Journal of Hepatology 1999; **31**: 656–663
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Munksgaard · Copenhagen

Journal of Hepatology
ISSN 0168-8278

Rapid normalization of hepatic glycogen metabolism in rats with long-term bile duct ligation after biliodigestive anastomosis

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Background/Aims: **Rats with chronic bile duct ligation have reduced hepatic glycogen stores and decreased activities of enzyme involved in glycogen metabolism. In the current studies, the reversibility of these changes following reversal of biliary obstruction by Roux-en-Y anastomosis (RY) was investigated.**
Methods: **Rats were studied after bile duct ligation for 4 weeks (BDL rats), or 5 or 14 days after relief of biliary obstruction by RY. Control rats were pair-fed to treated rats, and all rats were studied in the fed state.**
Results: **The liver glycogen content was decreased in BDL rats (198±167 *vs.* 753±315 mg/liver in BDL *vs.* control rats) and normalized within 5 days after RY. The total activities of glycogen synthase and phosphorylase were both reduced by 51% in BDL as compared to control rats. Five days after RY, the activity of glycogen synthase had increased significantly in comparison to BDL rats, whereas glycogen phosphorylase had remained unchanged. Fourteen days after RY, both enzyme activities had completely normalized. Northern blots revealed reduced hepatic mRNA levels in BDL rats, for glycogen synthase and phosphorylase. While the mRNA level for glycogen synthase normalized within 5 days after RY, the level for glycogen phosphorylase increased but did not normalize completely within 14 days after RY.**
Conclusions: **Hepatic glycogen stores are decreased in BDL rats but recover rapidly after relief of biliary obstruction. Reduced activity and mRNA levels of glycogen synthase suggest that impaired glycogen synthesis is the principal mechanism for decreased hepatic glycogen stores in BDL rats.**

Key words: **Glycogen; Glycogen phosphorylase; Glycogen synthase; Long-term cholestasis; Roux-en-Y anastomosis; Secondary biliary cirrhosis.**

GLYCOGEN, the storage form of carbohydrates in liver and skeletal muscle, is an important source of energy during early starvation and is essential for overall body glucose homeostasis (1,2). Glycogen synthesis and breakdown are tightly regulated by the two key enzymes glycogen synthase and glycogen phosphorylase, whose activity is controlled by phosphorylation and dephosphorylation. Glycogen synthase, the rate-limiting enzyme of glycogen formation, is activated by metabolites such as AMP (3) and glucose-6-phosphate (4), and by insulin, which stimulates protein phosphatases (2,5,6). Inactivation occurs by the action of protein kinases, which are stimulated by hormones such as glucagon, adrenaline or vasopressin (2,5). Glycogen phosphorylase, the rate-limiting enzyme of glycogen breakdown, is activated by phosphorylation (7). The protein kinases performing the phosphorylation steps are activated by cAMP-dependent hormones such as glucagon and β-adrenergic agonists, calcium-dependent agents such as vasopressin, and α_1-adrenergic or P_2-purinergic agonists (8–12). Glycogenolysis can also be stimulated by endotoxins which induce secretion of prostaglandin D_2 by Kupffer cells (13). Glycogenolysis is inhibited by insulin, which impairs both the cAMP- and the calcium-dependent pathways .

It is well established that rats and humans with different types of liver cirrhosis have reduced hepatic glycogen stores (14–16). Patients with alcohol-induced liver cirrhosis show a shift from the normally used

Received 19 January; revised 13 April; accepted 16 April 1999

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carbohydrates to fatty acids as the preferred energy source in the post-absorptive state and in early starvation (17,18), which may be a consequence of the reduced hepatic glycogen stores. Similarly, rats with carbon tetrachloride (CCl_4)-induced liver cirrhosis show an accelerated transition from the fed to the fasted state, which is a consequence of early exhaustion of the hepatic glycogen stores (15,18).

