Liver Hyperplasia and Paradoxical Regulation of Glycogen Metabolism and Glucose-sensitive Gene Expression in GLUT2-null Hepatocytes

FURTHER EVIDENCE FOR THE EXISTENCE OF A MEMBRANE-BASED GLUCOSE RELEASE PATHWAY*

(Received for publication, September 28, 1999, and in revised form, January 11, 2000)

Rémy Burcelin†, Maria del Carmen Muñoz‡, Marie-Thérèse Guillam‡, and Bernard Thorens¶

From the §Institute of Pharmacology and Toxicology, University of Lausanne, 27, rue du Bugnon, CH-1005 Lausanne, Switzerland and ‡Department of Biochemistry and Molecular Biology, University of Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain

We investigated the impact of GLUT2 gene inactivation on the regulation of hepatic glucose metabolism during the fed to fast transition. In control and GLUT2-null mice, fasting was accompanied by a ~10-fold increase in plasma glucagon to insulin ratio, a similar activation of liver glycogen phosphorylase and inhibition of glycogen synthase and the same elevation in phosphoenolpyruvate carboxykinase and glucose-6-phosphatase mRNAs. In GLUT2-null mice, mobilization of glycogen stores was, however, strongly impaired. This was correlated with glucose-6-phosphate (G6P) levels, which remained at the fed values, indicating an important allosteric stimulation of glycogen synthase by G6P. These G6P levels were also accompanied by a paradoxical elevation of the mRNAs for l-pyruvate kinase. Re-expression of GLUT2 in liver corrected the abnormal regulation of glycogen and l-pyruvate kinase gene expression. Interestingly, GLUT2-null livers were hyperplastic, as revealed by a 40% increase in liver mass and 30% increase in liver DNA content. Together, these data indicate that in the absence of GLUT2, the G6P levels cannot decrease during a fasting period. This may be due to neosynthesized glucose entering the cytosol, being unable to diffuse into the extracellular space, and being phosphorylated back to G6P. Because hepatic glucose production is nevertheless quantitatively normal, glucose produced in the endoplasmic reticulum may also be exported out of the cell through an alternative, membrane traffic-based pathway, as previously reported (Guillam, M.-T., Burcelin, R., and Thorens, B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12317–12321). Therefore, in fasting, GLUT2 is not required for quantitative normal glucose output but is necessary to equilibrate cytosolic glucose with the extracellular space. In the absence of this equilibration, the control of hepatic glucose metabolism by G6P is dominant over that by plasma hormone concentrations.

Hepatic glucose production is an essential physiological function required to prevent hypoglycemia in the postprandial or fasted states. The last two steps in hepatic glucose release consist of the hydrolysis of glucose-6-phosphate (G6P)1 into glucose and release of glucose from the cells. Hydrolysis of G6P is catalyzed by glucose-6-phosphatase, an enzyme located in the lumen of the endoplasmic reticulum (1). Classically, the pathway for glucose release has been viewed as involving glucose diffusion back into the cytosol through an as yet uncharacterized glucose carrier and release in the extracellular space by transport through the plasma membrane glucose transporter GLUT2 (2). Recently, however, we described the existence of an alternative pathway for glucose release, which coexists in normal hepatocytes with the GLUT2-dependent pathway (3). We showed that in the absence of GLUT2, although facilitated diffusion of glucose across the hepatocyte plasma membrane was decreased by greater than 95%, the rate of hepatic glucose production was normal and at least 10-fold higher than the remaining plasma membrane glucose transport capacity. These data therefore suggested the existence of an alternate route for glucose release. Our further observations suggested that this alternative pathway relied on membrane traffic originating from the endoplasmic reticulum and reaching the plasma membrane without transiting through the Golgi complex. Even though this pathway does not seem to be limiting for glucose release, our data showed that a fraction of the glucose produced in the endoplasmic reticulum remained inside the cells. This glucose has probably returned to the cytosol where it cannot diffuse directly into the extracellular space. It may, however, enter a futile cycle by being phosphorylated to G6P and re-entering the endoplasmic reticulum for hydrolysis to glucose. Therefore, in fasted GLUT2-null liver, when the gluconeogenic flux is maximal, a paradoxical accumulation of glucose and G6P in the cytosol may occur.

