Journal of Hepatology 1999; 30: 242–248
Printed in Denmark · All rights reserved
Munksgaard · Copenhagen

Copyright © European Association for the Study of the Liver 1999

Journal of Hepatology
ISSN 0168-8278

The liver carnitine pool reflects alterations in hepatic fatty acid metabolism in rats with bile duct ligation before and after biliodigestive anastomosis

Sandra Wächter[1], Lukas Krähenbühl[2], Markus Schäfer[2] and Stephan Krähenbühl[1]

Departments of [1]Clinical Pharmacology and [2]Visceral and Transplantation Surgery, University Hospital, Berne, Switzerland

Background/Aims: **Rats with long-term bile duct ligation (BDL rats) have impaired hepatic fatty acid metabolism and alterations in carnitine homeostasis. Analysis of the carnitine tissue and body fluid pools was used as a tool to study hepatic fatty acid metabolism in BDL rats and after reversal of bile duct ligation by Roux-en-Y anastomosis for 5 (RY5) or 14 days (RY14)**

Methods: **Control rats were pair-fed to treated rats, and all rats were studied after starvation for 24 h. Carnitine was analyzed by a radioenzymatic method and by high performance liquid chromatography.**

Results: **Both BDL and RY rats had decreased plasma β-hydroxybutyrate concentrations, whereas free fatty acid plasma concentrations were not different from control rats. Free carnitine plasma concentrations were not different between BDL or RY and control rats, whereas acetylcarnitine concentrations were decreased in BDL and RY rats, and showed a positive correlation with the plasma β-hydroxybutyrate concentrations. In comparison to control rats, the total hepatic carnitine content was increased in BDL and RY rats, both when expressed per g tissue and per total liver. This rise in the hepatic carnitine content was due to increases in both free and acylcarnitines, including acetylcarnitine. In comparison to control rats, the hepatic concentration of ß-hydroxybutyrate was decreased in BDL and RY rats, findings compatible with impaired formation of ketone bodies from acetyl-CoA. Urinary excretion of total carnitine was not different between treated and control rats.**

Conclusions: **Hepatic metabolism of fatty acids is impaired in BDL rats and does not recover during the 14 days after Roux-en-Y anastomosis. The increased hepatic carnitine content in BDL and RY rats can best be explained by decreased export of carnitine from the hepatocytes. The alterations in the hepatic carnitine pool and impaired hepatic fatty acid metabolism in BDL and RY rats are compatible with impaired ketogenesis.**

Key words: **Acylcarnitines; Carnitine; Cholestasis; Fatty acid metabolism; Ketogenesis.**

LIGATION OF the bile duct in rats is associated with liver fibrosis and cirrhosis with consecutive portal hypertension (1,2). The liver of these animals (BDL rats) not only has morphological changes, but also shows metabolic abnormalities. Glycogen metabolism is disturbed profoundly, showing a pronounced decrease in the liver glycogen content due to impaired glycogen synthesis (3,4). Previous studies have shown that the plasma concentration of β-hydroxybutyrate is decreased in fasted BDL compared to pair-fed control rats, suggesting impaired mitochondrial metabolism of fatty acids (5). These findings were confirmed by subsequent studies showing impaired production of ketone bodies by isolated hepatocytes and liver mitochondria from BDL rats in the presence of fatty acids with different chain lengths (6). Impaired activities of complex I and III of the electron transport chain were found in liver mitochondria isolated from BDL rats and considered to be responsible for the observed decrease in fatty acid metabolism and ketone body production (6).

Recently we have investigated the reversibility of these changes following Roux-en-Y anastomosis of BDL rats (7). In these studies, we confirmed the defects in the electron transport chain observed earlier and showed that they were reversible at least 14 days after

Received 14 March; revised 24 August; accepted 15 September 1998

Correspondence: Stephan Krähenbühl, Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne, Switzerland.
Tel: +41 31 632 31 91. Fax: +41 31 632 49 97.
E-mail: skraehen@ikp.unibe.ch

reversal of bile duct ligation. However, the plasma ketone body concentration was still decreased in fasted BDL rats 5 and 14 days after reversal of bile duct ligation, suggesting that the primary defect in fatty acid metabolism in BDL rats is not in the mitochondrial electron transport chain.

