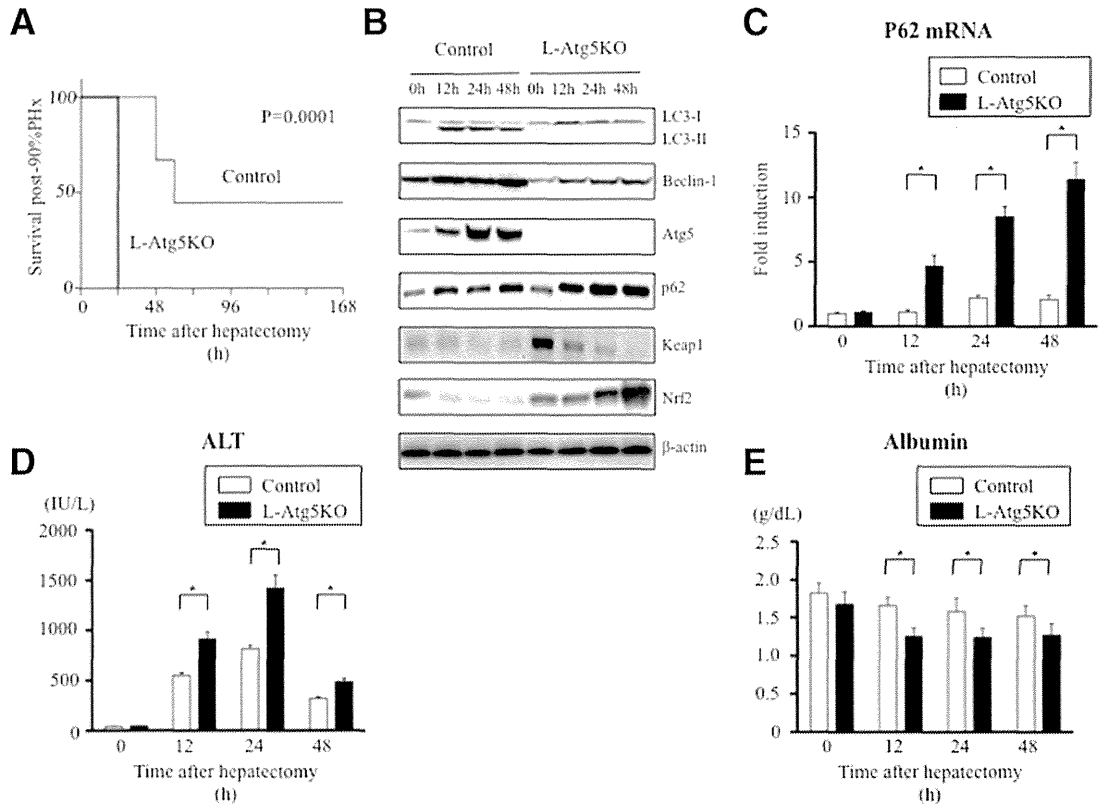


Fig. 2. Liver regeneration and survival after PHx is impaired in L-Atg5 KO mice. (A) Survival rate of control and L-Atg5 KO mice after 90% PHx. (B) Expression of autophagy-related proteins and Keap1/Nrf2 proteins in control and L-Atg5 KO mice at 0-48 hours after 70% PHx (representative western blottings are shown). (C) Fold change in p62 mRNA expression. (D and E) Changes in serum ALT and albumin levels after 70% PHx. $*P < 0.05$.

(Supporting Fig. 7A). These results indicate that autophagic flux is increased during liver regeneration. *In vitro*, the increase in LC3-II levels induced by HGF was also greater in the presence of CQ than in the absence of CQ (Supporting Fig. 7), indicating that HGF increases autophagic flux in hepatocytes.

***Impaired Recovery of Liver Regeneration in L-Atg5 KO Mice After PHx.*** All mice survived after 70% PHx in control and L-Atg5 KO mice. Excessive parenchymal damage by extended PHx has been proposed as the principal cause of hepatic failure, but little is known regarding the contribution of autophagic activity for primary deficiency in liver regeneration.[5] We developed a mouse model of 90% PHx to assess the effect of autophagic impairment on hepatic regenerative capacity of a critically small liver remnant. After 90% PHx, approximately 50% of control mice survived, whereas all L-Atg5 KO mice died after 24 hours (Fig. 2A). p62 expression after 70% PHx was higher in the regenerating liver in L-Atg5 KO mice than in control mice (Fig. 2B,C), which indicates that autophagic activity is essential to reorganize and modulate hepatocellular protein and organelle synthesis to achieve adequate regeneration. Serum ALT concentrations were significantly higher and serum albumin concentrations were lower after 70% PHx in L-Atg5 KO than in control mice (Fig. 2D,E and Supporting Fig. 8). These findings indicated that activation of autophagy in proliferative hepatocytes may be involved in cell survival and hepatic function after hepatectomy in the regenerating liver. In addition, Keap1 levels in liver of L-Atg5 KO mice were decreased by 70% PHx, and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) levels were higher in L-Atg5 KO mice than in control mice, as expected (Fig. 2B). These results indicated that the increase in p62 messenger RNA (mRNA) levels may have been mediated by compensatory Nrf2 activation in L-Atg5 KO mice.

***DNA Synthesis During Liver Regeneration Is Attenuated and Delayed in L-Atg5 KO Mice.*** DNA synthesis at the indicated times after PHx was determined by measuring bromodeoxyuridine (BrdU) incorporation, and the cell cycle was analyzed by propidium iodide PI staining. We found that the percentage of BrdU-positive cells in proliferating hepatocytes of control mice peaked at 36 hours after PHx, with a mean

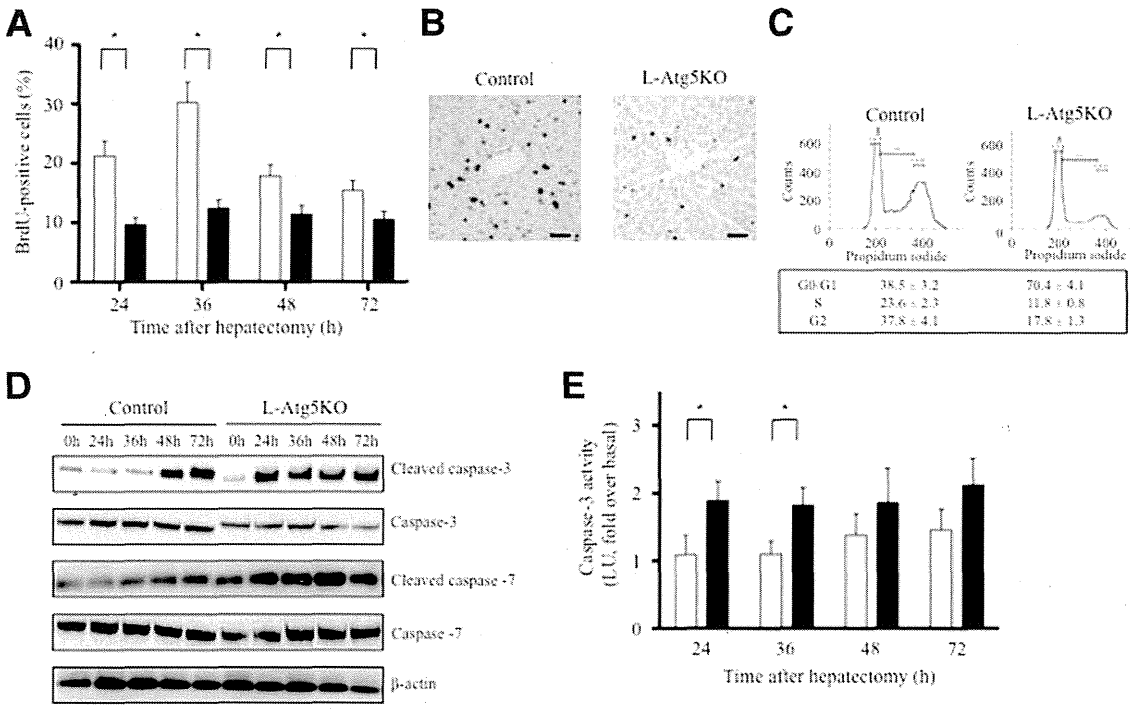


Fig. 3. DNA synthesis during liver regeneration is impaired in L-Atg5 KO mice. (A) BrdU-positive cell count at 24-72 hours after PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (B) BrdU staining of hepatocytes at 36 hours after PHx after 70% PHx in control and L-Atg5 KO mice. Scale bar, 50 $\mu$m. (C) Cell-cycle analysis of hepatocytes from control and L-Atg5 KO mice. (D) Changes in cleaved caspase-3 and caspase-7 expression levels at 0-72 hours after 70% PHx (representative western blottings are shown). (E) Changes in caspase-3 activity at 0-72 hours after 70% PHx in control and L-Atg5 KO mice. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$.

