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Reduced hepatic glycogen stores in patients with liver cirrhosis

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Abstract: *Background:* Patients with alcoholic liver cirrhosis have reduced hepatic glycogen stores but the mechanisms leading to this finding are not clear. *Methods:* We therefore determined the hepatic glycogen content in patients with alcoholic ($n = 9$) or biliary cirrhosis ($n = 8$), and in control patients undergoing liver surgery ($n = 14$). All patients were in the postabsorptive state. In addition, we performed a morphometric analysis of the livers, and measured activities and mRNA expression of several enzymes involved in glycogen metabolism. Cirrhotic and control patients were similar regarding age and body weight. *Results:* Cirrhotic patients had a reduced glycogen content per gram liver wet weight (17 ± 11 *versus* 45 ± 17 mg/g, $P < 0.05$), per milliliter hepatocytes (28 ± 16 *versus* 52 ± 21 mg/ml, $P < 0.05$) and per liver (28 ± 17 *versus* 64 ± 22 g, $P < 0.05$), the reduction being observed in both patients with alcoholic or biliary cirrhosis. Liver histology confirmed these findings and revealed that the decrease in liver glycogen in cirrhotic patients was not homogenous across cirrhotic lobules. Activities of glycogen synthase and phosphorylase (total activity and active form) were not different between cirrhotic and control patients, whereas hepatic mRNA expression was decreased in cirrhotics by approximately 50%. The activity of glucokinase was decreased in cirrhotic as compared in control patients (0.06 ± 0.30 *versus* 0.42 ± 0.21 U/ml hepatocytes, $P < 0.05$), the reduction being observed in both patients with alcoholic or biliary cirrhosis. *Conclusions:* We conclude that patients with alcoholic or biliary cirrhosis have decreased hepatic glycogen stores per volume of hepatocytes and per liver. Decreased activity of glucokinase may represent an important mechanism leading to this finding.

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Glycogen is the storage form of carbohydrates in liver and skeletal muscle and represents an important source of energy, in particular during exercise and during early starvation (1, 2). In accordance with the importance of glycogen metabolism for fuel homeostasis, glycogen synthesis and breakdown are tightly regulated. The two key enzymes responsible for this regulation are glycogen synthase and glycogen phosphorylase whose activities are controlled by phosphorylation and dephosphorylation. Glycogen synthase is activated by metabolites such as adenosine monophosphate (AMP) (3) or glucose-6-phosphate (4), and by insulin which stimulates protein phosphatases (2, 5, 6). Inactivation occurs by protein kinases stimulated by hormones such as glucagon, adrenalin or vasopressin (2, 5). Glycogen phosphorylase is activated by phosphorylation by phosphorylase kinase. Phosphorylase kinase is activated by cAMP-dependent hormones such as glucagon and β-adrenergic agonists, or calcium-dependent agents such as vasopressin, parathyroid hormone, angiotensin II, and α_1-adrenergic and P_2-purinergic agonists (7–11). Glycogenolysis is decreased by insulin which inhibits both the cAMP- and the calcium-dependent pathways (10).

Alterations in hepatic glycogen metabolism have been described in both rats and humans with liver cirrhosis. Rats with CCl_4-induced liver cirrhosis had a decreased hepatic glycogen content and an accelerated metabolic reaction to starvation in one study (12). In another study, the hepatic glycogen content was increased in rats with CCl_4-induced cirrhosis, probably due to a decreased activity of

glycogen phosphorylase (13). The causes leading to this discrepancy between these studies are not known. Rats with secondary biliary cirrhosis have reduced hepatic glycogen stores most probably due to decreased glycogen synthesis (14).

Humans with alcohol-induced liver cirrhosis have also been described to have reduced hepatic glycogen stores (15). Owen et al. studied eight patients with alcohol-induced liver cirrhosis and compared their results with values obtained in two control subjects. Similar to rats with CCl_4-induced cirrhosis (16), glycogenolysis following the administration of glucagon was found to be reduced in cirrhotic patients (17). Two possible reasons for this finding have been discussed: one of them is the reduction in the hepatic glycogen stores and the other one possible defects in signal transduction in hepatocytes of cirrhotic patients (18).