Carnitine pools of plasma, urine and liver have been shown to reflect the status of the hepatic energy metabolism under a variety of conditions (5,8,9). Tissue carnitine pools are in equilibrium with the respective coenzyme A pools (10), reflecting abnormalities in energy metabolism such as hepatic fatty acid oxidation. Accumulated tissue acyl-CoA's are converted to the respective acylcarnitines, subsequently transported into the plasma and finally excreted in the urine. Previous studies have shown alterations in liver and plasma carnitine pools of BDL rats, which were compatible with impaired hepatic mitochondrial β-oxidation of fatty acids (5). We therefore decided to study carnitine liver, plasma and urine pools in BDL rats and after reversal of biliary obstruction by Roux-en-Y anastomosis in order to answer the following questions: 1. Are the changes in carnitine homeostasis and hepatic fatty acid metabolism reversible after reversing bile duct ligation? and 2. Do the alterations in the carnitine pools allow predictions about the mechanisms leading to impaired hepatic fatty acid metabolism in BDL rats?

Materials and Methods

Animals

Male Sprague-Dawley rats obtained from the 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 (5) and studied after starvation for 24 h. The animal experiments had been approved by the Animal Ethics Board of the State of Berne.

Surgical procedures

Before surgery, rats were fasted overnight but had free access to water until immediately prior to and also after surgery. No antibiotics were administered.

All surgical procedures were performed under combined ether and pentobarbital anesthesia. Bile duct ligation was carried out as described previously (4). After a 3–4-cm midline incision, 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 exposed but not transected.

Roux-en-Y choledochojejunostomy (RY) was performed according to Zimmermann et al. (2). Briefly, after a 3–4-cm midline incision, 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

Three different treatments were performed, and for each group of treated animals a group of pair-fed control animals was studied. The first group were rats with bile duct ligation for 4 weeks (BDL; $n=11$). In the second group, bile duct ligation was reversed by Roux-en-Y anastomosis for 5 days (RY5; $n=7$). In the third group of animals, bile duct ligation was reversed by Roux-en-Y anastomosis for 14 days (RY14; $n=8$). 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 or suspected hepato-renal dysfunction.

Characterization of the animals and biochemical methods

The animals were characterized by their body weights at the end of the experiments, liver weight, spleen weight, activities of alkaline phosphatase and alanine aminotransferase (ALT) in plasma and by the plasma concentration of bilirubin. Alkaline phosphatase, ALT and bilirubin were analyzed on a COBAS analyzer (Hoffman-La Roche Diagnostics, Basel, Switzerland). The plasma bile acid concentration was determined using a commercially available RIA kit (Becton and Dickinson, Orangeburg, USA). The plasma glucose and free fatty acid concentrations were determined enzymatically (kits obtained from Sigma Chemicals, Buchs, Switzerland). The plasma and liver β-hydroxybutyrate concentrations were determined fluorometrically according to Olsen (11). For the determination in liver, the perchloric acid supernatant prepared for the determination of short-chain acylcarnitines was used directly (see below).

Sampling of body fluids and tissue

At the time points indicated in the Results section, rats were starved for 24 h in metabolic cages. After starvation for 12 h, urine was collected during the remaining 12 h of the starvation period. Then 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 previously cooled in liquid nitrogen. All liver biopsy specimens, plasma and urines were kept at $-70°$ C until analysis.

Carnitine determination

The carnitine concentration in plasma, urine and liver was determined by a radioenzymatic method originally described by Cederblad & Lindstedt (12) and modified by Brass and Hoppel (8). Urine was assayed directly for free and total acid soluble carnitine, whereas plasma was first treated with perchloric acid (final concentration 3%, v:v) and centrifuged for 2 min at 10 000 *g*. Liver was homogenized in 3% perchloric acid (v:v) and centrifuged for 2 min at 10 000 *g*. Long-chain acylcarnitines were determined in the resulting pellets after alkaline hydrolysis, and free and short-chain acylcarnitines in the perchloric acid supernatant. Addition of short chain acylcarnitine and free carnitine yields total acid soluble carnitine, and addition of total acid soluble and long-chain acylcarnitine yields total carnitine.

For comparison and specific detection of acylcarnitines such as acetylcarnitine, most determinations were also performed using the high-performance liquid chromatography (HPLC) method described by Minkler & Hoppel (13). The difference between the radioenzymatic and the HPLC methods was generally not larger than 10%. For acetylcarnitine, the values obtained by HPLC are given in the Results section.

