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DELAYED RECOVERY OF MITOCHONDRIAL FUNCTION IN RAT LIVER AFTER RELEASING BILIARY OBSTRUCTION

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ABSTRACT

In order to know the influence of obstructive jaundice on the function of the liver and also to clarify the underlying factors involved in the recovery process after releasing biliary obstruction, the author examined the following parameters:

— The respiratory function of the liver mitochondria, the activities of hepatic enzymes in serum and the ultrastructure of the liver.

Male Donryu rats were used for this investigation, in which the bile ducts of the rats were obstructed and, after a certain duration, were relased to allow for external bile drainage. The parameters mentioned above deteriorated due to obstructive jaundice, but recovered to some extent upon the release of the biliary obstruction. The recovery process depended on the length of the obstruction period. The longer the obstruction period persisted, the longer was the time required for recovery. Accordingly, it is desirable to perform biliary decompression as soon as possible, at least within a period of two weeks. Furthermore, after the release of obstructive jaundice, there existed a certain period of insufficient recovery of the mitochondrial function even when the biochemical data were completely normalized. This time lag of the recovery between mitochondrial function and biochemical data might reflect a reduced reserve function of the liver. Better understanding of the recovery process of the damaged liver is very important when considering major surgery after releasing obstructive jaundice.

Key Words: Liver mitochondria, Respiratory function, Ultrastructure, Obstructive jaundice, Bile drainage.

INTRODUCTION

Organs in general are affected in various ways under the condition of obstructive jaundice because of the stasis of bile components in blood and tissue.[1] Following cholestasis, feathery degeneration and bile infarct of hepatocytes occur[2] and, accordingly, morphological changes and dysfunction appear in the liver mitochondria.[3)-4)] These are aggravated as the biliary obstruction period is prolonged, until finally the liver reaches the point of hepatic insufficiency.[5] Those hepatic disturbances induced by biliary obstruction, however, are in some part reversible upon the release of biliary obstruction. Recently, percutaneous transhepatic bile drainage has been widely applied to cases of obstructive jaundice. This procedure was first executed by Kaplan[6] and has the merit of being less invasive to patients and easier to perform than surgical intervention. When the biliary obstruction is released by percutaneous transhepatic bile drainage, the disturbed hepatic function recovers gradually,[2),7)] but not much is known yet about the details. In cases of retarded obstructive jaundice, recovery from hepatic disturbance is sometimes difficult even if biliary

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decompression is applied.[3] It is therefore very important to know the influence of obstructive jaundice on the living body, especially on the liver, the central organ for metabolism, and to accurately grasp the recovery process after release of obstruction. This will serve as the basis on which to determine the timing and indications for radical opertion after biliary decompression and will be helpful in solving problems related to surgical clinics. Using rat models[7] the author investigated the function of the liver mitochondrion, which is a principal energy-producing organella, and attempted to clarify the effects of obstructive jaundice on liver function during and after complete biliary obstruction.

MATERIALS AND METHODS

Male Donryu rats aged 3 – 4 months were maintained longer than one month in an air-conditioned room preoperatively. The pelleted food (Oriental Kobo, Ltd., MF) was given with water *ad libitum*. After a median laparotomy without anesthesia, a vinyl tube with an outer diameter of 1 mm and an inner diameter of 0.5 mm (Hakko Denki Seisakusho, Ltd.) was inserted into the extrahepatic bile duct according to the cut-down technique in order to excrete bile. Complete obstructive jaundice was induced by ligating the end of the tube drawn out of the abdominal cavity. After either three days or two weeks duration of biliary obstruction, ligation of the vinyl tube was released to allow for external bile drainage. The rats were divided into 12 groups, each consisting of 5 – 9 animals, as follows:

i) Four obstructive jaundice groups, namely, the J-3 group (3-day obstruction), the J-1W group (one-week obstruction), the J-2W group (two-week obstruction) and the J-3W group (three-week obstruction), respectively, were made in reference to the duration of biliary obstruciton. A sham-operated group served as the control.

ii) After three days of biliary obstruction, biliary decompression was carried out and on the 1st, 3rd and 5th day, groups J-3-R-1, J-3-R-3 and J-3-R-5 were prepared.

iii) After two weeks of biliary obstruction, biliary decompression was carried out and on the 1st, 3rd, 5th and 10th day, groups J-2W-R-1, J-2W-R-3, J-2W-R-5 and J-2W-R-10 were prepared.