Due to the importance of hepatic glycogen metabolism in cirrhosis and the limited database currently available we decided to study hepatic glycogen metabolism in patients with different types of liver cirrhosis. We intended to answer the following specific questions: (i) can the findings obtained in patients with alcohol-induced liver cirrhosis be confirmed and are they also present in patients with biliary cirrhosis? and (ii) what are the principle mechanisms leading to reduced hepatic glycogen stores in cirrhotic livers: a reduced volume of hepatocytes and/or altered hepatocellular metabolism of glycogen?

Materials and methods

Patients

The studies have been reviewed and accepted by the Ethic's Committee of the State of Berne. Patients with liver cirrhosis ($n = 9$ patients with alcoholic and $n = 8$ patients with biliary cirrhosis) were studied during liver transplantation. Of the patients with biliary cirrhosis, five had primary biliary cirrhosis and three primary sclerosing cholangitis. Ten patients were in stage Child B (five with alcoholic and five with biliary cirrhosis) and seven in stage Child C (four with alcoholic and three with biliary cirrhosis). All Child C and four of the 10 Child B patients had variable amount of ascites which was estimated sonographically and/or determined during transplantation, and subtracted from the body weight determined at entry. In Table 1, the corrected body weight (ascites subtracted) is given. The time interval between the last meal and start of the operation was between 8 and 12 h.

Control patients ($n = 14$) underwent hepatic resection of a colorectal metastasis. None of these patients had a history of liver disease or increased alcohol consumption. Clinical signs of chronic liver disease were absent, and viral hepatitis, chronic cholestasis and autoimmune liver disease were excluded by laboratory analyses in all cases. The time interval between the last meal and surgery in control patients was 10–14 h. All patients (cirrhotics and controls) were treated with a diet containing 3.5 kcal/kg (25–30% as fat, 15% as amino acids and 55–60% as carbohydrates) for at least the last week prior to surgery. None of the patients (cirrhotics or controls) was treated with antidiabetic drugs. Routine inhalation anesthesia with enflurane or isoflurane was performed in all cases. From the liver tissue obtained during surgery a piece was rapidly frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for isolation of RNA, and determination of enzyme activities and glycogen content. The remainder of the biopsy (or the liver in case of the patients undergoing liver transplantation) was used for histological and stereological analysis as described below.

Characterization of the patients

Patients were characterized by their body weight, body mass index, activities of alanine aminotransferase (ALT) and alkaline phosphatase,

Table 1. Characterization of the patients

	Control ($n = 14$)	Cirrhosis ($n = 17$)	Alcohol ($n = 9$)	Biliary ($n = 8$)
Age	62 ± 12	57 ± 10	56 ± 9	58 ± 8
Body weight (kg)	72.8 ± 8.9	76.4 ± 12.7	78.6 ± 10.3	73.9 ± 15.2
Body mass index (kg/m^2)	25.3 ± 1.9	25.7 ± 3.2	25.5 ± 3.2	25.8 ± 3.3
Serum glucose (mmol/l)	9.5 ± 3.0	9.1 ± 4.8	7.9 ± 1.0	10.3 ± 7.0
Serum β-hydroxybutyrate (μmol/l)	0.24 ± 0.09	0.34 ± 0.18	0.36 ± 0.20	0.31 ± 0.17
Serum albumin (g/l)	36 ± 2	27 ± 3*	26 ± 3*	28 ± 3*
Serum bilirubin (μmol/l)	10 ± 7	47 ± 40*	37 ± 27*	59 ± 54*
Serum bile acids (μmol/l)	2 ± 2	35 ± 24*	25 ± 21*	46 ± 24*
Alkaline phosphatase (U/l)	135 ± 65	248 ± 78	177 ± 55	328 ± 82*
Alanine aminotransferase (U/l)	68 ± 32	98 ± 86	96 ± 67	101 ± 88