Calculations and statistics

Data are presented as mean±SD. Means were compared by ANOVA followed by Scheffé's test in the case of a significant ANOVA. An α-value <0.05 was considered to be statistically significant.

Results

Carnitine homeostasis was characterized in rats 4 weeks after bile duct ligation and after reversal of bile

TABLE 1

Characterization of the rats. Rats were either bile duct ligated (BDL), or studied 5 (RY5) or 14 days (RY14) after reversal of bile duct ligation by Roux-en-Y anastomosis. Control rats were pair-fed to treated rats and all rats were studied after starvation for 24 h

	CON (n=23)	BDL (n=11)	RY5 (n=7)	RY14 (n=8)
Body weight (g)	372±31	375+45	345±33	379±43
Liver weight (g)	9.7±1.5	22.2±4.2*	12.1+2.8*	11.3±1.9
Spleen weight (g)	0.65±0.09	1.86±0.40*	1.54±0.44*	1.56±0.52*
Bilirubin (μmol/l)	1±1	75±49*	29±28*	1±2
Bile acids μmol/l)	1±1	55±22*	15±9*	3+2
Alkaline phosphatase (U/l)	163±71	519±162*	402±245*	268±199
Alanine aminotransferase (U/l)	43±10	146±57*	49±27	47±11

* $p<0.05$ vs CON.

TABLE 2

Plasma concentrations of different metabolites. Rats were either bile duct ligated (BDL), or studied 5 (RY5) or 14 days (RY14) after reversal of bile duct ligation by Roux-en-Y anastomosis. Control rats were pair-fed to treated rats and all rats were studied after starvation for 24 h

	CON (n=12)	BDL (n=9)	RY5 (n=6)	RY14 (n=5)
Glucose (mmol/l)	7.7±3.2	7.1±1.6	8.3±1.5	8.5±1.7
β-Hydroxybutyrate (mmol/l)	1.03±0.33	0.19±0.11*	0.30±0.21*	0.19±0.11*
Free fatty acids (mmol/l)	0.98±0.19	0.91±0.15	0.84±0.32	0.82±0.29

* $p<0.05$ vs CON.

TABLE 3

Plasma carnitine concentrations. Rats were either bile duct ligated (BDL), or studied 5 (RY5) or 14 days (RY14) after reversal of bile duct ligation by Roux-en-Y anastomosis. Control rats were pair-fed to treated rats and all rats were studied after starvation for 24 h. Carnitine concentrations were determined radioenzymatically or by HPLC (acetylcarnitine) as described in Methods

	CON (n=23)	BDL (n=11)	RY5 (n=7)	RY14 (n=8)
Free carnitine (μmol/l)	50.2±7.0	55.9±12.6	47.1±11.3	51.5±6.9
Short-chain acylcarnitine (SCA) (μmol/l)	40.9±13.7	25.6±7.9*	27.2±10.4*	22.2±6.6*
Total acid soluble carnitine (TAS) (μmol/l)	91.1±15.7	81.5±13.7	74.3±15.7*	73.7±12.3*
Acetylcarnitine[1] (μmol/l)	36.2±8.1	16.4±5.2*	13.7±3.4*	17.1±5.0*
Long-chain acylcarnitine (μmol/l)	7.4±1.1	7.0±1.3	5.7±1.1*	6.7±1.4
Total carnitine (μmol/l)	98.5±16.3	88.5+14.2	80.0±16.2*	80.5±13.0*

* $p<0.05$ vs CON.
[1]Acetylcarnitine was determined in n=9 BDL, n=6 RY5, n=5 RY14 and n=17 control rats.

