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# Mechanism of primary graft non-function in a rat model for fatty liver transplantation

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**Abstract** We established a fatty liver model in rat suitable for the model of human liver with steatosis by cholesterol enriched chow, and investigated the mechanism of primary graft non-function in fatty liver transplantation (LTx) using this model. Grafts with steatosis caused primary graft dysfunction after LTx following even short cold preservation; however, no significant difference was recognized in mitochondrial function of the graft during preservation. Morphological findings were not different at 1 h after reperfusion between non-steatotic and steatotic livers. Focal necrosis of hepatocytes was seen and the sinusoidal endothelial cells were injured 24 h after reperfusion. In addition, the fluidity of the plasma membrane decreased in fatty liver. Our results indicate that deterioration of sinusoidal endothelial cells after reperfusion causes graft dysfunction in LTx of steatotic liver.

**Key words** Fatty liver transplantation · Cholesterol-rich chow · Primary graft non-function · Plasma membrane · Mitochondrial function · Sinusoidal endothelial cell

## Introduction

It has been reported that the prevalence of fatty liver among the potential donor population is high, ranging from 13 to 26% [2, 6], and it is well known that steatosis often leads to graft dysfunction after transplantation. However, only a few studies have been done to investigate the mechanism involved [8]. Clinically, fatty liver is caused by various conditions which include sustained alcohol intake, obesity, metabolic or endocrine disease or conditions such as diabetes mellitus, pregnancy, parenteral hyperalimentation, the use of certain drugs, and hepatitis C. Despite various causes of fatty liver, in most experimental models of fatty liver in rats, hepatic steatosis was produced by choline and methionine deficient chow, or ethanol intake; in addition, obese Zucker rats were used which bear a genetic mutation. Fatty liver produced by choline and methionine deficient chow and obese Zucker rats are not suitable as models of human liver with steatosis because of malnutrition and the presence of genetic mutation, respectively. Ethanol intake is only one reason out of many. No experimental transplantations of fatty liver induced by calorie overload has been done.

The purposes of this study were to establish a fatty liver model in rats, which was similar to steatotic liver in humans, induced by cholesterol rich chow, and to investigate the mechanism of graft dysfunction after fatty liver transplantation.

## Materials and methods

### Animals

Male Wistar rats, 4 weeks of age, were used for donors. The animals were devided into two groups. Rats were fed normal rat chow in the control group, and normal chow with 10% lard and 2.5% cholesterol in the fatty liver group for 4 weeks, respectively. Male Wistar rats, 8–9 weeks of age and weighing 250–350 g, were used as recipients. All animals were given free access to water. After liver transplantation, rats in both groups were fed normal rat chow.

### Liver transplantation procedure

The rats were anesthetized with ether, and liver transplantation was performed using the technique described by Zimmermann and Kamada with minor modification [22]. Briefly, heparin (300 IU) in 2 ml saline solution was injected into the penile vein, and a 4 mm long stent prepared from polyethylene tube was inserted into the common bile duct and secured with 6-0 sutures. Livers were flushed in situ with 5 ml saline solution at 4 °C followed by 10 ml of University of Wisconsin (UW) solution at 4 °C. Venous cuffs prepared from 14 and 12 gauge intravenous catheters were placed in the portal vein and infrahepatic vena cava, respectively, and grafts were stored in UW solution at 4 °C for 1, 6, 12, 18 and 24 h. For transplantation, the liver of the recipient was removed after clamping the suprahepatic vena cava, the portal vein, and the infrahepatic vena cava, and grafts were transplanted by connecting the suprahepatic vena cava with a running suture, then inserting cuff into the appropriative vessels and securing them with 6-0 sutures. The bile duct was anastomosed with an intraluminal stent. The transplantation procedure required less than 45 min, during which time the portal vein was clamped for 12–15 min. Five rats in each groups were transplanted and observed for 7 days and survival rate was calculated.

### Examination items

For evaluation of the graft viability before implantation, five rats were examined in each groups. Rats were anesthetized with ether, and livers were perfused through a cannula inserted in the portal vein with 5 ml saline solution at 4 °C followed by 10 ml UW solution at 4 °C and removed. At laparotomy, a specimen was taken and fixed by 10% formalin for hematoxylin and eosin stain. Another specimen was removed to measure the lipid content of liver tissue. Liver specimens were removed and frozen in liquid nitrogen at laparotomy and then every 6 h up to 24 h during cold preservation to measure the change of ATP and proton ATP ase activity of liver tissue. After liver transplantation following 1 and 6 h cold preservation, for evaluation of the function of sinusoidal endothelial cells, measurement of hyaluronic acid (HA) clearance and histological examination were carried out.

### Lipid content of liver tissue

Lipid content of liver tissue was measured as described by Folch et al. [5]. The weights of triglyceride, phospholipid, and cholesterol were measured by spectrophotometer using the commercial kit, triglyceride-Test-Wako, phospholipid-Test-Wako (Wako Pure Chemical Industries, Osaka, Japan), and F-kit-cholesterol (Roche Molecular Biochemicals, Tokyo, Japan), respectively.

