Effects of selective hyperglycemia and hyperinsulinemia on glucose transporters in fetal ovine skeletal muscle

MARIANNE S. ANDERSON,1 JING HE,2 JUDY FLOWERS-ZIEGLER,2 SHERIN U. DEVASKAR,3 AND WILLIAM W. HAY, JR.1
1Perinatal Research Center and Section of Neonatology, Department of Pediatrics, University of Colorado School of Medicine, Denver, Colorado 80262; 2Division of Neonatology and Developmental Biology, Department of Pediatrics, University of Pittsburgh, Magee Womens Research Institute, Pittsburgh, Pennsylvania 15213; and 3Division of Neonatology and Developmental Biology, Department of Pediatrics, University of California Los Angeles School of Medicine, Los Angeles, California 90095–1752

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Address for reprint requests and other correspondence: M. S. Anderson, Box B-195, Univ. of Colorado Health Sciences Center, 4200 E. Ninth Ave., Denver, CO 80262 (E-mail: marianne.anderson@uchsc.edu).

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STUDIES IN LATE-ESTATION fetal sheep have shown that fetal plasma glucose and insulin concentrations independently regulate fetal whole body glucose utilization rates (15–17). A central question from these studies is whether the effects of fetal plasma glucose and insulin concentrations to increase fetal glucose utilization rate are dependent on the process of glucose transport into fetal cells. Glucose transport is mediated by glucose transporter proteins, which consist of a family of facilitative transmembrane glycoproteins that move glucose into cells down a concentration gradient (4, 10, 24, 27–29). Of the different major isoforms, Glut 1 is found in all tissue types in the fetus, whereas Glut 4 is present primarily in insulin-responsive tissues, such as skeletal muscle, but in lower concentrations relative to the adult (31, 32, 34). Under normoglycemic and normoinsulinemic conditions, Glut 1 and Glut 4 reside primarily in the endosomal stores and in separate endosomal storage pools within the cytoplasm, respectively (12). Glut 1 is largely constituent in cell membranes, providing for basal glucose uptake. In contrast, Glut 4 is found primarily in the endosomal stores and much less at the cell membrane (1, 26). In adult human, animal, and insulin-responsive cell models, increased amount and translocation of Glut 1 and Glut 4 from intracellular storage pools to the cell membrane mediate increased rates of glucose uptake by cells in response to higher plasma concentrations of glucose and insulin, respectively (3, 7, 13, 36, 37). In contrast to these adult studies, there is minimal information in the fetus to correlate in vivo fetal glucose utilization rates with the amount of these transporters.

The purpose of the present study was to determine whether there are changes in Glut 1 and Glut 4 glucose transporter protein concentrations in skeletal muscle in fetal sheep under conditions of selective fetal hyperglycemia or hyperinsulinemia when glucose utilization rate is increased. We hypothesized that increased glucose utilization by the fetus and, in particular, by fetal skeletal muscle would be associated with increases in fetal skeletal muscle cell Glut 1 and Glut 4 transporter protein concentrations in response to selective hyperglycemia or hyperinsulinemia. We anticipated that the relative expression of Glut 1 would be increased by selective hyperglycemia and that the relative expres-
sion of Glut 4 would be increased by selective hyperinsulinemia. Skeletal muscle was chosen for study specifically because it contains both Glut 1 and Glut 4 (10, 28), shows increased rates of glucose uptake and utilization in response to increased plasma concentrations of glucose and insulin (2, 8, 41), and accounts for the majority of glucose consumption in the late-gestation fetus (15, 23, 30, 35).