Patients were studied in the postabsorptive state, 8–14 h after the last intake of food. Data are given as mean ± SD, *$P < 0.05$ *versus* control. Normal values are: serum albumin 35–52 g/l, serum glucose 3.8–6.1 mmol/l, serum bilirubin 5–18 μmol/l, serum bile acids < 6 μmol/l, alkaline phosphatase 31–108 U/l, alanine aminotransferase 10–37 U/l.

concentrations of albumin, bilirubin, bile acids, glucose and β-hydroxybutyrate in serum. Albumin, ALT, alkaline phosphatase and bilirubin were analyzed on a COBAS analyzer (Hoffman-La Roche Diagnostics, Basel, Switzerland). Bile acids were determined with a radioimmunoassay (Becton and Dickinson, Orangeburg, SC). The serum glucose concentrations were determined enzymatically (kit obtained from Sigma Chemicals, Buchs, Switzerland) and the serum β-hydroxybutyrate concentrations fluorimetrically using the method of Olsen (19). The liver weight was determined gravimetrically in the 17 patients undergoing liver transplantation. In the 14 comparison patients, liver weight was estimated according to body weight (liver weight = 2% of body weight) (20). This estimate was compared to the liver volume estimated by computerized tomography and found to differ by no more than 10% in the average.

Tissue preparation and histological analysis

For the stereological analysis, five pieces of liver tissue were obtained by random sampling from each liver biopsy or liver, and fixed in 5% buffered formalin. The samples were embedded into paraffin and five randomly chosen sections from each block were colored with Elastica van Gieson. On each of these sections, at least 100 points were counted (21) and classified as described before (22).

For the localization of hepatic glycogen, liver tissue was fixed with alcohol and embedded into paraffin. Several sections from each block were stained with Periodic Acid – Schiff (PAS) with and without previous treatment with diastase to destroy glycogen (negative control).

Liver glycogen content

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. (23) with the previously reported modifications (12). The glycogen content is expressed as milligram per gram liver wet weight or per milliliter of hepatocytes and as gram glycogen per whole liver.

Enzyme assays

For the determination of the activities of the glycogen synthase and glycogen phosphorylase, frozen liver was homogenized at 0 °C with nine 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. (24) and modified by Guinovart et al. (25). An aliquot of the supernatant was diluted 1:2 (vol:vol) with a solution containing 50 mmol/l 2-(N-morpholino)-ethanesulphonic acid (MES), 50 mmol/l potassium fluoride and 5 mmol/l dithiothreitol (pH 6.1). The resulting solution was assayed for total glycogen phosphorylase activity according to Gilboe et al. (26), and for the active form of glycogen phosphorylase according to Theen et al. (27).

Hexokinase activity was determined spectrophotometrically at glucose concentrations of 0.05 and 100 mmol/l as described by Agius and Tosh (28) and by Lowes et al. (29). Glucokinase activity was calculated as the difference between the hexokinase activities obtained at 100 mmol/l and 0.5 mmol/l glucose (29).

RNA isolation and reverse transcription

Total RNA was extracted from rat liver according to the general protocol of Sambrook et al. (30). The RNA concentration was determined by the absorbance at 260 nm, and the quality of the RNA was controlled by running an aliquot on a 1% agarose formaldehyde gel. Four μg of total RNA from rat liver were used as a template for first-strand cDNA synthesis with reverse transcriptase (Molony Murine Leukemia Virus reverse transcriptase; Gibco BRL, Life Technologies AG, Basel, Switzerland) and oligo (dT) primer.

