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Hepatic and skeletal muscle glycogen metabolism in rats with short-term cholestasis

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***Background/Aims*: To study the effect of short-term cholestasis on glycogen metabolism.**

***Methods*: Rats were bile duct ligated (BDL) for 4 or 8 days and compared to sham-operated control rats pair-fed to BDL (pair-fed CON) or fed ad libitum (ad libitum-fed CON).**

***Results*: Four days after surgery, the hepatic glycogen content was 21.6 ± 7.6 mg/g in BDL, 21.2 ± 8.5 mg/g in pair-fed CON and 72.9 ± 7.7 mg/g in ad libitum-fed CON, respectively. Eight days after surgery, the hepatic glycogen content was reduced in BDL as compared to pair-fed (31.2 ± 8.9 vs. 59.1 ± 5.4 mg/g) or ad libitum-fed CON (58.3 ± 4.7 mg/g). Similar findings were obtained with the glycogen content expressed per ml hepatocytes or per liver. Histological analysis of BDL livers showed that most hepatocytes were affected. As compared to CON, activities and mRNA levels of glycogen synthase and phosphorylase were reduced in BDL, whereas plasma glucagon and endotoxin levels were increased at both time points. In contrast to liver, skeletal muscle glycogen metabolism remained unaffected.**

***Conclusions*: While reduced intake of food explains the decrease in the hepatic glycogen stores in BDL and pair-fed CON 4 days after surgery, reduced glycogen synthesis, possibly related to endotoxinemia, is the most probable cause of the decrease in the hepatic glycogen content in BDL 8 days after surgery.**

***Keywords*: Acute cholestasis; Bile duct ligation; Endotoxin; Glycogen; Glycogen synthase; Glycogen phosphorylase**

1. Introduction

Hepatic and muscle glycogen metabolism are essential for glucose homeostasis and they represent important energy sources during early starvation and physical exercise [1–3]. Glycogen synthesis and breakdown are regulated by two key enzymes, glycogen synthase and glycogen phosphorylase [1,4,5]. The activity of these enzymes is controlled by phosphorylation and dephosphorylation, respectively [1,6]. Glycogen synthase, the rate limiting enzyme for glycogen synthesis, is activated either by metabolites such as glucose [3,7,8], AMP [9] or glucose-6-phosphate [10] or by insulin via stimulation of protein phosphatase 1-G [1,11–15]. Glycogen synthase is inactivated by various protein kinases that are activated by hormones such as glucagon, adrenalin or vasopressin [1,13,16–18]. The key enzyme for glycogen breakdown is glycogen phosphorylase, which is activated by phosphorylation [4,19,20]. The corresponding protein kinase is activated by hormones like glucagon, vasopressin or adrenalin by phosphorylation [1,17,21–24]. Endotoxins or acute phase proteins such as interleukin (IL)-1β stimulate glycogenolysis either by inducing secretion of prostaglandin D_2 by Kupffer cells [25,26] or by affecting glycogen phosphorylase directly [27]. Glycogenolysis is inhibited by insulin either via a cAMP- or a calcium-dependent pathway.

Bile duct ligation (BDL) for 2–4 weeks leads to progressive fibrosis and eventually cirrhosis in rats, similar to

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secondary biliary cirrhosis in humans [28–30]. We were recently able to demonstrate a progressive reduction in the hepatic glycogen content per g liver and per ml of hepatocytes [31], and a decrease in activity and expression of both glycogen synthase and phosphorylase [32] in rats with bile duct ligation for 2–4 weeks. These findings indicate that reduced glycogen synthesis is the major mechanism for reduced hepatic glycogen stores in rats with long-term cholestasis. However, whether cirrhosis or cholestasis is responsible for reduced glycogen stores in the liver is so far not clear investigated.

We therefore aimed to assess the effect of short-term cholestasis on hepatic and skeletal muscle glycogen metabolism in liver and skeletal muscle. The study should answer the following questions: (1) are the hepatic and skeletal glycogen contents reduced in rats with bile duct ligation for 4 or 8 days without cirrhosis? (2) if so, what are the potential mechanisms leading to alterations in glycogen metabolism?

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (BRL, CH-4414 Füllinsdorf, Switzerland) were housed individually on a 12:12-h dark/light cycle and were fed a standard rat chow with tap water ad libitum. All the animal experiments were approved by the State Animal Ethics Board and were performed according to these guidelines.