Each rat was relaparotomied without anesthesia, bled to death, and blood and fresh liver were collected. Total bilirubin was measured by Jendrassik-Cleghorn's method, GOT and GPT by the UV method, and mitochondrial GOT by the column method. Liver mitochondria were prepared by the method of Aoyama, *et al.*[8] Mitochondrial function was measured polarographically using an oxygen electrode (Beckman oxygen sensor 39550) according to the method of Chance and Hagihara.[9] Oxygen consumption by mitochondria was measured from State 1 to State 5 according to the definition of Chance and Williams.[10] These included the ratio of the rate of oxygen consumed in the presence of ADP to that in the absence of ADP (RCI) as a measure of tightness of coupling between respiration and high energy production, the ratio of moles of ADP phosphorylated to atoms of oxygen consumed (ADP/O) as a measure of efficiency of ATP production, the rate of oxygen-uptake in the presence of a substrate alone (State II respiration) as a measure of idling of respiration,[11] the rate of oxygen-uptake in the presence of ADP (State III respiration) as a measure of electron transport capability, and the rate of ATP formation (ATP formation). Mitochondrial protein was determined by the biuret method.

A part of the liver was used for electron microscopic observation. Fresh livers isolated from rats were fixed immediately in 2% glutaraldehyde followed by 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Epon 812. At least six blocks were made per rat. Ultrathin sections were double-stained with uranyl acetate and lead citrate and observed under the electron microscope (Hitachi H-500). Selecting a visual field at random,

the individual cut-surface area of 500 mitochondria was measured. (Mutoh Digigrammer)

The data were expressed as the mean ± standard deviation, and statistical analyses were made by the Student's t-test. When the P value was over 0.05, it was determined to have no significant difference.

RESULTS

1. Liver damage caused by persistent obstructive jaundice

Upon inducing biliary obstruction, the total bilirubin rapidly increased to a value in the J-3 group of more than 15 times that of the control, and in the J-1W group to nearly 25 times that of the control. After that, however, when the obstruction period was prolonged further, the value decreased somewhat to an almost stable level. There was no significant difference between the J-1W group and the J-2W group, nor between the J-1W group and the J-3W group. Transaminase values including mitochondrial GOT (m-GOT) also increased quite rapidly after biliary obstruction, and in the J-3 group they had already reached their peak, after which they remained at a plateau. No significant difference was found between the J-3 and the J-1W groups, between the J-1W and the J-2W groups, nor between the J-2W and the J-3W groups. The ratio between m-GOT and total GOT showed no fixed trend (Table 1). Mitochondrial function was disturbed by biliary obstruction in a short time, and even in the J-3 group RCI decreased significantly compared with the control, and kept decreasing as long as the obstruction was continued, but in the J-2W group the decrease bottomed out and no change was observed from that point on. ADP/O in the J-3 group was not affected, but from the J-1W group onward it began to decrease, and this continued in proportion to the prolongation of the obstruction period. However, there was no significant difference between the J-1W and the J-2W groups, nor between the J-2W and the J-3W groups. In State II respiration, compared with the control, a significant increase was observed even in the J-3 group, which increased up to the peak of the J-2W group, while the J-3W group showed a rather low value. State III respiration maintained a nearly fixed value. The rate of ATP formation began to decrease from the J-2W group onward (Table 2).

In electron microscopic observation for the J-2W group, the mean cut-surface area of 500 mitochondria was 0.68 ± 0.40 (μ^2), while that of the control was 0.58 ± 0.29 (μ^2), showing an obvious swelling (Fig. 1) with significant difference between them ($p < 0.001$).

2. Recovery from damage after release of biliary obstruction

From the above results, it was concluded that disturbance by obstructive jaundice was clearly observable already after 3 days of obstruction, and reached its peak in two weeks. In order to study the recovery processes from disturbance, obstruction was released at the points of 3 days and 2 weeks, and the state of mitochondrial function was examined.

In the J-3 group, when obstruction was released, the total bilirubin decreased with significant difference in 1 day, and returned to its normal range in 3 days. Transaminase values were nearly normalized within one day after release (Table 3). RCI increased upon release of the obstruction, and on the 5th day recovered up to about 85% of the value for the control. State II respiration returned nearly to normal in 3 days after release, and on the 5th day significant recovery was recognized (Table 4).

