and 96 h hyperglycaemic culture. The hyperglycaemia-induced alterations were specific for D-glucose, as they were not reproduced by osmotic control media containing equimolar D-mannitol.

In control experiments with pre-immune serum or in which the primary antibody was omitted, total gold labelling per cell profile was reduced to 10% or less (not shown) of the particle count under control conditions.

Western blotting identified GLUT1 proteins with apparent molecular weights between 55–65 kDa (not shown), further confirming the specificity of the antiserum used in this study.

Discussion

Hyperglycaemia (i.e. diabetes) during pregnancy has principally deleterious metabolic effects on both mother and fetus [24, 25]. The concept of fuel-medi-

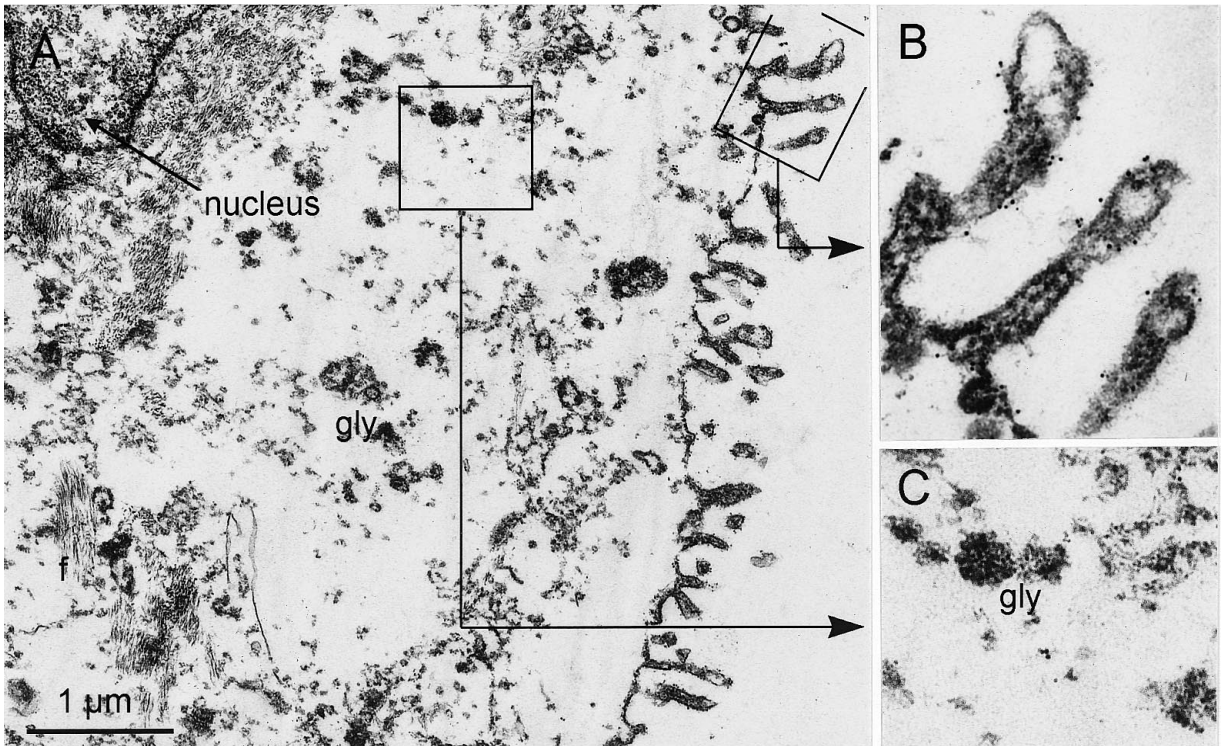

Fig. 2 A–C. Portion of trophoblast cultured in the presence of 5.5 mmol/l glucose (normoglycaemia) for 48 h. Immunogold stained for GLUT1 (**A**, magnification: × 23 000). Labelling was particularly prominent on the microvillous surface but within this membrane there was no preferential localization in the villi themselves (**B**, magnification: × 75 000). Only sparse cytoplasmic GLUT1 expression (**C**, magnification: × 50 000). Actin-like filaments (f), clusters of glycogen (gly)

ated teratogenesis [26] implicates the placenta as the key organ in transducing maternal metabolic changes to the fetus [27]. Alterations in transplacental glucose transport could lead to fetal hyperglycaemia which can cause serious and occasionally life-threatening anomalies in the growing fetus. Adaptative long-term down-regulatory effects of glucose on its own uptake system have recently been shown in the human term placental trophoblast after 24, 48 and 96 h culture under diabetes-like conditions [11, 12], as well as in midgestation rat trophoblast cells cultured with 100 mmol/l glucose, but only after 48 h [28]. The reported changes in GLUT1 expression are apparently, however, not the only means by which transport activity is regulated.