2.2. Surgical procedures

All surgical procedures were performed as described previously [31]. There was no mortality in the BDL rats studied 4 days after surgery or in either control group, whereas the mortality in the BDL group 8 days after surgery was 25%.

2.3. Study design

Six different groups of animals were investigated with all animals in the fed state. Animals were either bile duct ligated (BDL rats) for 4 ($n = 12$) or 8 ($n = 9$) days, or sham-operated on and pair-fed to BDL rats (pair-fed CON rats) for 4 ($n = 8$) or 8 ($n = 8$) days. Two other control groups were sham-operated and fed ad libitum for 4 ($n = 5$) or 8 ($n = 5$) days (ad libitum-fed CON rats).

2.4. Characterization of the animals

The rats were characterized by their body and spleen weights, activities of alkaline phosphatase and aspartate aminotransferase (AST) in plasma and by the plasma concentrations of glucose and bilirubin (analyzed on a COBAS analyzer, Hoffmann-La Roche Diagnostics, Basel, Switzerland), insulin and glucagon (by a radioimmunoassay, Linco Research Inc., St. Louis, MO, USA), bile acids (by a radioimmunoassay, Becton Dickinson, Orangeburg, SC, USA), and endotoxin (Limulus amebocyte lysate pyrogen test, Kinetic-QCL, Biowhittaker, Santa Clara, CA, USA).

2.5. Sample preparation

The rats were decapitated at the time points indicated above and two mixed venous/arterial blood samples were collected in heparinized tubes, or, for the determination of insulin or glucagon, in tubes containing the protease inhibitor Trasylol (Bayer Pharma, Zürich, Switzerland). The abdomen was opened quickly and freeze-clamped samples were obtained from liver and skeletal muscle (triceps femoris). All liver and muscle biopsy specimens were kept at −80 °C until analysis.

For the determination of portal and systemic endotoxin levels, additional rats (BDL rats for 4 and 8 days and pair-fed control rats for 8 days, $n = 6$ per group) were studied. Rats were kept in ether anesthesia and a systemic and portal blood sample were obtained under sterile conditions and immediately analyzed for endotoxin after heating to 75 °C for 10 min to denature endotoxin-binding proteins [25].

From the remainder of the liver, additional samples were obtained in 4% buffered formaldehyde for stereological analysis and in alcohol for histological analysis of glycogen.

2.6. Tissue glycogen content

The glycogen content in liver and skeletal muscle was determined enzymatically as glucose using a COBAS analyzer (Hoffmann-La Roche Diagnostics) after alkaline destruction of free glucose and enzymatic hydrolysis of glycogen as described by Lust et al. [33] with the modifications reported previously [31].

2.7. Enzyme assays

Glycogen synthase activity (active form and total activity) was determined as described originally by Thomas et al. [34] and modified by Guinovart et al. [35]. Total activity of glycogen phosphorylase was determined according to Gilboe et al. [36], and the active form according to Theen et al. [37].

2.8. Northern blotting

Total liver RNA was prepared according to Chomoczynski and Sacchi [38], 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, Bucks, UK) and hybridized after UV crosslinking. The cDNA probes obtained by reverse transcriptase–polymerase chain reaction and confirmed by sequencing corresponded to base pairs (bp) 192–621 of rat glycogen synthase (J05446) and bp 1307–1768 of rat glycogen phosphorylase (X63515). A 1.3 kb GAPDH cDNA was used as a control. The probes were labeled with [α-^{32}P]dCTP according to the general protocol of Sambrook et al. [52]. After a 2-h prehybridization period, the blots were hybridized overnight with 2×10^6 cpm/ml hybridization solution at 65 °C according to the general protocol of Sambrook et al. [52]. After washing (three times for 30 min with 2× SSC containing 0.5% SDS at 65 °C) the blots were exposed to an autoradiographic film (Kodak, X-Omat, Rochester, NY, USA) at −70 °C. Relative abundance of mRNA levels was determined by densitometric analysis of the film with a Sharp Scanner JX 325.

2.9. Stereological and histological analysis of the livers

The histological sections of the liver samples obtained randomly from all bile duct ligated and pair-fed control rats [39] were analyzed by stereological methods using the point-counting procedure described by Weibel [40]. Each point was classified as overlying either hepatocytes, connective tissue or 'other structures'. In Section 3, only the volume densities (V_v) and volumes per liver (V) of hepatocytes (hc) and connective tissue (ct) are reported.

