Time-dependent and tissue-specific effects of circulating glucose on fetal ovine glucose transporters

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1Division of Neonatology and Developmental Biology, Department of Pediatrics, Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, Pennsylvania 15213; 2Division of Neonatology, Department of Pediatrics, Health Sciences Center, St. Louis University, St. Louis, Missouri 63104; and 3Division of Neonatology, Department of Pediatrics, University of Colorado, Denver, Colorado 80217

Das, Utpala G., Robert E. Schroeder, William W. Hay, J r., and Sherin U. Devaskar. Time-dependent and tissue-specific effects of circulating glucose on fetal ovine glucose transporters. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R809–R817, 1999.—To determine the cellular adaptations to fetal hyperglycemia and hypoglycemia, we examined the time-dependent effects on basal (GLUT-1 and GLUT-3) and insulin-responsive (GLUT-4) glucose transporter proteins by quantitative Western blot analysis in fetal ovine insulin-insensitive (brain and liver) and insulin-sensitive (myocardium, skeletal muscle, and adipose) tissues. Maternal glucose infusions causing fetal hyperglycemia resulted in a transient 30% increase in brain GLUT-1 but not GLUT-3 levels and a decline in liver and adipose GLUT-1 and myocardial and skeletal muscle GLUT-1 and GLUT-4 levels compared with gestational age-matched controls. Maternal insulin infusions leading to fetal hypoglycemia caused a decline in brain GLUT-3, an increase in brain GLUT-1, and a subsequent decline in liver GLUT-1, with no significant change in insulin-sensitive myocardium, skeletal muscle, and adipose tissue GLUT-1 or GLUT-4 concentrations, compared with gestational age-matched sham controls. We conclude that fetal glucose transporters are subject to a time-dependent and tissue- and isoform-specific differential regulation in response to altered circulating glucose and/or insulin concentrations. These cellular adaptations in GLUT-1 (and GLUT-3) are geared toward protecting the conceptus from perturbations in substrate availability, and the adaptations in GLUT-4 are geared toward development of fetal insulin resistance.

maternal diabetes; maternal hyperglycemia; maternal hypoglycemia; fetal hyperglycemia; fetal hypoglycemia

GLUCOSE, an essential substrate for cellular oxidative metabolism, is transported into cells by a family of structurally related membrane-spanning glycoproteins termed the glucose transporters (1, 12). Of the six identified major isoforms of the facilitative type, GLUT-1 is the predominant fetal glucose transporter isoform (25, 29, 32, 42) that mediates basal glucose transport into rapidly growing cells (17, 20, 43). In all fetal tissues examined, GLUT-1 is expressed in relatively higher concentrations compared with the adult (25, 29, 32, 42). In contrast, tissue-specific glucose transporter isoforms such as GLUT-3 in brain/neurons (39), GLUT-2 in liver/pancreatic β-islet cells (29, 31), and GLUT-4 in insulin-responsive tissues (2, 21) are higher in the adult compared with the fetus (25, 29, 32, 42). In the adult, lower GLUT-4 concentrations reflect insulin-resistant states (15, 16, 23). In the fetus, lower levels of GLUT-4 (25, 29, 32, 42) are associated with limited insulin-sensitive glucose utilization by fetal tissues compared with adult tissues (3, 19).

Previous investigations in the adult rat have demonstrated that both hyperglycemia and hypoglycemia lead to time-dependent and tissue-specific responses expressed by GLUT-1, GLUT-3, and GLUT-4 (6, 15, 23, 38, 40). Studies in the rat fetus have demonstrated that both hyperglycemia and hypoglycemia cause adaptive changes in fetal insulin-insensitive and -sensitive tissue glucose transporter levels (34, 36). These changes consist of a decline in skeletal muscle and myocardial GLUT-1 and GLUT-4 (34, 37) with no associated change in brain GLUT-1 or GLUT-3 (35, 37). This demonstrates a tissue specificity of these responses. These previous rat studies, although insightful into the cellular adaptations, were limited by the use of a streptozotocin-induced maternal diabetic state to produce fetal hyperglycemia (34, 35) or were associated with fetal hypoxia, ischemia, and fetal hypoglycemia (36, 37). Thus the effect of fetal hyperglycemia or hypoglycemia per se on fetal glucose transporters in the absence of maternal diabetes or uterine artery ligation remains unknown. Further, investigations in the pregnant rat culminated in observations at a single gestational time point. Thus acute changes, or changes beyond the duration examined in the rat studies, could be different and may not have been detected in these previous investigations (34–37). To determine the acute and chronic time-dependent and tissue-specific effects of fetal hyperglycemia and hypoglycemia on glucose transporter protein concentrations, we employed the well-described chronically catheterized fetal sheep model (3, 19) and examined fetal GLUT-1, GLUT-3, and GLUT-4 protein concentrations in a time-dependent manner.

