Intrauterine Growth Retardation: Fetal Glucose Transport is Diminished in Lung but Spared in Brain

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ABSTRACT. "Uteroplacental insufficiency" often causes asymmetric fetal growth retardation. Glucose transporters control cell glucose utilization and thus may be critical in the control of fetal growth. We hypothesized that uteroplacental insufficiency might alter glucose transporter activity, protein, and gene expression and thereby affect discordant organ growth in small-for-gestational-age (SGA) fetuses. We performed bilateral uterine artery ligation in pregnant rats on d 19 of gestation (term-21.5 d) to cause uteroplacental insufficiency and obtained fetal brain and lung tissue on d 20. The brain mass of SGA fetuses did not differ from that of sham and normal fetuses, but lung mass was significantly diminished. Glucose transport, measured with [^{3}H]2-deoxyglucose, was similar in glial cells and brain tissue of SGA, sham, and normal fetuses. In contrast, type II pneumocytes, lung fibroblasts, and lung tissue of SGA fetuses had significantly decreased glucose transport. The intrinsic activity of the glucose transporter (Km) was not altered in the brain or lung of SGA fetuses. Total glucose transporter protein measured by cytochalasin-B binding and glucose transporter 1 mRNA was diminished in SGA lung tissue and type II pneumocytes, but not in SGA brain tissue or glial cells. We could not detect glucose transporter 3 mRNA in significant quantity in any tissue. With uteroplacental insufficiency, glucose transport is differentially altered in lung and brain. Glucose transporter protein and gene expression are diminished in the lung and normal in the brain of SGA fetuses. These changes may contribute to fetal growth retardation and the phenomenon of "brain sparing." (*Pediatr Res* 31: 59–63, 1992)

Abbreviations

SGA, small for gestational age
2-DG, 2-deoxyglucose

When "uteroplacental insufficiency" complicates a pregnancy, asymmetric fetal growth retardation develops. Under such conditions, overall somatic growth is retarded but gross brain growth remains normal. The mechanisms responsible for the phenomenon of "brain sparing" are not well understood.

In previous studies we quantitated factors responsible for fetal growth retardation by performing bilateral uterine artery ligation in the maternal rat (1). This technique, an extreme example of uteroplacental insufficiency, restricts glucose and amino acid availability and alters a number of physiologic variables in the fetus including gaseous exchange (2). Glucose is one of several important metabolic fuels for the fetus, and limitation of its availability and utilization is probably involved in retarding fetal growth.

In most fetal tissues, glucose uptake is controlled by a facilitated transport mechanism. Glucose transporters are a family of structurally related proteins encoded by distinct genes and expressed in a tissue-specific manner (3–5). cDNA encoding a glucose transporter have been cloned from both rat brain and human hepatoma (Hep G2) cell libraries (6, 7). This glucose transporter has been termed Glut 1 and is present in most fetal tissues including brain (8, 9). Glut 3, originally cloned from human fetal skeletal muscle, has been found in small quantities in fetal rabbit brain (9).

How uteroplacental insufficiency affects glucose transport in the fetus and thereby contributes to the development of growth retardation is unknown. Several *in vivo* studies using 2-deoxyglucose in the pregnant rat have demonstrated limited glucose transfer to the fetus after maternal uterine artery ligation (10, 11).

When substrate supply is limited to the fetus, tissue-specific regulation of glucose transporters might ration glucose availability to organs. In this manner, glucose transport and glucous transporter gene expression might differ between brain and other tissues, and this might in part be responsible for brain sparing in the SGA fetus. To address this possibility, we measured glucose transporter activity, total glucose transporter protein, and glucose transporter mRNA in freshly isolated brain and lung tissue and in cultured cell monolayers of brain and lung from SGA and normal rat fetuses. Glial cells, type II pneumocytes, and lung fibroblasts represent major cellular components of brain and lung and have important metabolic functions. We compared brain to lung because in the SGA fetal rat the brain has high metabolic activity and growth sparing, whereas the lung is less metabolically active and has decreased growth.

We found that glucose transporter activity, protein, and mRNA do not differ in the brain of SGA and normal fetuses. In contrast, lungs of SGA fetuses have significantly diminished glucose transport. These data suggest that under conditions of uteroplacental insufficiency, glucose transport and glucose transporter gene expression are differentially altered in the brain and lung. These changes along with other factors contribute to the development of intrauterine growth retardation and may in part be responsible for brain sparing.