of 30.2% (Fig. 3A,B), similar to previous reports.[5,15,16] Percentage of BrdU-positive cells was then decreased at 48-72 hours after PHx. Percentage of BrdU-positive cells was significantly lower in L-Atg5 KO than in control mice at all times after PHx. Percentages of hepatocytes in the S and $G_2$ phases were significantly lower in L-Atg5 KO mice (Fig. 3C). Western blotting analysis showed that expression levels of cleaved caspase-3 and cleaved caspase-7 were similar in L-Atg5 KO and control mice before 70% PHx, but their levels were greatly elevated after 70% PHx in L-Atg5 KO mice, compared to control mice (Fig. 3D). Caspase-3 activity was also elevated by 70% PHx in L-Atg5 KO mice (Fig. 3E). Hepatic mRNA and protein expression levels of cyclins A, B, D, and E, which regulate cyclin-dependent kinases, were significantly lower in L-Atg5 KO than in control mice at 36 hours after PHx (Fig. 4A-D, F). However, hepatic expression levels of cyclin D, which is initiated during $G_1$ and drives the $G_1$/S phase transition under the control of p21, were significantly lower in L-Atg5 KO than in control mice at 24 hours after PHx (Fig. 4E, F). Furthermore, hepatic mRNA and protein expression levels of p21 were higher in L-Atg5 KO than in control mice at 12 hours after PHx (Fig. 4F,G). These findings indicate that autophagy is involved in the mitotic response of hepatocytes after PHx, and is driven by down-regulation of p21, resulting in up-regulation of cyclin D. The proportion of dividing cells containing multipolar and lagging chromosomes was significantly lower in L-Atg5 KO than in control mice at 48 and 72 hours after PHx (Supporting Fig. 9). These findings indicate that impaired autophagy in the regenerating liver is associated with a decrease in altered nuclear ploidy levels in hepatocytes.

***Liver Hypertrophy and Hepatocyte Senescence in the Regenerating Liver After PHx in L-Atg5 KO Mice.*** Unlike the mitotic responses, the liver mass and liver weight/body weight ratio (LW/BW) at 24 hours after PHx was significantly higher in L-Atg5 KO than in control mice (Fig. 5A,B). The size of hepatocytes, an indicator of liver hypertrophy, was significantly greater at 24 hours after PHx in L-Atg5 KO than in control mice (Fig. 5C,D). Stress responses in senescent cells include up-regulation of p21 and cellular hypertrophy.[10,15,19] To confirm the involvement of p21 and cellular hypertrophy in hepatocyte senescence during liver regeneration in L-Atg5 KO mice, we analyzed the expression of SA-$\beta$-gal and specific senescence-associated secretory phenotype (SASP)

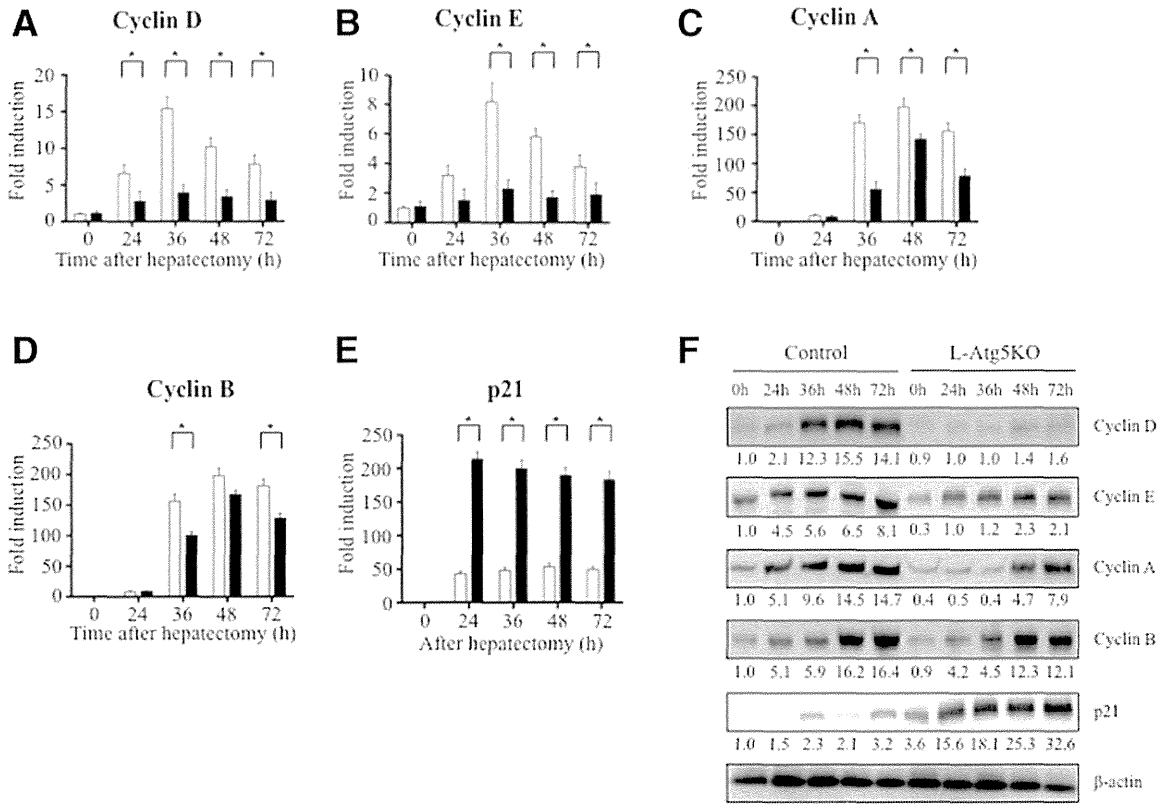


Fig. 4. Expression of cell-cycle-associated molecules in the regenerating liver in control and L-Atg5 KO mice. (A-E) Fold changes in cyclins D, E, A, and B as well as p21 mRNA levels in control and L-Atg5 KO mice at 0-72 hours after 70% PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (F) Changes in cyclins D, E, A, and B as well as p21 protein expression levels in control and L-Atg5 KO mice at 0-72 hours after 70% PHx. β-actin was used as a loading control, and these expression levels at 0 hours in control mice were defined as 1.0.

components. We found that levels of SA-β-gal and polyubiquitinated proteins at 24 hours after PHx were higher in L-Atg5 KO than in control mice (Fig. 5E,F), as were concentrations of the SASP components, interleukin (IL)-8 and IL-6 (Fig. 5G,H). These findings indicate that proliferating hepatocytes require activation of autophagy to prevent senescence, along with hepatic hypertrophy.

***Suppression of Autophagic Activity and Mitochondrial β-Oxidation.*** Hepatic ATP levels were significantly lower in L-Atg5 KO than in control mice (Fig. 6A). Fragmentation of hepatocyte mitochondria in L-Atg5 KO mice was demonstrated, but not in control mice (Fig. 6B). In addition, the proportion of hepatocytes with a low mitochondrial membrane potential ($\Delta\Psi_m$) was higher in L-Atg5 KO than in control mice (Fig. 6C). Relative to control mice, L-Atg5 KO mice had lower hepatocyte expression levels of the genes encoding medium chain acyl-coenzyme A dehydrogenase and carnitine palmitoyl transferase-1, which have a rate-controlling effect on β-oxidation, and liver-type fatty acid-binding protein, which plays a role in the transportation of free fatty acids (FFAs) to mitochondria. Moreover, hepatocyte expression levels of FAS, which is involved in the synthesis of FFAsa from acetyl-coenzyme A, were significantly higher in L-Atg5 KO mice (Fig. 6D). Hepatocyte levels of β-hydroxybutyrate, a final ketone body product, were significantly lower in L-Atg5 KO than in control mice (Fig. 6E). In addition, hepatocyte expression of extracellular signal-related kinase (Erk)1/2, an ATP-dependent mitogen-activated protein kinase, was significantly lower in L-Atg5 KO than in control mice, as was cyclin D expression (Fig. 6F). By contrast, expression levels of the upstream targets of AMPK were slightly increased in L-Atg5 KO mice as part of the compensation to KO of autophagic activation (Fig. 6F). Therefore, phosphorylation of the transcription factor, c-jun, at serine 63, another ATP-dependent protein, was significantly lower at 24 hours after PHx in L-Atg5 KO than in control mice (Fig. 6G), whereas there were no changes in expression of nuclear factor kappa B (NF-κB) or phosphorylation of c-jun at threonine 91 in the regenerating liver of L-Atg5 KO. These findings indicated that, after PHx, hepatocytes maintain intracellular ATP levels by