METHODS

Animal preparation. We studied 33 pregnant Columbia-Rambouillet ewes obtained from a commercial breeder (Nebecer Ranch, Santa Monica, CA) at 90% of a 147-day gestation. Surgery was performed at 120 days gestation to place indwelling polyvinyl catheters in the ewe and fetus. The ewes were fasted for 2 days before surgery. The ewes were sedated with a bolus injection (14 ml) of pentobarbital sodium solution (50 mg/ml) via an external jugular vein catheter. Anesthesia was provided by a single spinal injection of 2 ml tetracaine hydrochloride 1%. Sedation was maintained during surgery by intermittent 1- to 2-ml intravenous injections of pentobarbital sodium. With the use of a standard hysterotomy approach, fetal catheters for infusion were placed in the inferior vena cava via hindlimb pedal veins, and sampling catheters were placed in the umbilical vein directly and in the fetal aorta via hindlimb pedal arteries. A maternal femoral arterial sampling catheter and femoral venous infusion catheters were placed via a single groin incision. All catheters were filled with 0.9% (wt/vol) sodium chloride in water containing 100 U/ml of sodium heparin. The catheters were tunneled subcutaneously to exit through a flank incision and were kept in a plastic pouch secured to the ewe’s flank. The catheters were flushed every other day with the heparinized sodium chloride solution. For infection prophylaxis, the ewe was given intramuscular injections of gentamicin (80 mg; American Pharmaceutical Partners, Los Angeles, CA) and procaine penicillin G (600,000 U; Vedco, St. Joseph, MO) just before surgery, and the fetus was given intra-aminotic ampicillin (500 mg; Apothecon, Bristol-Meyers Squibb, New York, NY) at the end of surgery. A minimum 72-h recovery period follow surgery. During this time, the ewes had free access to food, minerals, and water. Sheep were kept in standard plastic cots, at least two in a room. The room was kept at 60 ± 2°F. Eight hours of darkness and 16 h of light were provided each day. This study was approved by the University of Colorado Health Sciences Center Institutional Animal Care and Use Committee. All procedures and studies were performed at the University of Colorado Health Sciences Center Perinatal Research Facility, which is accredited by the United State Drug Administration, National Institutes of Health, and the American Association for Accreditation of Laboratory Animal Care.

Study design. Animals were divided into two study groups. One group of fetuses was made hyperglycemic while maintaining normal plasma insulin concentrations, and the other was made hyperinsulinemic while maintaining normal plasma glucose concentrations (19). The two study groups were subdivided into three experimental time periods: 1, 2.5, and 24 h.

In both study groups, tritiated water was infused into the fetus to measure umbilical blood flow by the transplacental steady-state diffusion technique and to allow calculation of umbilical glucose uptake by the fetus using Fick principle methodology. At time 0, 0.1 ml of tritiated water mixed in 24 ml 0.9% (wt/vol) sodium chloride in water to make a concentration of 20.8 μCi/ml was infused intravenously into the fetus as a priming bolus (1 ml/kg fetal weight = 20.8 μCi/kg fetal weight) followed by a constant infusion at 1 ml-h⁻¹-kg⁻¹ (20.8 μCi-h⁻¹-kg⁻¹). Control-period blood samples for fetal arterial plasma glucose concentration, fetal arterial plasma insulin concentration, and plasma tritiated water counts in the fetal artery and umbilical vein were obtained at four times, 10 min apart, after 90 min of tritiated water infusion. Fetal euglycemia was defined as the mean fetal arterial plasma glucose concentration measured during the control sampling period.

Hyperglycemia studies. Hyperglycemia with normal fetal plasma insulin concentration was produced in study group 1 by infusing 50% dextrose in water (D50W) into the mother and somatostatin into the fetus to prevent increased fetal insulin secretion in response to the experimental hyperglycemia. Somatostatin (6 mg) was mixed in 0.9% (wt/vol) sodium chloride in water (20 ml) to a concentration of 300 μg/ml and was given to the fetus as a priming bolus (100 μg/kg) followed by a constant infusion (4 μg·min⁻¹·kg⁻¹). The dextrose infusion into the ewe was initiated 60 min after starting the somatostatin infusion into the fetus. The ewe received a priming bolus of D50W (~300 mg/kg) followed by an initial infusion rate of ~2.3 mg·kg⁻¹·h⁻¹. Glucose clamp technique (18) was used to keep fetal arterial plasma glucose concentration at ~40 mg/dl, twice the mean control period value. To establish a constant fetal glucose concentration, fetal arterial plasma glucose concentration was measured every 10 min, and the maternal dextrose infusion rate was adjusted until the fetal arterial plasma glucose concentration was stable at the target concentration. Once the clamp concentration was established, only infrequent measurements of fetal glucose concentration were necessary to verify continued stability.