Other liver samples were fixed in alcohol and stained with PAS to visualize tissue glycogen. Stains pretreated with diastase served as a negative control.

2.10. Statistical methods

All results are expressed as mean ± standard deviation (SD). Means

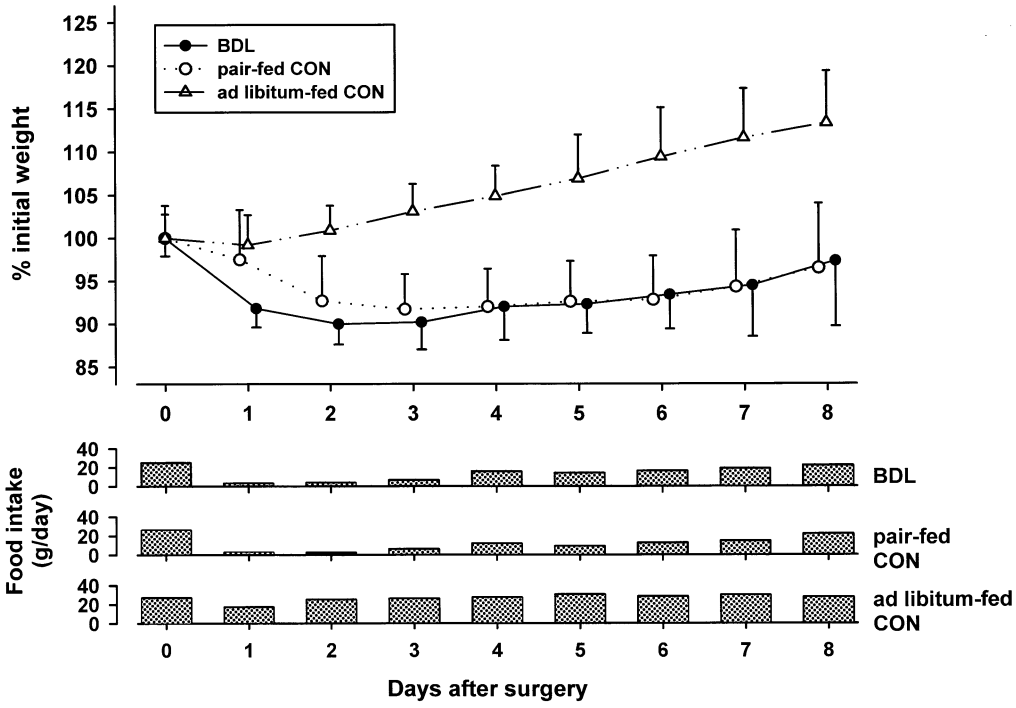

Fig. 1. Body weights and food intake. Body weights are expressed as the percentage of the initial body weights. All rats studied were included in the analysis. The initial body weights were 312 ± 6 g for BDL rats, 293 ± 11 g for pair-fed and 277 ± 8 g for ad libitum-fed CON rats. Data are given as mean ± SD.

were compared by ANOVA, followed by Scheffé's test. $P < 0.05$ was considered to be statistically significant.

3. Results

The body weights are given in Fig. 1 and Table 1. Both BDL and pair-fed CON rats showed an initial drop in body weight which reached its maximum two to three days after surgery (Fig. 1), whereas such a drop was not observed in ad libitum-fed CON rats. The spleen weights did not differ between BDL and CON rats 4 days after surgery, but were increased in BDL rats after 8 days (Table 1). The activities of alkaline phosphatase, AST and the plasma concentrations of bilirubin and bile acids all showed significant increases in BDL rats 4 and 8 days after surgery in comparison to CON rats. Endotoxin levels were elevated in BDL rats at both time points in plasma from portal and peripheral blood.