TABLE 4

Hepatic content of carnitine and β-hydroxybutyrate. Rats were either bile duct ligated (BDL), or studied 5 (RY5) or 14 days (RY14) after reversal of bile duct ligation by Roux-en-Y anastomosis. Control rats were pair-fed to treated rats and all rats were studied after starvation for 24 h. Carnitine concentrations were determined radioenzymatically or by high performance liquid chromatography (acetylcarnitine), β-hydroxybutyrate fluorometrically as described in Methods

	CON (n=23)	BDL (n=11)	RY5 (n=7)	RY14 (n=8)
Free carnitine (nmol/g)	226±68	318±48*	262±69	220±48
Short-chain acylcarnitine (SCA) (nmol/g)	163±47	298±47*	258±75*	290+82*
Total acid soluble carnitine (TAS) (nmol/g)	390±51	615±79*	520±134*	510±66*
Acetylcarnitine[1] (nmol/g)	32±22	114±21*	79±33*	70±25*
Long-chain acylcarnitine (nmol/g)	55±16	171±87*	171±44*	85±12*
Total carnitine (nmol/g)	444±52	786±147*	691±156*	595±65*
Acetylcarnitine/total carnitine	0.072±0.015	0.145±0.037*	0.114±0.026*	0.118±0.018*
Long-chain acylcarnitine/total carnitine	0.124±0.027	0.218+0.041*	0.247±0.066*	0.143±0.025
β-Hydroxybutyrate (nmol/g)	1890±460	740±260*	770±450*	1050±320*

* $p<0.05$ vs CON.
[1]Acetylcarnitine was determined in n=9 BDL, n=6 RY5, n=5 RY14 and n=17 control rats.

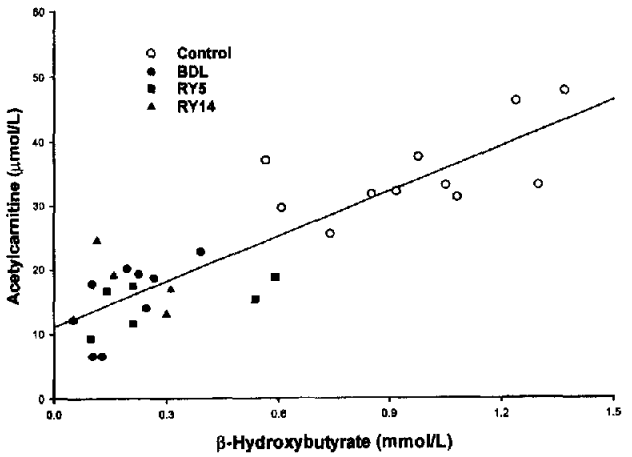

Fig. 1. Relationship between plasma β-hydroxybutyrate and acetylcarnitine concentrations. Rats were either bile duct ligated (BDL) or studied 5 (RY5) or 14 days (RY14) after reversal of biliary obstruction by Roux-en-Y anastomosis. All rats were studied after 24 h of starvation. There is a linear correlation between the two variables (y= 11.4+23.7 x, r=0.901).

