Myocardial gene expression of glucose transporter 1 and glucose transporter 4 in response to uteroplacental insufficiency in the rat

A E Tsirka, E M Gruetzmacher, D E Kelley, V H Ritov, S U Devaskar and R H Lane

Department of Pediatrics, Division of Neonatology and Developmental Biology, Magee–Women's Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, USA

1Department of Internal Medicine, Division of Endocrinology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213, USA

2Department of Pediatrics, Division of Neonatology and Developmental Biology, UCLA School of Medicine, Los Angeles, California 90095, USA

(Requests for offprints should be addressed to R H Lane, UCLA School of Medicine, Department of Pediatrics, Mattel Children's Hospital at UCLA, Division of Neonatology and Developmental Biology, B2–375 MDCC, 10833 Le Conte Avenue, Los Angeles, California 90095, USA; Email: rlane@mednet.ucla.edu)

Abstract

Uteroplacental insufficiency causes intrauterine growth retardation (IUGR) and subsequent low birth weight, which predisposes the affected newborn towards adult Syndrome X. Individuals with Syndrome X suffer increased morbidity from adult ischemic heart disease. Myocardial ischemia initiates a defensive increase in cardiac glucose metabolism, and individuals with Syndrome X demonstrate reduced insulin sensitivity and reduced glucose uptake. Glucose transporters GLUT1 and GLUT4 facilitate glucose uptake across cardiac plasma membranes, and hexokinase II (HKII) is the predominant hexokinase isoform in adult cardiac tissue. We therefore hypothesized that GLUT1, GLUT4 and HKII gene expression would be reduced in heart muscle of growth-retarded rats, and that reduced gene expression would result in reduced myocardial glucose uptake. To prove this hypothesis, we measured cardiac GLUT1 and GLUT4 mRNA and protein in control IUGR rat hearts at day 21 and at day 120 of life. HKII mRNA quantification and 2-deoxyglucose-uptake studies were performed in day-120 control and IUGR cardiac muscle. Both GLUT1 and GLUT4 mRNA and protein were significantly reduced at day 21 and at day 120 of life in IUGR hearts. HKII mRNA was also reduced at day 120. Similarly, both basal and insulin-stimulated glucose uptake were significantly reduced in day-120 IUGR cardiac muscle. We conclude that adult rats showing IUGR, as a result of uteroplacental insufficiency express significantly less cardiac GLUT1 and GLUT4 mRNA and protein than control animals (which underwent sham operations), and that this decrease in gene expression occurs in parallel with reduced myocardial glucose uptake. We speculate that this reduced GLUT gene expression and glucose uptake contribute towards mortality from ischemic heart disease seen in adults born with IUGR.


Introduction

Low birth weight or intrauterine growth retardation (IUGR) predisposes affected newborns towards long-term morbidity from many conditions, including ischemic heart disease (Barker et al. 1989, Fall et al. 1995, Barker 1997, 1999). Adults with IUGR at risk for ischemic heart disease are often characterized by dyslipidemia, hypertension, and non-insulin-dependent diabetes (Barker et al. 1993, Valez et al. 1994, Taittonen et al. 1996, Yarbrough et al. 1998). This constellation of findings constitutes an entity called Syndrome X. Individuals with Syndrome X demonstrate reduced insulin-stimulated glucose uptake by the myocardium (Botker et al. 1997a,b). However, the molecular basis for myocardial insulin resistance has not yet been identified.

Glucose transport across the cell membrane is a significant component of glucose homeostasis and a rate-limiting step for glucose entry into striated muscle (Goodman et al. 1974, Goodwin et al. 1998). Cellular glucose uptake is facilitated by the glucose transporters (GLUTs), a family of 5 structurally similar proteins (Devaskar & Mueckler 1992, Bell et al. 1993). The predominant striated muscle glucose transporters are GLUT1 and GLUT4. Heart GLUT1 gene expression peaks in the fetus and reaches a nadir in the adult (Santalucia et al. 1992). GLUT1 is responsible for basal, and to a lesser extent, for insulin-stimulated glucose uptake by the myocardium (Fischer et al. 1997). In contrast, gene expression of GLUT4 peaks in the adult, and GLUT4 is a significant determinant of insulin-stimulated cardiac glucose uptake (Santalucia et al. 1992, Zorzano et al. 1997). Glucose phosphorylation also
significantly contributes to myocardial glucose uptake, and hexokinase II (HKII) is the predominant hexokinase isoform in mature cardiac tissue (Postic et al. 1994, Osawa et al. 1995).