Rats with bile duct ligation for 2–4 weeks (BDL rats) show liver fibrosis and eventually cirrhosis, rendering this animal model clinically and histologically comparable to long-term extrahepatic cholestasis in humans (19–21). Early studies in BDL rats using electron microscopy suggested a decrease in the hepatic glycogen content even in the non-cirrhotic state (22). We recently investigated hepatic glycogen metabolism in rats with bile duct ligation for 2 and 4 weeks, and demonstrated a progressive decrease in the glycogen stores per g liver and per ml of hepatocytes associated with reduced activities of glycogen synthase and phosphorylase (14). As the hepatic glycogen pool is important for whole-body energy metabolism, we decided to study the reversibility of the alterations in hepatic glycogen metabolism observed in BDL rats after reversing biliary obstruction. Several studies have shown that the structural and functional changes observed in livers from BDL rats are reversible after relief of biliary obstruction (21,23,24). The current study was designed to answer the following specific questions: i) do the hepatic glycogen content, and activities and expression of glycogen synthase and phosphorylase recover after relief of biliary obstruction? ii) what is the time course of the recovery? and iii) what are the mechanisms impairing hepatic glycogen metabolism in BDL rats?

Materials and Methods

Animals

Male Sprague-Dawley rats (Süddeutsche Versuchstierfarm, Tuttlingen, Germany) were used for all experiments. Rats were housed individually in wire-bottom cages on a 12-h dark and light cycle. All animals were fed a standard rat chow with tap water *ad libitum*. Rats were pair-fed (one control rat to two BDL rats) as described previously (25) and studied in the fed state. The animal experiments had been approved by the Animal Ethics Board of the State of Berne, and were performed according to these guidelines.

Surgical procedures

The rats were fasted overnight before surgery but had free access to water until immediately before and after surgery. No antibiotics or vitamin K injections were administered prior to or after surgery. Bile duct ligation (BDL) and sham operation were performed as reported previously by this group (14). A 3–4-cm midline incision was made and the common bile duct was exposed, ligated with three sutures and transected between the two sutures closest to the duodenum. In sham-operated animals, laparotomy was performed and the common bile duct was exposed but not transected.

Roux-en-Y choledochojejunostomy (RY) was performed according to Zimmermann et al. (21). Briefly, after a 3–4-cm midline incision had been made, the dilated proximal common bile duct stump was exposed and minimally freed from surrounding tissue in order to avoid bleeding. The jejunum was divided 3 cm distal to the ligament of Treitz and a short 4-cm Roux-en-Y limb created. End-to-side jejunojejunostomy was performed prior to side-to-end choledochojejunostomy. Both anastomoses were performed with a 6–0 Prolene® (Ethicon, Spreitenbach, Switzerland) single-layer running suture. In sham-operated animals, laparotomy was performed and the abdominal cavity exposed without any surgical procedure.

Study design

Six different groups were investigated with all animals in the fed state. The first group of animals were rats with bile duct ligation for 4 weeks (BDL; $n=6$). In the second group of animals, bile duct ligation was reversed by Roux-en-Y anastomosis for 5 days (RY5; $n=8$). In the third group of animals, bile duct ligation was reversed by Roux-en-Y anastomosis for 14 days (RY14; $n=6$). Mortality was 12% for BDL, 5% for all sham-operated animals, 33% for RY5 and 40% for RY14, respectively, with no rats dying during surgery. In the RY groups, animals usually died within the first 48 h following biliodigestive anastomosis, mainly due to anastomotic leakage with subsequent biliary peritonitis, bleeding due to portal hypertension, or due to suspected hepato-renal dysfunction. Groups four to six were sham-operated control rats to each group of experimental rats. All sham-operated rats were pair-fed to the respective experimental rats.

Characterization of the animals

The animals were characterized by their body weights, liver and spleen weights, activities of alkaline phosphatase and aspartate aminotransferase (AST) in plasma as well as the plasma concentrations of bilirubin and bile acids. Alkaline phosphatase, AST and bilirubin were analyzed on a COBAS analyzer (Hoffman-La Roche Diagnostics, Basle, Switzerland). Bile acids were determined with a radioimmunoassay (Becton and Dickinson, Orangeburg, SC, USA). The plasma glucose concentrations were determined enzymatically (kit obtained from Sigma Chemicals, Buchs, Switzerland).