MATERIALS AND METHODS

Animals

Sheep. Columbia-Rambouillet mixed-breed pregnant ewes, each carrying a single fetus, were obtained from Nebeker Ranch (Santa Monica, CA). Sheep were kept in separate carts, but two sheep were kept in each room for company. All studies, animal surgery, and animal care procedures were in the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the University of Colorado Health Sciences Center Institutional Animal Care and Use Committee. The animals were cared for and studied in the University of Colorado Health Sciences Center Perinatal Research Facility, which is accredited by the NIH, the US Department of Agriculture, and the Association for the Assessment and Accreditation of Laboratory Animal Care.

Surgery. The late-gestation (125–140 days; term 145 days) ovine fetus, which has classically been used for fetal glucose kinetic measurements, was studied. Surgery was done at ~115–120 days of gestation to place maternal and fetal infusion and blood sampling catheters for acute and 2-wk-long experiments (16–18 days). In some animals the maternal and fetal infusion and sampling catheters were placed at 98–105 days gestation to undertake longer-term experiments (36–41 days). The ewes were fasted for 2 days before surgery. Surgery was conducted under ketamine anesthesia (12–15 mg/kg bolus induction followed by 0.3–0.5 mg·kg\(^{-1}\)·min\(^{-1}\) constant infusion) and intramuscular acepromazine (2 mg/kg) and intramuscular diazepam (10 mg) for muscle relaxation and sedation. Each ewe and fetus also received 1% lidocaine local anesthesia in all wounds. A maternal sampling catheter was placed into a femoral artery, and maternal infusion catheters were placed into a femoral vein, all through a single groin incision. The ewe's abdomen was opened through a midline laparotomy, and fetal hindlimbs were extracted through a uterine incision. Fetal sampling catheters were placed into the abdominal aorta via hindlimb pedival arteries, and fetal infusion catheters were placed into the abdominal aorta via hindlimb pedal arteries, and fetal infusion catheters were placed into the femoral veins via hindlimb saphenous veins. After closing of the uterine and abdominal wounds, the catheters were tunneled subcutaneously through a skin incision and kept in a plastic pouch attached to the ewe's flank. Each ewe received intramuscular gentamicin (80 mg) and penicillin G procaine (600,000 U), and the fetus was treated with intra-amniotic ampicillin (500 mg) at the time of surgery. Postoperatively, each ewe was maintained in its own cart and allowed ad libitum access to water, alfalfa pellets, and a mineral block. The ewes were paired in the same room to decrease psychological stress.