MATERIALS AND METHODS

Animals. We have described our surgical methods previously (1). In brief, time-dated, pregnant Sprague-Dawley rats were individually housed under standard conditions and allowed free access to standard rat food and water. On d 19 of gestation, the maternal rats were anesthetized with intraperitoneal Xylazine (Rompun Mobay Co., Shawnee, KS; 8 mg/kg) and ketamine (40 mg/kg), and both uterine arteries were ligated (SGA). We had two control groups: sham animals who underwent the identical anesthetic and surgical procedure except for ligation (sham), and animals who did not undergo any surgical procedure (normals). Rats recovered within a few hours and had *ad libitum* access to food and water. On d 20 the mothers were killed by cervical dislocation, the fetuses were immediately delivered and decapitated, and organs were quickly harvested. Litters from 21 maternal rats who underwent bilateral uterine artery ligation (SGA), 12 who underwent sham surgery (sham), and 19 who had no anesthesia or surgery (normals) were used for these studies.

Culture and isolation of glial cells, type II pneumocytes, and lung fibroblasts. The brains were removed under sterile conditions and dissociated with 0.25% trypsin and DNase (10 μg/mL) (12, 13). The cells were plated on poly-L-lysine coated plates and maintained at 37° C in a humidified atmosphere of 95% air and 5% CO_2. At 72 h, the plates were washed, and the resultant adherent cells were trypsinized, replated, and grown in Dulbecco's modified Eagle's medium with 10% FCS. The cells were incubated for 6 d in Dulbecco's modified Eagle's medium supplemented with 10% FCS before use in glucose transport experiments. Media was changed every 1–2 d. Determinations of purity of glial cell cultures by staining with glial fibrillary protein (95%) were made just before their use in experiments.

Lungs were removed and dissected under sterile conditions. The trachea and major airways were removed, and the remaining tissue was chopped into 1-mm³ cubes. Tissue from an entire litter was pooled. Explants were cultured for 24 h in serum-free Waymouth's media (2 mL/dish) at 37°C in a humidified atmosphere of 95% air and 5% CO_2 (10). The tissue was harvested, and a cell suspension was made by incubating the tissue with a trypsinizing solution (14, 15). A differential adherence method was used for separating type II pneumocytes from fibroblasts (14, 15). After 48 h culture in minimal essential medium supplemented with 10% FCS, the plates were rinsed with PBS and the confluent monolayers of pneumocytes were used for transport experiments. Hematoxylin-LiCO₃ staining was used to confirm purity (at least 90% pure) of the type II pneumocytes just before their use in experiments. Lung fibroblasts were grown in minimal essential medium supplemented with 10% FCS for 5–7 d before use in transport experiments. Media was changed daily in the type II pneumocyte and fibroblast cultures.

Membrane preparation. Membranes were prepared from uncultured brain and lung tissue and glial cells, type II pneumocytes, and lung fibroblasts. Brains and lungs were dissected from fetal rats and extensively washed to remove red blood cells. Tissues and cell monolayers were washed, placed in ice-cold isolation buffer (250 mM sucrose, 2 mM EDTA, 10 mM Tris-HCl, 1.5 mM phenylmethylsulfonylfluoride, 25 mM benzamidine, 2 μM leupeptin, 2 μM pepstatin, and 500 kallikrein inhibitor units/mL aprotinin), and homogenized with a small Polytron probe for 6 s (tissue) or a Virtis (Virtis Co., Gardiner, NY) sonicator (cell monolayers). The homogenates were centrifuged at 5000 × *g* for 5 min at 4°C, and the supernatant was centrifuged at 100 000 × *g* for 90 min at 4°C. The pellet was suspended in cold isolation buffer by several passes through a 22-gauge needle. Protein was determined by the method of Lowry (15).

