FETAL INTRAUTERINE GROWTH RETARDATION (IUGR) is a frequently occurring and serious complication of pregnancy. IUGR infants are at risk for numerous perinatal morbidities, including hypoglycemia in the neonatal period. This is thought to be secondary to poor hepatic glucose production. In IUGR newborns, hepatic glycogen stores are diminished, and gluconeogenic capacity is limited, thereby increasing the risk of neonatal hypoglycemia (15, 17).

Altered transport of glucose into the hepatocyte may be another factor contributing to abnormal hepatic glucose metabolism in the IUGR fetus and newborn. Glucose uptake into the cell is controlled by glucose transporters (GLUT). GLUT-2 is the major glucose transporter expressed in adult liver. In contrast, Levitsky et al. (12) have demonstrated that GLUT-1 is the predominant isoform expressed in cultured fetal hepatocytes. Because culturing cells can induce the expression of GLUT-1, it remains to be determined if this isoform is indeed the primary isoform expressed in fetal hepatocytes in vivo.

We have developed and characterized a model of IUGR in the rat (15). Transplacental supply of glucose to the IUGR fetus is impaired. During intrauterine life, the fetus is hypoglycemic and hypoinsulinemic (15). GLUT-1 expression and cellular transport of glucose by several organs, such as lung and muscle, are also decreased (21, 23). We hypothesized that GLUT-1 expression in IUGR rat fetal liver might also be decreased as well. We also wondered whether the changes in GLUT expression (decrease in GLUT-1; increase in GLUT-2) that occur during the transition between fetal and postnatal life might be altered in the IUGR rat.

To address these questions, we measured GLUT mRNA levels, with a modified method of RT-PCR (10), in IUGR fetal and postnatal rats. To determine if changes in mRNA abundance were due to alterations in transcription rate of GLUT genes, we performed nuclear run-on experiments. In addition, we measured glucose uptake and hepatic GLUT protein levels and determined the cellular localization of GLUT-1 and GLUT-2 by immunohistocytochemistry.

MATERIALS AND METHODS

Animals

Fetal experiments. We have described our surgical methods previously (15, 23). In brief, time-dated, Spraque-Dawley pregnant rats were individually housed under standard conditions and allowed free access to standard rat chow and water. On day 19 of gestation (term is 21.5 days), the maternal rats were anesthetized with intraperitoneal xylazine (8 mg/kg) and ketamine (40 mg/kg), and both uterine arteries were ligated (IUGR). Control animals underwent the identical anesthetic and surgical procedure except for ligation (sham). Rats recovered within a few hours and had ad libitum access to food and water. On day 20 (F20), the mothers were killed by pentobarbital overdose, the fetuses were immediately delivered and decapitated, and the livers were quickly harvested. Litters from 10 maternal rats who underwent bilateral uterine artery ligation (IUGR) and 9 who underwent sham surgery (sham) were used.

Neonatal-juvenile experiments. Maternal rats underwent surgery on day 19 of gestation as described in Fetal experiments. The maternal rats were allowed to deliver spontaneously, and the litter size was randomly reduced to six at birth. The pups remained with their mothers until being killed at either 1, 14, or 21 days of life (j 1, j 14, or j 21, respectively). Tissues were immediately harvested, and their wet and dry weights were determined. Litters from 15 maternal rats who underwent bilateral uterine artery ligation (IUGR) and 14 controls (sham operated) were used for these studies.

Hepatocyte Isolation and Culture

The culture procedure eliminates the hematopoietic population of the fetal liver. More than 90% of the cells are typical...
hepatocytes, and the remaining 10% are fibroblast-like cells (12, 18, 19). Livers were harvested from fetal rats and placed in ice-cold Hank’s balanced salt solution (HBSS) until harvest was complete. Livers were minced and then shaken for 5 min at 37°C in HBSS containing 5 mM EDTA. Minces were shaken for another 10 min in HBSS containing 2.5 mg/ml collagenase, 0.1 mg/ml DNase, and 5 mM CaCl2 at 37°C. Supernatant was removed, and the digestion process was repeated. The cell suspension was filtered through a 70-µm nylon mesh and centrifuged at 20 g for 1 min. The pellet was resuspended in 5 ml minimal essential medium (MEM) and centrifuged at 20 g for 1 min. The final pellet was resuspended and plated in DMEM supplemented with 5% fetal bovine serum, 1 mM proline, 0.2 mM serine, 0.2 mM L-aspartic acid, 2 mM glutamic acid, 1 mM pyruvate, 1 µg/ml hydrocortisone, and antibiotic-antimycotic solution. The final glucose concentration was 4.5 mM. Cell viability was determined by trypan blue exclusion. Cells were plated on untreated six-well culture plates until confluent (24–48 h). Medium was changed three times per day so that glucose concentration did not change over time. Uptake experiments were performed once cells were confluent.