Real-time quantitative PCR analysis glycogen synthase and phosphorylase

Real-time quantitative PCR analysis was performed with a PE Applied Biosystems 7700 Sequence Detector (PE Biosystems), which is a combined thermocycler and fluorescent detector. Sets of primers were chosen for glycogen synthase and glycogen phosphorylase to obtain a PCR product of less than 100 base pairs. A dual-labelled fluorogenic probe complementary to a sequence within the PCR product was added to the PCR reaction. The primers and the dual-labelled fluorogenic probe for GAPDH, which served as internal standard, were chosen accordingly. One fluorescent dye (6-carboxyfluorescein) serves as a reporter, and its emission is quenched by a second fluorescent dye (6-carboxy-tetramethylrhodamine). During elongation, the 5′ to 3′ exonuclease activity of the Taq DNA

polymerase hydrolyzes the probe, thus releasing the reporter from the quencher, resulting in increased fluorescence which is detected. The forward and reverse primers were: glycogen synthase: TTA AGA AAT TTT CAG CAG TGC ATG AG and TGA CCT CGA ACA AAA TCT TGG A with the probe TCA AAA TCT ACA TGC CAT GTA CAA GGC CAG A; glycogen phosphorylase: AGA GGA AGG AAG CAA AAG GAT CA and TGG ATT TTA GCC ACG CCA TT with the probe CAT CTC TGC ATT GTC GGT TCC CAT GC. For GAPDH, the internal standard, a predeveloped TaqMan Assay Reagent Control Kit was used. Primers and probes were custom-synthesized by PE Biosystems. Complementary DNA was amplified in a 50-µl volume containing 25 µl of the 2 × TaqMan Universal PCR Master Mix (PE Biosystems), 100 nmol/l probe and 300 nmol/l of each primer. After a denaturating step of 10 min at 95 °C, 40 cycles were performed: 95 °C for 15 s and 60 °C for 1 min.

The mathematical analysis of the results was performed according to the Ct method of calculation, where Ct stands for the cycle number at which the fluorescence of the sample crosses a given threshold (see PE Biosystems user bulletin #2). After individual normalization of all values obtained for GAPDH, the mean value for control livers was calculated, arbitrarily set at one and used as a normalization factor for the cirrhotic livers. Since the Ct values for GAPDH were not different between control and cirrhotic livers, GAPDH was considered to be a valuable internal standard.

Statistical evaluation

Results are expressed as mean ± SD unless specified otherwise. Means between two groups (control and cirrhotic patients) were compared by Student's *t*-test after having tested for normal distribution of the data. Subgroup analysis was performed by ANOVA, followed by Scheffé's test. Analysis of mRNA expression was performed by the Mann–Whitney *U*-test since the data showed no normal distribution. A $P < 0.05$ was considered to be statistically significant.

Results

Control and cirrhotic patients are characterized in Table 1. Age, body weights and body mass index were not different between cirrhotic and control patients. Metabolic characterization revealed no differences in glucose and β-hydroxybutyrate serum concentrations. The low values for β-hydroxybutyrate indicate that all patients were in the postabsorptive state but not starving. Cirrhotic patients had decreased albumin serum concentrations as well as higher serum levels of bilirubin and bile acids. The activity of alkaline phosphatase was increased in patients with biliary cirrhosis, while the activities of alanine aminotransferase were not different between cirrhotic and control patients.

A morphometric analysis of the livers was performed to ensure that control patients had a normal liver architecture and to determine the volume fraction (V_v) of hepatocytes, in order to be able to relate the glycogen content and enzyme activities to the volume of hepatocytes (Table 2). As expected from similar studies in rats with liver cirrhosis (14, 22), the volume fraction of hepatocytes was decreased in both types of liver cirrhosis by approximately 30%, while the volume fraction of connective tissue showed a corresponding increase. When expressed as an absolute value (volume per liver), a similar pattern emerged, since the liver volumes were not different between cirrhotic and control patients.

Table 2. Morphometric analysis

	Control ($n = 14$)	Cirrhosis ($n = 17$)	Alcohol ($n = 9$)	Biliary ($n = 8$)
Liver volume (l)	1.46 ± 0.18	1.57 ± 0.29	1.61 ± 0.26	1.53 ± 0.34
Hepatocytes				
V (ml)	1270 ± 190	988 ± 239*	1014 ± 244*	957 ± 247*
V_v (ml/ml)	0.87 ± 0.04	0.63 ± 0.09*	0.63 ± 0.12*	0.62 ± 0.07*
Connective tissue				
V (ml)	139 ± 59	508 ± 175*	535 ± 204*	478 ± 144*
V_v (ml/ml)	0.10 ± 0.04	0.32 ± 0.09*	0.33 ± 0.12*	0.31 ± 0.06*
Rest†				
V (ml)	47 ± 14	73 ± 40*	64 ± 33	94 ± 43*
V_v (ml/ml)	0.03 ± 0.01	0.05 ± 0.02*	0.04 ± 0.02	0.06 ± 0.02*