The stereological analysis of the livers revealed a decrease in the volume fraction of hepatocytes and a corresponding increase in the volume fraction of collagen in BDL

Table 1
Characterization of the animals[a]

	BDL		Pair-fed CON		Ad libitum-fed CON	
	4 days ($n = 12$)	8 days ($n = 9$)	4 days ($n = 8$)	8 days ($n = 8$)	4 days ($n = 5$)	8 days ($n = 5$)
Body weight (bw, g)	293 ± 11[a]	304 ± 24	268 ± 11[c]	283 ± 22[c]	292 ± 8	314 ± 17
Liver weight (g/100 g bw)	5.06 ± 0.36[ab]	5.23 ± 0.47[ab]	3.33 ± 0.30[c]	3.63 ± 0.36[c]	4.49 ± 0.23	4.28 ± 0.23
Spleen weight (g/100 g bw)	0.26 ± 0.04	0.30 ± 0.05[ab]	0.27 ± 0.02	0.24 ± 0.002	0.23 ± 0.05	0.24 ± 0.02
Plasma AST (U/l)	556 ± 178[ab]	509 ± 173[ab]	154 ± 40	175 ± 33	174 ± 41	261 ± 170
Plasma alkaline phosphatase (U/l)	486 ± 84[ab]	418 ± 100[ab]	172 ± 12	223 ± 28	208 ± 59	287 ± 124
Plasma bile acids (μmol/l)	89 ± 37[ab]	74 ± 54[ab]	0.9 ± 0.5[c]	1.0 ± 0.3[c]	1.8 ± 0.7	2.5 ± 0.7
Plasma bilirubin (μmol/l)	148 ± 25[ab]	145 ± 53[ab]	0.3 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.2
Plasma endotoxin (systemic, pg/ml)	41.9 ± 20.9[a]	78.5 ± 18.9[a]	n.d.	10.6 ± 4.6	n.d.	n.d.
Plasma endotoxin (portal, pg/ml)	57.8 ± 20.6[a]	95.2 ± 20.4[a]	n.d.	9.7 ± 3.9	n.d.	n.d.

[a] Rats studied were either bile duct ligated (BDL), sham-operated control rats pair-fed to BDL rats (pair-fed CON) or control rats fed ad libitum (ad libitum-fed CON). Data are given as mean ± SD. For endotoxin determinations, $n = 6$ animals for each group were studied. n.d., not determined. Significance: [a]$P < 0.05$, BDL vs. pair-fed CON; [b]$P < 0.05$, BDL vs. ad libitum-fed CON; [c]$P < 0.05$, pair-fed CON vs. ad libitum-fed CON.

Table 2
Stereological analysis of the livers[a]

	BDL		Pair-fed CON	
	4 days ($n = 12$)	8 days ($n = 9$)	4 days ($n = 8$)	8 days ($n = 8$)
Hepatocytes (hc)				
V_v (hc, liver) (ml hc per ml liver)	0.87 ± 0.04[a]	0.85 ± 0.04[a]	0.94 ± 0.03	0.95 ± 0.02
V (hc) (ml hc per liver)	12.9 ± 0.9[a]	13.9 ± 1.4[a]	8.3 ± 0.8	10.7 ± 1.2
Connective tissue (ct)				
V_v (ct, liver) (ml ct per ml liver)	0.09 ± 0.03[a]	0.06 ± 0.03[a]	0.04 ± 0.02	0.02 ± 0.01
V (ct) (ml ct per liver)	1.26 ± 0.09[a]	1.01 ± 0.10[a]	0.32 ± 0.03	0.23 ± 0.02

[a] Rats studied were either bile duct ligated (BDL) or sham-operated control rats pair-fed to BDL rats (pair-fed CON). Stereological analysis was performed by the point-counting procedure as described in Section 2. V_v stands for volume fraction, V for volume, hc for hepatocytes and ct for connective tissue. Data are given as mean ± SD. Significance: [a]$P < 0.05$ BDL vs. pair-fed CON.

as compared to pair-fed CON rats (Table 2). Due to the increase in liver weight mentioned above, both the volume of hepatocytes and the volume of collagen per liver were increased in BDL rats.

Four days after surgery, the hepatic glycogen content expressed per g liver or ml hepatocytes was not different between BDL and pair-fed CON rats, but was decreased in BDL compared to ad libitum-fed CON rats (Table 3). When expressed per liver, both BDL and pair-fed CON rats had a lower glycogen content than ad libitum-fed CON rats. Eight days after surgery, the glycogen content per g liver or per ml hepatocytes was lower in BDL rats than in both CON groups. Also, when expressed per liver, the glycogen content was lowest in BDL rats, but the difference reached statistical significance only in comparison to ad libitum-fed CON rats. The histological analysis of the livers shows that the loss of glycogen in BDL rats has no zonal distribution and affects the hepatocytes evenly (Fig. 2). In contrast to the liver, the glycogen content in skeletal muscle showed no significant difference between BDL and pair-fed or ad libitum-fed CON rats at both time points.