We hypothesized that GLUT1 and GLUT4 gene expression would be reduced in a well-characterized rat model of uteroplacental insufficiency and IUGR, because individuals with Syndrome X demonstrate cardiac insulin resistance (Botker et al. 1997a,b). The rat model of uteroplacental insufficiency and IUGR used in this study is bilateral uterine artery ligation of the pregnant rat. Length of gestation and litter size do not differ between control and IUGR groups (Kollee et al. 1985). At day 120 of adult life, female rats with IUGR have caught up with sham-operated control rats; however, male rats with IUGR continue to weigh significantly less (Lane et al. 1998a). At day 120 of adult life, fetal and neonatal rats in this model are significantly lighter than controls (which undergo identical anesthesia and also sham surgery), and this difference persists into young adulthood (Ogata et al. 1985b, Lane et al. 1998a). The rat fetus with IUGR (like equivalent human fetuses) is characterized by hypoxia, acidosis, altered insulin-like growth factor (IGF) levels, reduced branched-chain amino acids, hypoglycemia and hypoinsulinemia. These features normalize in the perinatal period (Ogata et al. 1985a,b, Lane et al. 1998a).

We measured the myocardial gene expression of GLUT1 and GLUT4 in sham-operated controls and animals with IUGR at day 21 of life (juvenile pre-weaning animals) and at day 120 of life (adult animals) to determine whether the intrauterine environment of uteroplacental insufficiency causes alterations in gene expression that persist throughout life or alterations that appear only with advanced age. To exclude the possibility that changes in GLUT gene expression were non-specific cardiac effects or technical artifacts, we measured carnitine palmitoyltransferase I (CPTI) and mitochondrial malate dehydrogenase (MMD) gene expression at day 120. We measured the glucose uptake of isolated myocardial cells from adult animals with IUGR and control adults (day 120) in vitro, with and without stimulation by insulin, to determine if changes in adult GLUT gene expression were associated with parallel changes in glucose uptake. Glucose uptake also affects HKII mRNA levels, which correspond to HKII function (Tsao et al. 1996). We therefore quantified HKII mRNA levels at day 120.

**Materials and Methods**

**Animals**

All procedures were approved by the Magee–Womens Research Institute Institutional Animal Care and Use Committee, Pittsburgh, Pennsylvania, USA.

Timed, pregnant Sprague–Dawley rats (Taconic Farms Inc., Germantown, New York, USA) were housed in individual cages and were exposed to a ratio of 12 h light:12 h darkness. All animals were provided with a supply of standard Purina (St Louis, Missouri, USA) rat chow and allowed to feed *ad libitum*. The animals were allowed at least 2 days of acclimatization before experimental handling.

On day 19 of gestation (term=21.5 days), all animals were sedated with ketamine (40 mg/kg) and xylazine (8 mg/kg) intraperitoneally. A midline incision was made and bilateral uterine artery ligation or sham surgery (control) was performed (*n*=12). Rats recovered within 2 h, and were allowed to drink water and feed on rat chow *ad libitum*. The maternal rats were allowed to deliver spontaneously, and litters were randomly culled to 6 on day 3 of life (to control for the effects of litter size upon growth).

At day 21 of life, animals were killed after sedation with isoflurane inhalation; the hearts were harvested and frozen immediately for glycogen, RNA and protein isolation and quantification. At day 120 of life, 6 control animals and 6 animals with IUGR animals were subjected to fasting for 12 h. Blood-glucose levels were determined using an i-STAT analyzer (i-STAT Corporation, East Windsor, New Jersey, USA). The remaining animals were killed during the first hour of their 12 h light cycle, and the hearts were harvested and weighed. Tissue was then used immediately for glucose-uptake studies or frozen for RNA and protein isolation. Whole hearts were used for our studies.

**RNA extraction**

Total tissue RNA was extracted as previously described, using the acid guanidium thiocyanate–phenol–chloroform (TRIzol; Gibco BRL, Gaithersburg, MD, USA) method (Chomczynski & Saachi 1987). RNA was quantified by measuring its absorbance at 260 nm, in triplicate, with a spectrophotometer (DU640; Beckman Coulter, Fullerton, CA, USA). The ratios between RNA absorbance and RNA pattern on agarose gel electrophoresis were used to evaluate RNA quality. We used *n*=12 for day–21 tissue and *n*=12 for day–120 tissue for both control and IUGR groups, representing a minimum of four litters at each age.