Glucose and G6P are regulators of glycogen synthesis and breakdown and may therefore profoundly influence glycogen metabolism. Glucose by binding to glycogen phosphorylase increases its susceptibility to glycogen phosphorylase phosphatase and, thereby, its rate of inactivation (4). On the other hand, G6P is an allosteric activator of glycogen synthase (5). Therefore, the combined effect of high cytosolic glucose and G6P should have a positive effect on glycogen accumulation. On the other hand, a paradoxical regulation of glucose sensitive genes such as those for l-PK, fatty acid synthase and Spot 14 could also be expected because G6P, or a downstream metabolite, plays a pivotal role in the regulation of their expression (6–11).

It was the aim of this study to evaluate hepatic glucose

---

1 The abbreviations used are: G6P, glucose-6-phosphate; RIP, rat insulin promoter; kb, kilobase; PEPCK, phosphoenolpyruvate carboxykinase; Mes, 4-morpholineethanesulfonic acid; l-PK, l-pyruvate kinase.
metabolism in GLUT2 knockout mice and draw conclusions on the role of this transporter. We were interested, in particular, in the transition from the fasted to fed state when increased plasma glucagon to insulin ratio normally stimulates glycogen breakdown and decreases expression of glucose-sensitive genes. We report here that in absence of GLUT2, fasting was associated with a normal up-regulation of PEPCK and glucose-6-phosphatase mRNAs, which are strongly controlled by plasma glucagon and insulin levels (12, 13) but with an abnormal degradation of glycogen stores and paradoxical stimulation of glucose-sensitive genes. This was correlated with persistent elevated intracellular G6P levels. These data indicate that the major role of GLUT2 in the fasted state is to equilibrate de novo synthesized, cytosolic glucose with the extracellular milieu. This is required to prevent accumulation of intracellular metabolites that have a dominant effect over the signals induced by the extracellular concentrations of glucagon and insulin. These abnormal regulations of metabolism and gene expression may also cause the observed liver hyperplasia.

**MATERIALS AND METHODS**

**Animals**

GLUT2-null mice die between 2 and 3 weeks of age (14). Transgenic re-expression of either GLUT2 or GLUT1 specifically in pancreatic β cells under the control of the rat insulin promoter (RIP) allows the mice to survive and breed (3). The present studies were therefore performed with RIPGLUT1×GLUT2−/− (referred to in the text as GLUT2−/−) mice at the age of 10–14 weeks. Control mice were of the RIPGLUT1, GLUT2−/− genotype. Mice were housed with an inverted light cycle with light on from 8:00 p.m. to 8:00 a.m. They were utilized either in the fed state (2:00 p.m.) or fasted for 6 or 24 h by removing food at 8:00 a.m. and 8:00 p.m., respectively. The animals were sacrificed in the fed state or under anesthesia with ether. They were used for glucose, glycogen, and insulin measurements. For RNA extraction, animals were sacrificed under ether anesthesia and homogenized in 1 M NaOH for 1 h at 37 °C under vigorous shaking in an ice-cold solution of 0.2 M Tris-HCl, pH 7.4, and 1 M KCl. The resulting homogenate was centrifuged at 10,000 × g for 20 min at 4 °C, and the supernatant was incubated in the presence of amyloglucosidase for 2 h at 37 °C under vigorous shaking in a 0.2 M sodium acetate buffer (pH 4.5) containing 0.06% Triton X-100. The extracts were centrifuged at 10,000 × g for 20 min at 4 °C, and the supernatant was precipitated with 200 μl of a 25% polyethylene glycol 6000 solution. The precipitate was washed twice with 250 μl of 95% ethanol and resuspended in a minimum of 20 μl of water. For RNA isolation, 20 μl of the resuspended RNA was incubated in the presence of 2 μl of glycogen type III, 52 μl of 66% ice-cold ethanol solution twice for 20 min under gentle stirring. The radioactivity remaining on the Whatman paper was then counted. One unit of phosphorylase is defined as the amount of enzyme that incorporates 1 nmol of glucose from glucose-1-phosphate into glycogen per minute.

**Glycogen Synthase—**Liver glycogen synthase activity was measured as described previously (18). This assay is based upon the measurement of the incorporation of [14C]UDP-glucose into glycogen. The total and active glycogen synthase activities are determined in the presence of 6.6 or 0.11 mM G6P, respectively. Briefly, the liver extracts were prepared as for the measurements of glycogen phosphorylase. The extracts were incubated in the presence of the assay buffer containing 50 mM Tris-HCl, 2 mM EDTA, 5 mM UDP-glucose, 0.5% rabbit liver glycogen type III, and 0.06 μCi/μmol of [14C]UDP-glucose. The synthesized [14C]glycogen was then precipitated and quantified as described for the phosphorylase activity.