The plasma glucagon concentrations were increased in BDL compared to pair-fed CON rats by approximately 50% at both time points. In contrast, the plasma insulin concentration was decreased by 50% in BDL rats 4 days after surgery, whereas after 8 days no difference between BDL and pair-fed CON rats could be detected.

In comparison to pair-fed or ad libitum-fed CON rats, the total activity and also the active part (a-form) of both glycogen synthase and phosphorylase was reduced in livers from BDL rats Table 4. There was no difference in the active fraction (a-form divided by total activity) between BDL and CON rats at both time points for both enzymes. In contrast to liver, the activity of both enzymes was not different between BDL and pair-fed CON rats in skeletal muscle.

To explain further the mechanism leading to the observed reduction in the activities of glycogen synthase and phosphorylase in BDL rats, Northern blots for both enzymes were prepared. As shown in Table 5, the hepatic mRNA levels were reduced in BDL as compared to pair-fed CON rats for both glycogen synthase and phosphorylase at both time points investigated.

Table 3
Metabolic characterization of the animals[a]

	BDL		Pair-fed CON		Ad libitum-fed CON	
	4 days ($n = 12$)	8 days ($n = 9$)	4 days ($n = 8$)	8 days ($n = 8$)	4 days ($n = 5$)	8 days ($n = 5$)
Tissue glycogen content						
mg/g liver	21.6 ± 7.6[b]	31.2 ± 8.9[ab]	21.2 ± 8.5[c]	59.1 ± 5.4	73.0 ± 7.7	58.4 ± 4.7
mg/ml hepatocytes	24.8 ± 8.7	36.9 ± 10.6[a]	22.7 ± 9.1	62.2 ± 5.8	n.d.	n.d.
mg/liver	324 ± 124[ab]	517 ± 160[b]	191 ± 81[c]	670 ± 122	960 ± 134	783 ± 72
mg/g skeletal muscle	5.2 ± 1.4	6.0 ± 1.9	5.2 ± 0.8	5.8 ± 1.4	n.d.	n.d.
Plasma metabolites and hormones						
Glucose (mmol/l)	7.4 ± 0.3[b]	7.6 ± 0.7	8.1 ± 0.7	7.7 ± 0.7	8.5 ± 0.9	8.2 ± 0.4
Insulin (ng/l)	923 ± 371[a]	1613 ± 669	1819 ± 499	1804 ± 466	n.d.	n.d.
Glucagon (ng/l)	142 ± 31[a]	147 ± 66[a]	74 ± 24	62 ± 14	n.d.	n.d.

[a] Rats studied were either bile duct ligated (BDL), sham-operated control rats pair-fed to BDL rats (pair-fed CON) or control rats fed ad libitum (ad libitum-fed CON). Tissue glycogen was determined as glucose after enzymatic hydrolysis and hormone concentrations by a RIA as described in Section 2. Data are given as mean ± SD. Significance: [a]$P < 0.05$, BDL vs. pair-fed CON; [b]$P < 0.05$, BDL vs. ad libitum-fed CON; [c]$P < 0.05$, pair-fed CON vs. ad libitum-fed CON.