The discrepancy between a 50 % lower V_{max} and a 20 % reduction in total cellular GLUT1 protein with no change in K_M [11] lead us to hypothesise the existence of mechanisms in addition to the reduction in steady-state GLUT1 protein concentrations. The results presented in this study strongly suggest that the post-translational regulation of trophoblast glucose transport activity by ambient glucose concentrations involves long-term changes in subcellular trafficking of the GLUT1 protein, thus generally providing morphological evidence for a redistribution of GLUT1 from the plasma membrane to intracellular sites in an insulin-insensitive tissue. Effects of hyperglycaemia for less than 24 h have not been investigated, therefore, the existence of short-term mechanisms cannot be ruled out.

As the nature of the intracellular storage compartments of GLUT1 in trophoblast cells is not clear, there are also no established markers allowing for the reliable identification of respective membranes with which GLUT1 might be associated. Therefore, quantitative studies into internalisation require other methods than subcellular fractionation. We studied this process at the electron-microscopical level in situ by immunogold labelling. Purity, viability and endocrine activity of the trophoblast cell preparations used were extensively characterised and validated. The results showed that the cells in culture were viable and highly purified trophoblast showing an intact plasma membrane and an internal architecture characteristic for trophoblast cells in situ [29]. According to current opinion, the increase in β-human chorionic gonadotropin (hCG) concentration during culture reflects a biochemical differentiation [30]. This was unaffected by hyperglycaemia, because hCG concentrations were unchanged. The presence of prominent endoplasmic reticulum and of well-developed Golgi apparatus indicates that the differentiated trophoblast in vitro possessed a high capacity for protein synthesis and secretion.

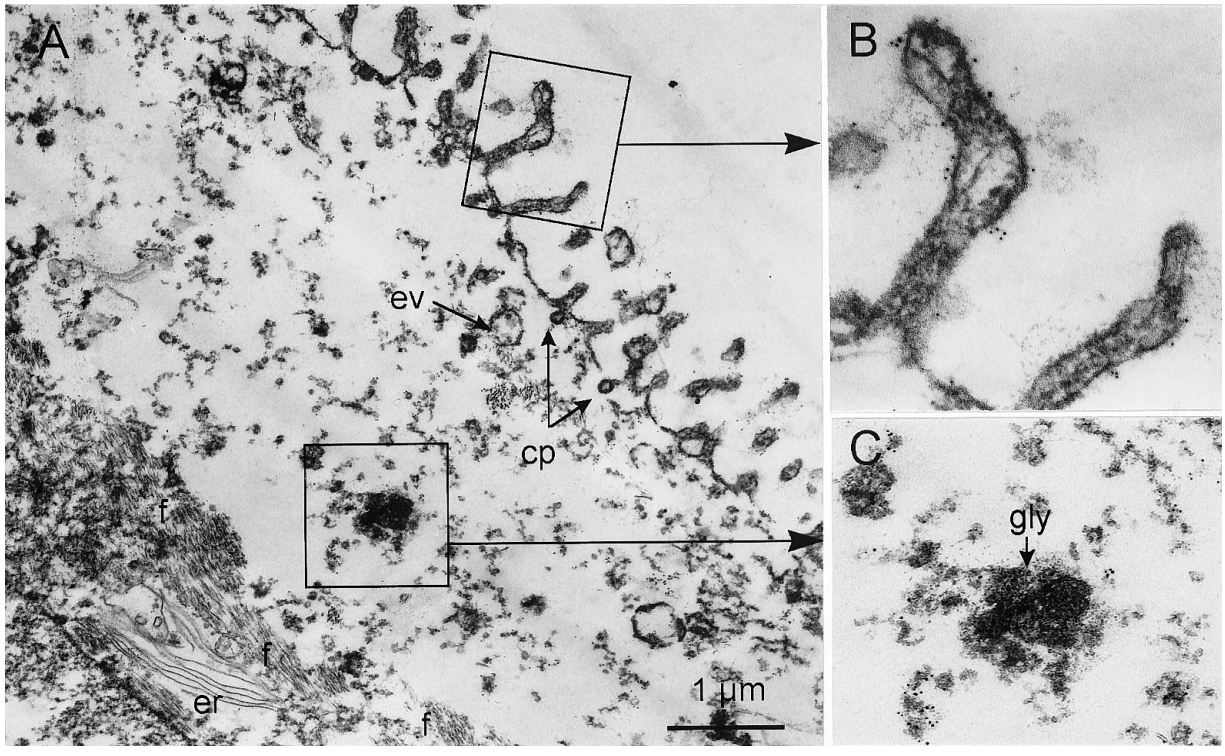

Fig. 3 A–C. Portion of trophoblast cultured in the presence of 25 mmol/l glucose (hyperglycaemia) for 48 h. Immunogold stained for GLUT1 (**A**, magnification: × 18000). The microvillous plasma membrane is less intensively labelled than under normoglycaemic conditions (**B**, magnification: × 50000). Numerous gold grains appear in the cells interior in response to hyperglycaemia (**C**, magnification: × 40000). Flattened cisternae of endoplasmic reticulum (er), actin-like filaments (f), coated pits (cp), endocytotic vesicles (ev), cluster of glycogen (gly)