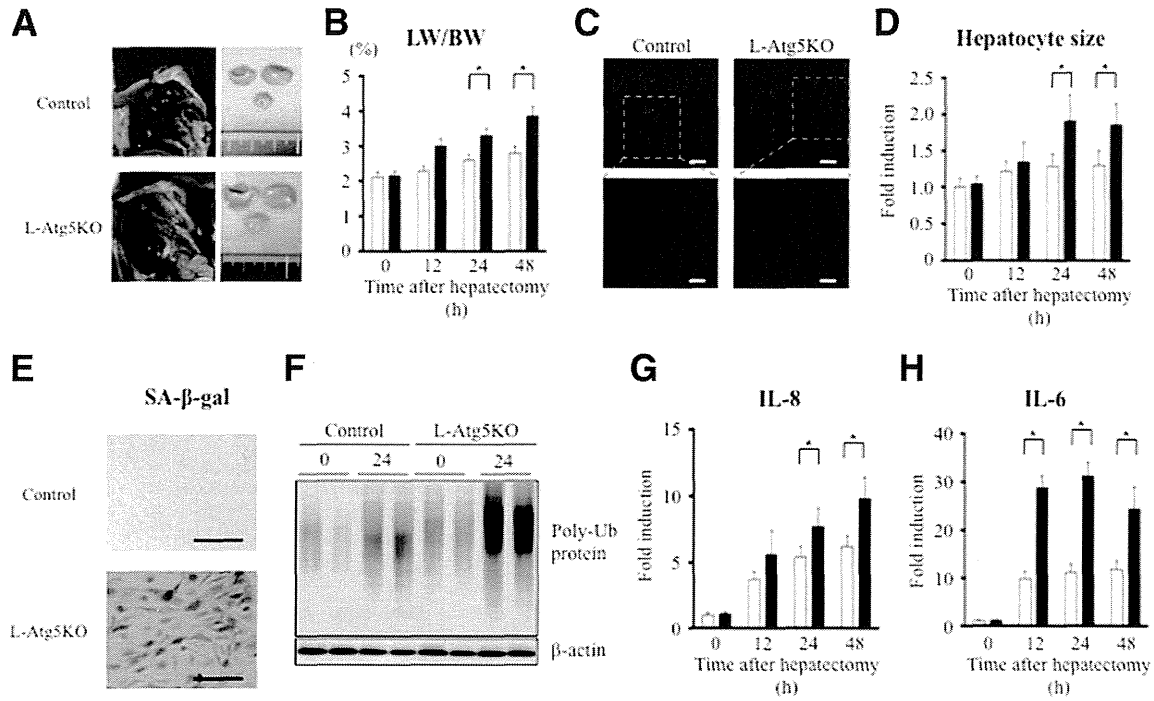


Fig. 5. Liver hypertrophy and hepatocyte senescence in the regenerating liver. (A) Macroscopic images of the liver after PHx. (B) LW/BW at 0-48 hours after 70% PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (C) Size of hepatocytes in control and L-Atg5 KO mice. Scale bar, 10 $\mu$m. (D) Fold change in hepatocyte size after 0-48 hours after PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (E) IHC staining of SA-$\beta$-gal in hepatocytes. Scale bar, 100 $\mu$m. (F) Expression of polyubiquitinated protein at 0 and 24 hours after PHx in control and L-Atg5 KO mice. (G and H) Fold changes in IL-8 (G) and IL-6 (H) mRNA expression at 0-48 hours after 70% PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$.

activating mitochondrial $\beta$-oxidation, possibly because of prompt removal of damaged mitochondria by activation of autophagy as a selective degradation system. Hepatocytes that fail to maintain their level of energy charge may become senescent.

## Discussion

We have used L-Atg5 KO mice to investigate the roles of autophagy-associated pathways in liver regeneration after PHx. We found that liver regeneration was severely impaired by 70% PHx in these mice. Their livers showed an impaired postoperative mitotic response, with quiescent hepatocytes becoming senescent and hypertrophic. Moreover, PHx was followed by considerable damage to mitochondria, reduced $\beta$-oxidation, and reduced intrahepatic ATP generation. Thus, autophagy during the early phase of liver regeneration is critical for maintaining healthy mitochondria capable of producing ATP and prevents hepatocytes from becoming senescent.

Growth factors, such as HGF and IL-6, activate phosphatidylinositol 3-phosphate, which, in turn, phosphorylates Akt at Thr308 and Ser473 in hepatocytes.[17,18] Akt also plays a critical role in proliferation by phosphorylating mTOR1, which suppresses autophagy by inhibiting TOR-dependent phosphorylation of Atg13 in a rapamycin-sensitive complex containing raptor.[17,18] During proliferation, Atg13 is phosphorylated by phosphorylated AMPK-$\alpha$, which is activated by an increase in the AMP/ATP ratio caused by cellular/environmental stressors, such as energy deficiency, hypoxia, and ischemia, during proliferation.[6-8] To date, a few reports have shown a relationship between HGF and autophagic activity. A recent report showed that HGF has a pivotal role in directly promoting autophagic activity for clearance of advanced glycation endproducts, which are involved in the pathogenesis of diabetic vascular complications, in primary mouse nonparenchymal cells (NPCs).[19] This previous report showed that LC3-II levels in NPCs are increased by HGF, similar to our results, and that this effect is inhibited by cotreatment with an anti-HGF neutralizing antibody.[19] In some ongoing clinical trials, pharmacological inhibitors of autophagy, such as CQ, which is an autophagolysosomal inhibitor, have been used for treating solid cancers (e.g., pancreatic adenocarcinoma and breast cancer), based on the knowledge that growth-factor–induced autophagic activity promotes proliferation of cancer cells through important

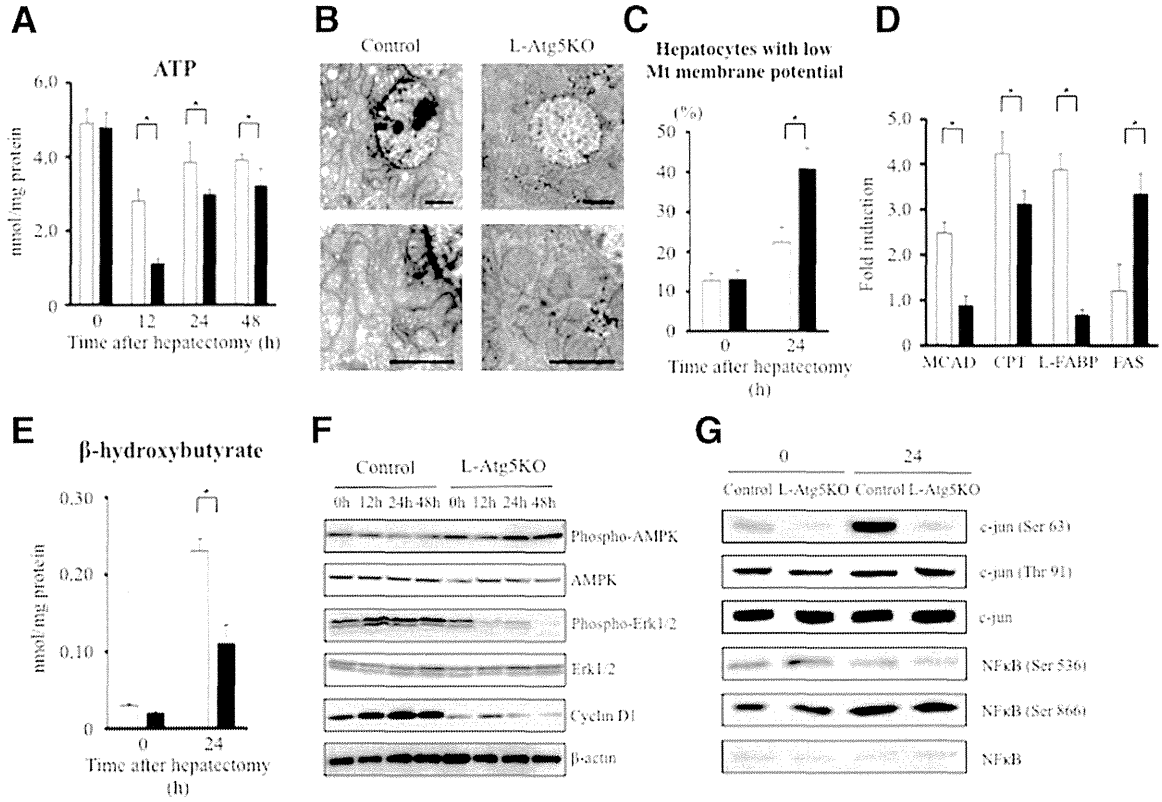


Fig. 6. Suppression of autophagy activity and mitochondrial β-oxidation. (A) Intrahepatic ATP concentrations at 0-48 hours after PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (B) Electron microscopic images of hepatocyte mitochondria at 24 hours after 70% PHx. Scale bar, 2.5 μm. (C) Proportion of hepatocytes with a low mitochondrial membrane potential ($\Delta\Psi_m$) at 24 hours after 70% PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (D) mRNA expression of mitochondrial β-oxidation-related genes at 0 and 24 hours after PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (E) β-hydroxybutyrate concentrations at 0 and 24 hours after PHx. Open bars, control mice; closed bars, L-Atg5 KO mice. $*P < 0.05$. (F) Changes in total and phosphorylated levels of AMPK, Erk1/2, and cyclin D1 at 0-48 hours after PHx in control and L-Atg5 KO mice. (G) Changes in total and phosphorylated levels of c-jun and NF-κB at 0 and 24 hours after PHx in control and L-Atg5 KO mice.

components of cellular metabolism.[20] In proliferating hepatocytes, the level of phosphorylated AMPK is increased, activating autophagy to overcome the burden of various stressors, especially those that cause endoplasmic reticulum stress or oxidative stress (OS).[4] Therefore, HGF indirectly increases LC3-II levels by enhancing phosphorylated AMPK levels.