**Hexokinase Activities—**The liver glucokinase and hexokinase activities were assessed as described previously (19). The glucokinase (HKIV) and hexokinase (I, II, and III) enzymes transform an excess of glucose and ATP into G6P and ADP. This assay is based upon the evaluation of the transformation of G6P into 6-phosphogluconate and NADPH by glucose-6-phosphate dehydrogenase in the presence of an excess of NADP+. The hexokinase activity is the rate-limiting step, and the amount of NADPH produced is proportional to the activity of the enyme. Quantification was performed by determining the absorbance at 340 nm. The hexokinase (I, II, and III) activities were determined in the presence of 0.5 mM glucose. The total hexokinase activities including glucokinase were determined in the presence of 100 mM glucose. The difference between the measurements corresponds to the glucokinase activity alone. Briefly, the livers were homogenized in an ice-cold buffer containing 50 mM triethylammonium, pH 7.3, 10 mM KCl, 1 mM dithiothreitol, 5% glycerol, 1 mM each EDTA and EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml each peptatin and leupeptin, 0.02% sodium azide, and 0.5% Triton X-100. The extracts were centrifuged at 10,000 × g for 20 min at 4 °C, and 1 ml of the supernatant was precipitated with 200 μl of a 25% polyethylene glycol 6000 solution. The resulting solution was centrifuged at 10,000 × g for 20 min at 4 °C, and the hexokinase activities were assayed from the supernatant in the presence of 150 mM triethylammonium, 20 mM MgCl2, 100 mM KCl, 1 mM dithiothreitol, 1% bovine serum albumin, 10 mM ATP, 1 mM NADP, and 1.5 units of glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (Roche Molecular Biochemicals). One unit of hexokinase transforms 1 nmol of glucose into G6P per minute at 37 °C.

**Analytical Techniques**

For intrahepatic glucose-6-phosphate measurements, livers were clamp frozen. After homogenization glucose-6-phosphate concentrations were determined as described previously (20). DNA was measured using bisbenzimidaol as described previously (21). Radioimmunoassays for blood detection of insulin and glucagon were performed using commercial kits (Linco, St. Louis, MO). Liver and muscle glycogen content was determined as described previously (22). Briefly, 100 mg of liver or muscle were dissolved in 8 volumes of 1 M NaOH at 55 °C and neutralized with 1 N HCl. The extract was spun down, and aliquots of the supernatant were incubated in the presence of amyloglucosidase for 2 h at 37 °C under vigorous shaking in an 0.2 M sodium acetate buffer. The glucose was then assayed by a glucose oxidase method (Trinder kit, Sigma).

**RESULTS**

**Blood Parameters—**Table I shows the characteristics of the mice analyzed. In the absence of GLUT2, the mice were slightly hyperglycemic in the fed state. Their glycemia decreased pro-

---

**Table I**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Body weight g</th>
<th>Glucose mg/ml</th>
<th>Lactate μM</th>
<th>FFA μM</th>
<th>Insulin pmol/ml</th>
<th>Glucagon ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>30.1 ± 0.8</td>
<td>8.1 ± 0.3</td>
<td>1.6 ± 0.1</td>
<td>534 ± 69</td>
<td>108 ± 19</td>
<td>68 ± 5</td>
</tr>
<tr>
<td>Fast 6 h</td>
<td>29.6 ± 0.5</td>
<td>6.4 ± 0.5</td>
<td>1.1 ± 0.1</td>
<td>1328 ± 182</td>
<td>11 ± 3</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>Fast 24 h</td>
<td>28.2 ± 0.5</td>
<td>6.3 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>897 ± 159</td>
<td>10 ± 3</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>GLUT2−/−</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>32.4 ± 0.7</td>
<td>9.2 ± 0.3</td>
<td>1.3 ± 0.1</td>
<td>364 ± 52</td>
<td>36 ± 6</td>
<td>132 ± 15</td>
</tr>
<tr>
<td>Fast 6 h</td>
<td>29.1 ± 0.7</td>
<td>6.7 ± 0.6</td>
<td>1.3 ± 0.2</td>
<td>1363 ± 50</td>
<td>2.5 ± 0.7</td>
<td>68 ± 2</td>
</tr>
<tr>
<td>Fast 24 h</td>
<td>29.4 ± 0.7</td>
<td>4.7 ± 0.4</td>
<td>1.3 ± 0.2</td>
<td>1226 ± 129</td>
<td>1.9 ± 0.8</td>
<td>63 ± 2</td>
</tr>
</tbody>
</table>

*Different from corresponding control value with p < 0.05.
versus the corresponding control value.