Glucose transport. 2-DG transport studies were performed in fresh brain and lung tissue, cultured glial cells, type II pneumocytes, and lung fibroblasts from SGA, sham, and normal fetal rats on d 20 of gestation. These methods have been described previously (16). Cultured monolayers of type II pneumocytes,

lung fibroblasts, and glial cells, were washed with 10 mL of 37°C PBS. Glucose transport was initiated by adding [³H]2-DG to the incubation media [incubation media: 900 μL PBS, 100 μL [³H] 2-DG (0.16 mM, 1 μCi/mL)]. 2-DG, a glucose analog, enters the cell and is phosphorylated but not further metabolized. Thus, the uptake of 2-DG into cells reflects both transport and phosphorylation of glucose. Transport rather than phosphorylation has been determined to be the rate-limiting step of 2-DG uptake in brain and lung (17, 18). Therefore, determination of 2-DG uptake into brain and lung cells is a valid measurement of glucose transport. Cells were incubated for different periods (0, 0.5, 1, 5, 10, 15, and 20 min). At the end of each period, transport was stopped by the addition of ice-cold PBS. Cells were washed and solubilized with 1% Triton. An aliquot from this was used for determination of radioactivity and for determination of DNA. Noncarrier-mediated transport was determined by adding 10 μM cytochalasin B (maximal inhibitory concentration in all three cell types), a fungal metabolite that is a specific and competitive inhibitor of glucose transport, to the incubation media. 2-DG uptake was then determined. The final concentration of ethanol in the solution was less than 1%. Nonspecific binding of glucose was determined by adding 1000-fold excess of unlabeled 2-DG to the incubation media. Nonspecific binding and noncarrier-mediated transport were subtracted to determine specific transport. The addition of cytochalasin B to the cell cultures inhibited transport of 2-DG by 85% in the brain cells, 65% in the type II pneumocytes, and 90% in lung fibroblasts. To determine the kinetics of glucose transport, the cell monolayers were incubated with 0.5, 1, 2, 5, 10, and 20 mM 2-DG, and glucose transport was determined as described above.

For glucose transport experiments done in uncultured tissue, brains and lungs were chopped and 100 mg of tissue were placed in 35-mL sterile plastic tubes and centrifuged for 5 min. Glucose transport was determined at 0, 10, 20, 40, and 60 min (*n* = 7 experiments). We used [³H]inulin to determine extracellular water space.

Transport was related to DNA and protein content. DNA content was determined by fluorometric assay (19).

Cytochalasin-B binding. We used cytochalasin-B binding studies to quantitate glucose transporter protein (20–22) in membranes of brain and lung tissue and cultured cells from SGA, sham, and normal fetuses. The assay was a modification of the procedure of Cushman and Wardzala (23). Brain and lung membranes (10 mg/μL) were mixed with 200 μL of 1% NaCl and 100 μL of 20 μM cytochalasin E in 1% NaCl. Aliquots (75 μL) were withdrawn and added to 40 μL of 2 M D-glucose or L-glucose and 20 μL [³H]cytochalasin B (DuPont-New England Nuclear, Boston, MA; 13.5 Ci/mmol). After incubation at room temperature for 15 min, the suspension was centrifuged at 100 000 × *g* for 1 h at 4°C. Aliquots of clear supernatant were withdrawn to determine the free concentration of cytochalasin B. The pellet was solubilized in 5 mL of scintillation fluid (Cytoscint; ICN, Plainview, NY) and counted. [¹⁴C]mannitol (64 μM; 52.0 mCi/mmol) was also included to correct for nonspecific binding of [³H]cytochalasin B.

Northern and slot-blot analysis. Total RNA was extracted using RNazol (guanidinium thiocyanate, phenol, 2-mercaptoethanol; Cinna/Biotecx, Friendswood, TX). RNA (10 μg) was fractionated by 1.2% agarose-6.6% formaldehyde gels and transferred to Nytran filters (Schleicher & Schell, Keene, NH) (24). The gel was stained with ethidium bromide and visualized under UV light. RNA was fixed to the filter by baking for 2 h at 180°C. The filters were prehybridized for 3 h at 42°C in 50% formamide, 5 × SSC (3 M NaCl, 3 M Na₃ citrate·2 H₂O), 1% glycine, 5 × Denhardt's solution (0.02% each of Ficoll 400, polyvinylpyrrolidone, and BSA), 100 μg of herring sperm DNA, and 100 μg torula yeast RNA. A full-length 2.8-kb rat brain D-glucose transporter cDNA (Glut 1) in the plasmid pGEM and the 493-bp Glut 3 cDNA (cloned from mouse) (both kindly provided by G. Bell) were labeled with ³²P dCTP (Amersham, Arlington Heights,