Mitophagy, the autophagy-dependent degradation of mitochondria, is a defensive mechanism that involves selective sequestration and subsequent degradation of dysfunctional mitochondria.[21] Occurrence of mitophagy in livers of patients with Reye's syndrome[22] suggests its importance for normal mitochondrial turnover and function.[23] Accumulation of mitochondria caused by disruption of autophagy is thought to lead to increased levels of reactive oxygen species and DNA damage, resulting in mitochondrial depolarization and permeability transition.[23-25] In this study, we showed that selective degradation of dysfunctional mitochondria in proliferating hepatocytes was impaired after PHx in L-Atg5 KO mice, resulting in a disruption of mitochondrial functions, such as maintenance of intracellular ATP levels, by mitochondrial β-oxidation. However, with the exception of hepatocyte senescence, which was accompanied by increased p21 expression, we were unable to detect any phenotypes of mitophagy in proliferating hepatocytes. Further detailed research is required to clarify the pivotal role of mitophagy in proliferating hepatocytes.

Recent studies have elucidated the mechanisms involved in cellular senescence in hepatocytes adapted to stress stimuli.[10,26-30] As a stress response, senescence is a dynamic process involving multiple effector mechanisms, whose combination ultimately determines cellular phenotype. This process is characterized by a number of biological, biochemical, and molecular changes, including cell hypertrophy, up-regulation of SA-β-gal, irreversible growth arrest, and expression of a specific SASP.[31,32] SASP components, such as IL-6, IL-8, and matrix metalloproteinases, can promote

tissue repair by preventing the generation of a persistent acute inflammatory response and by attracting immune cells that kill and clear senescent cells by adhesion molecules expressed on the latter. Stimuli, such as OS, can induce autophagy and senescence and may be part of the same physiological process, known as the autophagy-senescence transition.[33,34] However, the pivotal relationship between these two cellular responses has not been elucidated. In particular, it is unknown whether induction of autophagy is involved in the induction of senescence or vice versa.[26,27] The results of our study suggest that autophagy regulates cellular senescence during liver regeneration. A subset of autophagy-related genes was up-regulated during liver regeneration in association with negative feedback in p21-activated senescence. Furthermore, inhibition of autophagy augmented the senescence phenotype, including senescence-associated secretion of IL-6 and IL-8. We reasoned that rapid protein turnover, which involves autophagy coupled with active protein synthesis, may facilitate this process by allowing the remodeling of proteins needed for liver regeneration. Indeed, senescent cells are usually hypertrophic, and we confirmed that the fall into senescence was dependent on cellular quality maintained by autophagy. Thus, autophagy may be complementary to epigenetic regulation in preventing the specific biochemical alterations observed during acute induction of senescence in the regenerating liver. Our findings suggest that autophagy, and its maintenance of cellular quality by rapid protein turnover during hepatocyte regeneration, negatively regulates the acquisition of the senescence phenotype.

Several genetic and pharmacological interventions can suppress transient hepatic steatosis, a characteristic of the early regenerative response accompanied by hepatic energy deficiency, resulting in impaired liver regeneration.[35] Subsequent to liver injury, liver mass is maintained or recovers in proportion to body mass.[1,21] Moreover, hypoglycemia after PHx induces systemic lipolysis, which is followed by accumulation of fat derived from peripheral stores, during early regeneration.[35] These observations, and the central role of the liver as the principal intermediary between fatty acid metabolism and intrahepatic energy maintenance,[35,36] prompted us to investigate the regulation and functional role of autophagy, a systemic metabolic phenomenon, during liver regeneration after PHx. In our study, we found that hepatocytes with activated autophagy, without senescence, stimulated mitochondrial metabolism during liver regeneration by maintaining healthy mitochondria and the production of high-energy mitochondrial fuels to support hepatocyte proliferation. The remnant liver relies on mitochondrial oxidative phosphorylation to satisfy its energy demands, and defects in mitochondrial permeability transition have been reported in mitochondria isolated from remnant livers after PHx.[36] In addition, autophagy is critical for regulating hepatocellular lipid stores.[37] Future studies should address whether systemic adipose stores during normal liver regeneration are needed as fuel sources to support regeneration, as lipid precursors for membrane synthesis, and/or act as specific signals that initiate the regenerative response itself.[37,38] Mitochondrial oxidative damage is a fundamental component of liver regeneration, and multiple studies have shown that it is attenuated by autophagic activity to regulate hepatocellular lipid stores.[39] A recent study showed that sirtuin-1 (SIRT1), a nicotinamide adenine dinucleotide–dependent deacetylase, was involved in the protective effects of calorie restriction against hypoxia in the aged kidney, which is linked to calorie restriction-related longevity by mitochondrial autophagy.[40] Adult-onset and long-term calorie restriction in mice promoted increased SIRT1 expression in the aged kidney and attenuated hypoxia-associated mitochondrial and renal damage by enhancing mitophagy. Here, we have shown that autophagic activity in proliferating hepatocytes markedly diminished mitochondrial dysfunction in terms of increased mitochondria permeability transition and decreased $\beta$-oxidation. These data highlight the role of autophagy-dependent systemic lipolysis, including $\beta$-oxidation, in hepatocellular adaptation to mitochondrial oxidative damage, delineate a molecular mechanism of the autophagy-mediated antiaging effect, and could potentially direct the design of new therapies to promote liver regeneration after damage associated with OS and aging.

In our study, the peak percentage of Ki67-positive cells, as determined by immunohistochemistry (IHC), was 7.4% at 12 hours after PHx, 11.2% at 24 hours, and 31.2% at 36 hours (Supporting Fig. 10). These values are higher than those reported in previous studies.[5,15] All of the mice subjected to 70% PHx were 6 weeks old in our study, whereas other studies induced PHx at 10-12 weeks. Hepatocyte senescence strongly down-regulates cellular proliferation by decreasing farnesoid X receptor levels[41] or SIRT1 levels.[42] Therefore, the difference in the percentage of BrdU-positive cells at 12 hours after PHx between our study and previous studies may be the result of a difference in the effect of senescence on proliferating hepatocytes labeled with BrdU.[41,42]

Autophagy during the early phase of liver regeneration is critical for maintaining healthy mitochondria,

which can produce ATP through β-oxidation after hypoglycemia-induced hepatic steatosis. Therefore, autophagy may be essential for preserving cellular quality by preventing hepatocytes from becoming senescent and hypertrophic.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Original article

# Effect of laparoscopic splenectomy on portal haemodynamics in patients with liver cirrhosis and portal hypertension

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**Background:** The effect of splenomegaly in patients with liver cirrhosis and portal hypertension is not fully understood. This study was designed to determine the effect of laparoscopic splenectomy on portal haemodynamics in these patients.

**Methods:** Patients with liver cirrhosis and portal hypertension who underwent laparoscopic splenectomy in Kyushu University Hospital from January 2006 to March 2009 were evaluated retrospectively. Correlations between splenic size and portal haemodynamics, and changes in portal haemodynamics and in levels of the vasoactive agents endothelin (ET) 1 and nitric oxide metabolites (NOx) before and 7–10 days after laparoscopic splenectomy were analysed.