Morphometric analysis was performed by counting at least 500 points on five different sections of each liver biopsy as described in Methods. Data are given as mean ± SD, and reflect the volume per liver (*V*) or the volume fraction (V_v) of the respective compartment. The density of the liver was set at 1 g/ml for conversion of the liver weight to volume. *$P < 0.05$ *versus* control; †*Rest:* e.g. blood vessels such as sinusoids, portal and central veins, and bile ducts.

The hepatic glycogen content and activities of glycogen synthase and phosphorylase are given in Table 3. When expressed per gram liver wet weight, cirrhotic patients had an approximately 60% decrease in the hepatic glycogen content which was similar in both groups of cirrhotic patients studied. When expressed per milliliter of hepatocytes or per liver, the hepatic glycogen content was decreased by approximately 45% or 55%, respectively, in cirrhotic as compared to control patients. There were no significant correlations between the hepatic glycogen content and body weight, body mass index or Child score in cirrhotic patients.

In contrast to the hepatic glycogen content, total activities, activities of the active forms and the active fractions of glycogen synthase and phosphorylase were not different between cirrhotic and control patients. There were no significant correlations between enzyme activities and hepatic glycogen content of the patients.

As shown in Fig. 1, the hepatic mRNA expression of glycogen synthase and phosphorylase showed a large variation in control livers. In comparison, the variation was clearly smaller in cirrhotic livers. The median mRNA expression of both enzymes was decreased by 30–50% in cirrhotic as compared to control livers. This decrease reached statistical significance for all cirrhotic patients tested as one group and for patients with alcohol-induced cirrhosis for both enzymes.

As shown in Table 3, the activity of the low affinity hexokinase (glucokinase, determined in the presence of 100 mmol/l glucose) was

Table 3. Glycogen metabolism

	Control ($n=14$)	Cirrhosis ($n=17$)	Alcohol ($n=9$)	Biliary ($n=8$)
Glycogen				
mg/g liver wet weight	45 ± 17	17 ± 11*	17 ± 6*	18 ± 16*
mg/ml hepatocytes	52 ± 21	28 ± 16*	27 ± 6*	28 ± 24*
g/liver	64 ± 22	28 ± 17*	28 ± 10*	27 ± 23*
Glycogen synthase				
Total (mU/ml hepatocytes)	193 ± 78	249 ± 85	240 ± 114	258 ± 40
Active (mU/ml hepatocytes)	31 ± 15	36 ± 16	32 ± 17	41 ± 16
Active (% of total)	16 ± 5	14 ± 4	13 ± 3	15 ± 5
Glycogen phosphorylase				
Total (U/ml hepatocytes)	17.7 ± 7.2	21.0 ± 8.7	21.3 ± 10.0	20.7 ± 7.8
Active (U/ml hepatocytes)	15.3 ± 7.3	15.4 ± 7.9	15.1 ± 7.0	14.3 ± 6.6
Active (% of total)	79 ± 11	72 ± 13	72 ± 10	72 ± 16
Hexokinase/Glucokinase				
0.5 mmol/l glucose (U/ml hepatocytes)	1.56 ± 0.28	2.26 ± 0.96*	2.07 ± 0.54*	2.52 ± 1.40*
100 mmol/l glucose (U/ml hepatocytes)	1.98 ± 0.31	2.32 ± 0.97	2.18 ± 0.48	2.50 ± 1.47
Glucokinase (U/ml hepatocytes)	0.42 ± 0.21	0.06 ± 0.30*	0.11 ± 0.37*	−0.02 ± 0.15*

Glycogen and enzyme activities were determined by routine methods as described in the Method section. Activities of glycogen synthase and phosphorylase are expressed as total activity (a + b), and activity of the active part of the enzyme (a). Data are given as mean ± SD, *$P < 0.05$ *versus* control.