Glucose transporter protein assays. Thorougly washed ovine tissues, which included fetal brain (cerebral cortex), liver, myocardium (ventricle), skeletal muscle (hindlimb), or white fat (perirenal) were homogenized using a Tekmar Tissumizer (Cincinnati, OH) using two 50-s cycles of 5–7 W to ensure adequate homogenization of tissue. Protein content was determined by the Bio-Rad dye-binding assay (Bio-Rad, Richmond, CA). One hundred micrograms of protein in the ovine tissues homogenates were subjected to discontinuous 10% SDS-polyacrylamide gel electrophoresis followed by electrobol transfer to nitrocellulose (Nytran; Schleicher & Schuell, Keene, NH). Equal loading and efficiency of transfer were assessed by Coomassie blue staining of the gel and Ponceau S staining of the filters. The nitrocellulose filters were incubated for 4 h in 5% nonfat dry milk in PBS to decrease nonspecific binding of the antibody. This was followed by incubation for 16 h at 4°C with an affinity-purified rabbit anti-ovine GLUT-3 antibody (0.5 µg/ml) that was generated against the hemocyanin-limpted-linked rat GLUT-1, rat GLUT-4 COOH-terminal 16 amino acids (34, 35), or the ovine GLUT-3 COOH-terminal 10 amino acids (14), which were synthesized as oligopeptides (14). We characterized all three antibodies extensively for the respective isoform specificity using various tissues that expressed one particular isoform over the other and peptide blocking experiments. In additional detection of the ovine GLUT-1 and GLUT-4 isoforms with the respective anti-ovine glucose transporter isoform antibodies was confirmed previously by us (data not shown). In contrast, the tissue-specific GLUT-3 isoform (27, 28, 35) was not detected with the interspecies rabbit anti-mouse GLUT-3 (11, 33, 34) antibody, necessitating the use of an ovine species-specific anti-ovine GLUT-3 antibody (14). Similarly, because the available anti-rat GLUT-2 antibody (J ackson Pharm services; 22) did not detect the ovine GLUT-2 (data not shown) and no anti-ovine GLUT-2 antibody was available, this isoform was not examined. After washing in PBS-1% Triton X-100, the filters treated with the GLUT-1, GLUT-3, or GLUT-4 antibodies were incubated with 5 × 10\(^5\) counts·min\(^{-1}\)·ml\(^{-1}\) of 125I-labeled goat anti-rabbit IgG for 2 h at 23°C. After extensive washing, the blots were air-dried and subjected to autoradiography for 2–5 days. Because GLUT-3 amounts were low and undetectable by this method, the anti-GLUT-3 antibody-treated filters were incubated with the peroxidase-linked goat anti-rabbit IgG and subsequently exposed to a chemiluminescence reagent (Amersham Life Science, Little Chalfont, UK). The chemiluminescence was captured by autoradiography over an optimal period of time (1–5 min). Glucose transporter levels were assessed by quantitation of radioactivity or by densitometry. Both methods yielded similar results. The areas of the filters corresponding to the glucose transporter bands as seen on the autoradiograms were quantitated and reported as the percentage of total protein.
graphs were cut out, and the radioactivity was measured using a Beckman gamma counter. Background radioactivity was measured in blank areas of the filters and subtracted from the radioactivity within the glucose transporter band. For densitometry, the presence of linearity between the time of autoradiographic exposure and the optical density of the glucose transporter protein bands was initially ensured. The net radioactivity/optical density (OD) of each glucose transporter protein band was standardized against the OD of a constant internal control protein density to account for interlane loading variability. These ratios were then expressed as a percentage of the mean of the corresponding age-matched sham control values, which then represented the changes in glucose transporter protein levels (34).

Data analysis. All results are expressed as means ± SE. When two groups were compared, the Student's t-test (parametric) or the Wilcoxon's rank test (nonparametric) was used. Differences when comparing more than two time points were determined by the one-way ANOVA, and the intergroup differences were validated by the Newman-Keuls multiple-comparison test (parametric) or the Kruskal-Wallis with tied ranks followed by the Dunn's test (nonparametric). The nonparametric tests were employed to ensure that the conclusions drawn by parametric testing were not caused by either small numbers or a heteroscedastic distribution of observations.

RESULTS

Plasma Glucose and Insulin Concentrations

Hyperglycemia studies. The maternal arterial plasma glucose concentrations were, by experimental design, about twofold higher than in the age-matched sham control euglycemic group. This was paralleled by a twofold increase in fetal arterial plasma glucose concentrations associated with no change in fetal insulin levels (Table 1). This hyperglycemic group of animals was previously noted to be hyperinsulinemic during the first 48–72 h of glucose infusions, but by the end of the first week, despite persistent hyperglycemia, the fetal insulin concentrations returned to the normal range.