Tissue preparation

At the time points indicated in the Results section, the rats were decapitated and a mixed venous/arterial blood sample was collected in a heparinized tube. The abdomen was opened quickly and a freeze-clamped liver sample was obtained, with the clamps having previously been cooled in liquid nitrogen. All liver biopsy specimens were kept at −80°C until analysis.

Liver glycogen content and protein determination

The glycogen content in liver was determined enzymatically as glucose (using a commercially available reagent kit, Sigma Chemicals, Buchs, Switzerland) after alkaline destruction of free glucose and enzymatic hydrolysis of glycogen as described originally by Lust et al. (26) with the previously reported modifications (15). The glycogen content is expressed as mg per gram liver wet weight.

The hepatic protein content was determined according to Lowry et al. (27) using bovine serum albumin as a standard.

Enzyme assays

To determine of the activities of glycogen synthase and glycogen phosphorylase, frozen liver was homogenized at 0°C with 9 volumes of a solution containing 50 mmol/l potassium fluoride and 10 mmol/l EDTA (pH 7.0). The homogenate was centrifuged at 10 000 *g* for 10 min at 4°C, and the resulting supernatant was assayed directly for glycogen synthase activity (active form and total activity) as described originally by Thomas et al. (28) and modified by Guinoivart et al. (29). The final concentrations in the assay were 200 μmol/l for uridine 5′-diphosphoglucose, 150 μmol/l for glucose-6-phosphate (active form), and 6 mmol/l for glucose-6-phosphate (total activity). An aliquot of the supernatant was diluted 1:2 (vol:vol) with a solution containing 50 mmol/l 2-(N-morpholino)-ethanesulfonic acid (MES), 50 mmol/l potassium fluoride and 5 mmol/l dithiothreitol (pH 6.1). The resulting solution was assayed for total glycogen phosphorylase activ-

ity according to Gilboe et al. (30) and for the active form of glycogen phosphorylase according to Theen et al. (31). The final concentrations in the assay were 60 mmol/l for glucose-1-phosphate, 500 μmol/l for caffeine (active form), and 5 mmol/l for adenosine 5′-monophosphate (total activity).

Northern blotting

Total liver RNA was prepared according to Chomczynski & Sacchi (32), and mRNA was isolated using the PolyAtract system (Promega, Madison, WI, USA). Two micrograms of mRNA were separated by electrophoresis on a 1% agarose/formaldehyde gel, transferred to a nylon membrane (Hybond N, Amersham, Little Chalfont, Buckinghamshire, UK) and hybridized after UV crosslinking. The cDNA probes obtained by rt-PCR and confirmed by sequencing corresponded to bp 1307–1768 of rat glycogen phosphorylase (X63515) and to bp 192–621 of rat glycogen synthase (J05446). A 1.3-kb GAPDH cDNA was used as a control. The probes were labeled using a random prime labeling kit (High Prime DNA Labeling Kit, Boehringer, Mannheim, Germany) with [α-^{32}P]dCTP. After a 30-min prehybridization period, the blots were hybridized for 60 min with 2×106 cpm/ml Expresshabsolution (Clontech, Palo Alto, CA, USA) at 65°C. After washing (twice for 15 min with 2×SSC containing 0.1 % SDS at room temperature, and once for 20 min with 0.1×SSC containing 0.1% SDS at 60°C), the blots were exposed to autoradiographic film (Kodak, X-Omat, Rochester, NY, USA) at −70°C. Relative abundance of mRNA levels was determined by densitometric analysis of the films with a CAMAG TLC Scanner II (CAMAG, Muttenz, Switzerland).

Statistical evaluation

All results are expressed as mean±SD. Means were compared by ANOVA, followed by Scheffé's test. A $p<0.05$ was considered to be statistically significant.