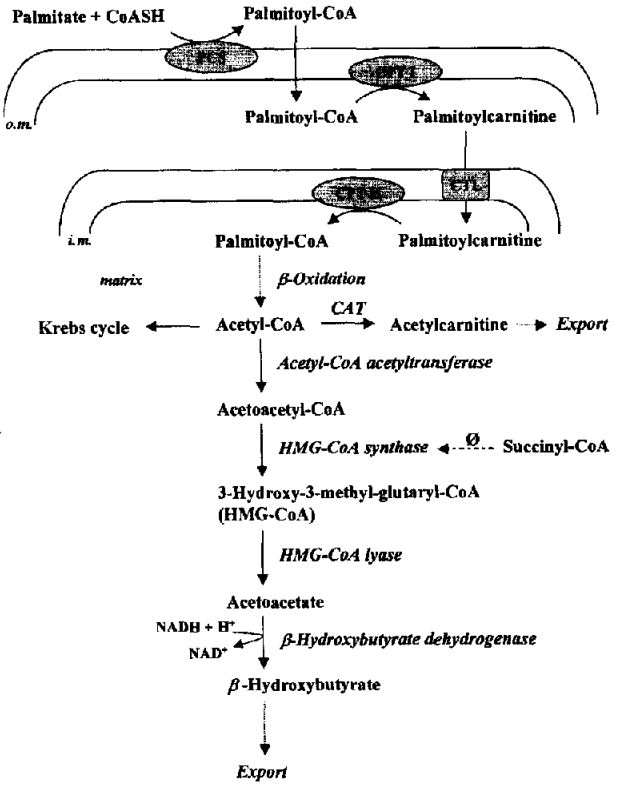

Fig. 2. Hepatic mitochondrial fatty acid metabolism and formation of ketone bodies. Long-chain fatty acids are activated on the outer mitochondrial membrane and transported into the mitochondrial matrix as the carnitine derivative. After reconversion to the CoA derivative, they undergo β-oxidation and acetyl-CoA is formed. Acetyl-CoA can be converted to acetylcarnitine or to ketone bodies. During starvation, degradation by the tricarboxylic acid cycle is negligible. The activity of HMG-CoA synthase is rate-limiting for ketogenesis and is inhibited by succinylation. Acetylcarnitine and β-hydroxybutyrate can be exported from the mitochondria and from the hepatocytes into the blood. PCS=palmitoyl-CoA synthase, CPT I=carnitine palmitoyltransferase I, CPT II=carnitine palmitoyltransferase II, CTL=carnitine:acylcarnitine translocase, o.m.=outer mitochondrial membrane, i.m.=inner mitochondrial membrane, matrix=mitochondrial matrix.

duct ligation by Roux-en-Y anastomosis for 5 or 14 days and compared to pair-fed control rats. Since the control rats at all three time points studied were not different from each other, they are given as one group of animals. The rats used in this study are characterized in Table 1. Body weight was not different between treated and control rats. In contrast, liver and spleen weights were both increased in BDL rats. Liver weight normalized after reversing biliary obstruction, whereas spleen weight did not normalize completely.

Serum bilirubin, alkaline phosphatase and alanine aminotransferase were all elevated in BDL rats but normalized completely 5 or 14 days after reversal of biliary obstruction.

Compatible with a previous study, the plasma glucose and free fatty acid concentrations (Table 2) were

TABLE 5

Urinary carnitine excretion. Rats were either bile duct ligated (BDL), or studied 5 (RY5) or 14 days (RY14) after reversal of bile duct ligation by Roux-en-Y anastomosis. Control rats were pair-fed to treated rats and all rats were studied after starvation for 24 h. Carnitine concentrations were determined radioenzymatically or by high performance liquid chromatography (acetylcarnitine) as described in Methods

	CON (n=14)	BDL (n=6)	RY5 (n=5)	RY14 (n=3)
Free carnitine (μmol/24 h)	1.15±1.07	0.84±0.48	1.52±1.01	0.86±0.31
Short-chain acylcarnitine (SCA) (μmol/24 h)	0.47±0.37	1.01±0.43*	1.39±0.56*	0.74±0.36
Total acid soluble carnitine (TAS) (μmol/24 h)	1.62±1.32	1.85±0.83	2.91±1.56	1.50±0.63
Acetylcarnitine (μmol/24 h)	0.03±0.02	0.03±0.01	n.d.	n.d.
SCA/TAS	0.35±0.21	0.56±0.09*	0.53±0.07*	0.49±0.13

* $p<0.05$ vs CON.

not different between BDL and control rats (5). However, the plasma β-hydroxybutyrate concentration was decreased in BDL rats, a finding suggesting decreased hepatic mitochondrial β-oxidation of fatty acids. After reversal of biliary obstruction, the plasma β-hydroxybutyrate concentration remained decreased in BDL rats.

Since it is known that the plasma, urine and liver carnitine pools are affected by metabolic disturbances such as impaired β-oxidation, carnitine homeostasis was characterized in detail in these animals. As shown in Table 3, treated rats had lower total acid soluble and total carnitine plasma concentrations when compared to control rats. This decrease was due to reduced short-chain acylcarnitines, in particular reduced acetylcarnitine. As illustrated in Fig. 1, the plasma ß-hydroxybutyrate concentration showed a positive correlation with the acetylcarnitine concentration. It is noteworthy that a similar correlation has been described in rats after different periods of fasting (8).

In agreement with a previous study (5), the total carnitine content expressed per g liver was increased in BDL rats by 77% (Table 4). When expressed per total liver, the carnitine content increased from 4.3 mmol/liver in control to 17.4 mmol/liver in BDL rats. This increase was due to both free and acylcarnitines, including long-chain acylcarnitines and acetylcarnitine. After reversal of bile duct ligation, the total carnitine content decreased, but did not normalize completely over 14 days. The free carnitine content had normalized already 5 days after reversal, whereas the acetylcarnitine and long-chain acylcarnitine contents remained elevated 5 and 14 days after biliodigestive anastomosis. The ratio acetylcarnitine/total carnitine was increased in livers from BDL rats and remained increased at both time points after biliodigestive anastomosis. The ratio long-chain acylcarnitine/total carnitine was increased in BDL rats and 5 days but not 14 days after Roux-en-Y anastomosis. Similar to the plasma concentrations, the hepatic content of β-hydroxybutyrate was decreased by a factor of 2 to 3 in treated as compared to control rats.