The GLUT1 glucose transporter protein is the major isoform expressed in the human placenta. It represents an asymmetric carrier and spans the lipid bilayer 12 times, with the carboxyl terminus exposed to the cytoplasmic face [4]. Our immunocytochemical data suggest that the penetration of antibodies beyond the surface membrane into the cell interior was not limited. Moreover, the efficiency of GLUT1 labelling in the plasma membrane and the intracellular organelles were obviously not different because, even though the total number of gold particles per cell profile decreased in response to hyperglycaemia, the intensity of intracellular labelling increased for cells treated with a high glucose concentration. Using SDS-PAGE followed by immunoblotting, trophoblast GLUT1 proteins migrated with apparent molecular weights between 55–65 kDa as a characteristically broad band which is due to their heterogeneous glycosylation and consistent with data reported earlier [19, 31–33].

It is well established, that the short-term hypoglycaemic effect of insulin in adipocytes, skeletal and cardiac muscle cells is achieved by the translocation of the insulin-regulatable transporter isoform GLUT4 from a cytoplasmic membrane fraction to the cell surface [34–39]. In contrast, GLUT1 trafficking upon short-term stimulation by insulin is controversial even in classical insulin-responsive tissues such as skeletal muscle [40]. In studies reporting on such effects, intracellular GLUT1, similar to GLUT4, translocates to the plasma membrane after short-term insulin stimulation in rat cardiac myocytes [39], in rat [41] as well as 3T3-L1 adipocytes [42] and after long-term insulin treatment in L6 skeletal muscle cells [43].

The results presented here indicate profound differences to the above conditions. The first is that, in contrast to non-stimulated GLUT4 that resides mainly in intracellular compartments [44, 45], GLUT1 was predominantly targeted to the trophoblast plasma membrane in control cells. The precise molecular basis of such isoform-specific intracellular sorting remains to be clarified but, apart from one study [46], there is now considerable agreement about a recruitment of GLUT1 to the cell surface under basal conditions in a number of cell types expressing it [20, 47–49]. Differential targeting information could reside in particular in the amino acid sequence of the four unique hydrophilic domains which display the highest degree of sequence heterogeneity among the GLUT family [21]. Moreover, specific post-translational modification of the molecules or interaction of the transporters with cytoskeletal elements could account for the distinct subcellular localisation of various glucose carriers [50].

Table 2. Subcellular distribution of GLUT1 labelling in human term placental trophoblast

	Number of gold particles		
	Normoglycaemia	Hyperglycaemia	Osmotic control
Microvillous membrane	644 (413–951)	355 (217–693)[a]	681 (440–741)
Basolateral membrane	197 (107–235)	152 (96–227)	206 (138–220)
Total plasma membrane	841 (539–1154)	507 (336–894)[a]	887 (588–945)
Cell interior	311 (238–612)	418 (207–784)[a]	293 (235–586)
Total gold particles	1152 (799–1705)	925 (574–1586)[a]	1180 (827–1498)

Cells were cultured in the presence of either 5.5 mmol/l glucose (normoglycaemia) or 25 mmol/l glucose (hyperglycaemia) or 19.5 mmol/l D-mannitol plus 5.5 mmol/l D-glucose (osmotic control), respectively, for 48 h. Data are medians and (range). [a] $p < 0.05$ vs normoglycaemia

The second difference is in the direction of hyperglycaemia-induced GLUT1 subcellular redistribution in placental trophoblast that is opposite to the insulin-induced GLUT1 and GLUT4 trafficking towards the plasma membrane described in other cells. Our data are consistent with translocation of the transporters from the cell surface to the intracellular compartment in response to hyperglycaemia.

Due to the limitations of the immunogold method, we cannot, however, entirely exclude the possibility that hyperglycaemia selectively caused a lower turnover rate of cytoplasmic transporters, nor can we provide conclusive information on the molecular machinery bringing about the GLUT1 internalisation. Cytoplasmic GLUT1 labelling showed no apparent association with definable intracellular membranes. Considering the entry mechanism of the ligand glucose into the cell [4], it comes as no surprise that no transporters were found within structures commonly related to receptor-mediated endocytosis, i.e. coated pits and coated vesicles. Indeed, GLUT1 itself, found in the cytoplasm, could have been taken up by non-specific fluid-phase endocytosis, which does not involve coated pits and coated vesicles. The general failure to find the transporters in vesicles of the endocytotic pathway could either reflect the low probability of rendering a specific protein visible during the endocytotic process in the very small areas that can be seen in the electron microscope. Alternatively it could be merely caused by the poor preservation of structure which is due to the obligatory compromise between maintenance of antigenicity and morphological integrity when using the immunogold procedure.

In conclusion, we postulate that in addition to down-regulating human term placental trophoblast GLUT1 expression, glucose exerts its autoregulatory effect on hexose transport by promoting GLUT1 subcellular trafficking in favour of carrier partitioning to intracellular sites.

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