Hyperinsulinemia studies. Hyperinsulinemia with euglycemia was produced in study group 2 by infusing insulin into the fetus and adjusting a D50W infusion into the ewe to keep fetal arterial plasma glucose concentration at the mean control period value. Regular insulin (100 U/ml) was mixed with normal saline to a concentration of 60 μU/ml. The fetus received a 30-mU/kg priming bolus of insulin followed by a constant infusion at 1 μU·min⁻¹·kg⁻¹. Fetal arterial plasma glucose concentration was measured every 10 min, and the maternal dextrose infusion rate was adjusted to keep the fetal arterial plasma glucose concentrations at the mean control period value.

In both study groups, 15 ml of maternal blood was drawn into a syringe containing 1 ml heparin (1,000 U/ml) before starting any infusions. This heparinized blood was used immediately following the control-period blood sampling to replace fetal blood loss from phlebotomy. Transfusion volume was sufficient to replace the estimated 5–6% of fetal blood volume lost for control blood draws and clamp measurements.

After clamp infusion periods of 1, 2.5, or 24 h, experimental fetal, maternal, and umbilical blood samples were obtained at four times, 10 min apart. Assays included fetal arterial plasma glucose, insulin, and tritiated water concentrations; umbilical venous plasma glucose and tritiated water concentrations; and maternal arterial plasma glucose and insulin concentrations. Fetal arterial oxygen saturation, fetal arterial oxygen content, and fetal hematocrit were measured at the beginning and at the end of the control and study sampling periods. Except for the 3H2O infusion, which was stopped at the end of the last sampling period, study infusions of dextrose into the mother and somatostatin or insulin into the fetus were maintained to necropsy, which occurred as soon as possible after the study.
At the end of the study, the ewes and fetuses were sedated to anesthetic level with pentobarbital sodium infused over 1 min. The fetus was removed intact, and fetal weight was measured. Biopsies of fetal biceps femoris skeletal muscle were obtained within 3 min of anesthesia induction. The ewe and fetus were then injected with lethal doses of euthanasia solution (Sleepaway pentobarbital sodium, Fort Dodge Laboratories, Fort Dodge, IA). Tissue samples were immediately snap-frozen in liquid nitrogen. Frozen samples were stored at −70°C until analysis. Tissues from normal, noninstrumented control animals with normal glucose and insulin concentrations were included to compare normal Glut 1 and Glut 4 concentrations at the same gestational age.

Biochemical analyses. Plasma glucose and lactate concentrations were measured in duplicate using a YSI model 2700 analyzer (Yellow Springs Instrument, Yellow Springs, OH). To measure plasma insulin concentrations, sampled blood was immediately centrifuged at 4°C for 3 min, and plasma was stored at −70°C until analysis with a Linco rat insulin RIA kit (St. Charles, MO) using ovine insulin standards (Elly Lily, Indianapolis, IN). Blood oxygen saturation, oxygen content, and hemoglobin concentration were measured using a Radiometer OSM3 hemoximeter (Copenhagen, Denmark).

Calculations. Umbilical blood flow was calculated by the transplacental steady-state diffusion technique using tritiated water ($^3$H$_2$O) as the tracer (40). Net umbilical (fetal) glucose uptake rate ($UGU_f$) was calculated by application of the Fick principle as $UGU_f$ = (mg kg$^{-2}$min$^{-1}$) = umbilical blood flow (ml/min) $\times$ Gv-Ga (mg/dl), where Gv and Ga are the umbilical venous and the fetal arterial plasma glucose concentrations, respectively. $UGU_f$ was considered equal to fetal glucose utilization rate, because there is no net fetal glucose production when the fetus receives normal or above-normal rates of glucose supply from the placenta and has normal to high plasma glucose and/or insulin concentrations (14, 21).