Next, obstruction was released in the J-2W group. Total bilirubin started to decrease rapidly, but it required 5 days to return to normal. Transaminase values also recovered in 5 days after release of the obstruction. Only m-GOT returned to normal in 1 day (Table 5). RCI gradually improved after release of the obstruction and on the 10th day returned to 85% or more of the normal value. No significant difference was found between the control and the J-2W-R-10 group. It took 10 days after removal of the obstruction for ADP/O to recover. State

Table 1. Biochemical Data with Obstructive Jaundice

Parameters		Control (9)	Duration of biliary obstruction			
			3 days (6)	1W (8)	2W (8)	3W (5)
T-Bil.	(mg/dl)	0.4 ± 0.1	7.6 ± 0.7†	9.2 ± 1.2†	6.7 ± 0.2†	7.2 ± 1.5†
GOT	(Unit)	103 ± 22	576 ± 71†	492 ± 155†	568 ± 95†	660 ± 96†
GPT	(Unit)	18 ± 7	91 ± 47††	42 ± 8†	57 ± 14†	90 ± 12†
m-GOT	(mU/ml)	11.0 ± 4.6	96.1 ± 15.1†	55.9 ± 2.7†	73.7 ± 26.3†	79.1 ± 20.6†

Data were expressed as the mean ± standard deviation. †$p<0.001$, ††$p<0.01$, compared with the control. Numbers in parentheses indicate numbers of animals.

Table 2. Mitochondrial Function with Obstructive Jaundice

Parameters	Control (9)	Duration of biliary obstruction			
		3 days (6)	1W (8)	2W (8)	3W (5)
RCI	5.45 ± 0.16	3.64 ± 0.21†	3.38 ± 0.51†	2.38 ± 0.38†	2.39 ± 0.51†
ADP/O	1.90 ± 0.10	1.95 ± 0.08	1.58 ± 0.11†	1.41 ± 0.12†	1.36 ± 0.16†
State III respiration (n Atoms/mg Prot./min.)	142.4 ± 11.9	133.8 ± 7.0	160.7 ± 13.2†††	145.6 ± 22.5	139.3 ± 38.7
State II respiration (n Atoms/mg Prot./min.)	26.2 ± 2.1	36.8 ± 1.7†	43.8 ± 9.3†	64.9 ± 7.3†	61.2 ± 17.0††
ATP formation (n moles/mg Prot./min.)	261.5 ± 19.0	277.1 ± 37.3	264.4 ± 29.7	197.8 ± 25.2†	182.2 ± 63.1†††

Data were expressed as the mean ± standard deviation. †$p < 0.001$, ††$p < 0.01$, †††$p < 0.05$ compared with the control. Numbers in parentheses indicate numbers of animals.

Table 3. Biochemical Data after Release of Biliary Obstruction

Parameters		Biliary obstruction	Release of biliary obstruction	
		3 days (6)	1st day (5)	3rd day (5)
T-Bil.	(mg/dl)	7.6 ± 0.7	0.9 ± 0.1†	0.4 ± 0.2†
GOT	(Unit)	576 ± 71	112 ± 21†	108 ± 5†
GPT	(Unit)	91 ± 47	22 ± 5††	18 ± 6††
m-GOT	(mU/ml)	96.1 ± 15.1	12.3 ± 7.4†	13.1 ± 0.3†

Data were expressed as the mean ± standard deviation. †$p<0.001$, ††$p<0.01$, compared with the pre-release group. Numbers in parentheses indicate numbers of animals.

Table 4. Mitochondrial Function after Release of Biliary Obstruction

Parameters	Biliary obstruction	Release of biliary obstruction		
	3 days (6)	1st day (5)	3rd day (5)	5th day (6)
RCI	3.64 ± 0.21	3.98 ± 0.33	4.26 ± 0.39†††	4.44 ± 0.27†
ADP/O	1.95 ± 0.08	1.97 ± 0.15	2.03 ± 0.24	2.00 ± 0.12
State III respiration (n Atoms/mg Prot./min.)	133.8 ± 7.0	166.4 ± 47.3	160.7 ± 19.6†††	119.4 ± 5.7††
State II respiration (n Atoms/mg Prot./min.)	36.8 ± 1.7	32.9 ± 3.6	27.0 ± 9.2	28.3 ± 2.5†
ATP formation (n moles/mg Prot./min.)	277.1 ± 37.3	288.2 ± 73.7	357.8 ± 40.5†††	245.1 ± 15.0

Data were expressed as the mean ± standard deviation. †$p < 0.001$, ††$p < 0.01$, †††$p < 0.05$ compared with the pre-release group. Numbers in parentheses indicate numbers of animals.