**Results:** Portal venous (PV) blood flow, PV cross-sectional area and PV congestion index correlated significantly with splenic size ($P < 0{\cdot}050$). All three were significantly reduced following splenectomy in 59 patients. The hepatic venous pressure gradient, measured in 18 patients, decreased by 25 per cent after splenectomy ($P < 0{\cdot}001$). Portal vascular resistance was also reduced, by 21 per cent ($P = 0{\cdot}009$). The peripheral blood concentration of ET-1 decreased from 2·95 to 2·11 pg/ml ($P < 0{\cdot}001$), and that of NOx tended to decrease (from 29·2 to 25·0 pg/ml; $P = 0{\cdot}068$). In hepatic venous blood, the level of ET-1 decreased from 2·37 to 1·83 pg/ml ($P = 0{\cdot}006$), whereas NOx concentration tended to increase (from 24·5 to 30·9 pg/ml; $P = 0{\cdot}067$).

**Conclusion:** In patients with liver cirrhosis and portal hypertension, splenectomy reduced portal venous pressure. A decrease in splanchnic blood flow, by eliminating splenic blood flow, and reduction in intrahepatic vascular resistance, by normalizing hepatic concentrations of ET-1 and NOx, may both have contributed.

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## Introduction

By the end of the 19th century splenectomy was already regarded as a therapeutic procedure for patients with Banti's disease, defined as the triad of splenomegaly, leucopenia and liver cirrhosis[1]. Splenectomy was reported to have beneficial effects in patients with Banti's syndrome and portal hypertension, including improvements in bleeding tendency, hypersplenism and gastrointestinal bleeding. During the 1940s and 1950s, however, many studies reported that splenectomy failed to stop oesophagogastric variceal haemorrhage in patients with portal hypertension, suggesting that splenectomy may not be curative in these patients[2]. Splenectomy has therefore been combined with other procedures, such as devascularization of the upper stomach and oesophageal transection, in order to control variceal haemorrhaging. Splenectomy alone is not proposed to treat portal hypertension, because of its serious risks, including surgical bleeding, thrombosis in the portal venous system and sepsis[3,4].

Technical advances in surgery, and the recognition of risk factors and effective treatments for portal venous (PV) thrombosis, have resulted in safer and less invasive forms of splenectomy, including the laparoscopic approach[5–7]. Owing to the development of treatments for chronic liver diseases, such as interferon (IFN) therapy for chronic hepatitis C virus (HCV) infection, laparoscopic splenectomy has again attracted attention as a treatment

for patients with liver cirrhosis and portal hypertension[4]. Currently, splenectomy is performed to improve thrombocytopenia in patients with chronic HCV infection, before treatment with pegylated IFN plus ribavirin, and in patients with cirrhosis undergoing treatment for hepatocellular carcinoma (HCC)[4,8]. Concurrent splenectomy has also shown beneficial effects in living-donor liver transplantation (LDLT), controlling excessive portal flow in small-for-size liver grafts and alleviating persistent thrombocytopenia[9].

Portal hypertension in patients with liver cirrhosis is characterized primarily by increased intrahepatic vascular resistance and increased splanchnic blood flow[10,11]. In this setting, splenomegaly is due not only to dilatation of the splenic sinus resulting from portal congestion induced by increased intrahepatic vascular resistance, but also to splenic tissue hyperplasia and fibrosis[3]. As splenic blood flow is indisputably increased, splenomegaly may have a role in the pathogenesis of portal hypertension by increasing splanchnic blood flow. However, the relationship between an enlarged spleen and PV pressure is unclear[3], as the splenectomy-associated reduction in PV pressure is not always substantial and splenectomy is usually performed in conjunction with other procedures such as oesophageal transection. As few reports have assessed haemodynamic changes after splenectomy alone, it is uncertain whether splenomegaly is involved in the pathogenesis of portal hypertension.

Recent studies have reported that, despite reducing PV blood flow by eliminating splenic blood flow, splenectomy often improves liver function[4,8]. This may imply that splenomegaly contributes actively to the pathophysiology of liver cirrhosis. This hypothesis is similar to that of Banti, that an enlarged spleen may secrete substances that injure the liver[1,3]. The present study analysed the effects of splenomegaly on the pathogenesis of liver cirrhosis with portal hypertension by investigating postsplenectomy changes in portal haemodynamics and vasoactive agents related to hepatic microcirculation, such as endothelin (ET) 1 and nitric oxide metabolites (NOx).

## Methods

All consecutive patients with liver cirrhosis and portal hypertension who underwent laparoscopic splenectomy at the Department of Surgery and Science, Kyushu University Hospital, from January 2006 to March 2009 were evaluated retrospectively. All patients had a detailed demographic, clinical and biochemical assessment. They all underwent Doppler ultrasonography and contrast-enhanced CT to evaluate portal haemodynamics and to screen for splanchnic vein thrombosis, before and 7–10 days after surgery. Upper gastrointestinal endoscopy was performed in all patients to assess the severity of oesophagogastric varices before and within 1 month of operation. Risky oesophageal varices were defined as moderate or huge in size with red colour signs, according to the criteria of the Japan Society for Portal Hypertension[12]. No patient enrolled in this study had thrombi in the portal venous system, and none had been administered portal pressure-reducing agents such as beta-blockers. The study protocol was approved by the local ethics committee, and informed consent was obtained from each patient.

### Operative procedures for laparoscopic splenectomy

Operative procedures for laparoscopic splenectomy were performed as described previously[5]. Each patient had preoperative CT to evaluate splenic volume and to determine the location and extent of collateral vessels. Patients whose spleen had a volume of 1000 ml or more, as assessed by CT volumetry, those with large perisplenic collateral vessels and/or patients with a Child–Pugh score of 9 or above underwent hand-assisted laparoscopic surgery (HALS); the remaining patients were operated on totally laparoscopically. Patients were placed in the supine position with the left flank elevated at a 60° angle. Splenic attachments were divided using electrocautery, ultrasound dissection and/or the LigaSure™ vessel sealing system (Covidien, Boulder, Colorado, USA). Once the upper pole of the spleen had been dissected from the diaphragm, the splenic hilar pedicle was transected with an endoscopic linear vascular stapler. The resected spleen was placed into a plastic bag, morcellated and extracted. Splenic size was based on the weight of the morcellated spleen.

### Doppler ultrasonography

After an overnight fast, patients underwent Doppler ultrasonography in the supine position using a duplex ultrasound device (ProSound SSD-5500SV; Aloka, Tokyo, Japan) with a 3·75-MHz probe provided with pulsed Doppler and a colour-flow mapping device. During the examination, patients were asked to hold their breath. All Doppler examinations were performed by two experienced examiners. PV blood flow (ml/min) was calculated from the PV velocity (cm/s) and the PV cross-sectional area ($cm^2$). The PV cross-sectional area was calculated using the formula: PV cross-sectional area $= \pi (R^2/4)$ (where R is

the diameter of the portal vein). The PV congestion index (cm·s) was calculated using the formula: PV congestion index = PV cross-sectional area/PV velocity[13]. Doppler shift signals were obtained from the centre of the portal vein. The angle between the ultrasonic beam and the longitudinal axis of the vessel never exceeded 60°. All measurements were repeated three times and averaged. The coefficients of variation of all parameters were less than 5 per cent.

### Measurement of hepatic venous pressure gradient

The hepatic venous pressure gradient (HVPG) was measured on the same days as the Doppler ultrasound examinations, before and after surgery, in a subset of patients. The patients were transferred to the angiography room after an overnight fast and placed in the supine position. Hepatic venous pressure ($cmH_2O$) was measured in triplicate with a high-sensitivity transducer (TC-704; Nihon Kohden, Tokyo, Japan) following catheterization of the main right hepatic vein under fluoroscopic guidance using a 6.5-Fr balloon catheter (B-RTO type II catheter; Create Medic, Tokyo, Japan)[14].

The zero reference point for each measurement was set at the mid-axillary line of the patient. The HVPG was calculated as the difference between the wedged hepatic venous pressure and the free hepatic venous pressure. The HVPG was used to assess the PV pressure. Intrahepatic portal vascular resistance was calculated using the formula: intrahepatic portal vascular resistance = HVPG/PV blood flow ($cmH_2O$ per ml per min)[15].

### Measurement of endothelin 1 and nitric oxide metabolites

ET-1 and NOx levels were measured in peripheral blood and in hepatic venous blood of some patients, before and after surgery. Plasma ET-1 concentration was determined by means of radioimmunoassay with a rabbit anti-ET-1 serum (Peninsula Laboratories, Belmont, California, USA)[16]. Plasma nitric oxide metabolites (nitrite and nitrate as NOx) were measured using high-pressure liquid chromatography[10,16]. Nitrate ($NO_3^-$) in each sample was reduced by the cadmium column to nitrite ($NO_2^-$), and the concentration of an azo dye compound formed from nitrite by the Griess reaction was measured spectrophotometrically at 540 nm.