Glucose transporter protein measurement. Thoroughly washed ovine fetal skeletal muscle samples were homogenized using a Tekmar Tissuemizer (Cincinnati, OH). The samples were then sonicated (60 sonic, Disembrator, Fisher Scientific, Pittsburgh, PA) using 2-s cycles of 5–7 W to ensure adequate homogenization of the tissue. Protein content was assessed by the Bio-Rad dye-binding assay (Bio-Rad, Richmond, CA), placed on a coverslip, and visualized using an Olympus microscope with an epifluorescence attachment using an affinity-purified rabbit anti-rat Glut 1 (1:500 dilution) or Glut 4 (1:200 dilution) IgGs in PBS at 4°C with an optimal concentration of the rabbit anti-rat Glut 1 linked with goat anti-rabbit secondary antibody (1:100; Sigma Chemical, St. Louis, MO) for 1.5 h at 23°C. After extensive washes in PBS, the sections were mounted in a commercially available mounting solution (Biomed, Foster City, CA), placed on a coverslip, and visualized using an Olympus microscope with an epifluorescence attachment using the appropriate filter. Cellular localization of Glut 1 and Glut 4 immunoreactivity was undertaken using the Simple 32 image analyzer software program (Compix, Imaging Systems, Cranberry Township, PA).

Data analysis. All results are expressed as means ± SE. When two groups were compared, the Student's t-test was used. Differences were determined by the Kruskal-Wallis test followed by a post hoc t-test when comparing more than two time points, given the small sample size limitation in sheep investigations.

RESULTS

There were no differences among groups in gestational age at surgery or study or fetal weight at study (Table 1). Fetal arterial blood oxygen saturation value was exposed to a chemiluminescence reagent (Amersham Pharmacia Biotech, Little Chalfont, UK). The chemiluminescence was captured by autoradiography over an optimal period of time (1–5 min). Glucose transporter protein concentrations were assessed by densitometry once the presence of linearity between the time of autoradiographic exposure and the optical density was established. The results were expressed as percentages of the means of the corresponding control for each experimental condition.

Subcellular localization studies. Cryostat (8 μm) sections of skeletal muscle were obtained at a cross-sectional level and mounted on Superfrost/Plus slides. A few sections were obtained in the longitudinal orientation. Comparable sections at the four different times (0, 1, 2.5, and 24 h) from the two experimental groups were all mounted on a single slide and subjected to immunohistochemical analysis to overcome interassay variability. The tissue sections were fixed in acetone for 10 min at 4°C and then washed with PBS (pH 7.4) for 10 min. The fixed tissue sections were incubated overnight with an optimal concentration of the rabbit anti-rat Glut 1 (1:500 dilution) or Glut 4 (1:200 dilution) IgGs in PBS at 4°C in a humidified chamber. Glut 1 or Glut 4 preabsorbed respective antibodies served as the appropriate negative control. The sections were subsequently incubated with FITC linked with goat anti-rabbit secondary antibody (1:100; Sigma Chemical, St. Louis, MO) for 1.5 h at 23°C. After extensive washes in PBS, the sections were mounted in a commercially available mounting solution (Biomed, Foster City, CA), placed on a coverslip, and visualized using an Olympus microscope with an epifluorescence attachment using the appropriate filter. Cellular localization of Glut 1 and Glut 4 immunoreactivity was undertaken using the Simple 32 image analyzer software program (Compix, Imaging Systems, Cranberry Township, PA).