Results

Hepatic glycogen metabolism was investigated in fed BDL rats 4 weeks after bile duct ligation and 5 and 14 days after relief of bile duct ligation by Roux-en-Y anastomosis, and compared to pair-fed, sham-operated control rats. The rats used in the current study are characterized in Fig. 1 and Table 1. As shown in Fig. 1, body weights did not differ between treated and sham-operated rats over the entire period of the study, reflecting the effect of pair-feeding. Following the first surgical procedure, the mean body weight decreased by about 9% in both groups within the first 4 postoperative days before a continuous regain started. The same phenomenon was observed following the second surgical procedure, which was either RY anastomosis or a second sham-operation, respectively. After the second operation, RY rats lost 11% of their body weight,

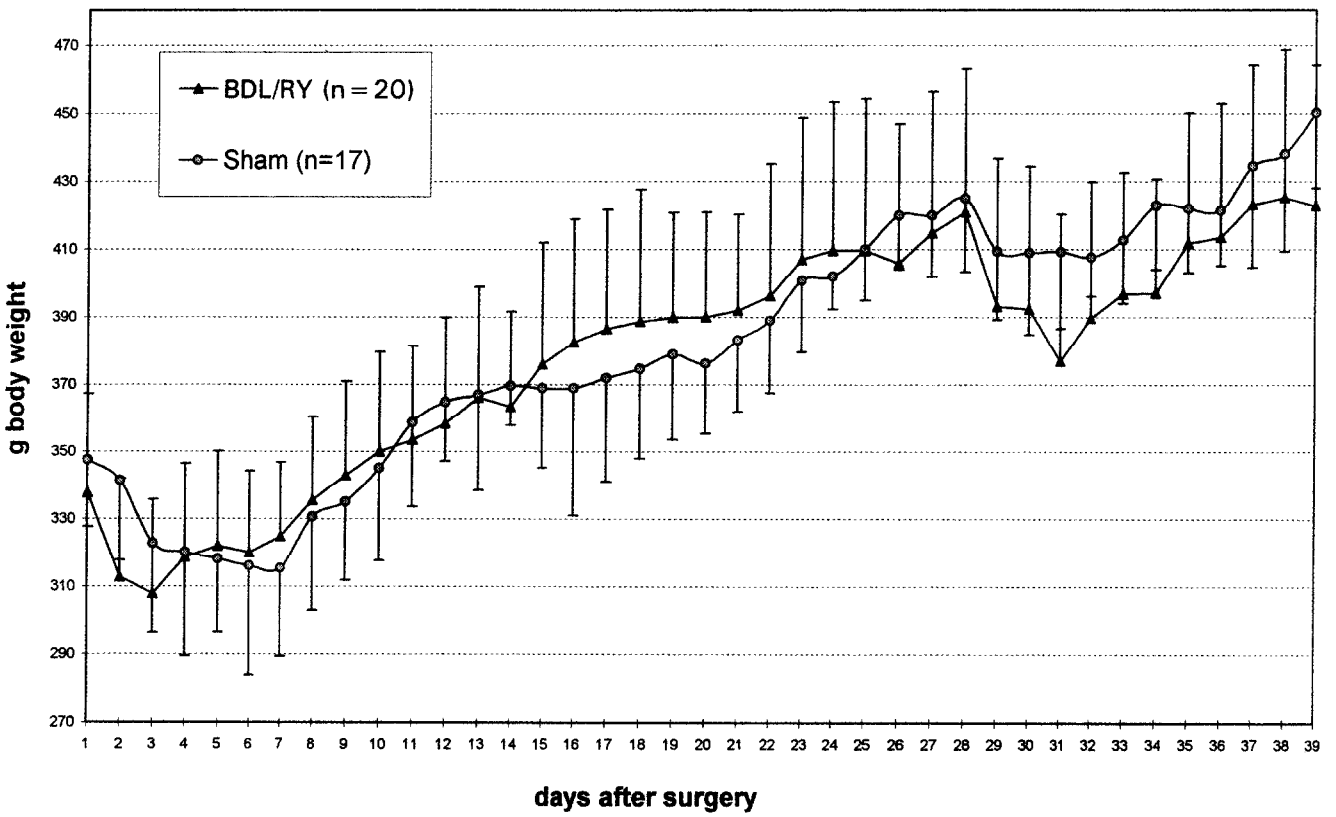

Fig. 1. Body weights of bile duct ligated rats (BDL), and rats 5 (RY5) or 14 days (RY14) after Roux-en-Y anastomosis, and sham-operated control rats. Bile duct ligation was performed at day 1 and Roux-en-Y anastomosis at day 28. Control rats were sham-operated at the same time points and pair-fed to treated rats throughout the study. The body weights are not significantly different between treated and control rats, whereas the drop in the body weight after Roux-en-Y anastomosis is significantly larger in BDL rats.

whereas only a 4% decrease was observed in the sham-operated control rats (significant difference at $p<0.05$). As shown in Table 1, at the end of the study, the body weights were not different between treated and sham-operated rats.

Liver and spleen weights were significantly higher in BDL compared to control rats, and decreased after 5 and 14 days following biliary decompression but remained significantly increased compared to controls. This is in agreement with other studies, where liver fibrosis and portal hypertension were