The data are the means \( \pm \) S.E. of \( n = 5–6 \). *, \( p < 0.05 \) versus fed; #, \( p < 0.05 \) versus corresponding control value.

TABLE II

<table>
<thead>
<tr>
<th>Mice</th>
<th>Glycogen phosphorylase</th>
<th>Glycogen synthase</th>
<th>Glucokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total active/total</td>
<td>total active/total</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25.4 ( \pm ) 3.0</td>
<td>5.4 ( \pm ) 1.7</td>
<td>0.20 ( \pm ) 0.05</td>
</tr>
<tr>
<td>Fasted</td>
<td>52.0 ( \pm ) 7.4</td>
<td>36.6 ( \pm ) 5.5</td>
<td>0.74 ( \pm ) 0.09</td>
</tr>
<tr>
<td>GLUT2(^{-/-})</td>
<td>15.6 ( \pm ) 1.8( a )</td>
<td>3.0 ( \pm ) 1.1</td>
<td>0.17 ( \pm ) 0.06</td>
</tr>
<tr>
<td>Fasted</td>
<td>48.8 ( \pm ) 1.0</td>
<td>46.2 ( \pm ) 2.4</td>
<td>0.94 ( \pm ) 0.04</td>
</tr>
</tbody>
</table>

\( a \) Different from corresponding control value with \( p < 0.05 \).

Glycogen Mobilization—To determine whether glycogen synthase or phosphorylase activities were normally regulated in GLUT2-null liver, these activities were measured in the fed state and after 24 h of fasting (Table II). The total and active glycogen synthase activities were 65% higher in fed and 75% higher in fasted GLUT2-null liver as compared with control livers. The total phosphorylase activity was 45% lower in fed mutant livers as compared with control but not significantly different in the fasted state. The phosphorylase to synthase ratio, which represents an index for the glycogen degradative activity, was increased by fasting in GLUT2-null livers even more than in control livers.

The glycogen stores were then measured in the fed state and after different periods of fasting: Fig. 1 shows that they were not decreased after 6 h of fasting in GLUT2-null livers and were still elevated after a 24-h fast, whereas they were almost completely mobilized within 6 h of fasting in control livers. In muscles, glycogen mobilization was, however, similarly efficient in both types of mice (Fig. 1).

Regulation of Gluconeogenic and Glycolytic mRNAs—To assess whether the elevation in plasma glucagon levels observed in fasting normally stimulated the expression of gluconeogenic genes, we measured the accumulation of the mRNAs for PEPCK and G6Pase. The Northern blots of Fig. 2 (A and B) show that the expression of these mRNAs was similarly increased by fasting in both control and mutant livers. Expression of the glucagon receptor mRNA was then assessed. This gene is induced by increased glycolytic and gluconeogenic fluxes (23). Fig. 2C shows that its expression in GLUT2-null liver was lower in the fed state but, upon fasting, higher than in control livers.

We next evaluated glucokinase mRNA and activity levels. These were similar in fasted control and GLUT2-null livers (Fig. 3 and Table II). Feeding induced the expected up-regulation of glucokinase mRNA and activity in control livers. However, in GLUT2-null liver no up-regulation of glucokinase mRNA and activity was induced by feeding. We then measured expression of the L-PK mRNA. This glucose-responsive gene is induced in the feeding period and suppressed by fasting, as observed in control livers (Fig. 4). However, in GLUT2-null liver the opposite regulation was observed. Indeed, even though the basal levels of expression in the fed state were similar in control and mutant livers, fasting induced a paradoxical increase in L-PK mRNA accumulation.

Glycogen Mobilization—Fasting induces a rapid degradation of glycogen stores in the liver of control or GLUT2-null mice. Glycogen stores were then measured in the fed state and after 24 h of fasting, and the glycogen content cannot be completely mobilized even after 24 h of fasting in GLUT2-null mice. Glycogen stores are normally mobilized in the hindlimb muscle of control or GLUT2-null mice. The data are the means \( \pm \) S.E., with \( n = 5–6 \). *, \( p < 0.05 \) versus fed; #, \( p < 0.05 \) versus corresponding control value.

Glycogen stores in the liver of GLUT2-null mice. Glycogen mobilization was, however, similarly efficient in both types of mice (Fig. 1).