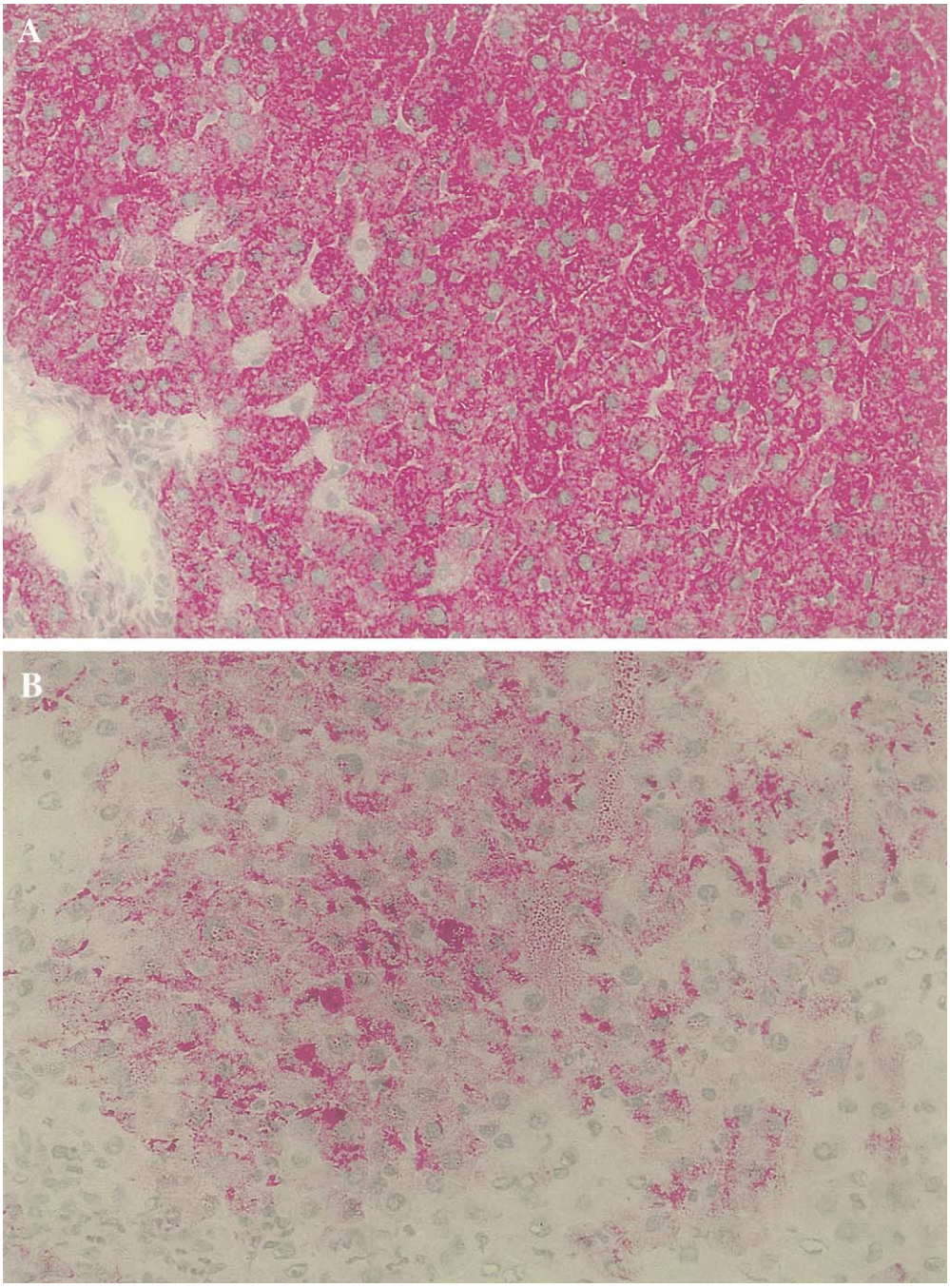

Fig. 2. Histological analysis of the hepatic distribution of glycogen. Liver tissue was fixed in alcohol and stained with PAS. Sections pretreated with diastase served as controls and showed no difference between livers from BDL and CON rats. (A) Liver tissue from a pair-fed CON animal 8 days after surgery. PAS reactivity for glycogen is diffuse (no zonal distribution) and equal for most hepatocytes. (B) Liver tissue from a BDL rat 8 days after surgery. PAS staining for glycogen in the parenchymal areas (center of the figure) is reduced in comparison to the control rat in (A). The adjacent non-hepatocyte tissue consists of fibrosed portal tracts and septa showing marked ductular proliferation.

4. Discussion

Our study shows that the hepatic glycogen content is decreased in rats with acute cholestasis as a consequence of both reduced food intake and cholestasis. In contrast to liver, skeletal muscle glycogen content is not affected.