**Reverse transcription/PCR (RT-PCR)**

To measure mRNA levels of GLUT1, GLUT4, HKII, CPTI and MMD, we used an RT-PCR method that utilizes bovine retinal RNA as an internal control for both reverse transcription and subsequent amplification (Lane et al. 1996). Bovine retinal RNA and the bovine rhodopsin gene were selected as internal controls because of their minimal similarity and homology to the genes expressed in rat RNA. In brief, 2·0 µg rat-heart RNA and 0·01 µg
Table 1 Sequences of each primer pair, and their location in sequences cited in GenBank

<table>
<thead>
<tr>
<th>Sequences of PCR primers</th>
<th>Sense primer (5’–3’)</th>
<th>Antisense primer (5’–3’)</th>
<th>Size of PCR product (bp)</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rGLUT1</td>
<td>TGGAGTTCGGCATAACACC</td>
<td>ACGCTCCCCACACATAATGC</td>
<td>371</td>
<td>X13979</td>
</tr>
<tr>
<td>rGLUT4</td>
<td>GGGCACTCTTCTCGTTGAG</td>
<td>ACGATGCACATTTGTTGAG</td>
<td>288</td>
<td>X14771</td>
</tr>
<tr>
<td>rHKII</td>
<td>TCTGCCAGATTGTGGACAG</td>
<td>AGTCCAGATCTGAGGCTCC</td>
<td>317</td>
<td>M68971</td>
</tr>
<tr>
<td>rCPTI</td>
<td>CCATACATGCGATCCCTG</td>
<td>GCACAGAGAGCAAGCCCTG</td>
<td>293</td>
<td>NM013200</td>
</tr>
<tr>
<td>rMMD</td>
<td>GTCCATCGGTATGCTGGAG</td>
<td>AGTCCCTGCGCTTTCTTGAC</td>
<td>250</td>
<td>X04240</td>
</tr>
<tr>
<td>Rhodopsin</td>
<td>TATTCTCTTGCTACGGGCAG</td>
<td>AGTGTTGAGATGTAGAAAC</td>
<td>180</td>
<td>M21606</td>
</tr>
</tbody>
</table>

Glucose transporter expression in IUGR myocardium · A E Tsirka and others

Bovine retinal RNA were reverse-transcribed using random hexamers and Superscript RT (Gibco BRL). cDNA was amplified by the PCR, using primers to the rat targets (GLUT1, GLUT4, HKII, CPTI, MMD), as well as bovine rhodopsin (a significant component of bovine retinal RNA) (Table 1). Each PCR was carried out in a reaction volume of 20 µl, using 0·5 U Ampli Taq Gold (Perkin Elmer, Norwalk, CT, USA). Reactions were replicated three times once the optimal PCR conditions had been determined, and the primer concentrations for each GLUT and HKII were identical across both ages and between study groups. With each set of reverse transcription and amplification, serial dilutions were run to demonstrate that both PCR products were being produced in the exponential phase of amplification. Products were separated on a 5% acrylamide gel, and radioactivity was quantified by phosphorimaging (Molecular Imager FX System; Bio-Rad Laboratories, Hercules, CA, USA).

Copper staining

Copper staining provided a rapid, reversible means of visualizing protein bands on SDS-polyacrylamide gels, and was used to standardize protein loading (Bickar & Reid 1992). In brief, gels were washed and equilibrated in double-distilled H₂O. Gels were then incubated for 5 min in the copper stain (1:10 dilution) and subsequently photographed. The photographs were subjected to densitometry using NIH IMAGE Version 1.6 software (available through the National Institutes of Health, Bethesda, MD, USA). After adequate destaining, the gels were equilibrated in a transfer buffer in preparation for Western blotting.