### Statistical analysis

All results are reported as mean(s.d.) or median (range). Linear regression analyses were used to assess the correlations between splenic size and each parameter. Data before and after surgery were compared using Student's *t* tests for paired data or the Wilcoxon signed-rank test, as appropriate. $P < 0{\cdot}050$ was considered statistically significant. All calculations were performed using the software package StatView® version 5.0 for Windows® (SAS Institute, Cary, North Carolina, USA).

**Table 1** Patient characteristics

| | No. of patients ($n = 59$) |
|---|---|
| Age (years)* | 57·5 (9·2) |
| Sex ratio (M : F) | 30 : 29 |
| Aetiology of cirrhosis | |
| HBV | 6 (10) |
| HCV | 48 (81) |
| Alcoholism | 3 (5) |
| Other | 2 (3) |
| Child–Pugh class | |
| A | 20 (34) |
| B | 30 (51) |
| C | 9 (15) |
| Child–Pugh score* | 7·4 (1·6) |
| Ascites | |
| Yes | 24 (41) |
| No | 35 (59) |
| Encephalopathy | |
| Yes | 0 (0) |
| No | 59 (100) |
| Platelet count ($\times 10^3/\mu l$)* | 50 (15) |
| Leucocytes (per $\mu l$)* | 2898 (948) |
| Oesophageal varices | |
| Yes | 26 (44) |
| No | 33 (56) |
| Splenic weight (g)* | 556 (318) |
| Indication | |
| Bleeding tendency† | 14 (24) |
| Difficulty in induction or continuation of IFN therapy | 33 (56) |
| Severe portal hypertension‡ | 12 (20) |

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). †Platelet count below $30 \times 10^3/\mu l$; ‡severe portal hypertensive gastropathy, endoscopic treatment-resistant oesophageal varices or refractory ascites. HBV, hepatitis B virus; HCV, hepatitis C virus; IFN, interferon.

## Results

A total of 97 patients with liver cirrhosis and portal hypertension underwent laparoscopic splenectomy in the study interval. Of these, 38 were excluded: five with persistent hypersplenism after LDLT, 15 who had concomitant treatment for HCC (radiofrequency ablation or hepatic resection), 11 who underwent concomitant occlusion of huge splenorenal shunts, and seven with postoperative PV thrombosis. Thus, 59 patients with liver cirrhosis and portal hypertension were included in the study (*Table 1*).

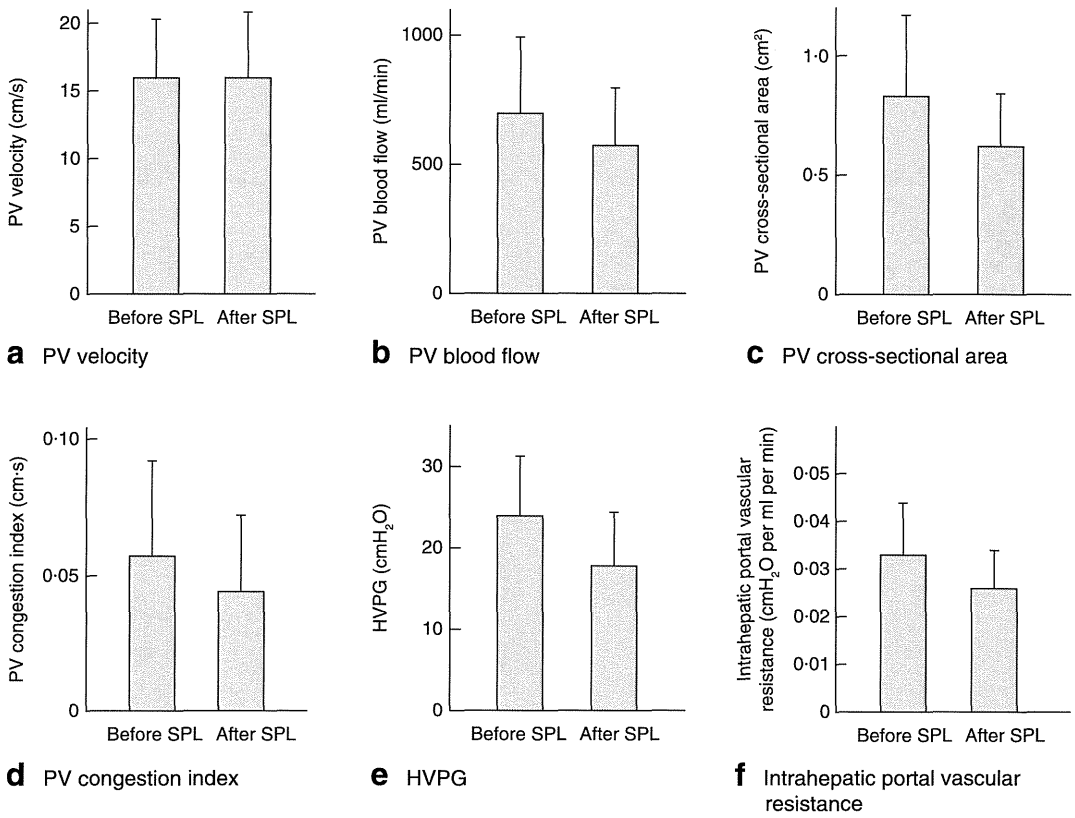



**Fig. 1** Comparison of **a** portal venous (PV) velocity, **b** PV blood flow, **c** PV cross-sectional area, **d** PV congestion index, **e** hepatic venous pressure gradient (HVPG) and **f** intrahepatic portal vascular resistance in patients before and after splenectomy (SPL). Values are mean(s.d.). **a** $P = 0{\cdot}928$, **b,c,e** $P < 0{\cdot}001$, **d** $P = 0{\cdot}035$, **f** $P = 0{\cdot}009$ (before *versus* after SPL, Student's *t* test)

## Surgical outcomes of laparoscopic splenectomy in patients with cirrhosis and portal hypertension

For safety reasons, HALS was performed in 23 (39 per cent) of the 59 patients. The remaining 36 patients had totally laparoscopic splenectomy; none required conversion to open splenectomy and there were no deaths related to laparoscopic splenectomy. The mean duration of surgery was 247(69) min and median blood loss was 143 (10–900) g. No patient had postoperative pancreatic fistula or postoperative bleeding requiring emergency haemostasis.

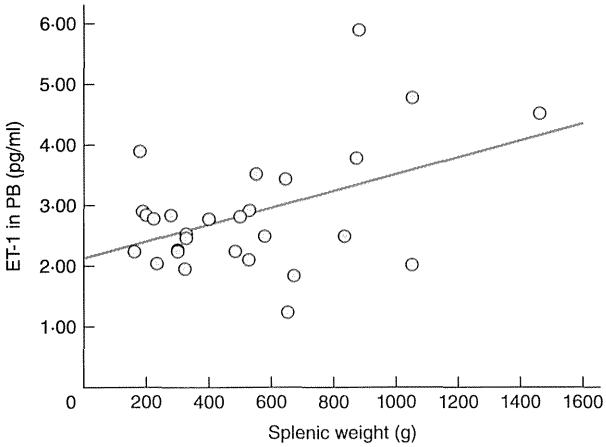
## Relationship between splenic size and portal haemodynamics

As the haemodynamic characteristics of the right and left portal veins and the main portal trunk were similar, only the characteristics of the right portal vein are shown (*Fig. S1*, supporting information). PV blood flow, cross-sectional area and congestion index were significantly correlated with splenic size, whereas PV velocity and HVPG were not.

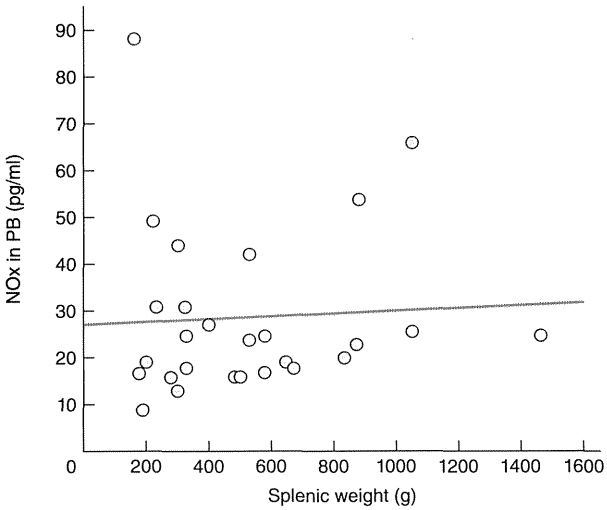
## Changes in portal haemodynamics after splenectomy

Haemodynamic changes in the right portal vein are shown in *Fig. 1*. Although PV velocity did not change significantly after splenectomy, PV blood flow, cross-sectional area and congestion index decreased significantly.