Table 5. Biochemical Data after Release of Biliary Obstruction

Parameters		Biliary obstruction	Release of biliary obstruction			
		2W (8)	1st day (6)	3rd day (6)	5th day (6)	10th day (6)
T-Bil.	(mg/dl)	6.7 ± 0.2	2.5 ± 1.2†	1.2 ± 0.1†	0.4 ± 0.2†	0.3 ± 0.2†
GOT	(Unit)	568 ± 95	213 ± 124†	205 ± 141†	127 ± 86†	145 ± 26†
GPT	(Unit)	57 ± 14	27 ± 20†	33 ± 12††	23 ± 11†	19 ± 9†
m-GOT	(mU/ml)	73.7 ± 26.3	9.1 ± 5.5†	12.7 ± 3.6†	13.0 ± 5.6†	13.5 ± 2.4†

Data were expressed as the mean ± standard deviation. †$p<0.001$, ††$p<0.01$, compared with the pre-release group. Numbers in parentheses indicate numbers of animals.

Table 6. Mitochondrial Function after Release of Biliary Obstruction

Parameters	Biliary obstruction	Release of biliary obstruction			
	2W (8)	1st day (6)	3rd day (6)	5th day (6)	10th day (6)
RCI	2.38 ± 0.38	2.58 ± 0.62	3.03 ± 0.49†††	4.01 ± 0.35†	4.67 ± 1.60††
ADP/O	1.41 ± 0.12	1.49 ± 0.57	1.51 ± 0.36	1.57 ± 0.38	1.89 ± 0.02†
State III respiration (n Atoms/mg Prot./min.)	145.6 ± 22.5	119.3 ± 5.1†††	136.6 ± 35.8	175.2 ± 7.9††	164.9 ± 31.6
State II respiration (n Atoms/mg Prot./min.)	64.9 ± 7.3	54.4 ± 10.0	55.9 ± 16.3	57.9 ± 29.9	39.6 ± 17.3†††
ATP formation (n moles/mg Prot./min.)	197.8 ± 25.2	202.5 ± 60.0	169.6 ± 26.3	208.8 ± 23.4	272.7 ± 49.9††

Data were expressed as the mean ± standard deviation. †$p < 0.001$, ††$p < 0.01$, †††$p < 0.05$ compared with the pre-release group. Numbers in parentheses indicate numbers of animals.