Western blot analysis

Whole hearts were used for protein isolation and quantification; 100 µg of day–21 (n = 8 representing control animals and animals with IUGR) and day–120 (n = 8 control animals and animals with IUGR animals) heart tissue were solubilized in lysis buffer (0·1 M NaCl, 0·01 M Tris, 0·1 M EDTA, 2 mg/l chymostatin, 2 mg/l leupeptin, 2 mg/l pepstatin, 100 mg/l phenylmethylsulfonylfluoride). Protein was isolated by centrifugation, and the content was determined by the Bio–Rad dye–binding assay; 50 µg protein was then separated by 10% SDS–PAGE gel. Molecular weight markers (Bio–Rad Laboratories) were run in an outside lane. The proteins were subsequently transferred to nitrocellulose, using the Trans-Blot Semi-Dry transfer cell (Bio–Rad Laboratories), and the filters were incubated overnight in Blotto solution (5% milk powder (w/v) in PBS, 3·2 mM Mgl₂ and 3 mM NaN₃; pH 7–4). The unwashed filters were then incubated with the respective primary antibodies (2 µg/ml protein Affinity purified rabbit anti–rat GLUT1 C–terminus antibody or 1 µg/ml rabbit anti–rat GLUT4 C–terminus peptide antibody) in a 1% Blotto solution at 4 °C for 16 h (Schroeder et al. 1997). The filters were then washed 3 times in PBS/1% Tween solution before incubation with secondary anti–rabbit antibody tagged with horseradish peroxidase (1:100 dilution). The filters were washed again. The Western blot detections were performed using chemiluminescence (ECL kit; Amersham Pharmacia Biotech, Piscataway, NJ, USA) and subsequent autoradiography. The products were quantified by densitometry using the NIH IMAGE software. Each blot was replicated four times.

Cardiac glycogen levels

Tissue glycogen was hydrolyzed according to the procedure of Passonneau & Lauderdale (1974). Tissue (10–15 mg) from day–21 and day–120 animals (n = 4 for each group representing different litters) were homogenized on ice in 1 ml 30 mM HCl. Homogenates were then incubated at 100 °C for 5 min. Each homogenate (100 µl) was then incubated with 83 µl 0·1 M sodium acetate plus 1 µl amyloglucosidase solution (10 mg/ml) (20 mM Tris–HCl, 0·2 mg/ml BSA) for 2 h at 30 °C to perform enzymatic hydrolysis of glycogen. Aliquots (10–20 µl) of hydrolysate and centrifuged muscle homogenate were mixed with 50 µl reagent cocktail (100 mM Tris buffer, 20 mM MgCl₂, 10 mM ATP, 2 mM NADP, 0·2 mM deeroxamine, 1 U/ml hexokinase and 1 U/ml glucose–6–phosphate dehydrogenase) and incubated for 10 min. This reaction mixture was added to
120 µl acetonitrile; after incubation for 5 min at room temperature, the samples were centrifuged at 13 000 g for 10 min to remove denatured protein. The supernatants were dried and resuspended in 100 µl H2O immediately prior to HPLC analysis of NADPH. The generation of NADPH was measured by HPLC separation of the deproteinized reaction mixture and UV detection of NADPH. Aliquots of deproteinized samples (20 µl) were deproteinized reaction mixture and UV detection of NADPH was measured by HPLC separation of the prior to HPLC analysis of NADPH. The generation of

Glucose-uptake studies

Glucose-uptake studies were performed on heart muscle as described by Fischer et al. (1991). Animals were killed using 100 mg (i.p.) pentobarbital per kg. Fresh heart tissue of 120-day-old animals (n=8 for controls and for animals with IUGR) was harvested and perfused retrograde with a Krebs–Henseleit buffer (KHB) containing 10 mM NaHepes, 5-5 mM glucose and 2 mM pyruvate previously equilibrated with 95% O2 and 5% CO2. Calcium-resistant cardiomyocytes were then prepared by incubating the tissue in 1:1 mg/ml collagenase, 0-05% trypsin, 0-7% BSA fraction V (fatty acid free), 15 mM 2,3-butanedione monoxime (BDM), and step-wise increases in calcium (100 µmol up to 1 mmol). Before the onset of the glucose-uptake assay, the cells were washed three times with 6 mM KCl, 1 mM Na3HPO4, 0-2 mM NaH2PO4, 1-4 mM MgSO4, 10 mM Hepes and 2% BSA (fatty acid free). A 1 ml aliquot of the cell suspension was subsequently incubated in this medium or in this medium with 10 mM insulin for 30 min. 2-Deoxy-d-glucose uptake was initiated by adding 2-deoxy-d-[^3H]glucose to the incubation medium and incubating for 30 min. This incubation time was chosen because glucose uptake is linear for up to 1 h in this preparation (Fischer et al. 1991). Uptake was stopped by washing with 100 µl of 6-8 mM phloretin, and the cells were centrifuged to separate the cells from the less-dense incubation media. The supernatant was removed and cells were then homogenized in 10% trichloroacetic acid solution. Aliquots were used for the determination of radioactivity (by scintillation counting) and protein content (Bio-Rad protein assay). Carrier-mediated uptake rates were calculated by subtracting the uptake in the presence of 400 µM phloretin from values in basal or insulin-stimulated cells and are presented as µmol glucose per g protein per min.

