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Reversibility of hepatic mitochondrial damage in rats with long-term cholestasis

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Background/Aims: **Long-term bile duct ligation in rats is associated with secondary biliary cirrhosis and metabolic alterations, e.g. mitochondrial dysfunction. We performed the current studies to characterize the reversibility of hepatic mitochondrial dysfunction after reversing biliary obstruction by Roux-en-Y anastomosis.**

Methods: **Rats were studied after 4 weeks of bile duct ligation, and after 5 or 14 days of reanastomosis. Control rats were pair-fed to treated rats and all rats were studied after starvation for 24 h. Mitochondria were isolated by differential centrifugation and enzyme activities determined by spectrophotometric methods.**

Results: **In comparison to controls, plasma β-hydroxybutyrate concentrations were decreased in bile duct ligated rats (200±70 vs. 790±200 μmol/l) and remained decreased after relief of biliary obstruction. In contrast, plasma free fatty acids were not different between controls and treated rats. Oxidative metabolism of L-glutamate, succinate and duroquinol was decreased in liver mitochondria from bile duct ligated rats. After relief of biliary obstruction, the metabolism of L-glutamate and duroquinol normalized quickly, whereas succinate metabolism remained impaired. Similar results were obtained for the mitochondrial oxidases in disrupted mitochondria. The activities of complex I, II, III and V of the respiratory chain were reduced in bile duct ligated rats. After relief of biliary obstruction, complex I and III normalized quickly, whereas complex II and V remained impaired. Oxidative metabolism of long-chain fatty acids by isolated liver mitochondria was decreased in bile duct ligated rats and did not recover after relief of biliary obstruction.**

Conclusions: **Long-term cholestasis in the rat is associated with a decrease in specific functions of liver mitochondria which recover only partially after Roux-en-Y anastomosis. The persistence of decreased mitochondrial fatty acid metabolism cannot be explained by impaired activity of the respiratory chain, but is more likely due to alterations in mitochondrial β-oxidation.**

Key words: **Biliary cirrhosis; Biliodigestive anastomosis; Cholestasis; Electron transport; Mitochondria; Reversibility.**

Chronic obstruction of the common bile duct is associated with proliferation of bile ducts, liver fibrosis and cirrhosis, and with portal hypertension in rats and humans (1–4). Ductular proliferation, fibrosis and portal hypertension are present already 2 weeks after bile duct ligation (1,5,6), whereas the development of secondary biliary cirrhosis is usually complete only after 4 weeks (1,6). Several studies have been published about the reversibility of these changes after relief of bile duct ligation by Roux-en-Y anastomosis. These studies have shown that ductular proliferation is almost completely reversible within 14 to 28 days after Roux-en-Y anastomosis, whereas portal hypertension does not resolve completely (2,7,8). The reasons for the slow normalization of portal pressure are not completely clear but may be due to proliferation of hepatic stellate cells (8).

In addition to the structural and vascular changes, chronic bile duct ligation is also associated with alterations in hepatic energy metabolism, in particular glycogen (1,7) and mitochondrial energy metabolism (5,9,10). After 4 weeks of bile duct ligation, hepatic glycogen stores are reduced, a finding which can most likely be explained by reduced glycogen synthesis (1).

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Recent studies have shown that the decreases in the hepatic glycogen content and glycogen synthase activity are completely reversible 5 to 14 days after relief of bile duct ligation (7,11). Changes in mitochondrial metabolism associated with long-term cholestasis include decreased activities of complex I, II and III of the electron transport chain (5,9,12), decreased activities of enzymes involved in β-oxidation (5) and impaired antioxidative defense mechanisms (13–15).

So far, the reversibility of alterations in mitochondrial metabolism in rats with chronic bile duct ligation has not been investigated. Since this question may also have clinical implications in patients with bile duct obstruction, we studied mitochondrial metabolism in rats with long-term bile duct ligation and 5 or 14 days after relief of biliary obstruction by Roux-en-Y anastomosis. The studies confirm the presence of changes in mitochondrial metabolism in bile duct ligated rats and show that some, but not all, of these alterations are reversible within 2 weeks after biliary reanastomosis.

Materials and Methods

Reagents

N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD), decylubiquinone, duroquinone, thenoyltrifluoroacetone, 2,6-dichloroindophenol (DCIP), rotenone, antimycin A and oligomycine were obtained from Sigma (Buchs, Switzerland). All other chemicals were of reagent grade.