IL) with random oligonucleotide primers (Pharmacia Oligo Labeling Kit; Pharmacia, Inc., Piscataway, NJ). The filters were hybridized with 1×10^6 cpm/mL of the labeled probe at 42°C overnight. After hybridization, the filters were washed twice for 5 min at 23°C in 5 × SSPE (sodium chloride, sodium phosphate, EDTA), 0.1% SDS, and 3 times for 30 min at 50°C with 0.1 × SSPE, 0.1% SDS. Autoradiography was done using Kodak XAR-5 film and two intensifying screens for 48 h at −70°C. In addition, slot blots with 10 μg total RNA were performed to determine mRNA abundance results. The relative amount of Glut 1 mRNA was quantitated by densitometry. The membranes were stripped and rehybridized to human B-actin cDNA as a control.

Statistical analyses were performed using analysis of variance and the t test (25). Data are reported as means ± SEM. These studies were approved by the Institutional Animal Care and Use Committee of Children's Memorial Institute for Education and Research.

RESULTS

The body mass of SGA fetuses, 2.96 ± 0.11 g, was significantly less than that of sham (3.16 ± 0.10 g) and normal (3.86 ± 0.08 g) fetuses. The body weight of sham fetuses was intermediate and differed significantly from SGA and normal fetuses. The brain mass of SGA fetuses (112.1 ± 11.0 mg) did not differ from that of sham (125 ± 15 mg) and normal (127.5 ± 23.2 mg) fetuses. However, the lungs from SGA fetuses were significantly lighter than those of sham and normal fetuses (SGA 96.6 ± 11.0, sham 119.4 ± 15.6, normal 134.5 ± 23.7 mg, $p < 0.05$). Lung mass was similar in sham and normal fetuses.

Total protein and DNA content did not differ significantly among SGA, sham, and glial cells, type II pneumocytes, and lung fibroblasts.

Transport of 2-DG in glial cells was linear at 37°C for at least 15 min and then reached saturation by 20 min (Fig. 1). 2-DG transport in SGA glial cells was similar to sham and normal values. Similarly, there was no difference in 2-DG transport in freshly isolated brain. By 60 min of incubation with 2-DG, glucose transport reached a plateau and was similar in SGA, sham, and normal brain tissue.

Neither the V_{max} nor the Km of glial cells differed among SGA, sham, and normal fetuses (Table 1). Therefore, the rate of 2-DG transport and its affinity for the glucose transporter are similar in SGA, sham, and normal glial cells.

The total amount of glucose transporter protein in whole brain and glial cell membranes, as measured by [³H]cytochalasin-B binding, did not significantly differ between SGA (glial cells 0.290 ± 0.079, brain tissue 0.569 ± 0.573 pM/μg DNA) and normal (glial cells 0.292 ± 0.092, brain tissue 0.573 ± 0.113 pM/μg DNA) fetal rats.
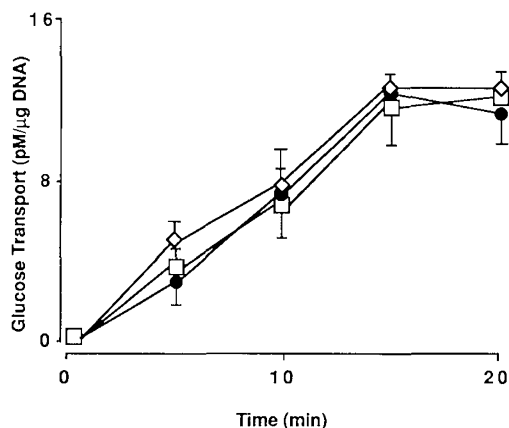

Fig. 1. 2-DG transport ($n = 7$ experiments) in glial cells from SGA (●), sham (◇), and normal (□) fetal rats on d 20 of gestation. No differences were noted among the three groups.