Regulation of Gluconeogenic and Glycolytic mRNAs—To assess whether the elevation in plasma glucagon levels observed in fasting normally stimulated the expression of gluconeogenic genes, we measured the accumulation of the mRNAs for PEPCK and G6Pase. The Northern blots of Fig. 2 (A and B) show that the expression of these mRNAs was similarly increased by fasting in both control and mutant livers. Expression of the glucagon receptor mRNA was then assessed. This gene is induced by increased glycolytic and gluconeogenic fluxes (23). Fig. 2C shows that its expression in GLUT2-null liver was lower in the fed state but, upon fasting, higher than in control livers.

We next evaluated glucokinase mRNA and activity levels. These were similar in fasted control and GLUT2-null livers (Fig. 3 and Table II). Feeding induced the expected up-regulation of glucokinase mRNA and activity in control livers. However, in GLUT2-null liver no up-regulation of glucokinase mRNA and activity was induced by feeding. We then measured expression of the L-PK mRNA. This glucose-responsive gene is induced in the feeding period and suppressed by fasting, as observed in control livers (Fig. 4). However, in GLUT2-null liver the opposite regulation was observed. Indeed, even though the basal levels of expression in the fed state were similar in control and mutant livers, fasting induced a paradoxical increase in L-PK mRNA accumulation.

Glycogen-6-phosphate Levels—To explain the observed lack of glycogen mobilization and paradoxical regulation of the L-PK genes in fasted GLUT2-null livers, we hypothesized that an abnormal regulation of G6P may cause these two dysregulations. Indeed, G6P is an allosteric activator of glycogen synthase and an obligatory intermediate in the stimulation of L-PK gene transcription. Thus, even though neosynthesized glucose can be released quantitatively normally from hepatocytes by a membrane traffic-based pathway (3), it is still possible that a fraction of glucose returns from the endoplasmic reticulum to the cytosol. Because glucose cannot diffuse out of the cytosol into the external milieu, it may be converted back to G6P by glucokinese. Fig. 5 shows that comparable levels of G6P were found in the fed state in both types of mice. However, whereas in control livers G6P levels progressively decreased, in the mutant livers these levels remained at the fed level even after 24 h of fasting.

Correction of Metabolic Abnormalities by Re-expression of GLUT2 in Liver—To confirm that the persistent accumulation of glycogen and the paradoxical regulation of L-PK mRNA were due to the absence of GLUT2 from hepatocytes, we re-expressed GLUT2 specifically in the liver of the GLUT2\(^{-/-}\) mice. This was achieved by crossing the RIPGLUT1 x GLUT2\(^{-/-}\) mice (referred to as GLUT2\(^{-/-}\) in this study) with transgenic mice expressing GLUT2 under the control of the liver-specific \( \alpha \)-1-antitrypsin (AAT) promoter. Following appropriate breed-
ing we obtained RIPGLUT1×AATGLUT2×GLUT2−/− mice. These are devoid of endogenous GLUT2 but express transgenic GLUT2 in liver (Fig. 6A). After fasting, glycogen levels were degraded to the same extent as in control livers (0.06 ± 0.01 versus 1.51 ± 0.54 μg/mg protein for fasted mutant and control livers, respectively; see Fig. 1 for other conditions). Expression of the L-PK mRNA was also normally suppressed by fasting (Fig. 6B), and PEPCK mRNA normally up-regulated. These data indicate the key role of GLUT2 absence in all these metabolic dysregulations.

Liver Hyperplasia—Microscopic examination of histological sections of GLUT2-null liver revealed a normal structure. Livers were, however, hyperplasic. Indeed, as presented in Table III, the weight of GLUT2-null livers were 40% greater than the control livers. This was correlated with a ~30% increase in total DNA content.

**DISCUSSION**

The present analysis was initiated following our study of glucose uptake and release from hepatocytes of GLUT2-negative mice (3). These previous results indicated that in the absence of GLUT2, glucose uptake was almost completely suppressed but glucose release was quantitatively normal and proceeded by a membrane traffic mechanism originating from the endoplasmic reticulum. A fraction of glucose nevertheless

These data are the means ± S.E., with n = 5. B, expression of the glucose-6-phosphatase mRNA detected by Northern blot analysis (upper panel) is increased severalfold in both the control and GLUT2-null livers. Quantitation of the Northern blot analysis is presented in the lower panel. These data are the means ± S.E., with n = 5. C, expression of the glucagon receptor mRNA detected by Northern blot analysis (upper panel) is increased severalfold in both the control and GLUT2-null livers. Quantitation of the Northern blot analysis is presented in the lower panel. These data are the means ± S.E., with n = 5. *, p < 0.05 versus fed state; #, p < 0.05 versus corresponding control value.