Glucose Uptake

2-Deoxyglucose (2-DG) uptake studies were performed in hepatocytes of day 20 fetal rats. The use of 2-DG to measure the rate of glucose uptake is preferred to the use of 3-O-methylglucose. Zero-trans kinetics can be more easily determined because 2-DG is trapped in the cell after transport. Hepatocytes were washed with 10 ml of 37°C PBS. 2-DG uptake was initiated by adding 2-[3H]DG (0.25 mM) to the incubation media (incubation media: 1,900 µl PBS, 100 µl 2-[3H]DG (1 µCi/ml)). Uptake of 2-DG was measured at 5, 10, 20, and 30 s, and at 30 min. Uptake was stopped by the addition of ice-cold PBS. Aliquots were used for determination of radioactivity and for determination of DNA (20) and RNA. Non-carrier-mediated uptake was determined by adding 10 µM cytochalasin B (maximal inhibitory concentration), a fungal metabolite that is a specific and competitive inhibitor of the glucose transporter, to the incubation media. The final concentration of ethanol in the solution was <1%. Non-specific binding of glucose was determined by adding 1,000-fold excess of unlabeled 2-DG to the incubation media. Non-specific binding and non-carrier-mediated uptake was subtracted to determine specific uptake. Uptake rates were calculated per microgram of DNA or milligram of protein.

Western Blot Analysis

Membrane proteins for Western blot analysis were prepared from liver, as described previously (25). The tissues were washed, placed in ice-cold buffer (in mM: 250 sucrose, 2 EDTA, 10 Tris HCl, 1.5 phenylmethylsulfonyl fluoride, pH 7.4), and homogenized. The homogenates were centrifuged at 13,500 rpm for 20 min at 4°C. The supernatant was collected, and an equal amount of 4 M KCl (final concn 0.8 M) was added for 30 min; this mixture was centrifuged at 200,000 rpm for 90 min at 4°C. The pellet was resuspended in cold isolation buffer. Protein was determined by the method of Lowry et al. (13).

Fifty micrograms of liver membrane proteins were loaded in duplicate onto a discontinuous 12% polyacrylamide gel and size-fractionated with a Protean IIxi slab cell (Bio-Rad, Richmond, CA). The separated proteins were electrophoretically transferred to Bio-Rad polyvinylidifluoride membranes with a Trans-Blot electrophoretic transfer cell (Bio-Rad). One membrane half, representing one-half of the gel, was stained with Coomassie R-250, and the other membrane half was used for chemiluminescence detection (Amersham) of GLUT-1 or GLUT-2 with a double-antibody system. The primary antibody (East Acres Biologicals, Southbridge, MA) was diluted 1:10,000 (for GLUT-1) or 1:2,000 (for GLUT-2). The second antibody was diluted 1:3,000 before use. Resulting signals were quantitated by densitometry. Sample loading corrections were made on the basis of densitometry data from the Coomassie-stained membrane. We used protein extracted from placenta as a positive control for GLUT-1, and protein from adult rat liver was used as a positive control for GLUT-2.