Table 1. *Kinetics of 2-DG transport in glial cells, type II pneumocytes, and lung fibroblasts of SGA, sham, and normal fetal rats**

	V_{max} (pM/μg DNA)	Km (mM)
Glial cells		
SGA	7.52 ± 1.23	2.08 ± 0.65
Sham	8.25 ± 2.09	2.19 ± 0.76
Normal	8.85 ± 1.12	1.92 ± 0.45
Type II pneumocytes		
SGA	2.80 ± 0.87†	2.09 ± 0.54
Sham	4.23 ± 0.75	1.95 ± 0.87
Normal	4.31 ± 1.01	2.15 ± 0.66
Lung fibroblasts		
SGA	3.10 ± 0.99†	2.98 ± 0.75
Sham	10.96 ± 3.46	3.01 ± 1.01
Normal	12.06 ± 2.35	2.54 ± 0.67

* V_{max} and Km were calculated using Lineweaver-Burk plots.

† $p < 0.05$ compared to corresponding values for sham and normal fetuses.

Northern and slot-blot analysis demonstrated that the amount of the 2.8-kb glucose transporter mRNA, Glut 1, was similar in uncultured brain tissue and cultured glial cells from SGA and normal fetuses (Figs. 2 and 3). A very weak Glut 3 mRNA signal was detected in cultured glial cells and uncultured brain tissue.

In contrast to the brain, lung 2-DG uptake was significantly diminished in SGA compared to sham and normal fetuses. Transport of 2-DG was linear up to 10 min in type II pneumocytes (Fig. 4) and lung fibroblasts. Of note, 2-DG uptake was inhibited only 65% by 10 μM cytochalasin B. Because cytochalasin B only inhibits facilitated glucose transport, it is likely that approximately 35% of 2-DG uptake is via Na⁺-coupled glucose transport. Na⁺-coupled glucose transport has been shown to occur in the lung and may account for a substantial proportion of glucose uptake by type II pneumocytes (26). 2-DG uptake was also significantly diminished in uncultured lung tissue from SGA fetuses, averaging 0.297 pM/min/μg DNA compared to 1.126 pM/min/μg DNA for shams and 1.524 pM/min/μg DNA for normals ($p < 0.05$).

SGA type II pneumocytes demonstrated a significantly lower V_{max} than shams and normals. The difference in V_{max} among SGA, shams, and normals was even greater in lung fibroblasts. V_{max} was four times less in SGA compared to normal fibroblasts. However, the Km values for type II pneumocytes and lung fibroblasts were similar in all three groups (Table 1).

[³H]cytochalasin-B binding was also significantly diminished in SGA fetal whole lung (0.044 ± 0.028 pM/μg DNA) and type II pneumocyte membranes (0.030 ± 0.011 pM/μg DNA) compared to normal fetuses (lung tissue 0.098 ± 0.031 pM/μg DNA, type II pneumocytes 0.058 ± 0.013 pM/μg DNA; $p < 0.05$).

Glut 1 was abundantly expressed in lung tissue, type II pneumocytes, and lung fibroblasts from SGA, sham, and normal fetuses. Northern and slot-blot analyses demonstrated that compared to normals, Glut 1 mRNA was diminished by approximately 50% ($p < 0.05$) in uncultured lung tissue and cultured type II pneumocytes of SGA fetuses (Figs. 2 and 3). There was a 3-fold decline in Glut 1 mRNA abundance in lung fibroblasts. We were unable to detect Glut 3 mRNA signal in type II pneumocytes or uncultured lung tissue from SGA, sham, or normal fetuses.

To ensure that the decrease in Glut 1 mRNA in SGA fetal lungs was specific for the D-Glut mRNA, the samples were probed with the cDNA B-actin. There were no differences in B-actin mRNA between SGA, sham, and normal fetuses (data not shown).