Table 1. Maternal glucose and fetal glucose and insulin concentrations

<table>
<thead>
<tr>
<th>Gestational Age, days</th>
<th>Days of Infusion</th>
<th>Hyperglycemia</th>
<th>Euglycemia</th>
<th>Hypoglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maternal arterial plasma glucose, mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>2</td>
<td>5.8 ± 0.27*</td>
<td>3.8 ± 0.09</td>
<td>2.2 ± 0.18*</td>
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<tr>
<td>107</td>
<td>7</td>
<td>7.2 ± 0.8*</td>
<td>4.0 ± 0.4</td>
<td>2.1 ± 0.1*</td>
</tr>
<tr>
<td>114</td>
<td>11</td>
<td>6.9 ± 1.1*</td>
<td>4.2 ± 0.3</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>121</td>
<td>21</td>
<td>7.3 ± 1.2*</td>
<td>4.0 ± 0.3</td>
<td>2.3 ± 0.2*</td>
</tr>
<tr>
<td>128</td>
<td>28</td>
<td>7.1 ± 1.3*</td>
<td>4.0 ± 0.3</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>135</td>
<td>35</td>
<td>7.1 ± 1.0*</td>
<td>3.9 ± 0.2</td>
<td>2.3 ± 0.1*</td>
</tr>
</tbody>
</table>

Fetal arterial plasma glucose, mM

<table>
<thead>
<tr>
<th>Gestational Age, days</th>
<th>Days of Infusion</th>
<th>Hyperglycemia</th>
<th>Euglycemia</th>
<th>Hypoglycemia</th>
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<tbody>
<tr>
<td>102</td>
<td>2</td>
<td>2 ± 0.07*</td>
<td>1.15 ± 0.04</td>
<td>0.83 ± 0.13*</td>
</tr>
<tr>
<td>128</td>
<td>28</td>
<td>3.5 ± 0.2*</td>
<td>1.1 ± 0.02</td>
<td>0.6 ± 0.05*</td>
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<tr>
<td>135</td>
<td>35</td>
<td>3.3 ± 0.2*</td>
<td>1.2 ± 0.02</td>
<td>0.7 ± 0.006*</td>
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Fetal arterial plasma insulin, pM

<table>
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<tr>
<th>Gestational Age, days</th>
<th>Days of Infusion</th>
<th>Hyperglycemia</th>
<th>Euglycemia</th>
<th>Hypoglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>28</td>
<td>75 ± 15</td>
<td>55 ± 8</td>
<td>25 ± 3*</td>
</tr>
<tr>
<td>135</td>
<td>35</td>
<td>55 ± 18</td>
<td>60 ± 4</td>
<td>28 ± 4*</td>
</tr>
</tbody>
</table>

Values represent means ± SE of 7 measurements performed once per day over 1 wk; n = 12 ewes and fetuses per group at each gestational age. *P < 0.05 vs. age-matched euglycemic control.

GLUT-1 Protein

Hyperglycemia studies. INSULIN-INSSENSITIVE TISSUES: BRAIN AND LIVER. Acute maternal hyperglycemia led to a 20% increase in brain GLUT-1 protein at 2–48 h (P = 0.02; Fig. 1, A and F) but did not lead to any significant change in liver GLUT-1 at the same time point (Fig. 1, B and G). Chronic maternal hyperglycemia, on the other hand, did not lead to significant changes in brain GLUT-1 (Fig. 1, A and F) but did cause a time-dependent decline in liver GLUT-1 of up to 60% at 15–20 days (P < 0.02; Fig. 1, B and G). INSULIN-SENSITIVE TISSUES: MYOCARDIUM, SKELETAL MUSCLE, AND ADIPOSE TISSUE. In response to maternal hyperglycemia, all three tissues showed no acute change at 2–48 h, but a time-dependent decline in GLUT-1 was observed in myocardium (Fig. 1, C and H), skeletal muscle (Fig. 1, D and J), and adipose tissue (Fig. 1, E and I). Myocardial GLUT-1 levels reached a nadir at 10–14 days (50% decline; P < 0.02; Fig. 1, C and H), skeletal muscle GLUT-1 demonstrated a 50% decrease at 15–20 days (P < 0.01; t-test; Fig. 1, D and J), and adipose tissue GLUT-1 showed a 45% decrease at 15–20 days (P < 0.05; Fig. 1, E and I).