Data analysis

All data are expressed as means ± s.e.m. Analysis of variance was determined using ANOVA (Fisher’s protected least significance difference). The comparison of blood-glucose levels, heart weights, RNA quantity and Western blot results were performed using the Student’s t-test. The analyses of the PCR and glucose-uptake results were performed using the non-parametric Wilcoxon Matched Pair Test.

Results

Fasting glucose levels

Fasting blood-glucose levels were determined in control animals and in rats with IUGR at day 120 of life. In the rats with IUGR, glucose levels were significantly raised (222-0 ± 10-0 mg/dl; P<0.05) relative to those of control animals (126-7 ± 7-2 mg/dl).

GLUT RNA levels

The quantity of total RNA isolated from heart muscle did not differ between controls and animals with IUGR (1-44 ± 0-13 µg/mg tissue for sham-operated animals; 1-45 ± 0-08 µg/mg tissue for animals with IUGR). GLUT1 mRNA levels were 51 ± 7% (P<0.05) of control values at 21 days of life, and 56 ± 5% (P<0.05) of control values at day 120 of life (Fig. 1A). GLUT4 mRNA levels were 29 ± 7% (P<0.05) of control values at day 21 of life, and this change persisted to adulthood, showing GLUT4 mRNA levels at 55 ± 6% (P<0.05) of control values at day 120 of life (Fig. 1B). Similarly, HKII expression at day 120 of life was significantly reduced to 65 ± 6% (P<0.05) of control values (Fig. 2).

The mRNA levels of CPTI and MMD were quantified further to exclude the possibility that changes in GLUT gene expression were non-specific cardiac effects or technical artifacts. In contrast to our GLUT and HKII finding, mRNA levels of CPTI were significantly raised (160 ± 14%; P<0.05) and MMD levels were unchanged (101 ± 8%) in the day-120 hearts from animals with IUGR.

GLUT protein quantification

GLUT1 protein levels were reduced to 56 ± 6% (P<0.05) of control values on day 21 and to 61 ± 7% (P<0.05) of control values on day 120 (Fig. 1A). Similarly, cardiac
GLUT4 protein levels were reduced to 43 ± 3% (P<0.05) of control values on day 21 and to 56 ± 5% (P<0.05) of control values on day 120 of life (Fig. 1B).

**Glycogen content and heart weight**

Glycogen content did not significantly differ between control and IUGR hearts at either day 21 (0.41 ± 0.04 versus 0.43 ± 0.04 μmol/g tissue) or day 120 (0.78 ± 0.20 versus 0.61 ± 0.10 μmol/g tissue). Similarly, heart weights of day-120 animals with IUGR were not significantly different from those of control animals (3.3 ± 0.5 g/kg for the control; 4.1 ± 1.3 g/kg animal weight for IUGR). There was no significant difference in this parameter for either male or female animals.

**Glucose uptake**

Basal glucose uptake was reduced to 58 ± 16% (P<0.05) of control values (1.7 ± 0.21 versus 0.98 ± 0.12 μg/g protein/min) in hearts from fasted adult rats with IUGR at day 120 of life in basal conditions. Similarly, in response to 10 nM insulin, the glucose uptake of the cardiac muscle from rats with IUGR was also significantly reduced to 55 ± 12% (P<0.05) of control values (2.7 ± 0.33 versus 1.62 ± 0.17 μg/g protein/min). The ratios of insulin-stimulated/basal uptake control (glucose uptake in the presence of insulin/glucose uptake during basal conditions) for control animals and animals with IUGR were 1.63 and 1.65 respectively. These values for glucose uptake and insulin-stimulated/basal uptake control ratios

