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ALTERED GLUCOSE TRANSPORTER mRNA ABUNDANCE IN A RAT MODEL OF ENDOTOXIC SHOCK

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To better understand molecular mechanisms of glucose transport in shock, we studied glucose transporter isoform mRNA abundance after injection of S. enteritidis endotoxin (40mg/kg) or saline. Six to 8 hours after injection, endotoxin-treated animals compared to controls became hypoglycemic (44± 6 vs. 111± 4 mg/dl) and lactacidemic (5.9± 0.5 vs. 1.3± 0.1). At such times, tissue RNA was isolated and hybridized to Riboprobes for GLUT1 (erythrocyte), GLUT2 (liver), and GLUT4 (muscle/fat) glucose transporter isoforms and expressed as percent of control. GLUT1 mRNA abundance was increased in fat (660%, $p<.05$), soleus muscle (314%, $p<.05$), and liver (871%, $p<.001$) of endotoxin-treated rats. Soleus muscle GLUT4 mRNA levels were increased (+33%, $p<.02$), while liver GLUT2 mRNA levels were markedly decreased (-58%, $p<.01$). The overall increase in GLUT1 mRNA abundance accompanied by lowered liver GLUT2 mRNA levels may either cause or reflect profoundly altered glucose transport.

Sepsis and endotoxemia are characteristically associated with a catabolic state that can proceed from cachexia to shock and death (1). Profoundly altered carbohydrate metabolism in sepsis may elicit initial hyperglycemia with increased glucose turnover and elevated lactic acid levels, and later progress to life-threatening hypoglycemia and lactacidemia (2-4). Specific metabolic consequences of sepsis include acceleration of glycogenolysis, depression of glycogen synthesis, and enhanced peripheral glucose utilization (5-7). In endotoxin-treated animals, such elevated glucose utilization has been reported for numerous tissues (8-12) including adipose (9), skeletal muscle (10,11), and liver (8,12).

Glucose entry into cells has been shown by recent studies to be facilitated by plasma membrane glucose transporting proteins belonging to a family of 5 homologous members (13,14). These glucose transporter isoforms each have specific tissue distributions and play specific roles to differentially regulate glucose uptake into various tissues (13-15). Key roles for overall glucose disposal have been suggested for 3 of these transporters (13). The GLUT1 isoform is present in many tissues and may be responsible for basal glucose uptake. The GLUT2 isoform mediates bidirectional transport of glucose by

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hepatocytes, serves as a glucose sensor in pancreatic β-cells, and is also at least partly responsible for transepithelial movement of glucose out of absorptive cells of the small intestine and kidney and into circulation. The GLUT4 isoform is expressed only in tissues where glucose uptake is regulated by insulin i.e. adipose, skeletal muscle, and heart, and insulin acutely stimulates glucose entry by recruiting these glucose transporters from intracellular pools to the plasma membrane (16,17).

Changes in tissue glucose transporter abundance may either cause or reflect altered carbohydrate metabolism. To enhance our understanding of the pathophysiology of glucose transport in sepsis and endotoxicosis, we sought to assess glucose transporter expression in tissues known to be involved in glucose disposal. Specific probing of blotted RNA from tissues of endotoxin- or saline-treated rats was used to evaluate changes in abundance of mRNA transcripts encoding GLUT1, 2, and 4 transporter isoforms. Compared to tissues from saline-treated control rats, GLUT1 abundance was markedly elevated in liver, fat, and muscle of endotoxin-treated rats. GLUT4 mRNA abundance was modestly increased in muscle in the endotoxin-treated rats, and GLUT2 mRNA was significantly reduced in liver. Such findings indicated that glucose transporter expression in different tissues was specifically, but differentially, altered in endotoxicosis.

Materials and Methods

Adult 300-400g male Sprague-Dawley rats (Holtzman Co., Madison WI) were treated with Salmonella enteritidis endotoxin (Difco Detroit, MI) after an overnight fast. Intraperitoneal injections of endotoxin were given at a dose of 40mg/kg, a dose that was a LD_{90} at 24 hours. Six to eight hours after endotoxin-treatment, brain, liver, soleus muscle and epididymal fat pads were rapidly removed, frozen in liquid nitrogen and stored at -70 ^{o}C for later RNA extraction. The experimental treatment interval was selected such that the endotoxin-treated rats were in the decompensated phase of shock, i.e. hypoglycemia and lactacidemia.