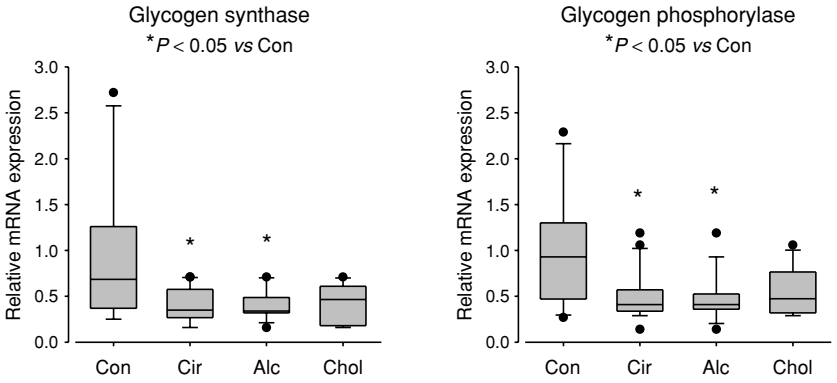

Fig. 1. Hepatic mRNA expression of glycogen synthase and phosphorylase. Steady state mRNA levels of the two enzymes were determined using a TaqMan as described in Methods. Data were first normalized individually to GAPDH mRNA expression. The mean of the values of control patients were then arbitrarily set at one and the values of cirrhotic patients normalized to it. Data analysis was performed by the Mann–Whitney *U*-test, since the values showed no normal distribution. Data are given as median, 25 and 75 percentiles (boxes), 10 and 90 percentiles (bars) and outliers.

significantly decreased in cirrhotic as compared to control patients. In contrast, the high affinity hexokinase activity (determined in the presence of 0.5 mmol/l glucose) was increased in cirrhotic livers.