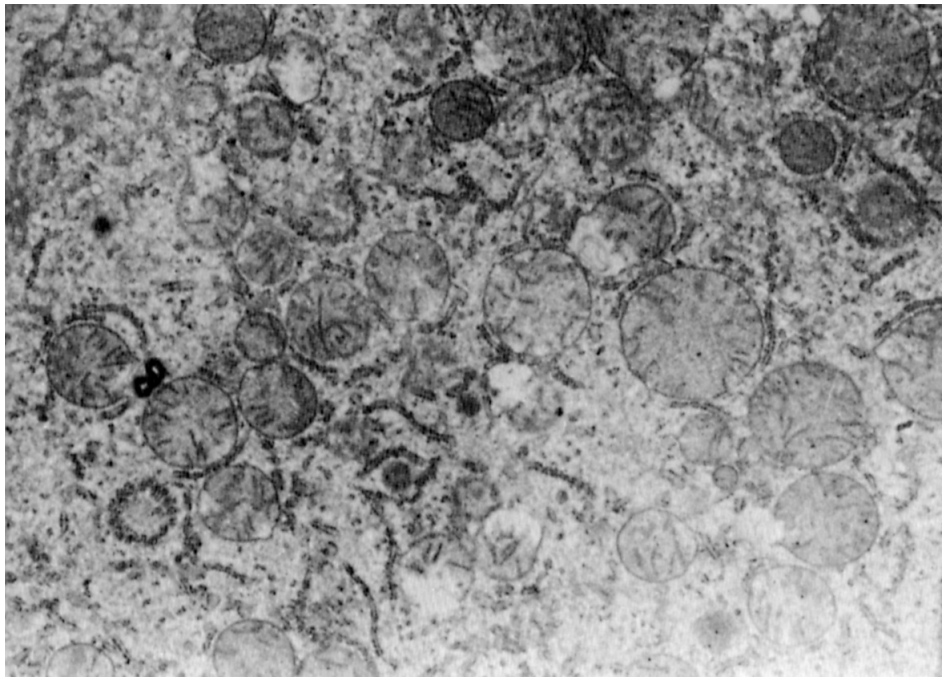

Fig. 1 Electron photomicrograph of liver cells after biliary obstruction of 2 weeks duration: Swelling of mitochondria is evident. Rough-surfaced endoplasmic reticulum shows decrease in number, and membrane-bound ribosomes are partially detached.
Magnification × 24,000.

II respiration showed no marked recovery up to the 5th day after removal of the obstruction but recovered to a considerable extent on the 10th day after release. However, the extent of recovery differed greatly from one individual rat to another. The rate of ATP formation also did not show a marked recovery until the 5th day after release, but on the 10th day it became normal (Table 6). There was no significant difference between the control and the J-2W-R-10 group.

The ultrastructure in the J-2W-R-10 group revealed that swelling of mitochondria was reduced but giant mitochondria were obvious (Fig. 2). The mean cut-surface area of mitochondria was 0.77 ± 0.45 (μ^2) with a significant difference from the control ($p < 0.001$).

The mortality rate of the rats was less than 15% in each group when biliary obstruction was kept within 3 weeks, but if it was continued for 6 weeks the mortality rate reached 80%, and at this point the liver apparently succumbed to cirrhosis. When obstruction was released in the J-3 group, no change was seen in the mortality rate of the J-3-R-5 group. But when obstruction was released in the J-2W group, the mortality rate was further increased, being 30% in the J-2W-R-5 group and 70% in the J-2W-R-10 group, with no further detection being feasible.

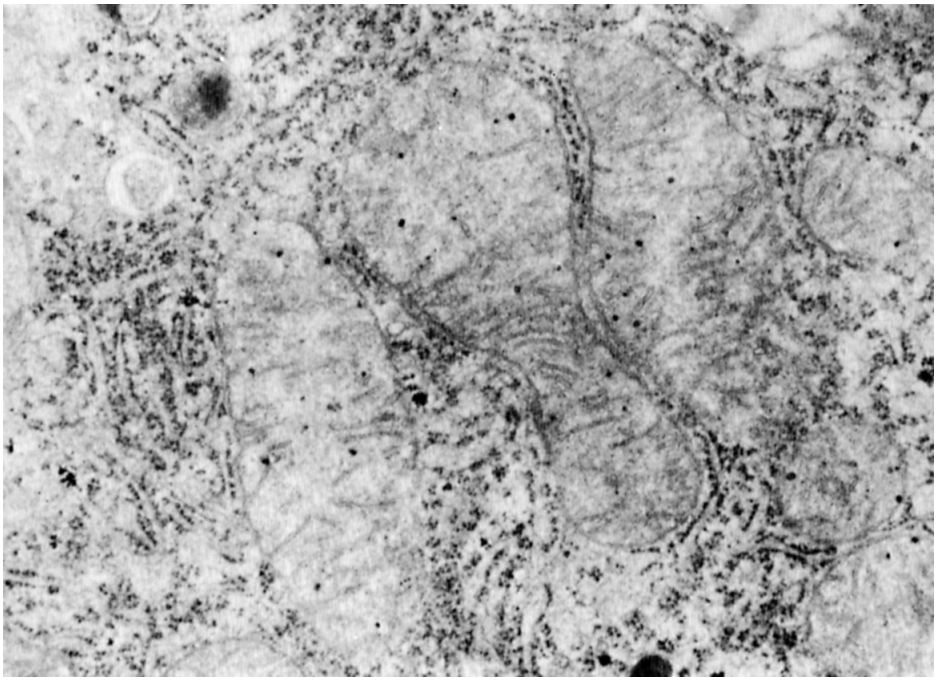

Fig. 2 Electron photomicrograph of liver cells on the 10th day after release of biliary obstruction of 2 weeks duration: Dumbbell-shaped giant mitochondria are present.
Magnification × 31,000.