Four days after surgery, the glycogen content expressed per ml hepatocytes or g liver was not different between BDL and pair-fed CON rats. However, in comparison to ad libitum-fed CON rats, both BDL and pair-fed CON rats had a significantly decreased hepatic glycogen content. Since the only difference between pair-fed and ad libitum-fed CON rats is the amount of food ingested (the surgical procedure was the same), reduced food intake has to explain the differ-

Table 4
Glycogen synthase and phosphorylase activity in liver and skeletal muscle[a]

	BDL		Pair-fed CON		Ad libitum-fed CON	
	4 days ($n = 12$)	8 days ($n = 9$)	4 days ($n = 8$)	8 days ($n = 8$)	4 days ($n = 5$)	8 days ($n = 5$)
Glycogen synthase						
Active (a) form (U/g liver)	0.036 ± 0.006[ab]	0.028 ± 0.013[ab]	0.062 ± 0.014	0.063 ± 0.010	0.061 ± 0.017	0.070 ± 0.007
Total (a + b) activity (U/g liver)	0.11 ± 0.02[ab]	0.10 ± 0.03[ab]	0.21 ± 0.03	0.21 ± 0.02	0.20 ± 0.02	0.21 ± 0.01
Active (a) form (U/g muscle)	0.075 ± 0.042	0.094 ± 0.043	0.089 ± 0.05	0.086 ± 0.037	n.d.	n.d.
Total (a + b) activity (U/g muscle)	0.25 ± 0.10	0.26 ± 0.08	0.25 ± 0.09	0.26 ± 0.10	n.d.	n.d.
Glycogen phosphorylase						
Active (a) form (U/g liver)	18.2 ± 2.5[ab]	13.3 ± 4.6[ab]	25.0 ± 3.6	25.4 ± 3.0	26.6 ± 3.9	28.8 ± 3.6
Total (a + b) activity (U/g liver)	19.7 ± 2.0[ab]	15.6 ± 4.3[ab]	28.5 ± 3.2	27.3 ± 2.8	31.3 ± 4.3	32.6 ± 3.1
Active (a) form (U/g muscle)	49.4 ± 22.6	47.0 ± 21.7	46.4 ± 19.8	56.8 ± 18.6	n.d.	n.d.
Total (a + b) activity (U/g muscle)	84.4 ± 28.7	86.2 ± 30.4	82.4 ± 25.7	91.4 ± 29.1	n.d.	n.d.

[a] Rats studied were either bile duct ligated (BDL), sham-operated control rats pair-fed to BDL rats (pair-fed CON) or control rats fed ad libitum (ad libitum-fed CON). Enzyme activities were determined using radioactive substrates as described in Section 2. Data are given as mean ± SD. Significance: [a]$P < 0.05$, BDL vs. pair-fed CON; [b]$P < 0.05$, BDL vs. ad libitum-fed CON.

ences in the hepatic glycogen content 4 days after surgery. The question remains why BDL rats ingest less food than ad libitum-fed control rats. This is not a consequence of anesthesia or laparotomy, since the CON rats had undergone exactly the same procedures as the BDL rats. Reduced food intake in BDL rats is therefore likely to be due to the specific surgical procedure (ligation of the common bile duct) and/or cholestasis.

Already 4 days after surgery, the hepatic mRNA expression and activity of glycogen synthase and phosphorylase were decreased in BDL as compared to CON rats. Since, as discussed above, the hepatic glycogen content per g liver was not different between BDL and pair-fed CON rats at this time point, the decreased activity of glycogen synthase was not associated with a significant decrease in the hepatic glycogen stores in BDL rats. It is possible that such a decrease was prevented by the contemporary decrease in the activity of glycogen phosphorylase, or, more likely, that it was overridden by the effect of the reduction in food intake.

Table 5
Hepatic mRNA content of glycogen synthase and phosphorylase[a]

	BDL		Pair-fed CON
	4 days ($n = 3$)	8 days ($n = 3$)	4 days ($n = 8$)
Glycogen synthase			
Relative density	0.15 ± 0.13[a]	0.22 ± 0.12[a]	0.67 ± 0.39
Glycogen phosphorylase			
Relative density	0.24 ± 0.06[a]	0.37 ± 0.05[a]	2.05 ± 0.72

[a] Rats studied were either bile duct ligated (BDL) or sham-operated control rats pair-fed to BDL rats (pair-fed CON). The values shown are the densities (unitless) of the bands on the Northern blots prepared as described in Section 2. Values were normalized to the values obtained for GAPDH. The control groups (4 days and 8 days after surgery) were pooled since the values were not different for the two time points. Data are given as mean ± SD. Significance: [a]$P < 0.05$, BDL vs. pair-fed CON.