### Measurement of ATP

Tissue samples were freeze clamped and stored at −80 °C before they were extracted in perchloric acid, and measured by high performance liquid chromatography.

### Measurement of mitochondrial proton ATP ase activity

Mitochondrial proton ATP ase activity was measured by the method of Seya et al. [7]. In brief, mitochondria were isolated from the liver by the high-yield differential centrifugation method. ATP ase activity was measured by a Jasco FP-777 fluorometer (Jasco, Tokyo, Japan) at 23 °C under stirring, with excitation of 625 nm and emission of 670 nm. A total of 100 µl mitochondria was added to 2 ml potassium buffer at pH 7.4 containing 225 mM potassium chloride, 5 mM magnesium chloride, 10 mM potassium diphosphate, and 2 mM triethanolamine. The fluorescence intensity of this mixture was regarded as baseline. Then, 100 µl of 50 mg/l diS-C3 in ethanol was added to the mixture. The difference between the fluorescence intensity of this mixture and the baseline was taken to represent a standard electric potential. Succinic acid 9 µmol and 1.25 µg antimycin A were added to the mixture. ATP 2 µmol was added to this medium, and the change in the fluorescence intensity was regarded as the ATP ase activity. We confirmed that the proton ATP ase activity measured in this procedure was inhibited by 25 µg oligomycin. ATP ase activity was calculated as follows: proton ATP ase activity (%) = change in the fluorescence intensity by ATP/standard electric potential × 100.

### Plasma membrane preparation

The membranes were isolated according to the procedure of Fremont et al. [7], modified by Epping and Bygrave [3], using a discontinuous Percoll density gradient (Pharmacia Biotech AB, Uppsala, Sweden). The liver specimens were removed at laparotomy, immersed in ice-cold N-[2-hydroxyethyl] piperazine-N'[2-ethanesulfonic acid] (HEPES) buffer, which contains 250 mM sucrose/ml and 0.5 mM ethylene glycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid(EGTA)/L and adjusted by KOH at pH 7.4, and then cut into small fragments with scissors and homogenized at 4 °C with 15 up-and-down strokes at 200 rpm in the same buffer (50% w/w). The crude suspension was filtered through nylon mesh, and the filtrate was centrifuged at 10000 g for 10 min at 4 °C. The supernatant, designated as the homogenate, was recovered after discarding floating lipids. Percoll solution was adjusted to pH 7.4 with HCl at 4 °C, and solution of Percoll (75%, 30%, 25%, 18%, and 10%) were mixed with HEPES buffer. The liver plasma membrane-enriched fraction was obtained in the layer of 10% Percoll with HEPES buffer after one centrifugation step at 48000 g for 4 min at 4 °C. Traces of remaining Percoll were eliminated by centrifugation (50000 g for 10 min at 4 °C) in a large volume of 10 mM TRIS-HCl/L buffer containing 150 mM NaCl adjusted at pH 7.4. The pellet of membrane was washed in HEPES buffer without sucrose and stored at −80 °C.

### Lipid extraction and analysis

Total lipid membrane was extracted with chloroform/methanol (2 : 1) containing 0.005% butylated hydroxytoluene, according to the method of Folch et al. [16]. Total membrane cholesterol, phospholipids, and triglycerides were determined by thin-layer chromatography and flame-ionization detector method (Iatroscan TH-10, Iatron Laboratories, Inc., Tokyo, Japan) in total lipid. Membranous phospholipids were eluted by aminopropyl column chromatography using methanol. After transmethylation, fatty acid components in the membranous phospholipids was determined by gas-liquid chromatography.

### Measurement of hyaluronic acid (HA) clearance

Rat was reanesthized with an intramuscular administration of ketamine (75 mg/kg), and an intravenous line was placed in the right jugular vein. A blood sample (200 µl) was taken for measurement

of serum HA at 1 h after reperfusion. Sodium hyaluronate (Sigma Chemical Co., St. Louis, Mo., USA) in sodium phosphate buffer (10 mg/ml) was then injected through the catheter (0.5 mg/kg). At 3 min and 30 min after injection, 200 μl of blood samples were collected, immediately followed by administration of 200 μl saline solution. HA was measured by a commercial ELISA assay kit (Reads Medical Products, Inc., Westminster, UK). All samples were stored at −20 °C until they were assayed. HA clearance was calculated as the ratio of the serum HA at 3 min to that at 30 min.

### Histological examination

After liver transplantation followed by 1 and 6 h cold preservation, the recipient rats were anesthesized with ether, and 100 IU heparin in 0.5 ml of saline solution was injected into the penile vein and specimen of transplanted livers were taken 1 and 24 h after reperfusion. A lobe was removed and a specimen was fixed by 10% formalin for hematoxylin and eosin stain. Another lobe was removed from the livers and the specimens were fixed by perfusion through a cannula inserted in the portal vein of 2–3 ml/min of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.3. The livers were cut into small cubes and immersed in the same fixatives used for perfusion-fixation and allowed to stand overnight. After postfixation in 1.0% osmium tetroxide, they were dehydrated by passing through a 50–100% gradient of ethanol, then passed through propylene oxide, and embedded in Epon 812. Ultrathin sections were counter stained with uranyl acetate and lead citrate, and observed under a transmission electronmicroscope (JEM-1010; Japan Electron Optics Laboratories, Tokyo, Japan).