DISCUSSION

The influence of obstructive jaundice on mitochondrial function has been reported,[3)-5)] and these studies suggest that a high energy production in mitochondria is one of the key factors in maintaining liver function. However, since it is generally difficult to reopen the experimentally obstructed bile duct sufficiently, the recovery processes after release of obstruction are less often studied.[2),12),13)] Using rat models,[7)] the author investigated the influence of obstructive jaundice and recovery processes after release of obstruction. This model was considered to be similar to the external biliary fistulation by percutaneous transhepatic bile drainage which is clinically applied today.

The serum level of bilirubin is most frequently used as an index of obstructive jaundice. The value increased to its peak in a week, but after that it either remained at a plateau or decreased somewhat. This was quite similar to the clinical phenomenon in which the total bilirubin level rose with the prolongation of the duration of the obstruction but became constant or fell off with a certain period as the boundary. Thus the state of the liver was not always reflected by the bilirubin levels. But longer time was required to normalize its value when the obstruction period was longer.[2),14)] Disturbance of the hepatic bilirubin excretion mechanism is considered to be reversible over a fairly long time. The values of transaminases increased gradually to a peak and then levelled off. The improvement of the biochemical tests after release of obstruction was rapid, indicating no more releases of the transaminases after opening the biliary tract. The m-GOT is located on the crista side of the membrane separating

the matrix of mitochondria from the crista. Bound firmly to the membrane, it is considered an enzyme which is not easily released.[15] Therefore, when m-GOT is released in the blood it is suspected that severe liver cell damage accompanied by breakage of the mitochondria has occurred, and/or an accelerated membrane permeability due to hypofunction of the mitochondria exists. That is, m-GOT is assumed to be strongly related to liver cell necrosis and disease in action.[16] In the author's investigation, m-GOT value was high only while the biliary obstruction persisted, and after release of obstruction it normalized in a short time. It is, however, impossible to presume the reserved mitochondrial function from the m-GOT value in the recovery process.

It has been generally confirmed that mitochondrial function is disturbed by bilirubin.[17),18] As stated above, although the bilirubin value arrived at a peak in one week after biliary obstruction and remained unchanged thereafter, mitochondrial function continued to decrease. Thus, based on the fact that bilirubin concentration was not parallel to mitochondrial function, liver mitochondrial dysfunction in obstructive jaundice should not be simply understood as the dysfunction caused when normal liver mitochondria were exposed to bilirubin.[12] In the present investigation RCI and ADP/O showed a decrease, which suggested that in dysfunction by bilirubin uncoupling of oxidative phosphorylation is considered a primary factor.[17] State III respiration maintained nearly a fixed value in spite of persistent biliary obstruction, which means that the electron transport system was hardly disturbed.[12]

Upon release 3 days after obstruction when early effects of biliary obstruction appeared, fairly good recovery was observed. But, even when the biochemical data including m-GOT were apparently normalized, liver mitochondrial function remained disturbed. Upon release of the obstruction after 2 weeks duration, too, liver mitochondrial function generally recovered well, although it was a little retarded in time compared with the group released from obstruction after 3 days. Biochemical data returned to normal range earlier than improvement of mitochondrial function after release of biliary obstruction, *i.e.* it was reconfirmed that both of them do not always recover in parallel. It must be emphasized that the recovery process is not estimated by the routine biochemical data but only by the mitochondrial fucntion.

The ultrastructure of jaundiced liver was investigated in detail by Hampton[19] and Steiner *et al.*[20] One of the features representative of the liver function is the volume of mitochondria. Under electron microscopic observation, swollen mitochondria decreased in number after release of obstruction, but still slightly swollen mitochondria were observed together with giant mitochondria. These findings were thought to express vigorous repair work from disturbance; that is, morphological changes of mitochondria were closely related to functional changes. It was also evident that the volumes of mitochondria represented the reserved function of the liver.

When obstructive jaundice is released after liver mitochondria have been seriously impaired, recovery cannot be expected to return to a level at which radical operation is applicable. Clinically, this is one of the reasons to execute biliary decompression as soon as possible for patients with obstructive jaundice. The present study also disclosed another aspect of risk factors after release of biliary obstruction. After release of obstructive jaundice, there exists a certain period during which there is insufficient recovery of liver mitochondrial function even when the biochemical data are apparently normalized. This is much importance when considering risk factors of major surgery after release of biliary obstruction.

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