not fully reversible within 5 and 14 days after reversal of biliary obstruction. The plasma concentrations of bilirubin and bile acids were significantly increased in BDL rats and normalized within 14 days after biliary decompression. In comparison, plasma alkaline phosphatase and aspartate aminotransferase showed a more rapid normalization after relief of biliary obstruction.

TABLE 1

Characterization of the animals. Rats studied were bile duct ligated for 4 weeks (BDL), or 5 (RY5) or 14 days (RY14) after relief of biliary obstruction by Roux-en-Y anastomosis. Sham-operated control rats (CON) were pair-fed to BDL or RY rats. Data are expressed as mean±SD

	BDL (n=6)	CON (n=5)	RY5 (n=8)	CON (n=6)	RY14 (n=6)	CON (n=6)
Body weight end of study (g)	435±51	414±31	427±18	424±15	423±41	450±22
Liver weight (g)	31.3±6.7*	15.8±1.6	19.9±3.4*+	14.9±3.4	19.5±4.5*+	14.9±1.1
Spleen weight (g)	2.7±0.7*	1.1±0.1	1.6±0.6*+	0.83±0.07	1.4±0.3*+	0.93±0.11
Plasma alkaline phosphatase (U/l)	630±192*	350±60	249±67+	291±82	260±73+	314±66
Plasma aspartate aminotransferase (U/l)	787±258*	260±113	178±50+	218±100	177±31+	179±32
Plasma bilirubin (μmol/l)	103±40*	0.6±0.09	4.5±3*+	0.3±0.4	1.0±0.6+	0.7±0.6
Plasma bile acids (μmol/l)	48±19*	0.7±0.4	2.6±2.4*+	0.5±0.4	1.3±0.5+	1.9±2.7

*$p<0.05$ *vs.* CON.
+$p<0.05$ *vs.* BDL.

TABLE 2

Hepatic glycogen content and plasma glucose concentration. Rats studied were bile duct ligated for 4 weeks (BDL), or 5 (RY5) or 14 days (RY14) after relief of biliary obstruction by Roux-en-Y anastomosis. Sham-operated control rats (CON) were pair-fed to BDL or RY rats. Data are expressed as mean±SD

	BDL (n=6)	CON (n=5)	RY5 (n=8)	CON (n=6)	RY14 (n=6)	CON (n=6)
Liver glycogen						
mg/g	6.4±5.5*	46.4±16.2	47.4±24.1+	57.3±24.0	50.9±19.1+	45.5±15.5
mg/liver	198±167*	753±315	921±528+	908±342	991±431+	683±246
Glucose in plasma						
mmol/l	5.1±1.2*	6.8±0.5	6.1±0.9+	6.0±0.9	6.7±0.5+	6.6±0.3

*$p<0.05$ *vs.* CON.
+$p<0.05$ *vs.* BDL.