Animals

The experiments had been reviewed and accepted by the Animal Ethics Committee of the State of Berne. Male Sprague-Dawley rats (obtained from the Süddeutsche Versuchstierfarm, Tuttlingen, Germany) were used for all experiments. Ligation and transection of the common bile duct and Roux-en-Y anastomosis were performed as described previously (2,16). Rats were studied either after 4 weeks of bile duct ligation (BDL rats), or after 4 weeks of bile duct ligation and subsequent Roux-en-Y anastomosis (RY rats) for 5 or 14 days. Control rats were sham-operated (laparotomy and manipulation of the bile duct) and pair-fed to treated rats throughout the study with normal rat chow (Kliba Futter, Basel, Switzerland). Mortality was 0% for control rats, 25% for BDL rats and 20% for Roux-en-Y anastomosis. All studies were performed with the rats starved for 24 h.

Rats were characterized by body, liver and spleen weight. In plasma, activities of alkaline phosphatase and alanine aminotransferase, and concentrations of bilirubin, glucose, free fatty acids and β-hydroxybutyrate were determined on a COBAS analyzer (Hoffmann-La Roche Diagnostics, Basel, Switzerland)

TABLE 1

Characterization of the rats. Rats were bile duct ligated for 4 weeks (BDL), or 5 days (RY5) or 14 days after relief of bile duct ligation (RY14) by Roux-en-Y anastomosis. Control rats (CON) underwent sham operation and were pair-fed to treated rats during the entire period of the study. Rats were investigated after starvation for 24 h. Data are presented as mean±SD

Day after relief of obstruction	Day 0		Day 5		Day 14	
	CON (n=8)	BDL (n=10)	CON (n=8)	RY5 (n=6)	CON (n=7)	RY14 (n=8)
Body weight (g)	363±27	372±54	351±30	351±34	377±22	388±37
Liver weight (g)	9.1±0.7	21.5±3.4*	8.8±1.5	12.2±2.5*	10.2±1.3	11.5±2.0^{+}
Spleen weight (g)	0.7±0.1	1.8±0.4*	0.6±0.1	1.4±0.5*	0.7±0.1	1.7±0.4*
Alanine aminotransferase (U/l)	43±13	137±64*	41±8	44±26^{+}	46±8	47±11^{+}
Alkaline phosphatase (U/l)	155±105	505±162*	205±68	354±232	143±60	268±199^{+}
Serum bilirubin (μmol/l)	0.3±0.2	75±49*	0.3±0.2	29±28*$^{+}$	0.3±0.1	1.1±1.7^{+}

*p<0.05 vs. control (CON). ^{+}p<0.05 vs. BDL.

TABLE 2

Plasma concentration of metabolites. Rats were bile duct ligated for 4 weeks (BDL), or 5 days (RY5) or 14 days after relief of bile duct ligation (RY14) by Roux-en-Y anastomosis. Control rats (CON) underwent sham operation and were pair-fed to treated rats during the entire period of the study. Rats were investigated after starvation for 24 h. Data are presented as mean±SD

Day after relief of obstruction	Day 0		Day 5		Day 14	
	CON (n=8)	BDL (n=10)	CON (n=8)	RY5 (n=6)	CON (n=7)	RY14 (n=8)
Glucose (mmol/l)	7.4±0.7	6.5±0.5*	8.5±2.9	8.3±1.3^{+}	8.4±5.7	10.2±4.7^{+}
Free fatty acids (μmol/l)	950±180	900±150	1030±240	790±260	880±260	720±290
β-Hydroxybutyrate (μmol/l)	790±200	200±70*	940±470	320±190*	650±340	190±110*

*p<0.05 vs. control (CON). ^{+}p<0.05 vs. BDL.