### Statistical analysis

All results were expressed as mean ± standard deviation (SD). The statistical significance of differences were determined by Student's *t*-test. A *P*-value of $< 0.05$ was considered statistically significant.

**Table 1** Survival rate on postoperative day 7 after LTx

| | Preservation time (h) | | | | |
|---|---|---|---|---|---|
| | 1 | 6 | 12 | 18 | 24 |
| Control group | 5/5 | 5/5 | 5/5 | 2/5 | 0/5 |
| Fatty liver group | 5/5 | 3/5 | 0/5 | 0/5 | 0/5 |

**Table 2** Lipid content of liver plasma membrane in control and fatty liver groups. Data were presented as mean ± SD. *PUFA*, polyunsaturated fatty acid

| | Control group ($n = 5$) | Fatty liver group ($n = 5$) |
|---|---|---|
| Triglyceride (mg/g protein) | 105.6 ± 33.7 | 58.6 ± 5.5[a] |
| Cholesterol (mg/g protein) | 125.2 ± 12.1 | 163.8 ± 17.5[b] |
| Phospholipid (mg/g protein) | 587.2 ± 30.0 | 555.0 ± 34.8 |
| PUFA (mg/g protein) | 318.8 ± 41.9 | 274.7 ± 17.8[a] |

[a] $P < 0.05$ vs control group; [b] $P < 0.01$ vs control group

**Table 3** Major fatty acid composition of phospholipids in liver plasma membrane in control and fatty liver groups. Data were presented as mean ± SD

| Fatty acid (wt%) | Control group ($n = 5$) | Fatty liver group ($n = 5$) |
|---|---|---|
| 16 : 0 | 17.0 ± 3.1 | 14.8 ± 0.7 |
| 18 : 0 | 22.9 ± 1.2 | 27.3 ± 1.0[a] |
| 18 : 1 | 5.1 ± 0.2 | 7.8 ± 0.6[a] |
| 18 : 2 | 13.9 ± 1.1 | 18.8 ± 1.0[b] |
| 20 : 4 | 29.1 ± 3.5 | 19.8 ± 0.9[c] |
| 20 : 5 | 0.1 ± 0.1 | 1.0 ± 0.1[b] |

[a] $P < 0.001$ vs control group; [b] $P < 0.0001$ vs control group; [c] $P < 0.01$ vs control group

## Results

### Body weight, liver weight and lipid content of liver tissue

Body weight of rats in each groups increased from about 90 to 250 g over 4 weeks and there was no significant difference. Liver weights of each group were 8.6 ± 0.5 g and 10.9 ± 0.4 g, respectively, and there was statistical significant ($P < 0.05$). The weights of triglyceride, cholesterol, and phospholipid of liver tissue in both groups were 17.7 ± 3.3 mg/g liver, 2.0 ± 0.1 mg/g liver and 23.7 ± 1.1 mg/g liver in the control group, and 56.4 ± 2.5 mg/g liver, 24.5 ± 3.5 mg/g liver and 27.3 ± 0.9 mg/g liver in the fatty liver group, respectively.

### Survival after liver transplantation

In the control group, all rats survived for 1 week after liver transplantation following cold preservation for 12 h. In contrast, in the fatty liver group, 40% of rats died within 1 week after transplantation following cold preservation for 6 h and all rats died within 2 days after transplantation following cold preservation for 12 h (Table 1).

### ATP and proton ATP ase activity during preservation

At laparotomy, in fatty liver group, ATP was significantly lower compared with the control group, but for the other determination point, there was no significant difference. ATP ase activity of both groups decreased in a time dependent manner and that of fatty liver group tended to fall; however, there was no significant difference except at 18 h cold preservation (Fig. 1).

### Lipid composition of plasma membrane

Total membrane lipid contents are shown in Table 2 and Table 3. In particular the polyunsaturated fatty acids