In the subgroup of 18 patients assessed for HVPG, the gradient decreased by a mean of 25(14) per cent after splenectomy ($P < 0{\cdot}001$) (*Fig. 1e*). In this subgroup, mean PV blood flow decreased from 716(245) to 623(206) ml/min ($P < 0{\cdot}001$) (mean reduction 12(18) per cent). Intrahepatic portal vascular resistance also decreased after splenectomy ($P = 0{\cdot}009$), with a mean reduction of 21(21) per cent (*Fig. 1f*). These patients were divided into those with mild (splenic weight less than 500 g) and those with severe (splenic weight 500 g or more) splenomegaly, and changes in portal haemodynamics were compared in these two groups. In the six patients with mild splenomegaly (mean splenic weight 229(52) (range 170–300) g), mean

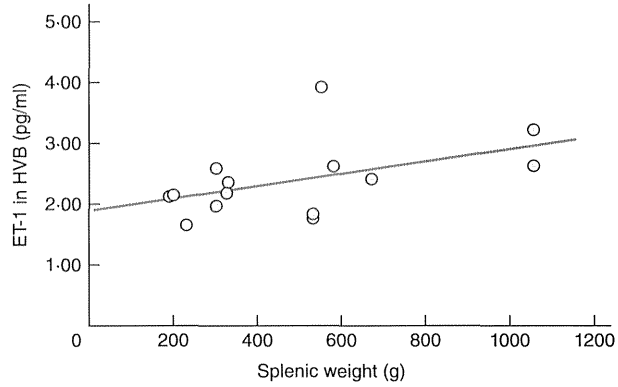



**a** ET-1 in PB and splenic weight

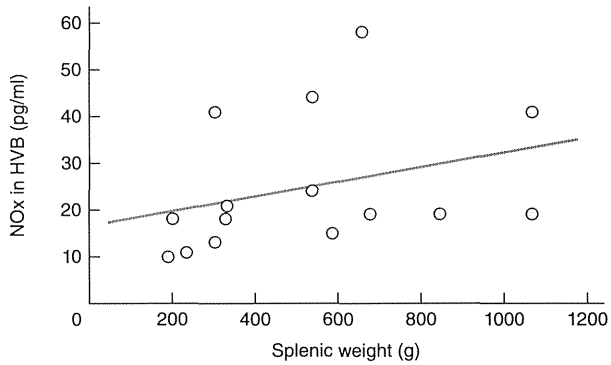



**b** NOx in PB and splenic weight

**Fig. 2** Correlation between splenic size and concentration of **a** endothelin (ET) 1 and **b** nitric oxide metabolites (NOx) in peripheral blood (PB). **a** $R = 0{\cdot}446$, $P = 0{\cdot}017$; **b** $R = 0{\cdot}049$, $P = 0{\cdot}807$ (linear regression analysis)




**a** ET-1 in HVB and splenic weight




**b** NOx in HVB and splenic weight

**Fig. 3** Correlation between splenic size and concentration of **a** endothelin (ET) 1 and **b** nitric oxide metabolites (NOx) in hepatic venous blood (HVB). **a** $R = 0{\cdot}483$, $P = 0{\cdot}080$; **b** $R = 0{\cdot}327$, $P = 0{\cdot}234$ (linear regression analysis)

PV blood flow decreased from 655(178) to 589(174) ml/min ($P = 0{\cdot}046$), intrahepatic portal vascular resistance decreased from 0·038(0·010) to 0·026(0·007) $cmH_2O$ per ml per min ($P = 0{\cdot}068$) and HVPG decreased from 23·7(9·0) to 19·6(8·0) $cmH_2O$ ($P = 0{\cdot}048$). In the 12 patients with severe splenomegaly (mean splenic weight 745(305) (range 500–1450) g), mean PV blood flow decreased from 777(290) to 657(235) ml/min ($P = 0{\cdot}007$), intrahepatic portal vascular resistance decreased from 0·031(0·011) to 0·024(0·008) $cmH_2O$ per ml per min ($P = 0{\cdot}048$) and HVPG decreased from 24·4(7·7) to 17·0(5·6) $cmH_2O$ ($P < 0{\cdot}001$). Although there was no difference in the reduction of intrahepatic portal vascular resistance between the two groups, the decreases in PV blood flow and HVPG were significantly greater in patients with severe splenomegaly ($P = 0{\cdot}045$ and $P = 0{\cdot}034$ respectively).

## Relationship between splenic size and levels of endothelin 1 and nitric oxide metabolites

The level of ET-1 in peripheral blood was correlated with splenic size ($R = 0{\cdot}446$, $P = 0{\cdot}017$) (*Fig. 2a*) and was reduced after splenectomy (from 2·95(0·90) to 2·11(0·47) pg/ml; $P < 0{\cdot}001$). The concentration of NOx in peripheral blood did not correlate with splenic size (*Fig. 2b*), but tended to decrease following splenectomy (from 29·2(18·6) to 25·0(10·4) pg/ml; $P = 0{\cdot}068$). The level of ET-1 in hepatic venous blood showed a slight correlation with splenic size ($R = 0{\cdot}483$, $P = 0{\cdot}080$) (*Fig. 3a*) and decreased after splenectomy (from 2·37(0·59) to 1·83(0·37) pg/ml; $P = 0{\cdot}006$). NOx concentration in hepatic venous blood did not correlate with splenic size (*Fig. 3b*), but tended to increase

**Table 2** Changes in haematological data and liver function after splenectomy

| | Child–Pugh class A ($n=20$) | | | Child–Pugh class B or C ($n=39$) | | |
|---|---|---|---|---|---|---|
| | Before SPL | 1 month after SPL | $P$† | Before SPL | 1 month after SPL | $P$† |
| Leucocytes (per μl) | 3250(1093) | 5505(1277) | <0·001 | 2661(810) | 5280(1323) | <0·001 |
| Platelet count ($\times 10^3$/μl) | 55(17) | 189(88) | <0·001 | 46(15) | 174(81) | <0·001 |
| Total bilirubin (mg/dl) | 1·0(0·4) | 0·8(0·3) | 0·001 | 1·7(0·6) | 1·1(0·5) | <0·001 |
| Albumin (g/dl) | 3·7(0·4) | 3·8(0·5) | 0·022 | 3·0(0·4) | 3·5(0·5) | <0·001 |
| Prothrombin time (%) | 86(10) | 87(20) | 0·828 | 66(9) | 77(11) | <0·001 |
| Ascites* | | | 1·000‡ | | | <0·001‡ |
| Yes | 0 (0) | 0 (0) | | 24 (62) | 7 (18) | |
| No | 20 (100) | 20 (100) | | 15 (38) | 32 (82) | |
| Child–Pugh score | 5·3(0·5) | 5·3(0·5) | 0·329 | 8·3(1·3) | 6·6(1·1) | <0·001 |

Values are mean(s.d.) unless indicated otherwise; *values in parentheses are percentages. SPL, splenectomy. †Student's t test, except ‡Wilcoxon signed-rank test.

**Table 3** Changes in portal haemodynamics

| | Child–Pugh class A ($n=5$) | | | Child–Pugh class B or C ($n=13$) | | |
|---|---|---|---|---|---|---|
| | Before SPL | After SPL | $P$* | Before SPL | After SPL | $P$* |
| PV blood flow (ml/min) | 709(344) | 572(206) | 0·010 | 693(262) | 577(233) | 0·005 |
| Intrahepatic portal vascular resistance ($cmH_2O$ per ml per min) | 0·025(0·016) | 0·026(0·019) | 0·586 | 0·036(0·009) | 0·025(0·005) | 0·004 |
| HVPG ($cmH_2O$) | 14·2(4·7) | 11·5(5·6) | 0·028 | 27·4(4·7) | 20·1(4·5) | <0·001 |
| ET-1 level (pg/ml) | | | | | | |
| In peripheral blood | 2·70(0·59) | 1·98(0·48) | 0·033 | 3·08(1·06) | 2·17(0·46) | 0·007 |
| In hepatic venous blood | 2·14(0·27) | 1·87(0·33) | 0·020 | 2·51(0·68) | 1·82(0·40) | 0·019 |
| NOx level (pg/ml) | | | | | | |
| In peripheral blood | 31·0(24·3) | 27·1(13·5) | 0·499 | 30·0(15·6) | 22·2(7·7) | 0·013 |
| In hepatic venous blood | 17·3(7·0) | 15·0(2·0) | 0·667 | 25·9(15·9) | 36·1(23·4) | 0·023 |
| Weight of spleen (g) | 438(241) | – | | 596(365) | – | |

Values are mean(s.d.). SPL, splenectomy; PV, portal venous; HVPG, hepatic venous pressure gradient; ET-1, endothelin 1; NOx, nitric oxide metabolites. *Student's $t$ test.

following splenectomy (from 24·5(14·4) to 30·9(20·7) pg/ml; $P=0·067$).