### Table 1. Gestational ages at surgery and study, fetal weight at autopsy, and fetal arterial blood oxygen saturations in the differently timed study groups (not significant differences)

<table>
<thead>
<tr>
<th>Test</th>
<th>1 h</th>
<th>2.5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at surgery, days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>118 ± 0.5(6)</td>
<td>125 ± 1.3(6)</td>
<td>120 ± 1.7(5)</td>
</tr>
<tr>
<td>Hyperinsulinemic</td>
<td>118 ± 0.5(5)</td>
<td>122 ± 2.0(6)</td>
<td>122 ± 2.0(5)</td>
</tr>
<tr>
<td>Fetal weight at autopsy, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>3.2 ± 0.2(6)</td>
<td>3.3 ± 0.2(6)</td>
<td>3.2 ± 0.2(5)</td>
</tr>
<tr>
<td>Hyperinsulinemic</td>
<td>3.3 ± 0.2(5)</td>
<td>3.4 ± 0.2(6)</td>
<td>2.9 ± 0.2(5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. in parentheses are no. of subjects.
ues, fetal arterial oxygen content, and fetal hematocrit were unchanged by experimental conditions (Table 2). The arterial oxygen saturation values and fetal arterial oxygen content were within the normal range for late-gestation fetal sheep (5, 16).

Immunolocalization of the fetal glucose transporter proteins did show Glut 1 transporter at the skeletal muscle cell sarcolemma under control conditions and at all experimental time points (Fig. 1). Glut 4 transporter was seen primarily in the intracellular space at all time points (Fig. 2). Changes in the basal distribution patterns of Glut 1 and Glut 4 under different experimental conditions were not significant.

Figure 3 shows evidence of selective hyperglycemia with normal fetal arterial plasma insulin concentrations in study group 1. The mean control period arterial plasma glucose concentration was 19.9 ± 0.7 mg/dl. The mean study period glucose concentrations were 43.2 ± 2.7 mg/dl at 1 h (n = 6), 37.8 ± 2.8 mg/dl at 2.5 h (n = 6), and 41.2 ± 2.0 mg/dl at 24 h (n = 5), all twice the mean control period glucose concentration (P < 0.05 for all). Mean fetal arterial plasma insulin concentration did not change over the study periods (Fig. 3), averaging 14.5 ± 1.5 μU/ml in the control period, 11.8 ± 2.2 μU/ml at 1 h, 12.9 ± 2.9 μU/ml at 2.5 h, and 18.0 ± 4.8 μU/ml at 24 h. Table 3 shows that the rate of fetal glucose uptake in this study was maximally increased at 1 h, remained significantly increased at 2.5 h, and had returned to the control rate by 24 h of glucose stimulation. Under these experimental conditions of fetal hyperglycemia and euinsulinemia, Glut 1 protein amount in the skeletal muscle (Fig. 4) increased fourfold at 1 h (P < 0.05) but was not significantly different than the control concentration at other study times, although a strong trend toward increase was evident at 24 h. Glut 4 protein amount was significantly increased twofold at 1 h (P < 0.05 vs. control).

Although the change in Glut 4 protein concentration did not show continued statistically significant elevation at 2.5 and 24 h, it remained above control at those time points.

Figure 5 shows evidence of selective hyperinsulinemia with maintenance of normal fetal arterial plasma glucose concentrations in study group 2. Mean basal fetal arterial plasma insulin concentration in the hyperinsulinemic-euglycemic group was 18.4 ± 2.0 μU/ml, which was significantly higher than the control concentration (P < 0.05). Mean fetal arterial plasma glucose concentrations were 36.6 ± 2.1 mg/dl (n = 6), 36.0 ± 2.0 mg/dl at 2.5 h (n = 6), and 36.5 ± 2.0 mg/dl at 24 h (n = 5), all significantly lower than the control concentration (P < 0.05 for all). Table 3 shows that the rate of fetal glucose uptake in this study was maximally increased at 1 h, returned to the control rate at 2.5 h, and had returned to the control rate by 24 h of glucose stimulation. Under these experimental conditions of fetal hyperinsulinemia and euinsulinemia, Glut 1 protein amount in the skeletal muscle (Fig. 6) increased fourfold at 1 h (P < 0.05) but was not significantly different than the control concentration at other study times, although a strong trend toward increase was evident at 24 h. Glut 4 protein amount was significantly increased twofold at 1 h (P < 0.05 vs. control). Although the change in Glut 4 protein concentration did not show continued statistically significant elevation at 2.5 and 24 h, it remained above control at those time points.