DISCUSSION

Diminished somatic growth with normal brain growth is characteristic of intrauterine growth retardation caused by uteropla-
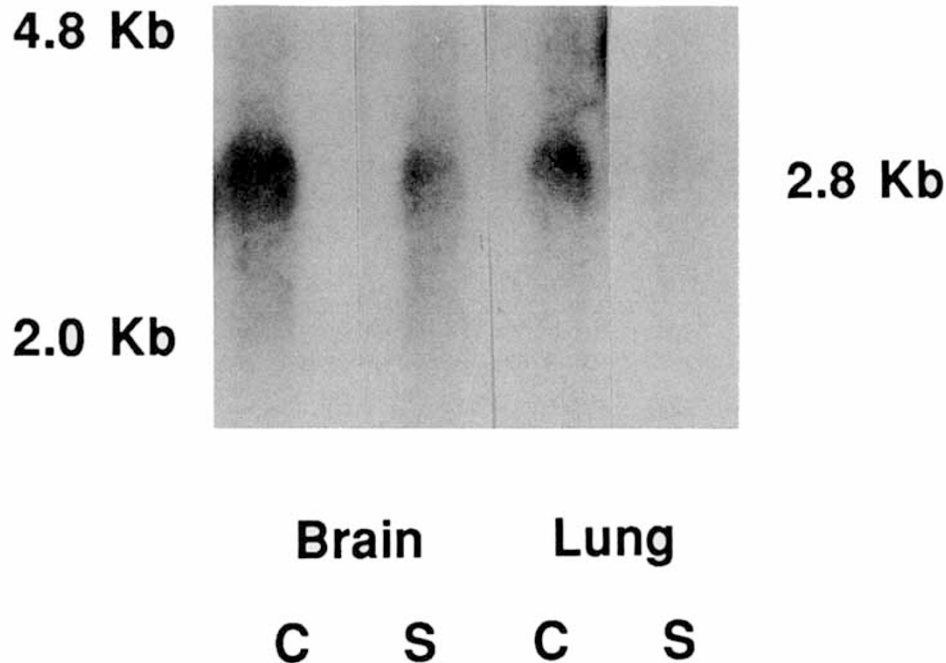
Fig. 2. Northern blot analyses of brain and lung tissue total RNA. An autoradiograph of a Northern blot probed with Glut 1 cDNA. There was no difference in intensity of bands between SGA (*S*) and normal (*C*) fetal brain tissue. In contrast, Glut 1 signal intensity in SGA lung tissue was significantly diminished.
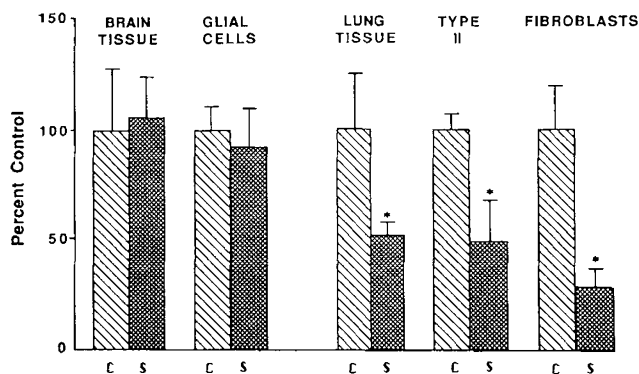

Fig. 3. Densitometry of slot blots probed with Glut 1. Each *bar* represents the mean ± SEM of four experiments. Data for SGA (*S*) were related to normal (*C*) values normalized as 100%. Glut 1 mRNA levels were similar in SGA and normal fetal brain tissue and glial cells. In contrast, Glut 1 mRNA levels were significantly diminished in SGA lung tissue type II pneumocytes and lung fibroblasts. *, $p < 0.05$.

Fig. 4. 2-DG transport in type II pneumocytes from SGA (●), sham (◇), and normal (□) fetal rats on d 20 of gestation (*n* = 7 experiments). Type II pneumocytes from SGA fetuses had significantly diminished transport at each point. *, $p < 0.05$.

cental insufficiency and other maternal disorders during pregnancy. The mechanisms at the cellular level responsible for impairing somatic growth but sparing brain growth are poorly understood. Because glucose is an important metabolic fuel for the fetus, we reasoned that glucose transport might be related to these phenomena. Our study demonstrates that glucose transporter activity, protein, and gene expression are normal in SGA fetal brain and diminished in lung under conditions mimicking uteroplacental insufficiency. Because glucose provision is important for cell metabolism and growth, these observations indicate a link between differences in tissue glucose transport and the asymmetric organ growth in intrauterine growth retardation.

We and others have found brain growth to be normal in SGA rat fetuses (1, 27). This study indicates that total glucose transporter activity, protein, and Glut 1 expression are also normal in the SGA fetal rat brain. Brain tissue and glial cell 2-DG uptake, cytochalasin-B binding, and Glut 1 mRNA did not differ between SGA, sham, and normal fetuses. These observations indicate that the numerous metabolic and physiologic variables
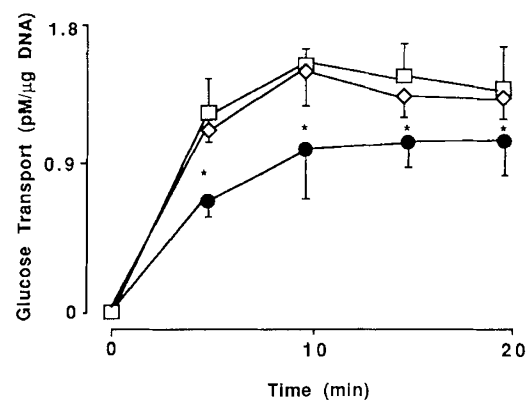
altered by maternal uterine artery ligation do not alter fetal brain glucose transport.