TABLE 3

Activities of glycogen synthase and glycogen phosphorylase. Rats studied were bile duct ligated for 4 weeks (BDL), or 5 (RY5) or 14 days (RY14) after relief of biliary obstruction by Roux-en-Y anastomosis. Sham-operated control rats (CON) were pair-fed to BDL or RY rats. Enzyme activities were determined using radioactive substrates as described in Methods. Data are expressed as mean±SD

	BDL (n=6)	CON (n=5)	RY5 (n=8)	CON (n=6)	RY14 (n=6)	CON (n=6)
Glycogen synthase						
Total activity (mU/g)	74.6±22.8*	151.3±15.8	119.8±26.3*+	206.1±33.7	155.4±20.0+	161.9±21.4
Active form (mU/g)	15.4±10.4*	54.4±9.4	34.8±12.8*+	83.2±8.3	55.1±10.0+	65.6±11.7
Active fraction (%)	19.3±8.0*	35.8±3.9	28.9±7.0*	41.0±5.6	35.6±6.0+	40.3±2.6
Glycogen phosphorylase						
Total activity (U/g)	11.2±4.1*	22.7±3.8	11.9±3.8*	26.5±6.0	21.9±3.8+	25.9±3.1
Active form (U/g)	9.0±3.0*	19.3±3.3	9.9±2.5*	23.3±5.2	18.8±2.9+	21.6±3.4
Active fraction (%)	81.3±9.5	85.5±6.2	84.4±5.8	88.3±6.5	86.0±4.8	83.2±3.6

*$p<0.05$ *vs.* CON.
+$p<0.05$ *vs.* BDL.

TABLE 4

Densitometric analysis of Northern blots for hepatic glycogen synthase and phosphorylase. Rats studied were bile duct ligated for 4 weeks (BDL), or 5 (RY5) or 14 days (RY14) after relief of biliary obstruction by Roux-en-Y anastomosis. Sham-operated control rats (CON) were pair-fed to BDL or RY rats. Values are corrected for GAPDH and are obtained from densitometric analysis of Northern blots as shown in Fig. 2. Data are expressed as mean±SD

	BDL (n=4)	CON (n=4)	RY5 (n=4)	CON (n=4)	RY14 (n=4)	CON (n=4)
Glycogen synthase	0.15±0.06*	0.35±0.10	0.21±0.06	0.33±0.10	0.26±0.11	0.37±0.15
Glycogen phosphorylase	0.15±0.05*	0.41±0.15	0.16±0.08*	0.39±0.11	0.26±0.06*+	0.41±0.09

*$p<0.05$ *vs.* CON.
+$p<0.05$ *vs.* BDL.

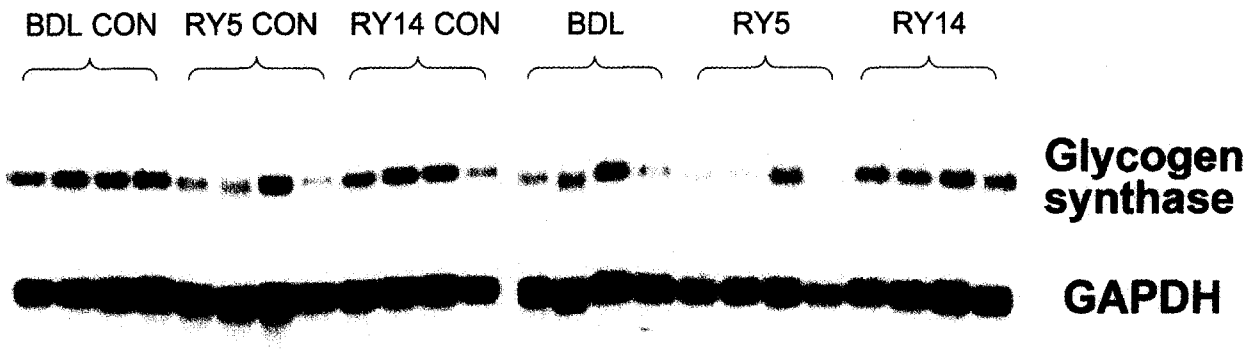

Fig. 2. Northern blot analysis of glycogen synthase. The Northern blot was performed using mRNA as described in Methods. GAPDH=glycerinaldehydephosphate dehydrogenase. In comparison to control rats (CON) the mRNA level of glycogen synthase is reduced in livers from BDL rats, but not 5 (RY5) or 14 days (RY14) after Roux-en-Y anastomosis. The results of the densitometric analysis are given in Table 4.