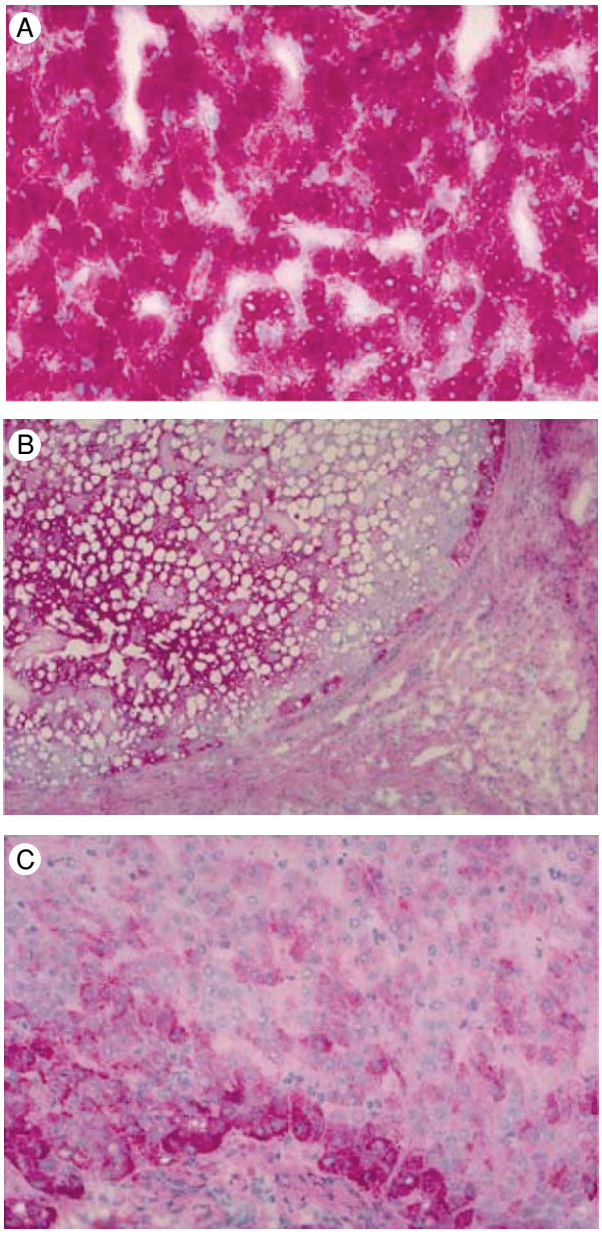

Fig. 2. Glycogen distribution in livers from control (A) and cirrhotic patients (B–C). (A) Shows control liver tissue with marked, diffuse and homogeneous PAS staining for glycogen. (B) Shows a part of a cirrhotic nodule with macrovesicular fatty change of a patient with alcoholic liver cirrhosis. In this situation, PAS reactivity for glycogen is heterogeneous, also in hepatocytes not showing steatotic change. (C) Shows a PAS stain of a patient with primary biliary cirrhosis. Note that marked PAS reactivity (indicating a high glycogen content) is seen in peripheral parts of a nodule, whereas more central parts show reduced glycogen staining. Similar changes were seen in patients with primary sclerosing cholangitis (not shown).

The distribution of glycogen within liver tissue is shown in Fig. 2. Figure 2A shows a liver from a control patient with normal liver histology. PAS reactivity is homogeneous and strong. Figure 2B shows the corresponding picture of a patient with alcoholic liver cirrhosis. In this situation, PAS reactivity is heterogeneous, irrespective of the presence of fat in hepatocytes. A similar picture emerges in patients with biliary cirrhosis (Fig. 2C), showing that hepatocytes in the periphery of a nodule have a clearly higher glycogen content than those in the center.

Discussion

Our study demonstrates that the hepatic glycogen content is reduced in patients with alcoholic or biliary cirrhosis and that two factors are responsible for this decrease: loss of hepatocytes and impaired hepatocellular glycogen metabolism.

Since liver tissue cannot be obtained from healthy persons due to ethical reasons, we had to use patients undergoing liver surgery for metastases as a control group. In order to assure that patients with liver cirrhosis and control patients were in a comparable metabolic condition, several precautions were taken. The diet ingested during the week before surgery was similar regarding calories and composition, and the time interval between the last meal and surgery was also tried to keep constant. This could not be realized completely for patients undergoing liver transplantation, for some of them the time interval was close to 8 h and not 10–14 h as for the control patients. If anything, however, the hepatic glycogen content would be higher in patients with a short interval between the last meal and surgery, what would accentuate the difference in the hepatic glycogen between cirrhotic and control patients even more. Since starvation is associated with an increase in the serum β-hydroxybutyrate concentrations (12, 31, 32), this metabolite was determined in serum of control and cirrhotic patients. Regarding the similarly low values obtained in all groups of patients, no patients were starving at the time point of surgery. Most importantly, control patients had an almost identical hepatic glycogen content as healthy control subjects in the postabsorptive state (15, 33, 34), showing that the control patients studied represent a valuable control group.

In agreement with the studies by Owen et al. (15), we could also demonstrate that the hepatic glycogen content expressed per gram liver is reduced in patients with alcoholic liver cirrhosis. Since liver cirrhosis is associated with replacement of hepatocytes by connective tissue (this study and

Ref. [14, 22]), a reduction in the hepatic glycogen could theoretically be explained by reduced liver parenchyma per gram liver only. In order to answer this question, it was necessary to perform a stereological analysis of the livers and to relate the glycogen content and enzyme activities to hepatocellular volume. Our results show clearly that loss of hepatocytes explains the reduction in hepatic glycogen in patients with alcoholic or biliary cirrhosis only partially and that additional mechanisms must be considered.