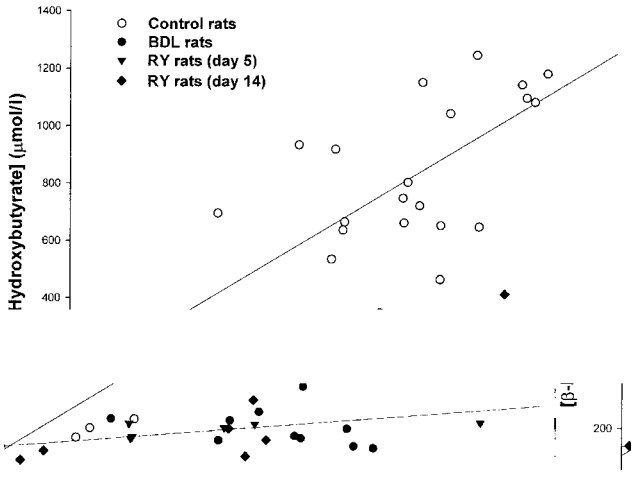

Fig. 1. Relationship between plasma free fatty acids and β-hydroxybutyrate concentrations in rats with bile duct ligation (BDL rats), rats after Roux-en-Y anastomosis for 5 or 14 days (RY rats) and control rats. Control rats were pair-fed to treated rats throughout the study, and all rats were studied after 24 h of starvation. There is a linear relationship between the two variables for both control and treated rats. The corresponding equations are: (i) for control rats $y=-268+1.08\times$ ($r=0.765$, $p<0.05$) and (ii) for treated rats $y=91+0.13\times$ ($r=0.421$, $p<0.05$). The two slopes are significantly different from each other ($p<0.05$).

using commercially available kits from Boehringer Mannheim (Mannheim, Germany).

Isolation of liver mitochondria

Liver mitochondria were isolated by differential centrifugation as described by Hoppel et al. (17). Rats were killed by decapitation and a mixed arterial/venous blood sample was collected into heparinized tubes. Plasma was obtained by centrifugation and stored at −20°C until analysis. The liver was removed, dried and weighed, and 10 g were placed in ice-cold MSM buffer (220 mmol/l mannitol, 70 mmol/l sucrose, 5 mmol/l 4-morpholinopropanesulfonic acid; pH 7.4). The piece of liver was rinsed, blotted, minced and washed with MSM buffer. A 10% suspension (wt/vol) of the minced liver was prepared in MSM buffer containing 2 mmol/l EDTA using a Potter-Elvehjem homogenizer with a loosely fitting pestle. Nuclei and cell debris were removed by centrifugation at 700 *g* for 10 min, and mitochondria were isolated by centrifugation of the supernatant at 7000 *g* for 10 min. The resulting mitochondrial pellet was washed twice with MSM buffer and diluted to contain approximately 50 mg of mitochondrial protein per milliliter. As shown previously, liver mitochondria isolated by this procedure have only minor contaminations of lysosomes or peroxisomes (5). The protein concentration was determined by the biuret method using bovine serum albumin as a protein standard (18).

Mitochondrial oxidative metabolism and activities of the enzyme complexes of the respiratory chain

Oxygen consumption by intact mitochondria was measured in a chamber equipped with a Clark-type

TABLE 3

Oxidative metabolism by isolated liver mitochondria. Rats were bile duct ligated for 4 weeks (BDL), or 5 days (RY5) or 14 days after relief of bile duct ligation (RY14) by Roux-en-Y anastomosis. Control rats (CON) underwent sham operation and were pair-fed to treated rats during the entire period of the study. Liver mitochondria were isolated by differential centrifugation from rats starved for 24 h. State 3 oxidation rates are expressed as natoms · mg mitochondrial protein^{-1} · min^{-1}. RCRs (respiratory control ratio), and ATP production rates were calculated as described in Methods. Data are presented as mean±SD

Day after relief of obstruction	Day 0		Day 5		Day 14	
	CON (n=8)	BDL (n=10)	CON (n=8)	RY5 (n=6)	CON (n=7)	RY14 (n=8)
L-Glutamate (20 mmol/l)						
State 3 oxidation	104±31	69±21*	104±46	75.6±20.3	90.5±46.4	85.0±25.1
RCR	8.3±4.2	7.5±3.7	10.1±4.7	7.3±1.5	7.3±3.0	6.6±3.3
ADP/O	2.8±0.4	2.4±0.8	2.3±0.4	2.4±0.8	2.9±0.4	2.7±0.5
Succinate (20 mmol/l)						
State 3 oxidation	220±32	151±39*	207±42	138±42*	194±38	134±53*
RCR	5.3±1.3	4.8±0.7	4.9±1.1	4.6±2.0	5.9±2.4	4.1±0.6
ADP/O	1.9±0.2	1.8±0.5	1.8±0.3	1.8±0.2	2.0±0.4	1.9±0.3
Duroquinol (1 mmol/l)						
State 3	50.8±7.4	32.5±11.5*	41.6±6.7	35.4±3.2	53.3±23.7	42.5±13.0
RCR	2.1±0.4	1.6±0.3	2.0±0.8	1.7±0.6	2.0±0.8	1.5±0.6
Ascorbate (7.2 mmol/l)/TMPD[1] (0.24 mmol/l)						
State 3	151±57	122±48	142±91	117±64	125±67	112±36
RCR	1.7±0.5	1.5±0.3	1.6±0.4	1.5±0.3	1.6±0.3	1.4±0.3

*$p<0.05$ vs control (CON). $^{+}p<0.05$ vs. BDL. [1]TMPD=N,N,N′,N′-tetramethyl-p-phenylenediamine.