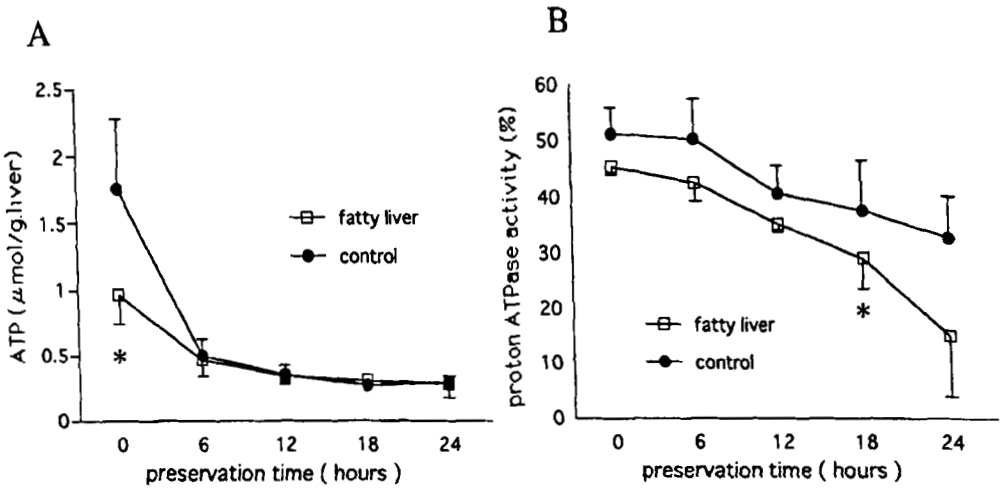



**Fig. 1** Change of ATP (**A**) and proton ATPase activity (**B**) during cold preservation. At laparotomy, in fatty liver group, ATP is lower significantly compared with control group, but the other determination point, there was no significant difference. Proton ATP ase activities of both groups decreased in time dependent manner and those of the fatty liver group were lower, compared with those of the control group; however, there was no significant difference except at 18 h (* $P < 0.05$)

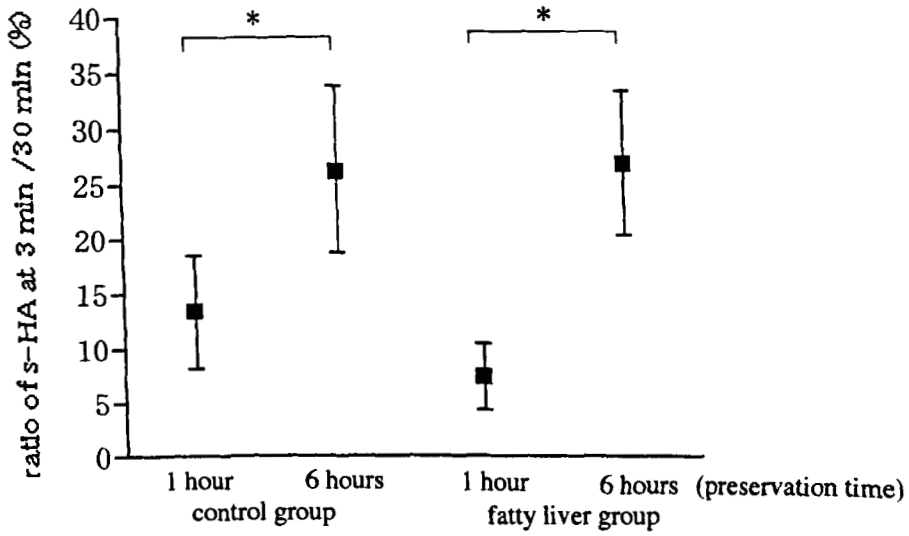



**Fig. 2** The clearance of hyaluronic acid *(HA)* after transplantation. HA clearance was presented as the ratio of the serum HA at 3 min and that at 30 min after injection of HA. In both groups, after transplantation following 6-h cold preservation, the rate of serum HA level increased compared with that after 1-h cold preservation; however, no significant differences were recognized between the steatotic and non-steatotic grafts (* $P < 0.05$)

(PUFA) and arachidonic acid decreased in fatty liver group.

## Hyaluronic acid (HA) clearance

In both groups, after transplantation following 6 h cold preservation, the rate of serum HA level (3 min/30 min after injection of HA) increased compared with that after 1 h cold preservation ($P < 0.05$); however, there was no significant difference between control and fatty liver group (Fig. 2).

## Histological findings by light microscope

In the control group, at laparotomy, hepatocytes contained few fat and sinusoidal lumens were widely opened. In fatty liver group, from zone 2 to zone 1, hepatocytes contained fat and fat droplets occupied most part of the cytosol and the sinusoidal lumens were remarkably narrow and irregular. In zone 3, hepatocytes contained few fat droplets and sinusoidal lumens were widely opened. Hepatocytes containing fat droplets were 30–60% of the total (Fig. 3).

At 1 h after reperfusion following cold preservation for 1 h, in the control group, sinusoidal lumens were widely opened and there was no degenerative change of hepatocytes (Fig. 4A). In the fatty liver group, from zone 2 to zone 1, sinusoidal lumens were narrowed by hepatocytes containing fat droplets. There was no degenerative change in hepatocytes (Fig. 4B). At 1 h after reperfusion following cold preservation for 6 h, the light microscopical findings did not differ from that following cold preservation for 1 h in both groups. However, 24 h after reperfusion following cold preservation for 6 h, in