Plasma glucose (mg/dl) and lactate (mM/L) concentrations were determined by glucose and lactate oxidase techniques (Yellow Springs, Instruments Yellow Springs, OH). Insulin levels were determined by radioimmunoassay utilizing reagents from Cambridge Diagnostics (Cambridge, Mass).

Total RNA was isolated from brain, liver, soleus muscle and epididymal fat pads by the method of Chomczynski and Sacchi(18) and separated by electrophoresis (10 μg/lane) on 1% agarose-formaldehyde gels essentially according to the technique of Fourney, et al (19). Gels were blotted onto nylon membranes and hybridized to [^{32}P]cRNA Riboprobes for the glucose transporters. GLUT1 Riboprobes were prepared from phGT-2 (1.8kb insert) in pGEM-4Z (20). GLUT4 Riboprobes were made from pSMID2 (1.5kb insert) in pBluescript (21), and GLUT2 Riboprobes were generated using pLGT-11 (0.9kb insert) in pGEM-4Z (22). Hybridization was performed for 16-20 hours at 65^{o}C in a solution of 10% dextran sulfate, 50% formamide, 5X SSPE (1X SSPE=180 mM NaCl, 10 mM $NaPO_4$, and 1 mM EDTA, pH 7.4), 5x Denharts solution (100x= 2% Ficoll, 2% bovine serum albumin, and 2% polyvinylpyrrolidone), 0.1% sodium dodecyl sulfate (SDS), and denatured, sheared salmon sperm DNA (100 μg/ml). Following hybridization, membranes were washed twice at room temperature in 2X SSC, 0.1%SDS, (1X SSC:300mM NaCl, 30mM Sodium Citrate, pH 7), twice at 70^{o}C in 2X SSC 0.1% SDS, and twice at 70^{o}C in 0.1X SSC, 0.1% SDS. Membranes were exposed to Kodak XAR-5 film at -70^{o}C with an intensifying screen (1.5 to 22 hours). The abundance of glucose transporter mRNA in total RNA samples from endotoxin-treated animals was quantitated by densitometry of autoradiographic bands using a Bio-Rad 620 Scanner (Richmond, CA). Signals were normalized for loading differences based on 28S ribosomal RNA abundance (23). Values for mean abundance of each glucose transporter isoform in endotoxin-treated animal groups were expressed as percent abundance in saline-treated control groups.

Glucose, lactate and insulin were expressed as mean± S.E.M. ANOVA was used to analyze differences in substrate and hormone concentrations. Student's t-test was used for mRNA abundance comparison. Differences were accepted as significant at $p<0.05$.

Results and Discussion

We studied abundance of mRNA transcripts for GLUT1 (in brain, soleus muscle, liver, and fat), GLUT2 (in liver), and GLUT4 (in fat and soleus muscle) in a rat model of shock from a highly lethal

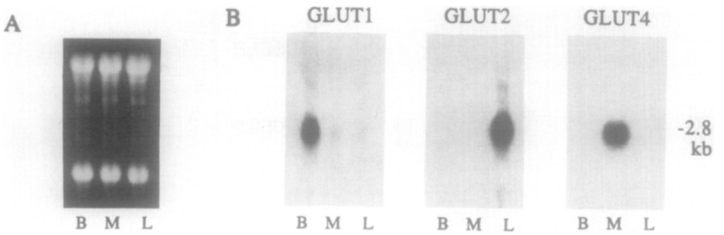

Figure 1A. **Representative ethidium bromide-stained total RNA from rat brain, soleus muscle, and liver.** Samples of total RNA extracted from brain (B), soleus muscle (M), and liver (L) of saline-treated control rats were loaded equally (10 μg/lane) and electrophoresed on 1% agarose-formaldehyde gels.