The hepatic glycogen content and the plasma glucose concentration are shown in Table 2. Livers of rats with 4 weeks BDL showed a reduction in the glycogen content of 86% expressed per g liver and by 74% expressed per liver. These values are in agreement with our previous studies (14). The plasma glucose concentration was decreased by 26% in BDL rats as compared to control rats. After decompression of the bile duct, both the hepatic glycogen content and the plasma glucose concentration normalized within 5 days, and remained normal after 14 days. The protein content per g liver was not significantly different between BDL, RY and control rats (results not shown).

In agreement with our previous study (14), both total activity and active forms of glycogen synthase and phosphorylase showed a significant decrease in livers from BDL as compared to control rats (Table 3). Five days after RY anastomosis, the activity of glycogen synthase started to recover, whereas the activity of glycogen phosphorylase remained decreased. Fourteen days after RY the activities of both enzymes, glycogen synthase and phosphorylase had completely normalized. For glycogen synthase, the active fraction was decreased in BDL rats and up to 5 days after RY, suggesting impaired activation. In contrast, for glycogen phosphorylase, the active fraction was not different between treated and control rats.

As a first step in determining the mechanisms for reduced activities of glycogen synthase and phosphorylase and for the reduction in the hepatic glycogen content, Northern blots for glycogen synthase and phosphorylase were performed. As shown in Table 4 and Fig. 2, the hepatic mRNA levels in BDL rats were reduced for both mRNA species investigated. After relief of the biliary obstruction for 5 days, the mRNA levels had normalized for glycogen synthase, but remained reduced for glycogen phosphorylase. After 14 days, the mRNA levels remained normal for glycogen synthase, and had significantly increased but not completely normalized for glycogen phosphorylase.

Discussion

The current studies confirm that the hepatic glycogen content and the activities of glycogen synthase and phosphorylase are decreased in BDL rats and demonstrate that these changes are reversible within 5 to 14 days after restoring bile flow.

As shown in Fig. 1, pair-feeding of the control to the

treated rats resulted in almost identical body weights between treated and control rats. It is interesting to note that surgery at day 28 was less well supported by BDL as compared to control rats, as evidenced by a more accentuated drop in the body weight and by a considerable mortality. While these findings may reflect the difference in the surgical procedures performed in BDL (biliodigestive anastomosis) and control rats (sham operation), chronic cholestasis may render rats more vulnerable to surgery. Our results show also that BDL rats have a large potential for recovery, as illustrated by the sharp increase in body weight during the first week after relief of biliary obstruction.

Reduced plasma glucose concentrations in BDL rats are most probably due to impaired hepatic glycogenolysis owing to reduced hepatic glycogen stores, or reduced activity of glycogen phosphorylase or glucose-6-phosphatase. Since control rats were pair-fed to BDL rats and all rats were studied in the fed state, reduced intake of food or impaired gluconeogenesis can be excluded. Because of the observations that the hepatic glycogen stores had recovered 5 days after RY and the activity of glycogen phosphorylase was still reduced at this time point, impaired activity of glycogen phosphorylase cannot explain hypoglycemia in BDL rats. Further studies are therefore needed to determine the mechanism for this finding.