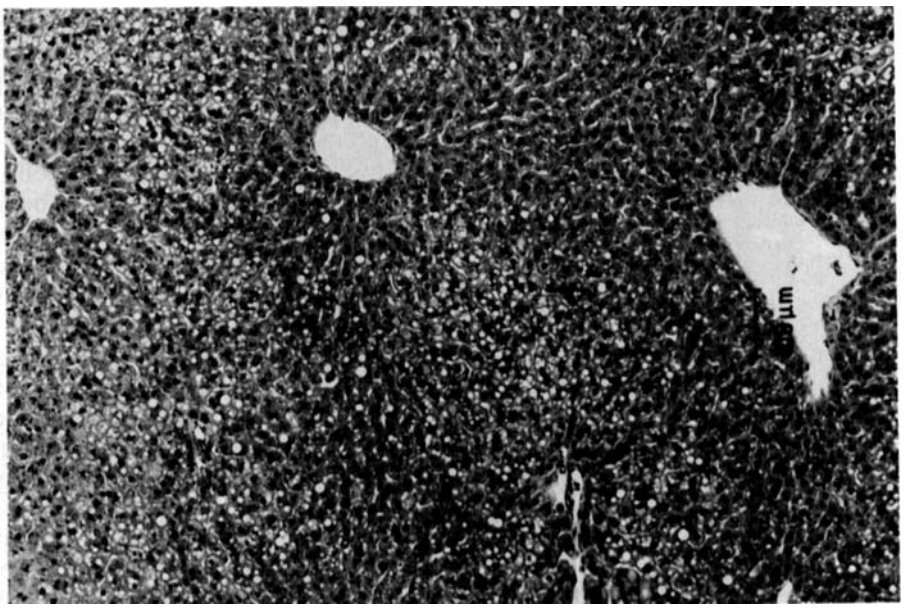


**Fig. 3** LM findings of fatty liver produced by cholesterol rich chow. From zone 2 to zone 1, hepatocytes contained fatty deposits and fat droplets occupied most of the cytosol and the sinusoidal lumens were narrow and irregular remarkably. In zone 3, hepatocytes contained few fat droplets and sinusoidal lumens were wide. Hepatocytes containing fat droplets were 30–60% of the total

the fatty liver group, there was focal necrosis of hepatocytes in zone 2. There was no degenerative change in the control group (Fig. 4C, D).

### Histological findings by transmission electron microscope

At 1 h after reperfusion following cold preservation for 1 h, sinusoidal endothelial cells were well preserved in both groups (Fig. 5A, B).

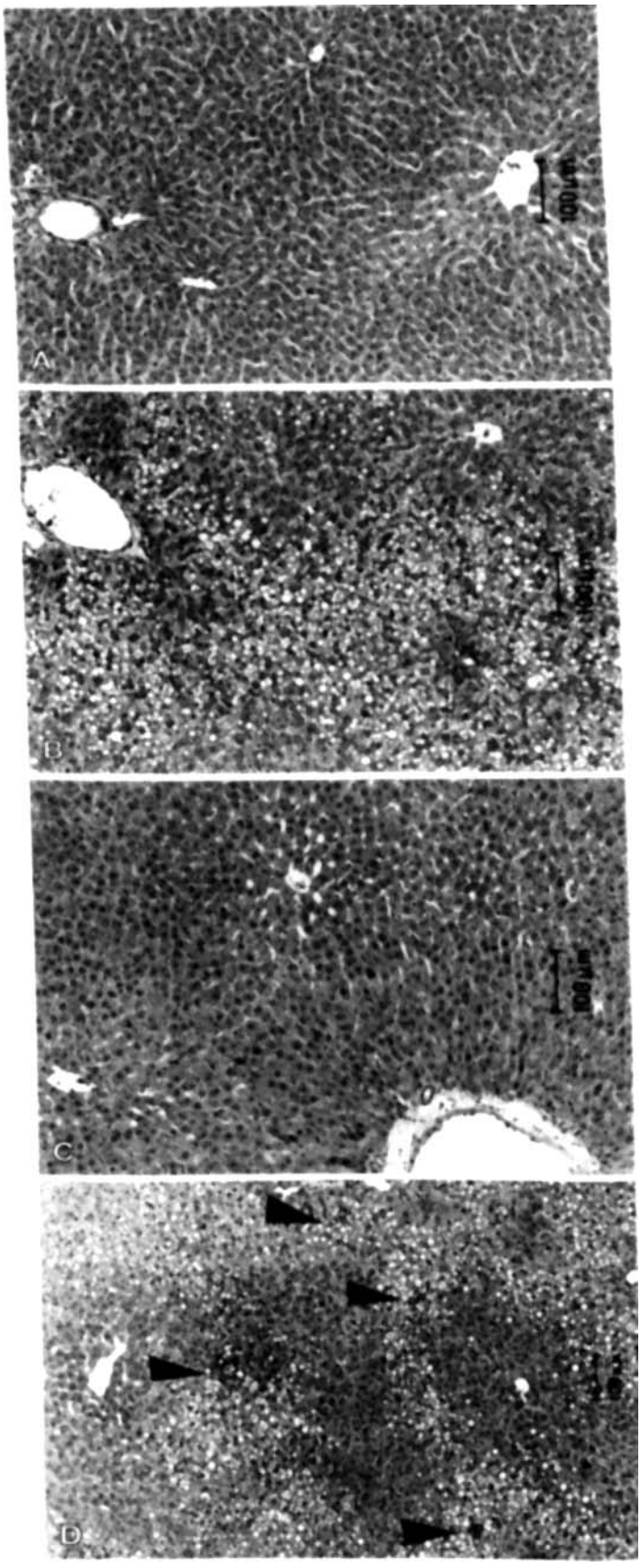
At 1 h after reperfusion following cold preservation for 6 h, sinusoidal lining cells were well preserved in control group (Fig. 5C); however, in the fatty liver group, there was discontinuity of the sinusoidal lining and destruction of sieve plates. Sinusoidal endothelial cells were round and irregular (Fig. 5D).