Figure 1B. **Specificity of cRNA probes for GLUT1 (brain), GLUT2 (liver), and GLUT4 (muscle) mRNA.** Northern blots of triplicate gels as in Figure 1A were each hybridized respectively with glucose transporter-specific cRNA Riboprobes for GLUT1, GLUT2, or GLUT4. Prints of resultant autoradiograms demonstrate the prevalence of GLUT1 mRNA transcripts in brain (B), GLUT2 mRNA in liver (L) and GLUT4 mRNA in soleus muscle (M).

endotoxin injection. Tissues were harvested from 5-12 control and 9-13 endotoxin-treated animals six to eight hours after the endotoxin injection. At the time the tissues were harvested, plasma glucose (mean ± S.E.M., 111±4 vs. 44±6mg/dl, $p<.001$), lactic acid (1.3±0.1 vs. 5.9±0.5mM/L, $p<0.001$), and insulin (16±2 vs. 10±2 μU/ml, $p.<.04$) concentrations revealed hypoglycemia and lactacidemia, with insulin concentrations that were slightly lowered in the endotoxin treated animals. These metabolic changes confirmed that the endotoxin-treated animals were in a state of altered glucose regulation and shock.

We sought to determine whether altered glucose utilization in endotoxic shock was associated with altered glucose transporter mRNA abundance. Samples of total RNA extracted from rat brain, soleus muscle, and liver were loaded and electrophoresed on agarose-formaldehyde gels. Ethidium-bromide staining of 28S and 18S ribosomal RNA bands demonstrated equal loading of samples (Figure 1A). Northern blots of these gels were hybridized with transporter-specific cRNA probes, and autoradiograms showed probe specificity and prevalence of GLUT1 mRNA in brain, GLUT2 mRNA in liver, and GLUT4 mRNA in muscle (Figure 1B). Autoradiograms of Northern blots showed increased GLUT1 mRNA abundance in fat, muscle, and liver in the endotoxin-treated rats when compared to controls (Figures 2A,B,C). Liver GLUT2 mRNA abundance was decreased in endotoxin-treated animals compared to saline-treated control animals (Figure 2D). GLUT1 mRNA abundance was also measured in the brain of the endotoxin-treated animal (data not shown), and no significant change was seen ($n=3$, saline and $n=4$, endotoxin-treated). GLUT1 mRNA abundance was significantly increased in fat (660%), soleus muscle (314%), and liver (871%) from endotoxin-treated animals when expressed as percent abundance of control animals (Figure 3). In soleus muscles from endotoxin-treated rats, GLUT4 mRNA abundance was modestly, but significantly, increased compared to saline-treated controls, while GLUT2 mRNA abundance was strikingly and significantly decreased in liver (-58%) (Figure 3). Such changes in glucose transporter mRNA abundance may be related to increased glucose transporter mRNA production or decreased degradation. To confirm that glucose transporter gene expression is altered in endotoxic shock, further studies are needed to define rates of transcription. It will also be necessary to verify that glucose transporter protein levels vary in consensus with the mRNA levels to demonstrate that altered glucose transport activity is linked to altered transporter expression.

Endotoxin-treatment was reported to preferentially increase glucose transport and consumption in tissues rich in mononuclear cells (8). Since endotoxin is a potent stimulant for mononuclear cells to