Table 2. Fetal arterial blood oxygen saturation, fetal arterial oxygen content, and fetal hematocrit in the differently timed study groups (experimental vs. control not significant)

<table>
<thead>
<tr>
<th></th>
<th>1 h</th>
<th>2.5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fetal arterial oxygen saturation, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>44.9 ± 5.3(6)</td>
<td>59.4 ± 2.5(6)</td>
<td>46.5 ± 5.0(5)</td>
</tr>
<tr>
<td>Hyperglycemic experimental</td>
<td>42.8 ± 6.5(6)</td>
<td>59.9 ± 1.6(6)</td>
<td>47.8 ± 3.7(5)</td>
</tr>
<tr>
<td>Control</td>
<td>47.6 ± 6.0(5)</td>
<td>57.8 ± 3.2(6)</td>
<td>46.9 ± 6.1(5)</td>
</tr>
<tr>
<td>Hyperinsulinemic</td>
<td>45.2 ± 6.5(5)</td>
<td>54.5 ± 3.2(6)</td>
<td>40.4 ± 6.2(5)</td>
</tr>
<tr>
<td><strong>Fetal arterial oxygen content, mM/l</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.6 ± 0.3(6)</td>
<td>3.5 ± 0.1(6)</td>
<td>2.3 ± 0.2(5)</td>
</tr>
<tr>
<td>Hyperglycemic experimental</td>
<td>2.5 ± 0.4(6)</td>
<td>3.4 ± 0.1(6)</td>
<td>2.3 ± 0.2(5)</td>
</tr>
<tr>
<td>Control</td>
<td>3.0 ± 0.3(5)</td>
<td>3.3 ± 0.2(6)</td>
<td>2.5 ± 0.4(5)</td>
</tr>
<tr>
<td>Hyperinsulinemic</td>
<td>2.7 ± 0.4(5)</td>
<td>3.2 ± 0.3(6)</td>
<td>2.1 ± 0.4(5)</td>
</tr>
<tr>
<td><strong>Fetal hematocrit, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>37.7 ± 2.5(6)</td>
<td>35.3 ± 0.6(6)</td>
<td>30.2 ± 1.2(5)</td>
</tr>
<tr>
<td>Hyperglycemic experimental</td>
<td>36.4 ± 2.3(6)</td>
<td>35.3 ± 0.7(6)</td>
<td>30.4 ± 1.1(5)</td>
</tr>
<tr>
<td>Control</td>
<td>39.3 ± 2.2(5)</td>
<td>35.8 ± 2.6(6)</td>
<td>32.8 ± 2.4(5)</td>
</tr>
<tr>
<td>Hyperinsulinemic</td>
<td>37.6 ± 1.7(5)</td>
<td>36.4 ± 2.7(6)</td>
<td>30.8 ± 2.2(5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; nos. in parentheses are no. of subjects.
μU/ml, not different from the mean basal insulin concentration measured in the hyperglycemic-euinsulinemic group. Insulin concentrations were increased two- to threefold at all study times, to 80.6 ± 8.5 μU/ml at 1 h (n = 5), 59.3 ± 7.6 μU/ml at 2.5 h (n = 6), and 53.1 ± 4.8 μU/ml at 24 h (n = 5; P < 0.05 for all). Fetal arterial plasma glucose concentration did not change significantly from the control period value of 21.9 ± 1.0 mg/dl, averaging 17.8 ± 3.9 mg/dl at 1 h, 23.0 ± 2.0 mg/dl at 2.5 h, and 19.5 ± 0.6 mg/dl at 24 h. The mean net fetal glucose uptake/utilization rate in the control period in this group was 6.4 ± 0.6 mg·min⁻¹·kg⁻¹, which was not different from the control fetal glucose uptake/utilization rate observed in the hyperglycemia study (Table 3). As shown in Table 3, glucose uptake/utilization rate was twice the control rate at all study times. Glut 1 protein amount in the skeletal muscle did not change with fetal hyperinsulinemia (Fig. 6). Glut 4 protein amount increased 1.5- to 2-fold at 1 and 2.5 h, achieving statistical significance only at 2.5 h of hyperinsulinemic stimulation (P < 0.05 vs. control; Fig. 6).