Immunohistocytochemistry

Fresh liver tissue blocks were fixed in 10% Formalin and then embedded in paraffin. Sections were deparaffinized in Hemo-De (Fisher) and then washed in ethanol. Endogenous peroxidases were blocked by incubating in 1% H2O2 in absolute methanol for 30 min. Sections were hydrated through descending grades of ethanol and rinsed in H2O and PBS. The slides were blocked by incubating in 1.5% normal goat serum and then incubated with the primary antibodies GLUT-1 (1:2,000) or GLUT-2 (1:2,000). After incubation, the slides were washed in PBS, and then sections were incubated with the secondary antibody, biotinylated anti-rabbit IgG. This incubation was followed by washing in PBS. The slides were then incubated with the avidin and biotinylated peroxidase complex (VECTOR, Burlingame, CA), washed in PBS, and incubated in 0.05% 3,3’-diaminobenzidine and 0.01% H2O2 in PBS to visualize the bound anti-GLUT antibodies. After the appearance of brown reaction product, slides were washed, counterstained with hematoxylin, dehydrated in graded alcohols, cleared in Hemo-De, and mounted. As immunohistochemical controls, sections were incubated without the primary antibody or with preimmune serum. To further check the specificity of the staining, sections were incubated with the anti-GLUT antibody in the presence of excess GLUT peptide (5 µg/ml).

RNA Isolation

Total RNA was extracted from tissues by the method of Chomczynski and Sacchi (3) and quantitated with ultraviolet absorbance at 260 nm. Bovine retinal RNA was prepared in a similar manner.

RT-PCR

These methods have been described in detail previously (10). DNA was synthesized with random hexamers and SUPERSCRIPT reverse transcriptase (GIBCO BRL, Gaithersburg MD) from 1.0 µg of hepatic RNA spiked with 0.02 µg of bovine retinal RNA (internal standard). After incubation at 37°C for 1 h, 0.75 N NaOH was added to the reaction mixture, which was then placed in a 95°C water bath for 5 min to hydrolyze any remaining RNA. The resulting cDNA underwent an ethanol precipitation with 20 µg of glycogen and was resuspended in water and stored at −20°C until use.

Amplification primers are listed in Table 1. Amplification of each GLUT isoform required a different ratio of rhodopsin and GLUT primers. The concentration of rhodopsin and GLUT primers was identical in a given reaction for one GLUT isoform. Reactions were performed in a DNA thermal cycler (Perkin-Elmer, model no. 480, Norwalk, CT). The PCR products were separated on a nondenaturing 5% acrylamide gel,
and the radioactivity incorporated into each specific fragment was determined with a phosphorimager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). To determine the specificity of the primers, the amplified products were sequenced and confirmed. The abundance of each specific GLUT transcript was quantified relative to that of the control rhodopsin band from the same reaction, which was assigned an arbitrary level of unity.

Run-on Transcription Assays

Frozen tissue was homogenized in 2 ml of homogenization buffer (20 mM tricine, pH 7.4, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 0.3 M sucrose, 0.5 mM dithiothreitol) with 20 strokes of a Dounce homogenizer. Homogenates were centrifuged at 1,000 rpm for 2°C. Nuclei were resuspended in suspension buffer (40% glycerol, 50 mM Tris·HCl, pH 8.3, 5 mM MgCl$_2$, and 0.1 mM EDTA) with 20 strokes of a Dounce mesh. The filtrate was washed and centrifuged for 5 min at 2°C. The nuclear pellet was resuspended in 2 ml of homogenization buffer and centrifuged 5 min at 4°C. The pellet was resuspended in 2 ml of homogenization buffer and centrifuged 5 min at 1,000 rpm at 2°C. The nucleic pellet was resuspended in 2 ml of homogenization buffer and centrifuged 5 min at 1,000 rpm at 4°C. The pellet was resuspended in 2 ml of homogenization buffer and centrifuged 5 min at 1,000 rpm at 2°C. Nuclei were resuspended in suspension buffer (40% glycerol, 50 mM Tris·HCl, pH 8.3, 5 mM MgCl$_2$, and 0.1 mM EDTA).

Run-on assays were then performed with the method of Cornelius et al. (4, 5).

To determine the apparent specificity of the primers, the amplified products were sequenced and confirmed. The abundance of each specific GLUT transcript was quantified relative to that of the control rhodopsin band from the same reaction, which was assigned an arbitrary level of unity.

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Statistical analyses were performed with ANOVA (Fisher’s protected least significance difference) and Student’s unpaired t-test (29). These studies were approved by the Animal Care Committee of Children’s Memorial Institute for Education and Research.

RESULTS

Animals

Body weight and wet and dry carcass weights were significantly reduced in IUGR animals compared with controls at all ages. However, neither wet nor dry liver weights were significantly reduced in IUGR animals until 3 wk after birth when liver weight was decreased by 30% compared with controls (P < 0.05) (Table 2).