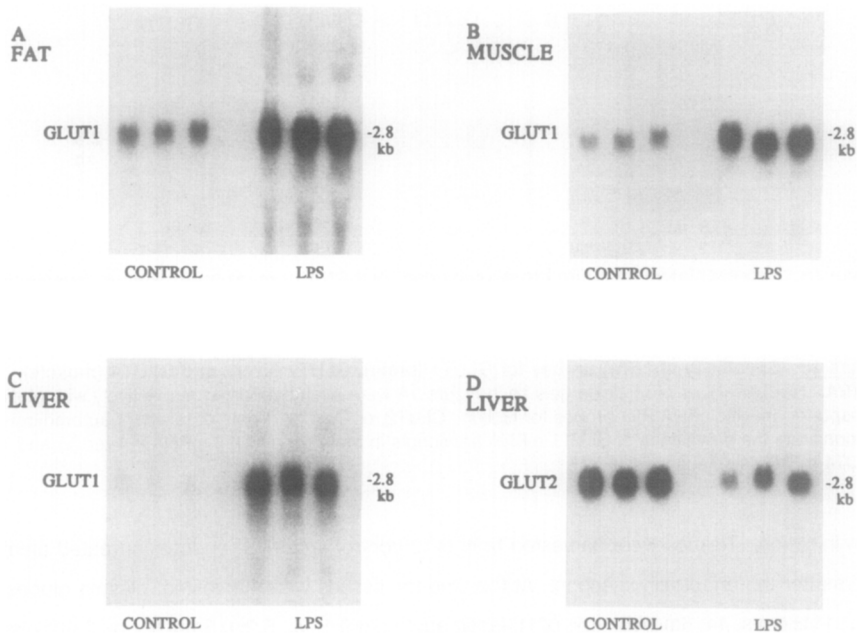

Figure 2. **Effect of endotoxin-treatment on GLUT1 and GLUT2 glucose transporter mRNA levels in fat, muscle and liver.** Adult male rats were treated with saline (control) or endotoxin (40 mg/kg, IP) as described in Materials and Methods. Total RNA was isolated from brain, liver, soleus muscle, and epididymal fat pad tissues and separated by electrophoresis on agarose gels (10μg/lane). Northern blots of these gels were hybridized to anti-sense cRNA Riboprobes specific for the glucose transporters GLUT1 and GLUT2. Representative autoradiograms of the northern blots reveal increased abundance of GLUT1 mRNA transcripts in epididymal fat (A), soleus muscle (B), and liver (C), and decreased abundance of GLUT2 mRNA transcripts in liver (D) following endotoxin-treatment.

produce many mediators including interleukin-1 (IL-1) and tumor necrosis factor (TNF), such findings suggested that IL-1 and TNF may be mediators responsible for altered glucose transport in endotoxin shock (8). Glucose transport was in fact found to be increased by IL-1 treatment of rat adipose cells (24) and fibroblasts (25). Also, TNF increased glucose transport in 3T3-L1 fibroblasts (26), and L6 muscle cells (27), and increased glucose uptake across the dog hind limb (28). The increased glucose

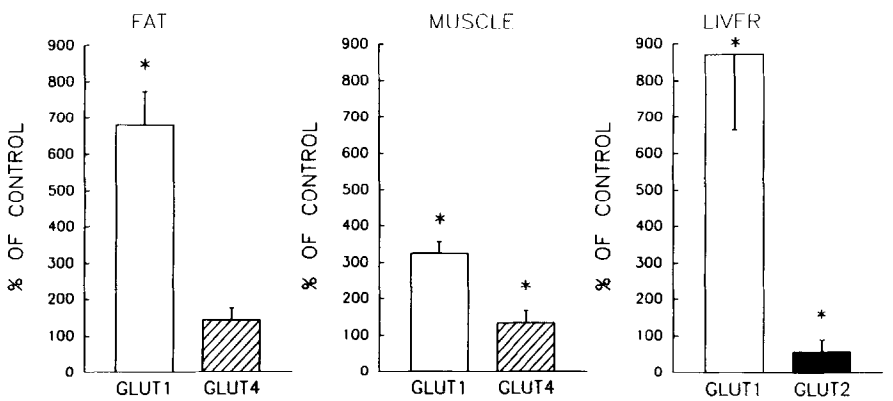

Figure 3. **Abundance of glucose transporter mRNA in fat, muscle and liver of endotoxin-treated rats.** Northern blot analyses were performed as described in Materials and Methods. Autoradiographic bands were quantitated by densitometry. Abundance of GLUT1, GLUT2, and GLUT4 mRNA in fat, muscle and liver of endotoxin-treated rats is expressed as a percent of saline-control. Data represent means ± S.E.M.

uptake across the canine hind limb likely resulted from a direct effect of TNF on the cellular glucose transport system (28). Our in vivo findings that endotoxin treatment increased GLUT1 mRNA abundance in muscle, fat, and liver are consistent with the in vitro observation of similarly increased GLUT1 mRNA abundance following TNF treatment of L6 myotube (29) and 3T3-L1 fibroblast (26) cell lines. We have extended prior observations of glucose transporter mRNA abundance changes by quantitating both GLUT1 and 4 mRNA abundance in adipose tissue and both GLUT1 and 2 mRNA abundance in liver.

In a rat model of shock, we have observed striking increases in GLUT1 mRNA abundance in muscle, fat and liver associated with profound hypoglycemia. Such changes were accompanied by significant reduction in GLUT2 mRNA abundance in liver, consistent with decreased liver glucose output associated with hypoglycemia. Further studies are required to precisely determine whether such changes in GLUT1 and GLUT2 mRNA abundance cause or reflect profoundly altered glucose metabolism in shock. Eventually, better understanding of cellular and molecular mechanisms of underlying altered glucose transport in shock should provide rationale for improved treatment strategies.

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