**FIG. 3.** Glucokinase expression in the liver of control and GLUT2-null mice. Northern blot analysis of glucokinase mRNA in the fed and 24-h fasted state in liver of control and GLUT2-null mice (upper panel). Cyclophilin mRNA hybridization of the same blots was used to verify equal loading of the samples. Quantitation of the Northern blot signal is presented in the lower panel. Data are the means ± S.E. of five determinations. *, p < 0.05 versus fed; #, p < 0.05 versus corresponding control value.

**FIG. 2.** Normal regulation of the mRNAs for PEPCK and G6Pase and of the glucagon receptor by fasting. A, expression of the PEPCK mRNA detected by Northern blot analysis (upper panel) is increased severalfold in both the control and GLUT2-null livers. Quantitation of the Northern blot analysis is presented in the lower panel.

Hepatic Glucose Metabolism without GLUT2

10933
remained inside the cells and probably returned to the cytosol. Because of its inability to diffuse into the extracellular space, this glucose may enter a futile cycle initiated by its phosphorylation and followed by re-entry of G6P in the endoplasmic reticulum for hydrolysis back to glucose and phosphate.

The data that we present here support and extend these observations. They are summarized in Fig. 7. First, in fasting, the glucagon to insulin ratio increased similarly in both the GLUT2-null and control mice, although the absolute values of these ratios were much higher in the mutant mice. The reason for this higher ratio is presently not known. However, it may not be due to a defect in glucose-stimulated insulin secretion because the mice used in these study re-express a glucose transporter in their β cells, which allows a normal insulin secretion in response to elevation in glucose concentrations. Furthermore, the glucagon-secreting pancreatic α cells do not

---

**Fig. 4.** Paradoxical regulation of the L-PK mRNA in the liver of fasted GLUT2-null mice. Expression of the L-PK mRNA detected by Northern blot analysis (upper panel) is increased severalfold in the livers of fasted GLUT2-null. This is in contrast to the normal down expression of this mRNA in the livers of fasted control mice. Quantification of the Northern blot analysis is presented in the lower panel. These data are the means ± S.E., with $n = 5$. *, $p < 0.05$ versus fed state; #, $p < 0.05$ versus control value.

---

**Fig. 5.** Glucose-6-phosphate levels in the liver of fed and 6- and 24-h fasted control or GLUT2-null mice. The data are the means ± S.E., with $n = 5–6$. Whereas glucose-6-phosphate levels decrease progressively with fasting in control livers, no reduction of the glucose-6-phosphate levels can be measured in GLUT2-null livers. *, $p < 0.05$ versus fed state; #, $p < 0.05$ versus corresponding control value.

---

**Table III**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Body weight</th>
<th>Liver weight</th>
<th>Protein/liver</th>
<th>DNA/liver</th>
<th>DNA/protein</th>
<th>DNA/liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g</td>
<td>mg/mg</td>
<td>µg/mg</td>
<td>µg/mg</td>
<td>µg/mg</td>
</tr>
<tr>
<td>Control</td>
<td>35.2 ± 0.2</td>
<td>1.5 ± 0.03</td>
<td>0.164 ± 0.005</td>
<td>4.67 ± 0.12</td>
<td>1.43 ± 0.06</td>
<td>7054 ± 154</td>
</tr>
<tr>
<td>GLUT2&lt;sup&gt;−&lt;/sup&gt;</td>
<td>35.8 ± 0.9</td>
<td>2.16 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.161 ± 0.002</td>
<td>4.22 ± 0.09</td>
<td>1.31 ± 0.04</td>
<td>9086 ± 335&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Different from control with $p < 0.05$. 