The urinary excretion of carnitine is shown in Table 5. Excretion of carnitine was not different between control and treated rats, primarily reflecting the effect of pair-feeding, since mammals ingest most carnitine from the diet and only a minor fraction is generated by biosynthesis (14). BDL rats excreted a significantly higher amount of short-chain acylcarnitines, of which only a small part was acetylcarnitine. Also, after 5 days of reversal of biliary obstruction, short-chain acylcarnitine excretion was still elevated, but had normalized after 14 days.

Discussion

The study demonstrates that BDL rats have an increased hepatic carnitine pool, whereas the hepatic content and the plasma concentration of β-hydroxybutyrate are both decreased. These changes persist for 14 days after reversal of biliary obstruction.

The increase in the hepatic carnitine pool of BDL rats was considerable, approximately by a factor 2 when expressed per g liver and by a factor of 4 when expressed per total liver. Although it cannot be excluded with certainty that this increase is due to proliferation of bile ducts observed in this model of biliary cirrhosis (1,2), this appears unlikely since bile ducts account for not more than approximately 20% of the hepatic volume after 4 weeks of bile duct ligation. Furthermore, the carnitine content of epithelia which are not directly involved in energy metabolism (such as bile duct cells) is considered to be lower than that of cells from tissues with a high content of mitochondria which are involved directly in energy metabolism (such as hepatocytes) (15). It is therefore much more likely that the observed increase in the hepatic carnitine content in BDL rats is due to accumulation of carnitine in hepatocytes. This is certainly the case after relief of bile duct obstruction, since bile duct proliferation is almost completely reversible 5 and 14 days after Roux-en-Y anastomosis (2).

Since in the rat, the liver is the most important organ for the conversion of butyrobetaine to carnitine (16–18), this increase could be due to increased carnitine biosynthesis. However, carnitine biosynthesis has been found to be reduced in BDL rats (14), excluding this possibility. Since the amount of carnitine or carnitine precursors provided by the diet was not different between treated and control rats (the rats were pair-fed), the observed increase in the hepatic carnitine content in BDL/RY rats may result from decreased hepatic metabolism and/or decreased hepatic excretion of carnitine. In the liver of mammals, carnitine can only be acylated but is not degraded (15), excluding the first possibility. On the other hand, studies with perfused rat livers have revealed a low affinity transport system which exports carnitine actively from hepatocytes to sinusoidal blood (19). In addition, carnitine is also excreted into bile (20), but this transport has so far not been characterized kinetically. In this context, it is important to note that the hepatic carnitine content per g liver had not completely normalized over 2 weeks after relief of biliary obstruction. At this time point, the serum bilirubin and bile acid concentrations had normalized completely, indicating that the function of the respective carrier systems in the canalicular membrane of the hepatocytes had recovered. This obser-

vation is compatible with the concept that long-term cholestasis affects carnitine excretion, not only across the canalicular, but also across the sinusoidal membrane of the hepatocytes.