The mechanisms responsible for normal glucose transporter activity, protein, and expression in the brain of SGA fetuses are unknown. Glucose transport might have been maintained as a result of sustained glucose availability. With maternal uterine artery ligation, glucose provision to the fetus is compromised; however, blood flow to the brain may be increased relative to other organs (28). This may maintain provision of glucose to the brain and guarantee normal brain glucose transport and growth.

We found that maternal uterine artery ligation reduces both glucose transporter activity, protein, and mRNA in fetal lung, an organ in which growth is retarded. This technique results in transient decreases in fetal glucose and amino acid concentrations and altered gaseous exchange to the fetus. Cellular energy and redox states are transiently altered (29), and plasma insulin and IGF concentrations are diminished (1, 2, 30, 31). The combination of these altered variables could affect glucose transport by tissue-specific regulation of glucose transporter gene expression, *i.e.* reducing lung but maintaining brain glucose

transport. It should also be pointed out that cell glucose transport might not actually modulate fetal growth but merely reflect intrinsic growth. The alterations in physical factors resulting from uteroplacental insufficiency could modulate tissue metabolism, which in turn could reduce glucose transport.

How the alterations in fetal metabolism resulting from maternal uterine artery ligation affect fetal glucose transport is unclear. In the adult rat, limited glucose availability resulting from fasting decreases glucose transport in adipose tissue (32, 33). Diminished insulin availability caused by streptozotocin treatment or fasting decreases adult rat muscle glucose transport (34–36). Acidosis can impair glucose transporter function and produce insulin resistance (32, 34). Little is known about the effects of these alterations on fetal glucose transporters.

Diminished glucose uptake in the SGA fetal lung could result from decreased Glut 1 gene expression or altered glucose transporter protein. We found that total glucose transporter protein as measured by cytochalasin-B binding and Glut 1 mRNA were similarly decreased in type II pneumocytes and lung fibroblasts of SGA, sham, and normal fetuses. However, the affinity of glucose transporters for glucose did not differ between SGA and normal lung. Therefore, the metabolic and physiologic variables altered in the SGA fetus appear to act at the transcriptional or posttranscriptional (altered mRNA stability) level rather than at the posttranslational level in lung.

Isoforms other than Glut 1 could contribute to fetal glucose uptake. Glut 3 has been found in small amounts in fetal and newborn rabbit brain (8). Our data suggest that little Glut 3 (as measured by the mouse cDNA probe) is expressed in the fetal rat brain and even less is expressed in lung.

In vitro conditions could have affected measurements of cell glucose transport. We performed 2-DG studies in type II pneumocytes after 3 d and in glial cells after 7 d of culture. This difference was unavoidable because type II pneumocytes dedifferentiate with longer incubation. In contrast, the sufficient growth of glial cell monolayers requires at least 7 d, and dedifferentiation does not occur. Glucose transport was similar in SGA and normal uncultured brain tissue and in cultured glial cells. Glucose transport, cytochalasin-B binding and Glut 1 expression were similarly diminished in uncultured lung tissue and in cultured type II pneumocytes and lung fibroblasts from SGA fetuses. These similarities confirm that the *in vitro* conditions for cell monolayer culture did not alter the *in vivo* effects of maternal uterine artery ligation upon fetal brain and lung glucose transport.

These data suggest that under conditions of uteroplacental insufficiency, which results in numerous metabolic and physiologic alterations in the SGA fetus, glucose transport is differentially altered in the brain and lung. To the extent that glucose is an important metabolic fuel for the fetus, this limitation of glucose transport may affect the growth of such organs as the lung. The maintenance of glucose transport in the brain may be one mechanism to assure normal brain growth and may contribute to the brain sparing phenomenon seen in SGA fetuses.

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