Table 2. Fetal and neonatal carcass and liver weights of control and IUGR rats

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Carcass, g</th>
<th>Liver, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>IUGR</td>
</tr>
<tr>
<td>F20</td>
<td>1.63 ± 0.08</td>
<td>1.26 ± 0.05*</td>
</tr>
<tr>
<td>J4</td>
<td>4.97 ± 0.18</td>
<td>3.25 ± 0.25*</td>
</tr>
<tr>
<td>J14</td>
<td>18.70 ± 1.35</td>
<td>14.47 ± 1.84*</td>
</tr>
<tr>
<td>J21</td>
<td>32.09 ± 1.24</td>
<td>25.43 ± 3.00*</td>
</tr>
</tbody>
</table>

Values are means ± SE. IUGR, intrauterine growth retardation. F20, fetal day 20; J4, J14, and J21, neonatal (juvenile) days 4, 14, and 21, respectively. *P < 0.05 vs. control for each day.

Fetal Hepatocytes

Glucose uptake. Glucose uptake was linear between 5 and 30 s (y = 0.058x + 1.419, r$_2$ = 0.997 for IUGR; and y = 0.39x + 0.834, r$_2$ = 0.998 for controls) and was maximal at 30 min. Uptake of 2-DG by IUGR fetal hepatocytes was greater than controls at all time points (P < 0.05) (data expressed in nmol/mg protein): at 5 s, 1.71 ± 0.22 for IUGR vs. 1.01 ± 0.12 for controls; at 10 s, 2.02 ± 0.35 for IUGR vs. 1.25 ± 0.15 for controls; at 20 s, 2.53 ± 0.43 for IUGR vs. 1.60 ± 0.15 for controls; at 30 s, 3.18 ± 0.40 for IUGR vs. 2.0 ± 0.29 for controls; and at 30 min, 48.18 ± 7.15 for IUGR vs. 30.64 ± 5.39 for controls. The addition of cytochalasin-B to the incubation media inhibited glucose uptake in both IUGR and control hepatocytes by ~90%.

GLUT protein and mRNA. Abundance of GLUT-1 protein and mRNA increased 1.5-fold during the 4-h culture period in both IUGR and control fetal hepatocytes (P < 0.05). In contrast, GLUT-2 protein and mRNA levels did not change during culture. These results are similar to those observed by Levitsky et al. (12).

Liver

GLUT protein. The photomicrograph in Fig. 1 shows the distribution of GLUT-1 protein in control (Fig. 1A) and IUGR (Fig. 1B) fetal liver on day 20 of gestation. GLUT-1 protein was localized to hepatocytes and to erythropoietic elements in fetal rat liver. GLUT-1 immunostaining was increased in hepatocytes and erythropoietic elements in IUGR compared with control fetal liver. After birth, GLUT-1 immunostaining declined dramatically in both IUGR and control animals, and by day 4, GLUT-1 staining was undetectable. In liver sections treated with either preimmune serum or GLUT-1 antibody preabsorbed with GLUT-1 peptide, there was no significant immunoreactivity, a finding that demonstrates the staining was specific for GLUT-1 (Fig. 1C).

In contrast to the abundant localization of GLUT-1 in the fetal liver, GLUT-2 was only expressed in limited quantities in hepatocytes. The photomicrographs show the distribution of GLUT-2 protein in IUGR (Fig. 1D) and control (Fig. 1E) fetuses at 20 days of gestation. GLUT-2 immunoreactivity is decreased in IUGR fetal liver. After birth, GLUT-2 immunoreactivity increased substantially with age in both IUGR and control liver; however, no differences in GLUT-2 immunoreactivity were observed between IUGR and control animals (data not shown). GLUT-2 staining was not observed in tissue...
sections treated with preimmune serum or GLUT-2 antibody preabsorbed to GLUT-2 peptide, demonstrating the specificity of GLUT-2 immunoreactivity (Fig. 1).

Western blot analysis was done to quantitate the changes observed in the immunohistochemistry experiments. Levels of GLUT-1 protein were approximately twofold ($P < 0.05$) greater in IUGR F20 fetal liver compared with controls (Fig. 2). After birth (J 1 and J 4), GLUT-1 levels remained elevated in IUGR liver compared with controls (Fig. 2); however, the abundance of GLUT-1 protein in postnatal animals was one-third that of fetal levels ($P < 0.05$). By day 14 of life, no GLUT-1 protein could be detected in IUGR or controls by Western blot analysis.