Hypoglycemia studies. INSULIN-INSSENSITIVE TISSUES: BRAIN AND LIVER. Maternal hypoglycemia did not lead to any significant change in brain GLUT-1 protein levels at 4–48 h or 21–41 days. However, at 5–15 days, a 20% increase in brain GLUT-1 was observed (P < 0.03; Fig. 2, A and F). Liver GLUT-1 levels showed no statistically significant change until 5–15 days, only to be followed by a 60% decline at 21–41 days (P < 0.02; Fig. 2, B and G). INSULIN-SENSITIVE TISSUES: MYOCARDIUM, SKELETAL MUSCLE, AND ADIPOSE TISSUE. Acute and chronic hypoglycemia did not significantly change myocardium, skeletal muscle, or adipose tissue GLUT-1 levels (Fig. 2, C–E, respectively).

GLUT-3 and GLUT-4 Protein

Hyperglycemia studies. INSULIN-INSSENSITIVE TISSUES: BRAIN. No statistically significant changes in fetal brain GLUT-3 levels were noted at 2–48 h, 10–14 days, or 15–20 days (Fig. 3A).

INSULIN-SENSITIVE TISSUES: MYOCARDIUM, SKELETAL MUSCLE, AND ADIPOSE TISSUE. Similar to the GLUT-1 changes, myocardium (Fig. 3, B and E) and skeletal muscle (Fig. 3, C and F) GLUT-4 showed a 50% decline (P < 0.02 and 0.05, respectively) at 15–20 days, with no time-dependent change in adipose tissue GLUT-4 levels (Fig. 3D).
Fig. 1. Quantitative analysis of GLUT-1 protein levels in fetal ovine tissues in response to maternal and fetal hyperglycemia of varying durations. Values are means ± SE, expressed as percentage of mean of euglycemic age-matched sham control values (dashed line). A: brain (n = 13, 9, 13, and 8 sheep for control, 2-48 h, 10–14 days, and 15–20 days, respectively; *P = 0.02 vs. control). B: liver (n = 10, 9, 13, and 8 sheep for control, 2-48 h, 10–14 days, and 15–20 days, respectively; *P < 0.02 vs. control). C: myocardium (n = 10, 2, 4, and 9 sheep for control, 2-48 h, 10–14 days, and 15–20 days, respectively; *P < 0.02 vs. control). D: skeletal muscle (n = 12, 9, 13, and 8 sheep for control, 2-48 h, 10–14 days, and 15–20 days, respectively; *P < 0.01 vs. control). E: adipose tissue (n = 13, 9, 12, and 8 sheep for control, 2-48 h, 10–14 days, and 15–20 days, respectively; *P < 0.05 vs. control). Corresponding representative Western blots demonstrating 50-kDa GLUT-1 band in fetal ovine brain (F), liver (G), myocardium (H), adipose tissue (I), and skeletal muscle (J) are shown. C, control.
Hypoglycemia studies. **INSULIN-INSSENSITIVE TISSUES: BRAIN.** The time-dependent changes in brain GLUT-3 protein levels consisted of a 50% decline ($P < 0.01$; t-test) at 4–48 h followed by no change at 5–15 days and 21–41 days (Fig. 4, A and E).

**INSULIN-SENSITIVE TISSUES: MYOCARDIUM, SKELETAL MUSCLE, AND ADIPOSE TISSUE.** All three tissues demonstrated no change in GLUT-4 levels in response to hypoglycemia with hypoinsulinemia (Fig. 4, B–D).