---

**Figure 1** (A) Quantification and representative phosphorimages (RNA) and Western blots (with associated protein copper staining) of GLUT1 in control and IUGR cardiac muscle at day 21 and at day 120 (n=12 for RNA; n=8 for protein). In each inset, the control specimen is on the left and the specimen from IUGR heart is on the right. (B) Quantification and representative phosphorimages (RNA) and Western blots (with associated protein copper staining) of GLUT4 in control and IUGR cardiac muscle at day 21 and at day 120 (n=12 for RNA; n=8 for protein). In each inset, the control specimen is on the left and the specimen from IUGR heart is on the right. GLUT and bovine internal control (rhodopsin) RT-PCR products were quantified by phosphorimage analysis (MOLECULAR ANALYST software; Bio-Rad Laboratories). GLUT protein was quantified by densitometry by using NIH IMAGE software. Results are expressed as mean percentages ± S.E.M. relative to the mean of the age-matched control. *P<0.05 vs control.
are similar to those previously reported (Eckel et al. 1983, Chen et al. 1985).

**Discussion**

The present study found reduced myocardial GLUT1 and GLUT4 mRNA and protein expression, reduced HKII mRNA expression, and a parallel decrease in myocardial glucose uptake in adult rats that have IUGR secondary to uteroplacental insufficiency. Individuals with IUGR are at high risk for Syndrome X and ischemic heart disease (Barker et al. 1993, Valez et al. 1994, Taittonen et al. 1996, Yarbrough et al. 1998). These findings are particularly significant because even though the adult heart uses fatty acids as the predominant carbon source for oxidative phosphorylation, the myocardium shifts towards glucose metabolism during ischemia by increasing the GLUT1 and GLUT4 expression (Randle & Tubbs 1979, Liedtke 1981, Barker et al. 1993, Stanley et al. 1994, Brosius et al. 1997, Goodwin et al. 1998). This shift involves both glycolysis and glucose oxidation. Glycolysis stabilizes the myocardial cell, and increased glucose oxidation raises the ATP/O₂ ratio (Opie 1990, Vanoverschelde et al. 1994, Goodwin et al. 1998). Our present set of observations leads to the speculation that reduced GLUT expression and function contributes to the increased morbidity, from ischemic heart disease, observed in low-birth-weight individuals (Barker et al. 1989, Fall et al. 1995, Barker 1997, 1999).

Reduced myocardial glucose uptake is found in individuals with Syndrome X and in some individuals with non-insulin-dependent diabetes mellitus (Ohtake et al. 1995, Botker et al. 1997a,b). Botker et al. (1997a) reported a 50% decrease in basal myocardial glucose uptake and insulin-stimulated glucose uptake that was independent of myocardial blood flow in patients with Syndrome X, thereby demonstrating no change in insulin-stimulated:basal uptake in individuals with Syndrome X compared with controls (Botker et al. 1997a). Our data mimic this study in humans because we also found no difference in the cardiac insulin-stimulated:basal uptake control ratio between control rats and rats with IUGR, consistent with the decrease in both GLUT1 and GLUT4 expression, as well as HKII gene expression, in hearts from rats with IUGR.

Despite these changes in gene expression, cardiac glycogen levels were not significantly different at either day 21 or day 120 of life. This suggests that altered glycogen metabolism is not compensating for depressed glucose uptake. Furthermore, although this study did not quantify glucose-transporter translocation, the observed decrease in glucose uptake parallels the decrease in GLUT gene expression. This suggests that the translocation machinery functions normally, and that the primary defect causing depressed glucose uptake is reduced expression of the GLUT genes and HKII.

Altered fetal and neonatal GLUT gene expression has been reported for non-cardiac tissues from growth-retarded animals exposed to uteroplacental insufficiency, though altered adult myocardial gene expression of GLUT1 and GLUT4 has not been previously reported in this or other models of IUGR (Simmons et al. 1992, Sadiz et al. 1999). However, rat models that induce IUGR by maternal malnutrition, particularly those employing isocaloric low-protein maternal diets, provide insights into the significance of our findings.