**DISCUSSION**

These results show that selective increases in fetal plasma glucose or insulin concentrations in late-gestation fetal sheep have independent in vivo effects on fetal glucose uptake/utilization rate and on Glut 1 and Glut 4 glucose transporter concentration within skeletal muscle cells. The effects of glucose and insulin are independent, time dependent, and glucose transporter-isofrom specific.

Significant changes in variables other than glucose and insulin concentrations, such as fetal arterial oxygen saturation or oxygen content, which could affect fetal glucose transporter concentration or intracellular location, were not seen in these studies. Doses for insulin infusion in this study were well below the pharmacological doses used in some previous studies (20, 41) and resulted in fetal hyperinsulinemia only two- to threefold above control concentrations. Also, the fetal sheep were transfused
with maternal blood after the control blood draws to avoid the hypoxemia and acidosis that have been reported previously with anemia and hemodilution by infusates (16). As shown in Table 2, there was no experimental effect on fetal oxygen saturation, fetal arterial oxygen content, or hematocrit with either hyperglycemia or hyperinsulinemia at any time point. In a similar hyperglycemic clamp experiment using somatostatin (11), no significant changes in fetal blood flow, fetal umbilical oxygen uptake, or fetal arterial oxygen content were measured as a result of somatostatin infusion alone.

Under basal conditions of euglycemia and euinsulinemia, immunofluorescence assays showed an expected association of Glut 1 primarily with the cell membrane/sarcolemma and Glut 4 primarily within the cells. The lack of significant and/or consistent change in these distribution patterns, particularly when correlated with the protein concentration changes, was surprising. This may represent a limitation of this type of assay, especially in light of the relatively low concentrations of Glut 4 in fetal tissue at any time or under any condition. Subcellular fractionation procedures may yield a more quantitative assessment of the translocation process.

There was a clear temporal relationship between the initial increase in the rate of fetal glucose utilization and the total amount of Glut 1 and Glut 4 protein during experimental hyperglycemia. This early increase in net glucose uptake in response to hyperglycemia probably represents the acute response to increased substrate availability, because transport of glucose is energy independent and follows its concentration gradient from the plasma into tissues and cells. The acute and immediate increase in fetal skeletal muscle Glut 1 and Glut 4 protein concentrations in response to a perturbation in circulating glucose concentrations has been reported here for the first time. This increase reflects a net balance between glucose transporter protein synthesis and degradation, processes that will require investigation in the future.

The relative decrease in skeletal muscle sarcolemma-associated Glut 1 protein concentration at 24 h of the hyperglycemic condition compared with the 1-h concentration correlated with the return of net fetal glucose utilization to control. Downregulation of the ovine fetal skeletal muscle Glut 1 transporter protein with associated decreases in glucose transport has been previously observed with more sustained hyperglycemia (9), an observation that correlates with decreases in fetal glucose utilization (6). Under acute hyperglycemic conditions, such a downregulation from control values was not evident, but rather an absence of a change or increase was noted. In contrast, Glut 4 concentrations are either unchanged or increased from baseline at 24 h. This increase in intracellular Glut 4 protein does not correlate with fetal glucose utilization. Thus it appears that while acute fetal hyperglycemia correlates with an increase in fetal glucose utilization and increased Glut 1 and Glut 4 protein, Glut 1 may be active in transporting glucose via its sarcolemmal association, whereas intracellular Glut 4, in the absence of hyperinsulinemia, may be inactive.