**DISCUSSION**

We have for the first time shown a time-dependent and tissue- and isoform-specific effect of fetal hyperglycemia with normoinsulinemia and of fetal hypoglycemia with hypoinsulinemia on fetal tissue glucose transporter proteins. Fetal hyperglycemia caused no acute change in fetal tissue glucose transporter levels, including the insulin-responsive isoform GLUT-4 and the neuronal isoform GLUT-3, except in the case of brain GLUT-1, where a transient mild increase was noted. This change in brain tissue or lack thereof in other tissues reflects the presence or absence of an immediate compensation by tissue glucose transporters in handling the increased glucose load. A chronic increase in fetal circulating glucose concentrations over a period of 2 wk with normoinsulinemia led to a decline in hepatic, myocardial, skeletal muscle, and adipose tissue GLUT-1 levels with no change in brain GLUT-1 or GLUT-3 concentrations. These observations are consistent with adaptive mechanisms aimed at protecting nonneural fetal cells from excessive basal glucose entry, which could lead to cellular toxicity (44). Thus chronically elevated circulating glucose concentrations appear to partially shut the portal of entry of glucose into growing cells in all peripheral fetal tissues examined. In contrast, a similar decline in basal glucose transporting mechanisms was not apparent in the central nervous system. GLUT-4 levels also declined in myocardium and skeletal muscle with no change in adipose tissue. This differential effect between skeletal muscle and adipose tissue glucose transporter protein levels has previously been described in the adult rat (9). The decline in myocardial and skeletal muscle GLUT-4 concentrations suggests the emergence of fetal insulin resistance, which is characteristic of the progeny of a diabetic pregnancy exposed to intrauterine hyperglycemia. This may also reflect the importance of the skeletal muscle with respect to insulin responsiveness in the fetus compared with the adult counterpart (43). The absence of a change in fetal adipose tissue GLUT-4

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**Fig. 2.** Quantitative analysis of GLUT-1 protein levels in fetal ovine tissues in response to maternal and fetal hypoglycemia of varying durations. Values are means ± SE, expressed as percentage of mean of euglycemic age-matched sham control values (dashed line). A: brain ($n = 14$, 7, 6, and 9 sheep for control, 4–48 h, 5–15 days, and 21–41 days, respectively; $*P < 0.03$ vs. control). B: liver ($n = 11$, 7, 6, and 9 for control, 4–48 h, 5–15 days, and 21–41 days, respectively; $*P < 0.02$ vs. control). C: myocardium ($n = 14$, 6, 5, and 9 for control, 24–48 h, 10–15 days, and 21–41 days, respectively). D: skeletal muscle ($n = 14$, 6, 5, and 9 for control, 24–48 h, 10–15 days, and 21–41 days, respectively). E: adipose tissue ($n = 14$, 5, 6, and 9 for control, 24–48 h, 10–15 days, and 21–41 days, respectively). Corresponding representative Western blots demonstrating 50-kDa GLUT-1 band in fetal ovine brain (F) and liver (G) alone are shown. C, control.
levels suggests an absence of insulin resistance in this tissue with a propensity toward ongoing insulin-induced glucose transport and utilization in the presence of excessive substrate, thereby leading to obesity. Alternatively, white fat may not play a major role in insulin-induced glucose utilization, inasmuch as ovine fetal lipogenesis is derived more from lactate and acetate than from glucose (10).

Our present results are in keeping with previous studies in the fetal rat, in which maternal diabetes-induced chronic fetal hyperglycemia caused a pronounced decline in myocardial and skeletal muscle GLUT-1 and GLUT-4 levels (34). These studies in the rat demonstrated the existence of GLUT-1 on the plasma membrane of the myocytes in both tissues, whereas GLUT-4 was intracellular (34). Although fetal hyperglycemia in the rat (34) and sheep led to a decline in GLUT-1 and GLUT-4, the effect of hyperglycemia on the unmasking or translocation of GLUT-1 and GLUT-4 to the transverse tubules or sarcolemmal membrane in the fetal sheep remains unknown. Because GLUT-4 and to some extent GLUT-1 function is dependent on their unmasking and/or translocation to the sarcolemmal membrane (24, 30, 41), future studies remain to be done targeting the effect of fetal hyperglycemia on the ability of GLUT-1 and GLUT-4 to unmask its COOH terminus or translocate to the sarcolemmal membrane in order to assess the biological significance of a suppression in the total glucose transporter protein concentrations in insulin-sensitive fetal tissues.

Insulin-induced fetal hypoglycemia did not acutely affect brain GLUT-1 (blood-brain barrier and glial cells) but led to a decline in neuronal GLUT-3 levels. This change suggests a decline in neuronal glucose transport in the presence of diminished substrate. In contrast, no change in hepatic, skeletal muscle, or fat GLUT-1 levels was noted. Chronic hypoglycemia led to a slight but transient increase in brain GLUT-1, with
no change in GLUT-3 concentrations at 5–15 days. Thus a transient compensation toward preserving brain glucose transport during hypoglycemia was noted. In contrast, a decline in hepatic GLUT-1 levels was observed at 21–41 days. Whether this GLUT-1 decline occurs in the hematopoietic component of the fetal liver or in the hepatocytes is unknown. In other species, such as the rat, GLUT-1 is localized to the hematopoietic cells and hepatocytes present in the fetal liver (31). Depending on the cell type expressing this decline in GLUT-1, a diminution in glucose entry can interfere with the process of hematopoiesis (31) or the late-gestation buildup in glycogen stores (4, 5).