GLUT-2 protein levels in IUGR fetal liver were approximately 50% ($P < 0.05$) of control values (Fig. 3). However, after birth, the difference between IUGR and control GLUT-2 abundance decreased, and by day 4 of life, GLUT-2 protein levels in IUGR liver were not significantly different from controls. In both IUGR and control liver, the abundance of GLUT-2 increased dramatically during the newborn period, and levels were eightfold higher in J 4 newborns compared with fetuses (Fig. 3).

We were unable to detect GLUT-3 protein in the liver. This may be due to the use of a mouse GLUT-3 antibody, which may have less affinity for rat GLUT-3. However, very concentrated amounts of anti-GLUT-3 were used in our experiments, and this antibody has detected small amounts of GLUT-3 protein in fetal rat brain. Whereas GLUT-3 mRNA was detected in small quantities, the absence of GLUT-3 protein suggests that this transporter does not play an important role in glucose metabolism in the fetal and neonatal liver.

GLUT mRNA. We quantitated relative GLUT mRNA levels in IUGR and control liver of F20 fetuses and J 1, J 4, J 14, and J 21 juvenile rat pups with our modified RT-PCR method (10).

Hepatic GLUT-1 mRNA levels were three- to fourfold greater in IUGR compared with controls at fetal F20 and juvenile J 1 ($P < 0.05$) (Fig. 4). GLUT1 mRNA levels decreased dramatically with age in both IUGR and control pups and by day 4 of life were diminished by a factor of 7 compared with fetal values (Fig. 4).

GLUT-2 mRNA abundance was significantly less in IUGR F20 fetal and J 1 newborn rats compared with controls ($P < 0.05$) (Fig. 5). However, as was the case for GLUT-2 protein, by 14 days of age this difference disappeared, and GLUT-2 mRNA levels were similar in IUGR and control juvenile rat pups (Fig. 5). In contrast to the decline in GLUT-1 mRNA levels, abundance of GLUT-2 mRNA (Fig. 5) increased dramatically with age and by 21 days of life levels were sevenfold greater than in F20 IUGR and control fetal rats ($P < 0.05$).

GLUT-3 mRNA levels were three times greater in F20 IUGR fetal rats compared with controls ($P < 0.05$).
GLUT-3 abundance remained elevated in IUGR liver after birth. By 14 days of age, GLUT-3 levels significantly declined and were one-tenth those of the fetus in both IUGR and control pups \((P, 0.05)\). At 21 days, GLUT-3 mRNA was not detectable.

GLUT-5, a fructose transporter, was not found in fetal liver but was expressed in juvenile liver. Levels were elevated in IUGR animals \((P < 0.05)\) and increased with age in both groups.

To determine whether the changes in GLUT-1 and GLUT-2 mRNA levels were due to an altered rate of synthesis, we measured the transcription rates of these
The densitometric signal for each transcript was normalized to the signal generated on hybridization of in vitro-transcribed RNA to β-actin and lactate dehydrogenase 42 cDNA. Surprisingly, despite the marked differences in GLUT mRNA levels between IUGR and control liver, the rate of transcription of GLUT-1 and GLUT-2 did not differ between IUGR and control at any age (Fig. 6).

Whereas IUGR had no effect on the transcription rate of GLUT-1 and GLUT-2, transcription of these two genes did appear to be developmentally regulated. The rate of GLUT-1 transcription dramatically declined after birth in control and IUGR liver and by day 21 of life was ~20% of fetal values (P < 0.01). In contrast, transcription of GLUT-2 increased with age. By day 21 of life, there was a fourfold increase in transcription rate over fetal values (P < 0.01) (Fig. 6).