---

Importantly, this elevated hormone ratio was sensed by the hepatocytes as revealed by the increase in active glycogen phosphorylase and decrease in glycogen synthase activities and by the induction of the mRNAs for PEPCK and G6Pase. Surprisingly, however, glycogen store mobilization in GLUT2-null livers was strongly impaired. This could not simply be explained by an impaired activation of glycogen phosphorylase or inactivation of glycogen synthase because these were normally regulated during the fed to fast transition. Actually, the ratio of glycogen phosphorylase to glycogen synthase, which can be taken as a measure of the glycogen degradative activity of the cells, is increased to a greater extent in the GLUT2-null liver. This indicates that solely based on these activity ratio, one would expect a faster degradation of glycogen in the mutant than in the control livers. We believe that this apparent contradiction can be resolved when we take into the account the intracellular levels of G6P. These are indeed not decreased by fasting in the

---

**Hepatic Glucose Metabolism without GLUT2**
be a critical signaling molecule. It should, however, be noted metabolism at least to the glucokinase step, and G6P appears to action is stimulated by a mechanism that requires glucose me-
digm for glucose-sensitive genes (6, 24–26), and its transcrip-
tion is stimulated by a mechanism that requires glucose me-
tabolism and paradoxical regulation of the L-PK gene observed in fasted GLUT2-null mice: (i) G6P generated from gluconeogenesis and/or glycogen degradation enters the endoplasmic reticulum to be hydrolyzed into glucose and phosphate, (ii) the glucose produced can be released from the cells by the membrane traffic pathway (left) but a fraction of this glucose can also return into the cytosol (right), (iii) because this cytosolic glucose cannot diffuse out the cell, it is phosphorylated back to G6P, (iv) the relatively elevated G6P levels then stimulate glycogen synthase and L-PK gene expression, (v) these stimulatory effects of G6P are dominant over the glucagon action, which normally should lead to glycogen degradation and inhibition of L-PK gene expression, and (vi) glucagon signal is, however, efficiently transmitted as shown by increased PEPCK and G6Pase expression. All of the above abnormal regulations are corrected by re-expression of GLUT2 in the plasma membrane. This indicates that the major role of GLUT2 in fasted liver is to allow equilibration of glucose with the external milieu. Our data also indicate that intracellular glucose metabolites have a dominant effect over the plasma concentration of glucagon and insulin in the control of glycogen metabolism and expression of glucose-sensitive genes.

absence of GLUT2 but remain at the fed levels. Because G6P is a strong allosteric stimulator of glycogen synthase (5), we favor the hypothesis that the prevailing G6P levels stimulates glycogen synthase so that the equilibrium between glycogen degra-
dration and synthesis prevents degradation.

Another striking observation that can in part be explained by the persistent G6P levels was the paradoxical stimulation of L-PK mRNA accumulation. Indeed, the L-PK gene is a para-
digm for glucose-sensitive genes (6, 24–26), and its transcription is stimulated by a mechanism that requires glucose me-
tabolism at least to the glucokinase step, and G6P appears to be a critical signaling molecule. It should, however, be noted that the observed increase in L-PK mRNA occurred in the presence of G6P levels, which remained constant during the fed to fast transition. Therefore G6P cannot be the only regulator of this gene expression. Other metabolites of G6P and/or other transcriptional or posttranscriptional regulatory mechanisms controlled by hormones and/or glucose may participate in this regulation. This abnormal regulation was also observed for expression of the glucose-sensitive Spot14 gene (not shown). These data on L-PK gene expression are in agreement with those of Antoine et al. (27), who reported that the transcriptional control of the L-PK gene by glucose was dependent on the level of GLUT2 expression in a panel of hepatoma cell lines. They suggested that GLUT2 was required for L-PK gene extinction rather than for its stimulation. They proposed that GLUT2 normally allowed a fast exit of glucose from the cytosol in the extra-
cellular medium. In the absence of this glucose exit, glucose could be phosphorylated into G6P, which maintains an elevated level of L-PK gene expression. Our data agree with their proposal, except that we showed that hepatic glucose production proceeded quantitatively normally in the absence of GLUT2.

Why do G6P levels remain elevated in fasted GLUT2-null livers? Formally, this G6P can originate from gluconeogenesis and/or glycogen degradation and accumulate in the cytosol because of a limited capacity to enter the endoplasmic reticulum or to be hydrolyzed (Fig. 7). Our Northern blot analysis indicated that G6Pase was increased in fasting to the same extent in GLUT2-null and control livers and hepatic glucose production in 6-h fasted GLUT2-null or control mice was identical (~25 mg/kg/min) (3). Therefore, the flux of glucose from cytosolic G6P to the endoplasmic reticulum and the extracellular space via the membrane traffic pathway does not appear to be limited. Cytosolic G6P may thus come in large part from glucose diffusing back into the cytosol after its production in the endoplasmic reticulum. Because glucokinase activity was measurable in both the fed and fasted states, the production of G6P from glucose was therefore possible at all times.