In contrast to the hyperglycemic condition, only Glut 4 protein concentration in the skeletal muscle was increased with hyperinsulinemia. The increase in Glut 4 protein peaked at 2.5 h and was transient, despite ongoing hyperinsulinemia and a sustained increase in glucose uptake and utilization above control. This observation suggests that sustained hyperinsulinemia fails to alter fetal skeletal muscle Glut 1 or Glut 4 concentrations. However, no long-term investigations characterizing the chronic effects of fetal hyperinsulinemia on fetal skeletal muscle glucose transporter proteins exist.

Temporal changes in glucose uptake in organs other than skeletal muscle could contribute to the overall decline in fetal glucose uptake with hyperglycemia and the sustained increase in glucose uptake with hyperinsulinemia. Thus glucose transporter expression in the skeletal muscle alone would not necessarily translate into whole fetus glucose flux. Support for this in the fetus comes from previous observations of an acute increase in the amount of Glut 1 transporter protein in fetal myocardial muscle, brain, and liver at 2–48 h of experimental hyperglycemia followed by decreased amounts of transporter protein after chronic periods of hyperglycemia (9). Additionally, experiments in adult rats have shown time dependency of insulin action on...
skeletal muscle and adipose tissues (25). Hence, whereas whole fetal glucose utilization rates provide a surrogate for fetal skeletal muscle glucose utilization, it does not replace the in vivo skeletal muscle glucose transport and uptake assessments, which are necessary in future investigations.

The results of these studies indicate that substrate- and hormone-induced changes in the expression of Glut 1 and Glut 4 in ovine fetal skeletal muscles cells play a significant role in regulating the effects of hyperglycemia and hyperinsulinemia on fetal glucose uptake. It is interesting that whereas Glut 1, as anticipated, responded only to glucose stimulation, Glut 4 transporter protein responded to both hyperglycemia and hyperinsulinemia. Both transporters are found in skeletal muscle tissues but, in the adult, are selectively responsive to either glucose or insulin. This pattern of response by late-gestation fetal glucose transporters is quite different from that seen in the adult. It also has been shown that insulin stimulation of glucose transport is developmentally regulated (22, 38) and that Glut 1 is the predominant isoform in most fetal rat tissues (39). Thus the function of Glut 4 in fetal tissues, as opposed to its insulin responsiveness and rate-limiting role in adult tissue glucose uptake (28), remains to be determined. Further studies in the fetus are indicated to more specifically determine the temporal and kinetic relationships among changes in plasma glucose and insulin concentrations, Glut 1 and Glut 4 subcellular localization, and skeletal muscle glucose uptake.

Perspectives

More definitive quantitative subcellular localization of Glut 1 and Glut 4 proteins in the sarcolemma vs. endosomal storage compartment under basal euglycemic and euinsulinemic conditions and in response to selective hyperglycemia or hyperinsulinemia will assist in determining the relationship between the skeletal muscle glucose transporters and glucose uptake. In our present study, we observed Glut 1 in the fetal skeletal muscle sarcolemma and Glut 4 in an intracellular endosomal compartment (punctate staining). This distribution pattern was described previously in the fetal rat myocardium and skeletal muscle and is similar to that of the adult (34). Whereas the hyperglycemia-induced changes in the sarcolemma-associated Glut 1 have a functional significance, the role of a change in the intracellular Glut 4 concentrations in the fetus remains elusive. Our studies of immunolocalization failed to detect a visible change in subcellular distribution of Glut 4 in response to hyperglycemia or hyperinsulinemia. However, immunolocalization may lack the sensitivity to detect translocation of fetal Glut 4 to the sarcolemma, requiring additional investigations in the future. What remains unknown is whether hyperglycemia or hyperinsulinemia can induce translocation of fetal Glut 4.

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