## Discussion

It is well known that severe fatty infiltration often causes graft dysfunction after liver transplantation in humans [21]. To investigate the mechanism of graft dysfunction in fatty liver transplantation, we established a fatty liver model in rats by cholesterol-rich chow. In our established fatty liver model, livers showed moderate macrovesicular steatosis and were considered suitable for the LTx experiment because the degree of steatosis, i.e., moderate macrovesicular steatosis, is considered to be the borderline for use of steatotic liver grafts in humans [4]. In this model, steatosis was located mainly in zone 2; however, steatosis is usually located in zone 3 in humans. Although there was a difference in localization of the fatty deposits, we used this fatty liver model in rats for investigation of primary graft nonfunction after transplantation.

It was reported that the amount of ATP in liver graft was strongly associated with the results of liver transplantation [12]. Recently this concept was resised and it was necessary to measure the recovery rate of mitochondrial ATP synthesis pre- and post-reperfusion. We reported that proton ATP ase activity, the key enzyme of mitochondrial ATP synthesis, was useful for the evaluation of mitochondrial energy productive capacity [15]. In this study, there was no difference in ATP of the graft except at laparotomy. Proton ATP ase activity of both groups decreased in a time dependent manner and that of the fatty liver group seemed to fall compared with that of the control group. There was no significant difference except at 18 h cold preservation. These results indicated that mitochondrial function in the moderately steatotic liver was preserved during cold preservation for up to 12 h.

In ischemia-reperfusion injury of the liver, the sinusoidal endothelial cells are mainly injured and the degree of injury determine the graft viability [9, 18]. Teramoto et al. noted that sinusoidal microcirculatory disorder and blood cell adhesion to the endothelial cells increased after transplantation using a model fed a diet deficient of choline and methionine [20]. Fukumori et al. reported that injury of sinusoidal endothelial cells during cold preservation markedly increased in fatty liver of Zucker rats [8]. In this study, transmission electron microscopical findings indicated that the sinusoidal endothelial cells were injured not in cold preservation but mainly after reperfusion.




HA was cleared by sinusoidal endothelial cells and the clearance of HA reflected the function of sinusoidal endothelial cells [18]. In our study, there was no significant difference in HA clearance 1 h after reperfusion between control and fatty liver group. One hour after reperfusion following 6-h preservation, HA clearance decreased in both groups compared with that following cold preservation for 1 h. No definite injury was observed by transmission electron microscope in either group. These results indicated that no morphological injury of sinusoidal endothelial cells was recognized; however, functional deterioration of these cells seemed to exist. The sinusoidal endothelial cells at 24 h after reperfusion following cold preservation for 6 h were injured in the steatotic graft and 40% of animals died within 7 days after transplantation in the fatty liver group. The cause of the high mortality rate was associated with the fact that the injury of sinusoidal endothelial cells progressed after reperfusion, probably because of an increase in free radicals and cytokines from activated Kupffer cells and neutrophils [16, 17].

Fluidity is one of most important physical properties of membranes, and the lipid content of the plasma membranes influences on the fluidity. The major factor influencing membrane fluidity is the degree of unsaturation of the acyl chain [11, 14, 19]. Fukumori et al. reported that the lipid content of plasma membrane in steatotic liver differed from that of non-steatotic liver, and that polyunsaturated fatty acids decreased in steatotic liver compared with non-steatotic liver in Zucker rats [8]. The stiffening effect on the membrane can be counteracted in various ways [14], and polyunsaturated fatty acid in particular could play an important role in controlling the fluidity of membranes. In our studies, the amount of polyunsaturated fatty acids significantly decreased in steatotic liver. The results indicated that steatotic liver was susceptive to cold preservation-reperfusion injury because of the decreased fluidity of the membrane. In addition, Fukumori et al. reported that arachidonic acid (20 : 4) decreased in the plasma membrane in fatty liver [8]. On the other hand, Phinney et al. reported that the arachidonic acid was decreased

**Fig. 4** LM findings of the transplanted graft. **A** Non-steatotic graft, 1 h after reperfusion following 1-h cold preservation. Sinusoidal lumens were widely opened and no degenerative change of hepatocytes were observed. **B** Steatotic graft, 1 h after reperfusion following 1-h cold preservation. From zone 2 to zone 1, sinusoidal lumens were narrowed by hepatocytes containing fat droplets. No degenerative change in hepatocytes were observed. **C** Non-steatotic graft, 24 h after reperfusion following 6-h cold preservation. Sinusoidal lumens were widely opened and no degenerative change of hepatocytes were observed. **D** Steatotic graft, 24 h after reperfusion following 6-h cold preservation. In addition to the narrowing of the sinusoidal lumen, focal necrosis of hepatocytes were observed in zone 2 (arrowheads)