Hepatic glycogen is an important source of energy, in particular in the early phase of starvation. For instance, in rats with CCl_4-induced cirrhosis, which have reduced hepatic glycogen stores, the metabolic transition from the fed to fasted state (e.g. the transition from glucose to fatty acid metabolism) occurs much earlier after cessation of food intake as compared to control rats (15,18). Since patients and rats with liver cirrhosis preferentially use fatty acids as a fuel, impaired function of hepatic β-oxidation could be detrimental in this situation. In rats with secondary biliary cirrhosis due to long-term bile duct ligation, hepatic mitochondrial fatty acid metabolism is impaired, as indicated by reduced production of ketone bodies by perfused livers or isolated hepatocytes (33,34) and reduced ketogenesis during starvation (24,25). Nevertheless, these animals support starvation for 24 h with only mild, apparently symptomless hypoglycemia, suggesting that hepatic fatty acid metabolism is still sufficient to meet the energy needs of the liver. It is unclear, however, how longer periods of starvation are supported by these animals and how organs which depend on ketone bodies as a fuel during starvation (e.g. brain) support fasting for longer periods.

Reduced hepatic glycogen stores may contribute to the catabolic metabolism often observed in patients and animals with liver cirrhosis (16,17,21,35), since hepatic production of glucose must be maintained by gluconeogenesis, which uses amino acids derived from skeletal muscle breakdown. It is therefore important to know whether the changes in hepatic glycogen metabolism are reversible after removing the cause of liver cirrhosis and how fast reversibility is achieved. Our study shows that the hepatic glycogen content had already normalized 5 days after relief of bile duct ligation, a finding which was confirmed 9 days later. Five days after relief of biliary obstruction, glycogen synthase activity had increased in comparison to BDL rats but had not normalized. This increase may be sufficient, however, to explain the observed normalization of the hepatic glycogen pool 5 days after surgery, since the activity of glycogen phosphorylase was still low at this time point. In comparison with the corresponding enzyme activities, the mRNA levels of glycogen synthase showed approximately the same time course, whereas the mRNA levels of glycogen phosphorylase clearly normalized more slowly. These findings suggest that the activity of glycogen phosphorylase is determined not only on the level of transcription, but most probably also on the protein level, i.e. translation and/or protein breakdown.

Considering glycogen synthase, the activity was decreased in BDL rats not only because of a reduced total activity, but also because of a reduction in the active fraction. Glycogen synthase is activated by metabolites including glucose-6-phosphate and AMP, and also by insulin (2–6). Inactivation of glycogen synthase is achieved by phosphorylation of various phosphorylation sites, mediated by hormones such as glucagon, vasopressin and adrenaline (8–12). While the concentration of glucose-6-phosphate is not known in livers from BDL rats, the concentration of AMP has been found to be unchanged compared to control rats (S. Krähenbühl and J. Reichen, unpublished results 1988). On the other hand, it is well established that rats and humans with liver cirrhosis have increased serum concentrations of insulin, glucagon, vasopressin and adrenalin (14,36–40). One possible explanation for the observed reduction in the activation of glycogen synthase in BDL rats is therefore that the effect of the factors promoting glycogen synthase phosphorylation overrides insulin-mediated dephosphorylation. In support of this concept, the activation of glycogen phosphorylase, which is achieved by phosphorylation mediated basically by the same hormones as phosphorylation of glycogen synthase (2,3,11), is maintained in BDL rats. Alternatively, chronic cholestasis could be associated with a decrease in the activity of the glycogen synthase phosphatase due to mechanisms

independent of the action of insulin, such as reduced expression. Independently of the mechanisms involved, the study confirms our previous hypothesis that the reduction in the hepatic glycogen content in BDL rats results from decreased glycogen synthesis and not from increased breakdown.

In conclusion, our studies demonstrate that rats with chronic bile duct ligation have a reduced hepatic glycogen content which predisposes them to a catabolic state in particular in situations with metabolic stress. The liver has an enormous capacity to recover from the metabolic sequelae of chronic cholestasis, as evidenced by the rapid normalization of the hepatic glycogen stores after relief of biliary obstruction.

Acknowledgements

We thank K. Krusch for caring for the animals, and A. Jeger and Y. Bloemhard for performing biochemical analyses.

The studies were supported by grants from the Swiss National Science Foundation to L.K. (3200–051127.97–1), B.H. (31–45677.95) and S.K. (31–46792.96). B.H. has a fellowship from the Cloetta Foundation.

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