**DISCUSSION**

In this study, we determined the localization of GLUT-1 and GLUT-2 in situ in the fetal liver and investigated the effects of IUGR on glucose uptake and hepatic GLUT gene expression. Our in situ immunohistochemistry experiments confirm earlier in vitro studies (12) demonstrating that GLUT-1 protein is abundantly expressed in hepatocytes of F20 gestation fetal liver. GLUT-2 is also expressed in hepatocytes of fetal liver, and there does not appear to be any difference in localization between GLUT-1 and GLUT-2, as both isoforms are distributed throughout the liver. GLUT-1 is expressed in erythropoietic elements that make up <15% of the F20 fetal liver. Although abundance of GLUT-1 and GLUT-2 protein is altered in liver of IUGR fetuses and newborns, localization of the two isoforms is not affected.

Our studies demonstrate that the metabolic and physiological factors that cause IUGR also alter glucose transport in fetal liver. We found that hepatic GLUT-1 protein and mRNA levels are higher in IUGR compared with control fetal rats, whereas GLUT-2 protein and mRNA content are diminished in IUGR fetuses.

Glucose uptake by IUGR hepatocytes is increased but to a lesser degree than the observed increase in GLUT-1 protein (50% for glucose uptake vs 100% for GLUT-1 protein). At the low levels of glucose used in our experiments (0.25 mM), glucose uptake is largely facilitated by GLUT-1, and there is probably little contribution to uptake by GLUT-2. The discrepancy between glucose uptake and GLUT-1 protein levels indicates that a posttranslational process results in less uptake than would be predicted from the increase in synthesis of GLUT-1 in IUGR hepatocytes. This could be explained by a change in translocation of GLUT-1 or a change in intrinsic activity of the transporter. It is unlikely that translocation of GLUT-1 is responsible for the discordance in uptake and protein levels in IUGR hepatocytes, because our immunohistochemistry studies and studies done by Levitsky et al. (12) demonstrate that GLUT-1 resides on the plasma membrane in hepatocytes. This suggests that a decrease in GLUT-1 activity is likely to be responsible for the difference in uptake and protein levels. Several mechanisms, such as glycosylation and phosphorylation, can affect intrinsic activity of glucose transporters (7, 9, 11). Our Western blot analyses did not detect additional bands or a change in molecular weight of GLUT-1 protein in IUGR compared with control hepatocytes, thus making it less likely that glycosylation of the transporter is altered in IUGR animals. However,
additional experiments need to be done to rule this out and to determine the exact mechanism of posttranslational modifications of GLUT-1 that appear to be occurring in IUGR liver.

The liver of the IUGR fetus may have an increased need for glucose for a variety of reasons. Most are related to the increased anaerobic metabolism caused by the intracellular conditions responsible for IUGR. Oxidative phosphorylation is decreased in IUGR fetuses (16), a state that must be compensated by increased glycolysis. IUGR is also associated with a decreased hepatic ATP-to-ADP ratio and profoundly altered cytosolic and mitochondrial redox states (16). Hepatic cellular energy and redox states are uncoupled, resulting in less ATP generated per unit of glucose. These alterations further increase the demand of the liver for glucose.

Several mechanisms may underlie the differences in hepatic GLUT-1 expression between IUGR and control fetal rats. The increased GLUT-1 expression in IUGR fetal liver may be a response to the low glucose levels in this model of fetal IUGR (15). Transplacental provision of glucose is impaired, and GLUT-1 may be upregulated to compensate for this impairment. We have demonstrated that low levels of glucose in vitro in other fetal tissues, such as lung and muscle, are associated with increased rates of glucose uptake, as well as increased levels of GLUT-1 protein and mRNA (22). Glucose availability has been shown to have an effect on GLUT expression in adult liver. GLUT-1 in adult rats is normally localized to a single ring of hepatocytes surrounding the central vein, and transporter levels in these cells increase fourfold with diminished glucose availability (27).

The role of GLUT-2 in glucose metabolism in fetal rat liver is not known. Not only are GLUT-2 levels low in fetal liver, GLUT-2 has a low affinity for glucose. However, studies done by Levitsky et al. (12) do demonstrate that GLUT-2 contributes to glucose uptake by isolated fetal hepatocytes, under conditions of higher glucose concentrations. Thus, in IUGR fetuses where glucose concentrations are low, glucose uptake into the hepatocyte is predominantly facilitated by GLUT-1.