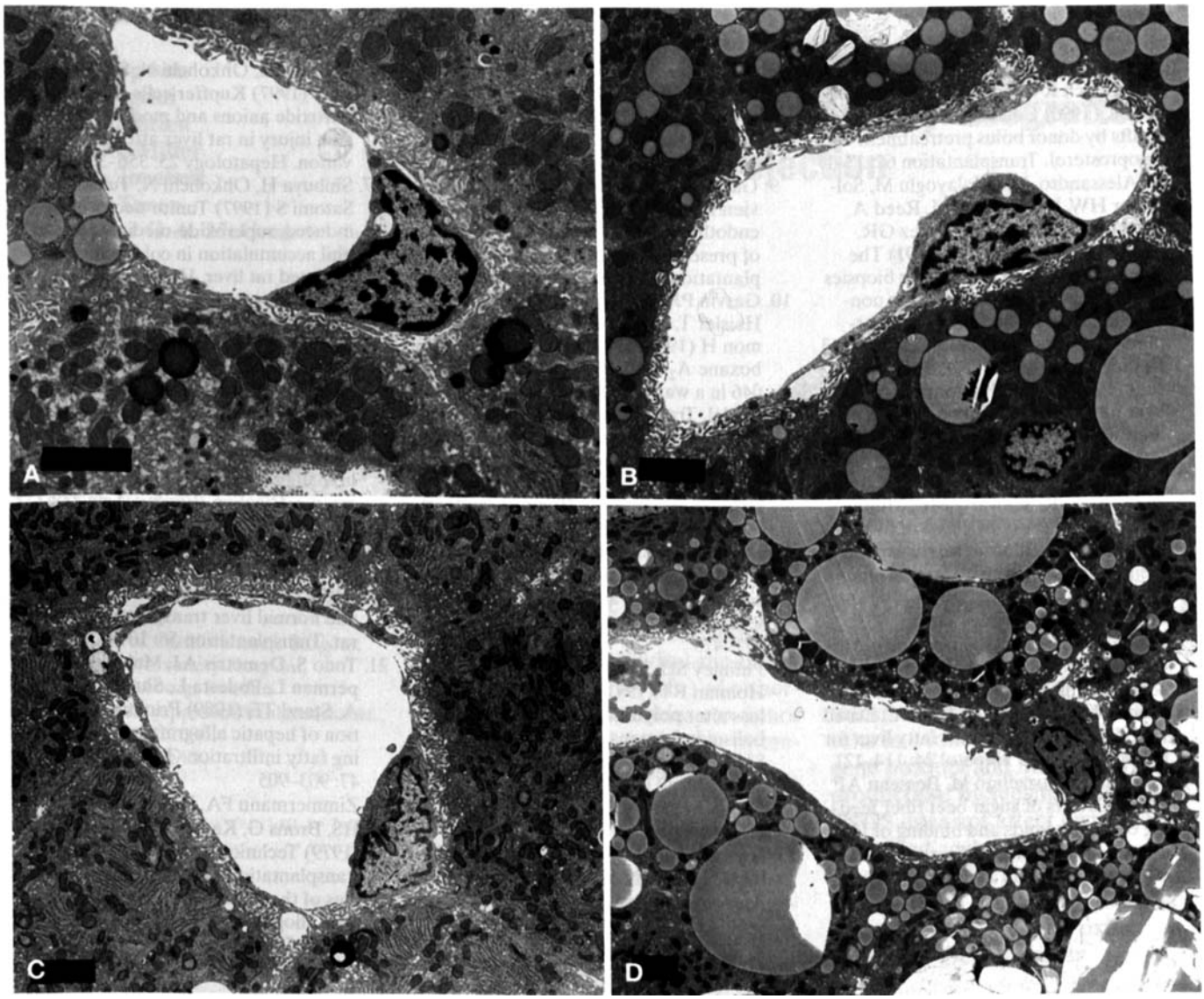



**Fig. 5** Ultrastructical findings by transmission electron microscope. **A** Non-steatotic graft, 1 h after reperfusion following 1-h cold preservation. Sinusoidal endothelial cell was well preserved. **B** Steatotic graft, 1 h after reperfusion following 1-h cold preservation. Sinusoidal endothelial cell and sinusoidal lining were well preserved. **C** Non-steatotic graft, 24 h after reperfusion following 6-h cold preservation. Sinusoidal endothelial cell was still well preserved. **D** Steatotic graft, 24 h after reperfusion following 6-h cold preservation. Discontinuity of the sinusoidal lining and destruction of sieve plates were observed. Sinusoidal endothelial cells were round and irregular. Each bar indicates 3 μm

in the serum of obese humans [13]. Arachidonic acid is released from the plasma membrane by various stimuli and is a precursor of eicosanoids. Several studies have indicated that thromboxane $A_2$ synthetase inhibitor or prostaglandin $I_2$ analog, which are the metabolic products from arachidonic acid, protected the liver from ischemia-reperfusion injury [1, 10]. In our study, arachidonic acid of plasma membrane decreased significantly in steatotic liver. Therefore, the abnormal low content of arachidonic acid in the plasma membrane is likely to be associated with the preservation-reperfusion injury of fatty livers after transplantation.