As in our previous studies, we observed a decreased plasma concentration of β-hydroxybutyrate in BDL rats, whereas the plasma free fatty acid concentration was not different from control rats, compatible with impaired hepatic fatty acid metabolism (5,7). The increases in the hepatic long-chain acylcarnitine content and in the long-chain acylcarnitine/total carnitine ratio in BDL rats indicate that long-chain fatty acids reached the liver in sufficient amount and could be converted into their carnitine derivative. A significant impairment of the function of carnitine palmitoyltransferase I, a rate-limiting enzyme of mitochondrial fatty acid oxidation which converts long-chain acyl-CoAs to their carnitine derivative (see Fig. 2), appears therefore not to be the cause of reduced fatty acid oxidation in BDL rats. This is in agreement with the observation that the hepatic content of acetylcarnitine and the acetylcarnitine/total carnitine ratio were increased in BDL rats, suggesting that the production of acetyl groups from long-chain fatty acids was not significantly impaired in livers of BDL rats. The most likely location of the metabolic block in hepatic metabolism of fatty acids in BDL rats is therefore the formation of ketone bodies from acetyl-CoA. As shown in Fig. 2, hepatic ketogenesis is catalyzed by the mitochondrial enzymes acetyl-CoA acetyltransferase, HMG-CoA synthase, HMG-CoA lyase and β-hydroxybutyrate dehydrogenase. Short-term regulation of ketogenesis is achieved by inhibition by insulin and vasopressin and by stimulation by glucagon, and long-term regulation by control of the expression of the rate-limiting enzyme, HMG-CoA synthase (21,22). It is well established that glucagon activates HMG-CoA synthase by decreasing the extent of succinylation of the enzyme, a reaction mediated by the cAMP-dependent type 2 glucagon receptors (22,23). The precise mechanisms by which insulin and vasopressin increase succinylation of HMG-CoA synthase are less well established, but may involve Ca^{2+}- dependent pathways in the case of vasopressin (22). Since humans and rats with liver cirrhosis have increased serum concentrations of all of these three hormones mentioned (4,24–26), the inhibitory effect of insulin and vasopressin may override the stimulation by glucagon, resulting in a reduced activity of HMG-CoA synthase, and consequently impaired ketogenesis. Further studies are under way to elucidate the mechanism of impaired ketogenesis in BDL rats on the molecular level.

As shown in Fig. 1, we observed a positive correlation between the plasma acetylcarnitine and β-hydroxybutyrate concentrations. A similar relationship has been described in rats fasted for different periods of time (8). During starvation, production of acetyl-CoA increases in liver mitochondria due to accelerated β-oxidation of fatty acids. Since mitochondrial acetyl-CoA is the precursor of both, acetylcarnitine and ketone bodies such as β-hydroxybutyrate, a positive correlation between these two metabolites can be expected. When the formation of ketone bodies is impaired, more mitochondrial acetyl-CoA can enter the Krebs cycle and can also be converted to acetylcarnitine (see Fig. 2), leading to an increase in the hepatic acetylcarnitine content, as observed in livers of BDL rats Acetylcarnitine is exported from the mitochondria into the cytoplasm, and from the cytoplasm of hepatocytes into the blood across the sinusoidal membrane (19,27). Similar to free carnitine, the observed decrease in the plasma acetylcarnitine concentration in BDL rats is therefore explained best by impaired transport of acetylcarnitine across the sinusoidal membrane of hepatocytes. Increased uptake and metabolism of acetylcarnitine by extrahepatic tissues offers an additional possibility, but this mechanism would not explain the observed increase in the hepatic concentration of acetylcarnitine. An increase in renal excretion of acetylcarnitine was ruled out directly by the determination of the acetylcarnitine excretion in the 24-h urine (see Table 5). Following this argument, the positive correlation between the plasma acetylcarnitine and β-hydroxybutyrate concentration in BDL and RY rats can be explained best by the concept that long-term cholestasis inhibits both formation of ketone bodies and hepatic excretion of acetylcarnitine.

In conclusion, hepatic fatty acid metabolism is impaired in BDL rats and remains decreased up to 14 days after Roux-en-Y anastomosis. Analysis of the hepatic carnitine pool reveals that impaired ketogenesis is the most likely mechanism. Further studies are necessary to characterize this defect in more detail on the molecular level.

Acknowledgements

We thank Alex Jeger and Monika Ledermann for technical assistance. The study was supported by grants from the Swiss National Science Foundation to S.K. (31–46792.96) and to L.K. (3200–051127.97–1).

References

1. Gross JB, Reichen J, Zeltner TB, Zimmermann A. The evolution of changes in quantitative liver function tests in a rat model of biliary cirrhosis: correlation with morphometric measurement of hepatocyte mass. Hepatology 1987; 7: 457–63.