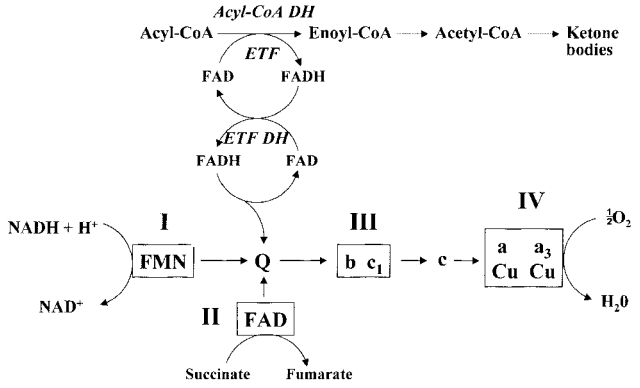

Fig. 2. Mitochondrial fatty acid β-oxidation and electron transport chain. The electron transport chain is located in the inner mitochondrial membrane and is composed of four enzyme complexes (indicated with roman numerals) and of the electron shuttles ubiquinone (Q) and cytochrome c (c). F_1F_0-ATPase (not shown) is a fifth enzyme complex generating ATP from the proton gradient across the inner mitochondrial membrane. The electron transport chain and F_1F_0-ATPase together are called the respiratory chain. The cytochromes are indicated with small letters (a, a_3, b, c, c_1). The electron transport complexes are NADH: ubiquinone oxidoreductase (I), succinate:ubiquinone oxidoreductase (II), ubiquinol:ferricytochrome c oxidoreductase (III) and ferrocytochrome c:oxygen oxidoreductase (IV). The flow of the electrons across the electron transport chain is indicated with solid arrows. Substrates producing NADH (e.g. L-glutamate) feed the electron transport chain at complex I, succinate at complex II, duroquinol at complex III, ascorbate at complex IV and molecular oxygen accepts the electrons at complex IV. Dehydrogenation of fatty acids by the mitochondrial acyl-CoA dehydrogenases (Acyl-CoA DH) yields FADH which transports the electrons to ubiquinone (Q) via the electron transferring flavoprotein (ETF) and the electron transferring flavoprotein dehydrogenase (ETF DH). Further metabolism of the resulting enoyl-CoA yields NADH (entering the electron transport chain at complex I) and acetyl-CoA. Acetyl-CoA can be used for the production of ketone bodies.

oxygen electrode (Yellow Springs Instruments, Yellow Springs, OH, USA) at 30°C as described previously (19). Respiratory control ratios (RCR's) were calculated by dividing state 3 by state 4 oxidation rates, and ADP/O ratios by dividing the amount of ADP added by the amount of oxygen used to convert it to ATP (20).

Activities of mitochondrial oxidases were determined at 30°C with the oxygen electrode using freeze-thawed mitochondria as originally described by Blair et al. (21) with the modifications described previously (19).

Activities complex I (NADH:decylubiquinone oxidoreductase), complex II (succinate:2,6 DCIP oxidoreductase), complex III (decylubiquinol:ferricytochrome c oxidoreductase), complex IV (ferrocytochrome c: oxygen oxidoreductase) and complex V (F_1F_0-ATPase) were determined at 30°C by spectrophotometric methods using freeze-thawed mitochondria as described previously (19,22,23). Net activities were calculated by subtracting the activity in the presence from the activity in the absence of a specific inhibitor (rotenone for complex I, thenoyltrifluoroacetone for complex II, antimycine A for complex III, sodium azide for complex IV and oligomycine for complex V).

Statistics

Results are given as mean±SD. Means were compared by ANOVA followed by Student's *t*-test for unpaired observations with Bonferroni's correction for multiple comparisons. A $p<0.05$ was considered to be statistically significant.