The activity of glycogen synthase and phosphorylase is regulated short-term by phosphorylation and long-term by altered expression [1,6]. Since the active fractions of both glycogen synthase and phosphorylase were not altered in BDL as compared to CON rats, decreased expression but not acute changes in the phosphorylation state of these enzymes is the likely cause of reduced activity. This explanation is in agreement with the results of the Northern blots, which revealed reduced hepatic mRNA levels of both enzymes at 4 and 8 days after surgery in BDL rats. These findings are in agreement with those from rats with long-term cholestasis [32] and indicate that cholestasis and not secondary biliary cirrhosis is the primary cause of decreased expression of these enzymes. Interestingly, contrary to hormonal short-term regulation, which increases the activity of one and decreases the activity of the other enzyme [1], cholestasis decreases mRNA expression and activity of both glycogen synthase and phosphatase at the same time, suggesting a common regulatory pathway.

Similar to humans [41,42] and rats [31] with liver cirrhosis, acute cholestasis was associated with increased plasma concentrations of glucagon in BDL rats, whereas the plasma insulin levels were either decreased or unchanged. Since glucagon accelerates glycogen breakdown in the liver [1,13,16], increased plasma concentrations of this hormone could theoretically explain a decrease in the hepatic glycogen content. If this were the case, however, an increase in the active fraction of glycogen phosphorylase and a corresponding decrease of glycogen synthase would have been expected in BDL rats [1], which was not the case. Further studies are needed to explain why glycogen phosphorylase is irresponsive to glucagon in livers of cholestatic rats. Interestingly, glucagon failed to stimulate glucose production in perfused livers from rats with long-term cholestasis [43].

Eight days after surgery, the glycogen content per ml of hepatocytes or per g liver was clearly decreased in BDL rats if compared to pair-fed or ad libitum fed CON rats, but not

different between the two control groups. Since the intake of food (and also body weight) was not different between BDL and pair-fed CON rats at this time point, this decrease in the hepatic glycogen content is due to cholestasis, which impairs expression and activity of glycogen synthase and phosphorylase. In accordance with these findings and interpretation, our studies in rats with bile duct ligation for 2–4 weeks showed a progressive decrease in the hepatic glycogen content with prolonged duration of cholestasis [31]. A possible explanation for reduced hepatic glycogen stores in BDL rats is endotoxinemia, which has been described in BDL rats also in other studies [44], and which is known to impair hepatic glycogen synthesis [25,45]. Since prostaglandins produced from Kupffer cells mediate the hypoglycogenic effect of endotoxins in the liver [25], the skeletal muscle glycogen stores in BDL rats are maintained despite elevation of endotoxins in peripheral blood.

Physiologically more important than the glycogen content per g liver or ml of hepatocytes, is the glycogen content per whole liver. When the hepatic glycogen stores are exhausted, the body glucose needs must be met by gluconeogenesis, which depends on amino acids mainly from skeletal muscle [46]. Since long-term cholestasis and/or liver cirrhosis are associated with low hepatic glycogen stores [47,48], this may be one of the mechanisms leading to muscle wasting in patients with chronic liver disease [49,50]. Four days after surgery, both pair-fed and BDL rats had a lower total liver glycogen content than ad libitum-fed CON rats, whereas 8 days after surgery, total liver glycogen was equal in both CON groups but lower in BDL rats. Due to an increase in liver weight, the reduction in glycogen per liver was less pronounced than the reduction per g liver tissue or volume of hepatocytes in BDL rats. An increase in the volume of hepatocytes per liver at least partially compensates for loss of hepatic glycogen stores during the early phase of bile duct ligation in rats, and may therefore be regarded as a way for the liver to compensate for reduced function.

In contrast to liver, the skeletal muscle glycogen content and the activities of glycogen synthase and phosphorylase did not differ between BDL and CON rats at both time points. These findings are in contrast to patients with alcoholic liver cirrhosis [51] or rats with long-term bile duct ligation [31] where the activity of glycogen synthase or the glycogen content, respectively, have been found to be reduced. This finding indicates that local rather than systemic factors contribute to the alterations in hepatic glycogen metabolism in rats with acute cholestasis. As suggested by the histological pattern, showing that the glycogen content of most hepatocytes was reduced, such factors affect the whole liver and may therefore include high hepatic concentrations of bile acids and/or of endotoxin.

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