These starvation models mimic poor maternal nutrition, which, like uteroplacental insufficiency, is a common

---

**Figure 2** Quantification and representative phosphorimages (RNA) of HKII, CPTI, and MMD in control and IUGR cardiac muscle at day 120 (n=12 for RNA). In each inset, the control specimen is on the left and the specimen from IUGR heart is on the right. HKII, CPTI, MMD and bovine internal control (rhodopsin) RT-PCR products were quantified by phosphorimage analysis (MOLECULAR ANALYST software; Bio-Rad Laboratories). Results are expressed as mean percentages ± S.E.M. relative to the mean of the age-matched control. ∗P<0·05 vs control.
cause of IUGR that results in long-term morbidity and mortality (Ravelli et al. 1998). Rat pups with IUGR in low-protein starvation models demonstrate systemic hypertension during maturity (Langley-Evans et al. 1999). The heart responds to overload secondarily to conditions such as hypertension with cardiac hypertrophy. Cardiac hypertrophy in humans is associated with reduced myocardial glucose uptake secondary to altered GLUT expression (Paternostro et al. 1999). Interestingly, GLUT4-null mice and mice suffering cardiac-specific deletion of GLUT4 demonstrate cardiac hypertrophy, suggesting that GLUT4 can play a primary role in hypertrophic cardiac disease (Katz et al. 1994, Abel et al. 1999). The insult imposed on the fetal rat in both models of IUGR is severe and specific, and creates reproducible phenotypes. In contrast, the range of etiologies and pathophysiology of IUGR in humans represent a continuum, and the human life experience is confounded by both genetic and environmental variables. As a result, the different models of IUGR, as well as transgenic animals, contribute unique characteristics that allow us to piece together the complex and manifold pathological patterns seen in humans with IUGR.

In adult rats with IUGR, fasting glucose levels are significantly increased in this study. Cardiac GLUT1 and GLUT4 expression is reduced by diabetes (Kainulainnen et al. 1994). Interestingly, previous studies at day 21 and day 35 of life showed no significant difference before and after a 24 h fast in serum glucose, insulin, and glucagon between control rat pups and those with IUGR (Ogata et al. 1985b). However, GLUT1 expression and GLUT4 expression are reduced at this age. This suggests that our finding of reduced GLUT expression at both day 21 and day 120 is a result of programming that was initiated by the altered intrauterine environment.

Because cardiac tissue, by itself, may not affect whole-body glucose homeostasis, these findings also suggest that glucose metabolism may be altered in other tissues, such as skeletal muscle and adipose tissue. Cardiac muscle and skeletal muscle can exhibit divergent patterns of glucose metabolism, so similar changes in glucose metabolism cannot be presumed in other muscles, let alone other tissues (Kainulainnen et al. 1994, Nuutinen et al. 1994, Schroeder et al. 1997). Future studies should target the effects of uteroplacental insufficiency upon other tissues and compare the results with those for other models of growth retardation and human uteroplacental insufficiency.

Uteroplacental insufficiency leading to programmed changes in adult myocardial GLUT expression and function is consistent with Barker’s ‘Fetal Origins Hypothesis’ (Dennison et al. 1997, Barker 1999). This hypothesis proposes that fetal adaptation to a deprived intrauterine milieu leads to permanent changes in cellular biology and whole-body physiology. These adaptations ensure the survival of the immature animal under adverse conditions, but may be detrimental to the adult.

In summary, we found that adult rats with IUGR expressed significantly less cardiac GLUT1 and GLUT4 mRNA and protein than sham-operated control rats, and this decrease in gene expression occurred in parallel with reduced myocardial glucose uptake. We speculate that if a similar reduction occurs in humans, this could contribute to the reduced cardiac glucose uptake and increased mortality from ischemic heart disease associated with low birth weight and subsequent development of Syndrome X.

Acknowledgements

This research was supported by NICHD grants P30HD-28836–05 (RHL) and 5P30-DK46204–05 (RHL), as well as the very generous support of the Magee–Womens Hospital ‘25’ Club and The Lemieux Foundation.

References


www.endocrinology.org
A E TSIRKA and others

Glucose transporter expression in IUGR myocardium


Lane RH, Devaskar SU, Turka A & Gurtzmacsher EM 1998b Diverse effects of intracellular growth retardation (IUGR) upon mitochondrial gene expression and function in male and female d120 soleus and extensor digitalis longus (EDL) muscle. *Pediatric Research* **43** 79A.


Received 7 May 2000
Accepted 19 December 2000