In contrast to that in the liver, fetal hypoglycemia caused no significant change in myocardial, skeletal muscle, or adipose tissue GLUT-1 and GLUT-4 levels. In the presence of limited substrate availability (26), this global absence of change in fetal tissue GLUT-1 levels supports no impedance to cellular glucose entry within saturability of the glucose transporter isoform. Similarly, no significant changes in GLUT-4 levels were observed in myocardial, skeletal muscle, or adipose tissue, attesting to no major change in fetal insulin sensitivity. These findings contrast with previous studies in the adult rat, in which, in response to starvation, skeletal muscle and adipose tissue GLUT-4 levels respond differently, increasing in muscle and decreasing in fat (23). This differential response might represent the means by which available glucose is conserved for tissues that require it for function (skeletal muscle) at the expense of stores (adipose tissue). Our present studies demonstrate that hypoglycemia-induced changes are mainly confined to the brain and liver and are not as dramatic in the insulin-sensitive tissues. These differences may stem from the severity of hypoglycemia induced by starvation in the adult rat studies (23) as opposed to that induced by insulin in our present investigation. Alternatively, the absence of hypoglycemia-induced compensatory changes in fetal glucose transporters within insulin-responsive tissues may suggest the presence of sufficient glucose delivery to fuel the energy metabolism, thereby presenting no additional need to further protect the tissue glucose supply. This in turn may compromise the intrauterine growth potential.

In summary, perturbations in fetal glucose concentrations caused transient and minimal changes in brain glucose transporter levels. Fetal hyperglycemia led to a decline in other fetal tissue GLUT-1 concentrations, preventing excessive glucose entry into developing cells and thereby preventing glucose toxicity. Whereas a decline in myocardial and skeletal muscle GLUT-4 levels may determine the onset of insulin resistance, the lack of a change in adipose GLUT-4 levels may...
herald the onset of obesity. In contrast, although fetal hypoglycemia suppressed GLUT-1 levels primarily in the liver, it led to no major changes in GLUT-1 or GLUT-4 concentrations in other tissues. This supports the hypothesis that minimal to no compensation is necessary to protect the peripheral tissue glucose supply in the presence of limited substrate. Thus fetal hyperglycemia or hypoglycemia causes time-dependent and tissue- and isoform-specific changes in fetal glucose transporter levels. Whether these fetal changes persist postnatally and alter the adult responses to hyperglycemia and hypoglycemia remains to be investigated.

Perspectives

The implications of our observations are that perturbations in maternal and fetal circulating glucose concentrations have a time-dependent and tissue- and isoform-specific differential effect on fetal glucose transporter protein levels. In both cases, the changes (or lack thereof) in tissue glucose transporter protein levels are targeted at protecting the glucose supply necessary to fuel the energy metabolism of a tissue. In the case of excessive glucose, a downregulation of glucose transporter proteins in most tissues protects cells from glucose toxicity. In contrast, limited substrate availability causes no major change because of the lack of a need to upregulate the fetal glucose transporters to increase the intracellular glucose supply above that of euglycemic conditions. Instead, glucose is delivered intracellularly, within the saturability of a given glucose transporter isoform, to match the euglycemic conditions despite limited glucose availability. Our present results also support a more general hypothesis that cellular mechanisms that support energy metabolism and growth in the fetus, such as the steady-state expression of glucose transporters, are modulated in the presence of energy substrate (glucose) excess or deficiency to preserve normal cellular metabolism at the expense of energy substrate. Thus fetal hyperglycemia or hypoglycemia causes time-dependent and tissue- and isoform-specific changes in fetal glucose transporter levels. Whether these fetal changes persist postnatally and alter the adult responses to hyperglycemia and hypoglycemia remains to be investigated.

REFERENCES