Analysis of mice re-expressing GLUT2 in liver indicated that restoration of glucose facilitative diffusion across the plasma membrane allowed normal degradation of glycogen stores and regulation of L-PK mRNA. Therefore, this is in support of the hypothesis shown in Fig. 7 that in the absence of GLUT2 (i) de novo synthesized glucose returns into the cytosol but cannot equilibrate with the extracellular space and is thus phosho-
rylated into G6P and (ii) the primary cause of dysregulated glycogen metabolism and L-PK gene expression is the relative increase in intracellular G6P levels.

One unexpected finding was that the liver of the GLUT2-null mice was larger than that of control mice. The 40% increase in liver weight was correlated with a 30% higher total DNA con-
tent. Increased liver mass is thus due in great part to an increase in cell number. This hyperplasia may be caused by

FIG. 7. Hepatic glucose metabolism in the liver of fasted GLUT2-null mice. In the absence of GLUT2, there is no remaining facilitated diffusion of glucose across the plasma membrane, but hepatic glucose production is quantitatively normal. We proposed that this release occurs through a membrane traffic-dependent pathway originating from the endoplasmic reticulum (3). Our data support the following scheme to explain the abnormal regulation of glycogen metabolism and paradoxical regulation of the L-PK gene observed in fasted GLUT2-null mice: (i) G6P generated from gluconeogenesis and/or glycogen degradation enters the endoplasmic reticulum to be hydrolyzed into glucose and phosphate, (ii) the glucose produced can be released from the cells by the membrane traffic pathway (left) but a fraction of this glucose can also return into the cytosol (right), (iii) because this cytosolic glucose cannot diffuse out the cell, it is phosphorylated back to G6P, (iv) the relatively elevated G6P levels then stimulate glycogen synthase and L-PK gene expression, (v) these stimulatory effects of G6P are dominant over the glucagon action, which normally should lead to glycogen degradation and inhibition of L-PK gene expression, and (vi) glucagon signal is, however, efficiently transmitted as shown by increased PEPCK and G6Pase expression. All of the above abnormal regulations are corrected by re-expression of GLUT2 in the plasma membrane. This indicates that the major role of GLUT2 in fasted liver is to allow equilibration of glucose with the external milieu. Our data also indicate that intracellular glucose metabolites have a dominant effect over the plasma concentration of glucagon and insulin in the control of glycogen metabolism and expression of glucose-sensitive genes.

2 R. Burcelin and B. Thorens, unpublished data.
up-regulation of autocrine growth/proliferation factors, which are regulated similarly to the glucose-sensitive L-PK gene. Differential display analysis of mRNA from the livers of control and GLUT2-null mice may allow identification of these genes.

Together, our studies showed that in the absence of GLUT2, although glucose production was quantitatively normal, there was important dysregulations in hepatic glucose metabolism. As summarized in Fig. 7, these appear to result from a lack of equilibration of cytosolic glucose with the extracellular milieu. The major role of GLUT2 in periods of stimulated glucose output is thus to allow equilibration of cytosolic glucose with the external space. In the absence of this equilibration, G6P levels cannot decrease normally during fasting. This leads to abnormal regulation of glycogen metabolism and expression of glucose-sensitive genes. This suggests, therefore, that the control of hepatic glucose metabolism by intracellular glucose metabolites is dominant over that by circulating hormone concentrations. Also, because glucose can still be released efficiently in the absence of GLUT2 and therefore in the absence of facilitated diffusion of glucose across the plasma membrane, these data further support our previous observations suggesting that this glucose release was by a membrane traffic-based pathway.

Based on these observations, one can further speculate that, in normal hepatocytes, the membrane pathway for glucose release is essential for compartmentalization of glucose away from the cytosol. High cytosolic glucose, which could diffuse into the nucleus, may activate glucokinase by displacing it from its regulatory protein (28, 29), and a subsequent increase into the nucleus, may activate glucokinase by displacing it from

REFERENCES

Liver Hyperplasia and Paradoxical Regulation of Glycogen Metabolism and Glucose-sensitive Gene Expression in GLUT2-null Hepatocytes: FURTHER EVIDENCE FOR THE EXISTENCE OF A MEMBRANE-BASED GLUCOSE RELEASE PATHWAY
Rémy Burcelin, Maria del Carmen Muñoz, Marie-Thérèse Guillam and Bernard Thorens

doi: 10.1074/jbc.275.15.10930

Access the most updated version of this article at http://www.jbc.org/content/275/15/10930

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 29 references, 17 of which can be accessed free at http://www.jbc.org/content/275/15/10930.full.html#ref-list-1