In summary, we have established a fatty liver model in rats, which was suitable for the experiment to investigate the mechanism of primary graft non-function after steatotic liver transplantation. The steatotic liver was susceptible to graft dysfunction after transplantation even following short cold preservation. It was also clarified that narrowing of sinusoidal lumen and injury of sinusoidal endothelial cells caused graft dysfunction after transplantation of a liver with steatosis.

## References


1. Anthuber M, Farkas S, Rihl M, Menger MD, Jauch KW, Schildberg FW, Messmer K (1996) Conditioning of liver grafts by donor bolus pretreatment with epoprosterol. Transplantation 62: 13–17
2. D'Alessandro AM, Kalayoglu M, Sollinger HW, Hoffmann RM, Reed A, Knechtle SJ, Pirsch JD, Hafez GR, Lorentzen D, Belzer FO (1991) The predictive value of donor liver biopsies for the development of primary nonfunction after orthotopic liver transplantation. Transplantation 51: 157–163
3. Epping RJ, Bygrave FL (1984) A procedure for the rapid isolation from rat liver of plasma membrane vesicles exhibiting $Ca^{2+}$ transport and $CA^{2+}$ATPase activities. Biochem J 223: 733–745
4. Fishbein TM, Fiiel MI, Emre S, Cubukcu O, Guy SR, Schwartz ME, Miller CM, Sheiner PA (1997) Use of livers with microvesicular fat safely expands the donor pool. Transplantation 64: 248–251
5. Folch J, Lees M, Sanley GHS (1957) A sample method for isolation and purification of total lipids from animal tissue. J Biol Chem 226: 497–509
6. Franco T, Alessandra C, Paolo C, David H (1996) The use of donor fatty liver for transplantation. J Hepatol 24: 114–121
7. Fremont L, Gozzelino M, Bosseau AF (1993) Effects of sugar beet fiber feeding on serum lipids and binding of low density lipoproteins to liver membrane in growing pig. Am J Clin Nutr 57: 524–532
8. Fukumori T, Ohkohchi N, Tsukamoto S, Satomi S (1999) The mechanism of injury in a steatotic liver graft during cold preservation. Transplantation 67: 195–200
9. Gao W, Bentley RC, Madden JF, Clavien PA (1998) Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation. Hepatology 27: 1652–1660
10. Garvin PJ, Niehoff ML, Robinson SN, Heisler T, Madrigal L, Contis J, Solomon H (1996) Evaluation of the thromboxane $A_2$ synthetase inhibitor OKY-046 in a warm ischemia-reperfusion rat model. Transplantation 61: 1429–1434
11. Hazel JR, Williams EE (1990) The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Prog Lipid Res 29: 167–227
12. Lanir A, Jenkens RL, Caldwell C (1988) Hepatic transplantation survival: correlation with adenine nucleotide level in donor liver. Hepatology 8: 471–475
13. Phinney SD, Davis PG, Johnson SB, Holman RT (1991) Obesity and weight loss alter polyunsaturated lipid metabolism in humans. Am J Clin Nutr 51: 831–838
14. Schachter D (1984) Fluidity and function of hepatocyte plasma membrane. Hepatology 4: 140–151
15. Seya K, Ohkohchi N, Watanabe N, Shibuya H, Taguchi Y, Mori S (1994) Rapid fluorometric assay for mitochondrial proton adenosine triphosphatase activity for assessment of viability of liver graft tissue. J Clin Lab Anal 8: 418–423
16. Shibuya H, Ohkohchi N, Seya K, Satomi S (1997) Kupffer cells generate superoxide anions and modulate reperfusion injury in rat liver after cold preservation. Hepatology 25: 356–360
17. Shibuya H, Ohkohchi N, Tukamoto S, Satomi S (1997) Tumor necrosis factor-induced, superoxide-mediated neutrophil accumulation in cold ischemia/reperfused rat liver. Hepatology 26: 113–120
18. Shimizu H, Wei H, Ping G, Dziadkoviec I, Miyazaki M, Falk RE (1994) Serum hyaluronate in the assessment of liver endothelial cell function after orthotopic liver transplantation in the rat. Hepatology 20: 1323–1329
19. Shinitzky M (1984) Membrane fluidity in malignancy adversative and recuperative. Biochim Biophys Acta 738: 251–261
20. Teramoto K, Bowers JL, Kruskal JB, Clouse ME (1993) Hepatic microcirculatory change after reperfusion in fatty and normal liver transplantation in the rat. Transplantation 56: 1076–1082
21. Todo S, Demetris AJ, Makowka L, Teperman L, Podesta L, Shaver T, Tzakis A, Starzl TE (1989) Primary nonfunction of hepatic allografts with preexisting fatty infiltration. Transplantation 47: 903–905
22. Zimmermann FA, Butcher GW, Davis HS, Brons G, Kamada N, Turel O (1979) Techniques of orthotopic liver transplantation in the rat and some studies of the immunologic response to fully allogenic liver grafts. Transplant Proc 1: 571–557