Results

The rats used in this study are characterized in Table 1. Body weights were not different between treated (BDL rats, RY rats) and sham-operated rats (control rats), reflecting the fact that the rats were pair-fed throughout the study. Liver and spleen weights were both increased in BDL rats. After relief of bile duct ligation, liver weight normalized within 14 days, whereas spleen weight did not normalize, indicating persisting portal hypertension. Alanine aminotransferase, alkaline phosphatase and serum bilirubin were all increased in BDL rats and normalized within 5 or 14 days after relief of bile duct ligation.

The plasma concentration of metabolites is illustrated in Table 2. The concentration of glucose was decreased in BDL rats but normalized after relief of biliary obstruction. The concentrations of free fatty acids were not different between treated and control rats, whereas the plasma β-hydroxybutyrate was decreased in BDL rats and remained decreased also after relief of bile duct ligation, suggesting persistently impaired mitochondrial fatty acid metabolism. As shown in Fig. 1, there was a linear relationship between the plasma concentrations of free fatty acids and β-hydroxybutyrate for both control and treated rats. The slope was significantly steeper in control as compared to treated rats, again demonstrating impaired mitochondrial fatty acid metabolism in treated rats.

In order to study mitochondrial metabolism in more detail, oxidative metabolism of different substrates was determined in isolated liver mitochondria (Table 3). As in previous studies (5), state 3 oxidation rates for L-glutamate, succinate and duroquinol were decreased in

BDL rats, whereas oxidative metabolism of ascorbate remained unaffected, indicating normal function of complex IV of the respiratory chain (see Fig. 2 for explanation of the respiratory chain and mitochondrial fatty acid metabolism). After relief of biliary obstruction, state 3 oxidation rates of L-glutamate and duroquinol normalized within 5 days, whereas succinate oxidation did not recover, suggesting a defect either in succinate transport or at complex II. ADP/O ratios for L-glutamate and succinate were not different between treated and control rats.

In order to answer the question whether the observed decrease in state 3 oxidation rates in intact mitochondria was due to transport of substrates and/or decreased activity of enzyme complexes of the respiratory chain, mitochondrial oxidases and activities of the different enzyme complexes of the respiratory chain were determined. As shown in Table 4, activities of mitochondrial oxidases showed a pattern similar to the state 3 oxidation rates obtained in intact mitochondria. Activities of NADH, succinate and duroquinol oxidase were all decreased in BDL rats, whereas cytochrome c oxidase was not affected. After relief of the ligation, NADH and duroquinol oxidase normalized, whereas succinate oxidase did not recover. As shown in Table 5, this result was confirmed by direct measurement of the activities of the enzyme complexes of the respiratory chain. Activities of complex I, II, III and V were all reduced in BDL rats. While the activities of complex I and III recovered within 5 days after relief of the obstruction, the activities of complex II and V remained decreased, also 14 days after Roux-en-Y anastomosis.

Since decreased activities of complex II and V do not necessarily explain the observed decrease in hepatic fatty acid metabolism in RY rats, oxidative metabolism of long-chain fatty acid derivatives was studied in isolated, intact mitochondria (Table 6). State 3 oxidation rates were decreased in BDL rats for both palmitoyl-CoA and palmitoyl-L-carnitine and remained decreased also after reversal of the bile duct for 14 days. In contrast, state 4 oxidation rates were not different between mitochondria from treated and control rats.

Discussion

The current studies show that some mitochondrial functions, e.g. activities of complex I and III, normalize rapidly after reversal of bile duct ligation whereas

TABLE 4

Activities of mitochondrial oxidases. Rats were bile duct ligated for 4 weeks (BDL), or 5 days (RY5) or 14 days after relief of bile duct ligation (RY14) by Roux-en-Y anastomosis. Control rats (CON) underwent sham operation and were pair-fed to treated rats during the entire period of the study. Liver mitochondrial were isolated from rats starved for 24 h, and mitochondrial oxidases were determined using oxygen electrode as described in Methods. Units are natoms · mg mitochondrial protein^{-1} · min^{-1}. Data are presented as mean±SD

Day after relief of obstruction	Day 0		Day 5		Day 14	
	CON (n=8)	BDL (n=10)	CON (n=8)	RY5 (n=6)	CON (n=7)	RY14 (n=8)
NADH oxidase	283±82	146±56*	249±43	219±32^{+}	219±54	184±53
Succinate oxidase	168±71	73.7±12.4*	146±40	44.5±24.5*$^{+}$	138±30	62.7±28.6*
Duroquinol oxidase	306±46	238±32*	267±27	265±89	259±69	269±92
Cytochrome c oxidase	509±126	503±127	383±77	472±86	502±126	408±46

*p<0.05 vs. control (CON). ^{+}p<0.05 vs. BDL.