The factors responsible for the decrease in GLUT-2 mRNA and protein levels in liver of IUGR fetuses are not clear. Whereas it has been shown that high levels of glucose decrease GLUT-2 protein in isolated fetal hepatocytes (30), the effect of limited glucose availability on the expression of GLUT-2 in fetal liver is unknown. In adult liver, limited glucose and insulin availability (induced by starvation) appears to have no effect on GLUT-2 protein (2, 8, 26, 28). If the response to limited glucose is similar in the IUGR fetal liver, then other factors such as hypoxia and acidosis may play a more important role than glucose and insulin in downregulating GLUT-2 in the IUGR fetus.

After birth, GLUT-1 protein expression in hepatocytes rapidly decreases, so that in the adult, GLUT-1 is expressed in only very small quantities in perivenous hepatocytes (27). The rise in expression of a bidirectional glucose transporter, GLUT-2, is necessary, because after birth the liver becomes the primary glucose producing organ. This isoform plays a much larger role than GLUT-1 in modulating hepatic glucose homeostasis after birth. In the IUGR newborn, limited GLUT-2 production may contribute to abnormal glucose homeostasis after birth. We found that the abundance of GLUT-2 mRNA and protein remains decreased in IUGR compared with control liver immediately after birth (1).

IUGR newborns are often transiently hypoglycemic, and this is thought to be because of diminished glycogen stores and limited gluconeogenic capability (2). Decreased GLUT-2 levels in the IUGR neonate may also contribute to neonatal hypoglycemia by limiting the efflux of glucose from the hepatocyte. Diminished GLUT-2 levels may be an adaptation by the IUGR liver to keep glucose for its own metabolic needs.

Usually the return to normal from an abnormal metabolic status is accompanied by a return of mRNA levels to normal values (6, 8). However, in IUGR animals just after birth (1 day), the recovery of various metabolic variables (oxygen status, acid-base status, glucose, and insulin levels) to normal levels is not accompanied by the recovery of GLUT-1 and GLUT-2 mRNA and protein levels to normal values. These abnormal levels of mRNA and protein are transitory; after a delay of several days, the levels return to the normal values for each GLUT, i.e., near zero for GLUT-1 and higher levels for GLUT-2. Thus, in the fetal liver, IUGR conditions cause a lag in the compensatory mechanisms that return GLUT-1 and GLUT-2 levels to normal. This delay in decrease of GLUT-1 and increase of GLUT-2 represents another consequence of IUGR that extends into the postnatal period. Other such consequences include a delay in induction of hepatic phosphoenolpyruvate carboxykinase and poor postnatal growth (2).

Liver mass was similar in IUGR animals and controls until day 21 of life when liver weight was significantly diminished in IUGR pups. A reduction in liver weight in growth-retarded juvenile animals has also been found in previous studies (2). Hepatic protein and DNA levels were lower in IUGR liver, indicating that the decrease in liver mass is due to a decrease in cell size as well as in cell number (data not shown). Interestingly, although glycogen stores are lower in IUGR newborn pups compared with controls, by 3 wk of age glycogen stores are equivalent between the two groups (2). It is possible that there is insufficient energy for both hepatic growth and metabolic processes in the growing IUGR animal, although this remains to be determined.

Although GLUT-1 and GLUT-2 mRNA levels were altered in IUGR liver, the transcription rate of these genes remained unchanged compared with that of controls. We can speculate from these data that IUGR induces changes in mRNA stability rather than in the transcription process. A number of investigators have shown that GLUT-1 mRNA stability is enhanced when cellular energy stores (ATP) are depleted by various factors (1, 14, 24). In the IUGR model, glucose depriv-
tion and inhibition of oxidative phosphorylation occur (16, 22), and these changes result in lower ATP levels. If GLUT-1 mRNA stability is somehow enhanced under these conditions, more transporters could be produced and glucose uptake and subsequent ATP production could help compensate for depleted energy stores in the cell. No such parallel studies have been done examining GLUT-2 mRNA stability under various metabolic conditions. However, our data suggest that a decrease in GLUT-2 mRNA stability may be another mechanism regulating GLUT-2 expression.

In conclusion, our studies demonstrate that IUGR results in a remarkable alteration of hepatic GLUT-1 and GLUT-2 expression. IUGR also affects the normal sequence of events in neonatal and postnatal GLUT expression. These alterations in GLUT-1 and GLUT-2 may contribute to the abnormal glucose homeostasis observed in the IUGR newborn.

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