Glycogen synthase and phosphorylase are two key enzymes for glycogen formation and breakdown, respectively, and are both under the control of kinases and phosphatases whose activity depends on hormonal (systemic) and local factors (1). The maintained or even higher activities of glycogen synthase and phosphorylase (total activities and active fractions) in cirrhotic patients suggest that the differences in the hepatic glycogen content between cirrhotic and control patients cannot be explained by mechanisms acting through activation/deactivation of these enzymes. However, it has to be taken into account that the patients studied were in the postabsorptive phase. Since non-starved cirrhotic patients have higher glucagon serum concentrations than non-starved healthy persons (35), it is possible that patients with liver cirrhosis have a reduced activity of glycogen synthase and/or an increased activity of glycogen phosphorylase in the fed state, explaining the observed reduction in the hepatic glycogen stores. Due to ethical and logistical reasons (the liver samples could be obtained only during surgery), we could study our patients only in the postabsorptive state. In contrast to the respective activities, hepatic mRNA expression of glycogen synthase and phosphorylase was decreased in cirrhotic patients, suggesting an increased translational efficiency and/or increased stability of these two enzymes in cirrhotic livers.

Similar results (reduced hepatocellular glycogen stores and maintained activities of glycogen synthase and phosphorylase) have been obtained in rats with CCl_4-induced liver cirrhosis (12). In contrast, in rats with secondary biliary cirrhosis due to bile duct ligation for 4 weeks, both hepatic glycogen stores and activities of glycogen synthase and phosphorylase were reduced (14), suggesting that impaired synthesis is the most important mechanism for reduced hepatic glycogen stores in this animal model of liver cirrhosis. In contrast to rats with secondary biliary cirrhosis, patients with biliary cirrhosis had maintained activities of glycogen synthase and phosphorylase.

Interestingly, patients with liver cirrhosis had a reduced activity of glucokinase (glucose-6-phosphate formation at 100 mmol/l glucose minus formation at 0.5 mmol/l glucose), whereas the total glucose-6-phosphate formation capacity at 100 mmol/l glucose was not different from control patients. Phosphorylation of glucose in position six represents the first step in the synthesis of glycogen from glucose. Importantly, glucose-6-phosphate, the product of this reaction, is not only a substrate for glycogen synthesis but is also able to stimulate the activity of glycogen synthase. In agreement with our findings, a reduced activity of glucokinase has been described also in other patients with liver cirrhosis (29). Interestingly, the hexokinase activity (in the presence of 0.5 mmol/l glucose) was increased in cirrhotic patients, apparently compensating for the decreased activity of glucokinase. It has to be taken into account, however, that the formation of glucose-6-phosphate is compartmentalized and that glucose-6-phosphate for glycogen synthesis appears to be formed almost exclusively by glucokinase (36). Therefore, although the glucose-6 phosphorylation capacity at 100 mmol/l glucose was not different between cirrhotic and control livers, the reduced activity of glucokinase may be an important factor leading to reduced hepatic glycogen synthesis in patients with liver cirrhosis.

Regarding the pattern of glycogen distribution in liver biopsies, showing on one hand a general decrease per hepatocyte and on the other hand areas almost completely devoid of glycogen, local factors could also contribute to glycogen loss in cirrhotic patients. One possibility, which could account for the observed heterogeneous loss of glycogen in cirrhotic livers, is loss of metabolic zonation due to the formation regenerative nodules (37). It is well established that the hepatic glucose metabolism shows zonal differences across the hepatic lobules, with glucokinase showing a centroacinar and the gluconeogenetic enzymes a periportal distribution (38).

A reduction in the hepatic glycogen content is associated with important metabolic consequences. The transition from the fed to the fasted state develops earlier, since the hepatic glycogen stores are exhausted more rapidly after onset of starvation (12). Therefore, fatty acids become important substrates for energy production in cirrhotics also in the postabsorptive state (15, 39), a time period during which subjects without liver disease have still high hepatic glycogen levels and can produce glucose and energy from hepatic glycogen (18, 39). When the hepatic glycogen stores are exhausted, the glucose needs for glucose-dependent tissues such as erythrocytes or brain have to be met by gluconeogenesis. Important substrates for gluconeogenesis are amino

acids, many of them originating from skeletal muscle. Loss of hepatic glycogen with subsequent accelerated gluconeogenesis may therefore represent a mechanism for muscle wasting in patients with liver cirrhosis (40, 41).

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