2. Zimmermann H, Reichen J, Zimmermann A, Sägesser H, Thenisch B, Höflin F. Reversibility of secondary biliary fibrosis by biliodigestive anastomosis in the rat. Gastroenterology 1992; 103: 579–89.
3. Krähenbühl L, Krähenbühl S. Rapid normalization of the hepatic glycogen metabolism in rats with biliary cirrhosis after biliodigestive anastomosis. J Hepatol 1997; 26(Suppl 1): 75.
4. Krähenbühl L, Talos C, Reichen J, Krähenbühl S. Progressive decrease in tissue glycogen content in rats with long-term cholestasis. Hepatology 1996; 24: 902–7.
5. Krähenbühl S, Brass EP. Fuel homeostasis and carnitine metabolism in rats with secondary biliary cirrhosis. Hepatology 1991; 14: 927–34.
6. Krähenbühl S, Talos C, Reichen J. Mechanisms of impaired hepatic fatty acid metabolism in rats with long-term bile duct ligation. Hepatology 1994; 19: 1272–81.
7. Krähenbühl L, Schäfer M, Krähenbühl S. Reversibility of hepatic mitochondrial damage in rats with long-term cholestasis. J Hepatol 1998; 28: 1000–7.
8. Brass EP, Hoppel CL. Carnitine metabolism in the fasting rat. J Biol Chem 1978; 253: 2688–93.
9. Brass EP, Hoppel CL. Relationship between acid-soluble carnitine and coenzyme A pools *in vivo*. Biochem J 1980; 190: 495–504.
10. Pearson DJ, Tubbs PK. Carnitine and derivatives in rat tissues. Biochem J 1967; 105: 953–63.
11. Olsen C. An enzymatic fluorometric micromethod for the determination of acetoacetate, β-hydroxybutyrate, pyruvate and lactate. Clin Chim Acta 1971; 33: 293–300.
12. Cederblad G, Lindstedt S. A method for the determination of carnitine in the picomolar range. Clin Chim Acta 1972; 37: 235–43.
13. Minkler PE, Hoppel CL. Quantification of free carnitine, individual short- and medium-chain acylcarnitines, and total carnitine in plasma by high-performance liquid chromatography. Anal Biochem 1993; 212: 510–8.
14. Krähenbühl S, Brass EP, Hoppel CL. A specific renal defect in carnitine biosynthesis in rats with secondary biliary cirrhosis. J Hepatol 1992; 16: S71.
15. Bremer J. Carnitine: metabolism and functions. Physiol Rev 1983; 63: 1421–66.
16. Cox RA, Hoppel CL. Carnitine and trimethylaminobutyrate synthesis in rat tissues. Biochem J 1974; 142: 699–701.
17. Lindstedt G. Hydroxylation δ-buytrobetaine to carnitine in rat liver. Biochemistry 1967; 6: 1271–82.
18. Zaspel BJ, Sheridan KJ, Henderson LM. Transport and metabolism of carnitine precursors in various organs of the rat. Biochem Biophys Acta 1980; 631: 192–202.
19. Sandor A, Kispal G, Melegh B, Alkonyi I. Release of carnitine from the perfused rat liver. Biochim Biophys Acta 1985; 835: 83–91.
20. Hamilton JJ, Hahn P. Carnitine and carnitine esters in rat bile and human duodenal fluid. Can J Physiol Pharmacol 1986; 65: 1816–20.
21. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 1997; 244: 1–14.
22. Guzman M, Geelen MJH. Regulation of fatty acid oxidation in mammalian liver. Biochim Biophys Acta 1993; 1167: 227–41.
23. Quant PA, Tubbs PK, Brand MD. Glucagon activates mitochondrial 3-hydroxy-3methylglutaryl-CoA synthase *in vivo* by decreasing the extent of succinylation of the enzyme. Eur J Biochem 1990; 187: 169–74.
24. Kim JK, Summer SN, Howard RL, Schrier RW. Vasopressine gene expression in rats with experimental cirrhosis. Hepatology 1993; 17: 143–7.
25. Kruszynska YT, Home PD, McIntyre N. Relationship between insulin sensitivity, insulin secretion and glucose tolerance in cirrhosis. Hepatology 1991; 14: 103–11.
26. Letiexhe MR, Scheen AJ, Gerard PL, Bastens BH, Pirotte J, Belaiche J, et al. Insulin secretion, clearance, and action on glucose metabolism in cirrhotic patients. Clin Endocrinol Metab 1993; 77: 1263–8.
27. Sandor A, Cseko J, Kispal G, Alkonyi I. Surplus acylcarnitines in the plasma of starved rats derive from the liver. J Biol Chem 1990; 265: 22313–6.