TABLE 5

Activities of the enzyme complexes of the mitochondrial respiratory chain. Rats were bile duct ligated for 4 weeks (BDL), or 5 days (RY5) or 14 days after relief of bile duct ligation (RY14) by Roux-en-Y anastomosis. Control rats (CON) underwent sham operation and were pair-fed to treated rats during the entire period of the study. Liver mitochondria were isolated from rats starved for 24 h, and the activities of the enzyme complexes of the respiratory chain were determined using spectrophotometric assays as described in Methods. Units are nmol · mg mitochondrial protein^{-1} · min^{-1}. Data are presented as mean±SD

Day after relief of obstruction	Day 0		Day 5		Day 14	
	CON (n=8)	BDL (n=10)	CON (n=8)	RY5 (n=6)	CON (n=7)	RY14 (n=8)
Complex I	40.7±15.9	21.6±8.5*	38.5±10.7	46.6±4.7^{+}	34.1±10.7	33.2±7.7^{+}
Complex II	10.1±1.7	6.4±1.5*	10.8±2.5	5.7±1.5*	14.0±1.4	7.8±3.8*
Complex III	1440±290	1150±150*	1460±270	1680±540	1880±580	1690±490^{+}
Complex IV	311±34	255±74	317±81	280±82	348±68	273±97
Complex V	57.1±12.3	34.3±14.5*	81.3±14.6	38.1±15.3*	59.6±11.6	35.7±13.7*

*p<0.05 vs. control (CON). ^{+}p<0.05 vs. BDL.

TABLE 6

Oxidative metabolism of fatty acid derivatives by isolated liver mitochondria. Rats were bile duct ligated for 4 weeks (BDL), or 5 days (RY5) or 14 days after relief of bile duct ligation (RY14) by Roux-en-Y anastomosis. Control rats (CON) underwent sham operation and were pair-fed to treated rats during the entire period of the study. Liver mitochondria were isolated by differential centrifugation from rats starved for 24 h. State 3 and state 4 oxidation rates are expressed as natoms · mg mitochondrial protein^{-1} · min^{-1}. RCRs (respiratory control ratio) were calculated by dividing state 3 by state 4 oxidation rates. Data are presented as mean±SD

Day after relief of obstruction	Day 0		Day 5		Day 14	
	CON (n=8)	BDL (n=10)	CON (n=8)	RY5 (n=6)	CON (n=7)	RY14 (n=8)
Palmitoyl-CoA (80 μmol/l)[1]						
State 3	93.0±18.28	56.1±24.4*	105±20	54.4±18.4*	99.1±29.7	42.8±14.3*
State 4	22.6±6.9	18.5±2.9	19.8±7.8	18.0±7.6	19.6±3.6	16.6±4.2
RCR	4.4±1.6	3.2±1.7	5.3±2.4	3.9±2.9	5.1±2.5	2.7±1.3*
Palmitoyl-L-carnitine (80 μmol/l)[1]						
State 3	111±24	66.4±29.9*	87.4±25.5	43.9±25.9*	85.0±31.4	37.4±20.9*
State 4	19.8±6.3	14.3±5.3	18.5±7.2	15.6±5.7	18.7±4.9	15.9±9.0
RCR	6.1±2.3	5.0±2.9	5.2±2.1	2.2±1.4*$^{+}$	4.4±2.0	2.4±1.1†

*p<0.05 vs. control (CON). ^{+}p<0.05 vs. BDL. [1] Incubations contained 5 mmol/l malate.

others, in particular fatty acid oxidation, do not normalize up to 14 days after Roux-en-Y anastomosis.

Our studies confirm the findings reported previously, namely that the activities of complex I, II and III of the respiratory chain and mitochondrial β-oxidation are reduced in livers of BDL rats (5). Since bile duct ligation for 4 weeks is associated with proliferation of bile ducts in rats, reaching a volume fraction of 20–25% (1,2), these observations could potentially be caused by contamination with mitochondria from biliary epithelial cells. However, in comparison to hepatocytes, biliary epithelial cells are less abundant and contain less mitochondria in BDL rats (24), suggesting that the contamination with biliary mitochondria is probably negligible.

Since complex I and III can be rate-limiting for the activity of the electron transport chain and are important for mitochondrial fatty acid metabolism (25,26), our earlier studies favored the conclusion that decreased activities of complex I and III are primarily responsible for impaired hepatic fatty acid metabolism in BDL rats. However, the current studies demonstrate that mitochondrial fatty acid metabolism is still impaired 2 weeks after restoring bile flow, a time point when the activities of complex I and III have normalized completely. The current studies therefore suggest that reduced hepatic mitochondrial fatty acid metabolism in BDL rats results primarily from impaired β-oxidation, and not from decreased activity of the mitochondrial electron transport chain. Since the plasma free fatty acid concentrations were not different between treated and control rats, reduced lipolysis can be excluded as a cause for impaired fatty acid metabolism in rats with long-term cholestasis.

In contrast to complex I and III, the activities of complex II and V (F_1F_0-ATPase) did not normalize up to 14 days after Roux-en-Y anastomosis. Complex II is responsible for the conversion of succinate to fumarate, a reaction of the Krebs cycle which does not directly affect fatty acid oxidation. On the other hand, a decrease in the activity of F_1F_0-ATPase could impair fatty acid metabolism by reducing the flow of protons from the intermembranaceous space into the mitochondrial matrix, with consecutive impairment of the function of the electron transport chain. However, the ADP/O ratio for L-glutamate and succinate, a measure for the degree of coupling between the electron transport chain and ATP production (20), was not different between treated and control rats. This finding indicates that the activity of F_1F_0-ATPase was not limiting ATP production in mitochondria from treated rats and therefore probably was also not responsible for impaired fatty acid oxidation.

Important hepatotoxic principles associated with chronic cholestasis include accumulation of substances normally excreted by the bile, for instance bilirubin (27,28), hydrophobic bile acids (29,30) and cholesterol (9), and a decrease in antioxidative mechanisms (13–15). While bilirubin and hydrophobic bile acids can impair the function of the enzyme complexes of the respiratory chain directly, cholesterol is incorporated into mitochondrial membranes and increases their rigidity (31), leading to a possible decrease in the function of membrane-bound enzymes (32). As shown recently, a reduction in mitochondrial antioxidative mechanisms, in particular a decrease in the mitochondrial content of glutathione and ubiquinone, may also be an important mechanism of hepatotoxicity associated with chronic cholestasis (13,14). The rapid recovery of the activities of the enzyme complexes of the

respiratory chain after Roux-en-Y anastomosis is compatible with the concept that reversal of biliary obstruction removes one or more toxins with a fast mode of action. This may, for instance, be due to a decrease in the hepatic concentration of bilirubin and bile acids, as suggested by the rapid normalization of their plasma concentration. Increasing the hepatic concentrations of antioxidants, in particular of glutathione, may also be a fast process (33), whereas removing cholesterol from mitochondrial membranes may last longer than the 5 days which elapsed before normalization of the activities of complex I and III.

In contrast to the respiratory chain, mitochondrial β-oxidation is only partially membrane-bound and some reactions are catalyzed by soluble enzymes located in the mitochondrial matrix. Palmitoyl-CoA synthase and CPT I are located in the outer, acylcarnitine translocase, CPT II, very-long-chain acyl-CoA dehydrogenase and the trifunctional enzyme in the inner mitochondrial membrane (34,35), whereas the acyl-CoA dehydrogenases, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and β-ketoacyl-CoA thiolase are all soluble enzymes in the mitochondrial matrix. The results of the current investigations are compatible with a defect distal to the activation of palmitate and the formation of palmitoyl-CoA. This finding is in agreement with our previous observations in BDL rats, showing markedly reduced activities of the matrix enzymes short-chain acyl-CoA dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and β-ketoacyl-CoA thiolase (5). However, these enzymes are soluble and therefore toxins affecting the physical properties of the mitochondrial membranes such as cholesterol are unlikely to affect their function. The observation that hepatic mitochondrial β-oxidation is still impaired 14 days after Roux-en-Y anastomosis militates against the possibility that bilirubin, bile acids and reduced antioxidative defense mechanisms are responsible for the observed decrease in the function of these enzymes. Further studies are therefore necessary to identify the precise mechanisms leading to the observed reduction in hepatic β-oxidation in rats with chronic cholestasis.

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