## Changes in haematological data, liver function and oesophageal varices after splenectomy

Leucocyte and platelet counts were significantly higher 1 month after splenectomy compared with preoperative values (*Table 2*). Improvements in total bilirubin and albumin concentrations were observed in patients with Child–Pugh A and those with Child–Pugh B/C, and improvements in prothrombin time occurred in patients with Child–Pugh B/C 1 month after splenectomy. The probability of ascites requiring diuretics was lower 1 month after than before splenectomy. None of the 59 patients had encephalopathy before or after splenectomy; thus, none showed worsening of liver function, and patients with Child–Pugh B/C had improvements in the Child–Pugh score. Even in the nine patients with Child–Pugh C (score 10), mean total bilirubin concentration decreased from 2·2(0·1) to 1·2(0·6) ($P=0·009$), albumin increased from 2·6(0·2) to 3·0(0·5) ($P=0·032$), prothrombin time increased from 61(7) to 71(11) ($P=0·002$), and the Child–Pugh score decreased from 10(0) to 7·9(1·1) ($P=0·002$).

Risky oesophageal varices, observed in 26 (44 per cent) of the 59 patients before splenectomy, were found in only 12 patients (20 per cent) after splenectomy ($P<0·001$). All 12 patients underwent successful endoscopic variceal ligation.

## Changes in portal haemodynamics categorized by liver function

HVPG was significantly reduced after splenectomy both in patients with Child–Pugh A and in those Child–Pugh B/C (*Table 3*). There was a greater percentage reduction in the latter group, owing to the significant decrease in intrahepatic portal vascular resistance, despite the similar reduction in PV blood flow in the two groups. The ET-1 concentration in peripheral blood and hepatic venous blood was significantly lower after splenectomy in both groups, whereas the level of NOx was significantly reduced in peripheral blood and significantly increased in hepatic venous blood after splenectomy only in the Child–Pugh B/C group.

## Discussion

Orthotopic liver transplantation is the best therapeutic option for liver cirrhosis and portal hypertension, but many patients are currently awaiting this operation because of the lack of liver donors. This problem is especially serious in Japan, because LDLT is the main form of liver transplantation. Recent advances in laparoscopic surgery and the treatment of PV thrombosis have made splenectomy more safe and less invasive, even in patients with liver cirrhosis and portal hypertension[5–7]. In Japan, splenectomy is of increasing importance for patients with liver cirrhosis and portal hypertension, and as a bridging therapy to LDLT[4]. The present study was therefore designed to assess the role of splenomegaly in the pathogenesis of liver cirrhosis and portal hypertension, and the impact of laparoscopic splenectomy on portal haemodynamics.

During laparoscopic splenectomy, the splenic artery and vein were transected simultaneously using an endoscopic linear vascular stapler without intraoperative ligation of the splenic artery. It was assumed that the weight of the resected spleen was representative of splenic volume. Patients in this study had liver cirrhosis of any stage (Child–Pugh score 5–10), except for end-stage disease, along with portal hypertension. All patients underwent laparoscopic splenectomy safely using a standardized technique[5], with no serious complications. Therefore, this population of patients with liver cirrhosis and portal hypertension was considered suitable for investigating the role of splenomegaly and the portal haemodynamic effects of splenectomy as a single operation.

Splenic size was associated with increases in PV blood flow and congestion index, but not HVPG, consistent with previous findings[3]. Portal hypertension in liver cirrhosis is characterized by increased intrahepatic vascular resistance and increased splanchnic blood flow, which may explain the lack of correlation between splenic size and HVPG. Splenectomy was followed by reductions in PV blood flow, congestion index and HVPG, suggesting that splenomegaly contributes actively to the pathogenesis of portal hypertension by increasing splanchnic flow (active congestion).

In liver cirrhosis, ET-1 concentration is increased in both the splanchnic and the systemic circulation[17–19]. ET-1 in liver cirrhosis is thought to derive from hepatic stellate cells and the splanchnic bed, including the spleen. The ET-1 concentration in peripheral blood correlated, and the level in hepatic venous blood tended to correlate, with splenic size, with ET-1 levels in both peripheral and hepatic venous blood decreasing significantly after splenectomy. These findings suggest that the spleen may be a major source of ET-1 in patients with liver cirrhosis, and that spleen-derived ET-1 could reach the hepatic and systemic circulation via the splanchnic circulation.

Splenectomy reduced HVPG by 25 per cent, more than that expected from the reduction in PV blood flow. Intrahepatic portal vascular resistance was also reduced, by 21 per cent following splenectomy. Intrahepatic portal vascular resistance is regulated by the contractility of hepatic stellate cells, which is regulated by the balance between vasoactive agents such as ET-1 and vasorelaxing agents such as nitric oxide (NO). ET-1 has dual vasoactive effects, mediating vasoconstriction by binding to endothelin A ($ETR_A$) and endothelin B ($ETR_B$) receptors on hepatic stellate cells and vasodilatation by binding to $ETR_B$ on sinusoidal endothelial cells, resulting in the production of NO[11,17]. In liver cirrhosis, ET-1 production is increased in the liver and splanchnic circulation, upregulating ETR–rho-kinase signalling and resulting in the contraction of hepatic stellate cells[17,18]. In sinusoidal endothelial cells of liver cirrhosis, NO production is reduced by impaired signalling of $ETR_B$-mediated NO production[10,11,20]. Decreased sinusoidal circulation reduces shear stress on sinusoidal endothelial cells, leading to a further decrease in NO production[11]. Thus, in cirrhotic liver, increased levels of ET-1 and decreased levels of NO contribute to the contraction of hepatic stellate cells and increased intrahepatic vascular resistance. In the present study, splenectomy significantly reduced the level of ET-1 and increased the level of NOx in hepatic venous blood. Splenectomy, by eliminating spleen-derived ET-1, may therefore lead to relaxation of hepatic stellate cells and a reduction of intrahepatic portal vascular resistance. Subsequent improvements in the sinusoidal circulation may increase the shear stress on sinusoidal endothelial cells, restoring NO production.

The splanchnic and systemic hyperdynamic circulation in liver cirrhosis and portal hypertension is characterized by increased NO production in endothelial cells and decreased response to vasoconstrictive agents such as ET-1. Despite the increase in ET-1 concentration, splanchnic and systemic vascular tone is reduced, possibly owing to increased $ETR_B$-mediated NO production in endothelial cells and the decreased responses to ET-1 resulting from impaired ETR–rho-kinase signalling in vascular smooth muscle cells[11,17,21,22]. The resulting increases in splanchnic and systemic blood flow result in additional NO overproduction, worsening the hyperdynamic circulation. As a result, raised levels of ET-1 and NO are associated with splanchnic and systemic hyperdynamic circulation. Splenectomy reduced the levels of both ET-1 and NOx in peripheral blood, the former significantly. Splenectomy may decrease systemic ET-1 concentration

by eliminating spleen-derived ET-1, subsequently reducing $ETR_B$-mediated NO production in endothelial cells. Although increasing levels of NO in hepatic venous blood and decreasing levels of NO in peripheral blood after splenectomy are seemingly contradictory findings, these results suggest that splenectomy may improve not only intrahepatic portal vascular resistance, but also splanchnic and systemic hyperdynamic circulation.

Long-term clinical studies have consistently reported that reducing HVPG below 12 mmHg, or by at least 20 per cent, markedly lowers the risk of variceal bleeding[14]. In the present study, splenectomy reduced HVPG by 25 per cent, surpassing the therapeutic goals for oesophageal varices. Risky oesophageal varices were eradicated by laparoscopic splenectomy alone in most patients. However, several papers from the 1940s and 1950s reported that splenectomy failed to control variceal bleeding[2]. In contrast, splenectomy with oesophageal transection was able to control variceal haemorrhage[3,4], because the procedure has two effects in treating varices: reducing PV pressure and obliterating varices by devascularization of perioesophagogastric collateral vessels. Following splenectomy, reduced PV pressure can lead to a transient diminution in oesophageal varices, but these varices may cause problems without further treatment. Endoscopic treatments for oesophagogastric varices have been shown to be effective, safe and easy to perform[23]. Therefore, laparoscopic splenectomy in combination with endoscopic treatment should have the same effects as oesophageal transection, with the former being less invasive and safer. Currently, in the present authors' institution, laparoscopic splenectomy is not considered first-line therapy for risky oesophageal varices, but is regarded as an alternative or adjunctive procedure.

Interestingly, liver function improved after splenectomy, with more pronounced improvements in patients with Child–Pugh B/C (score 7–10). The improvements in intrahepatic portal vascular resistance, together with the normalization of ET-1 and NOx concentrations may explain, at least in part, the mechanisms underlying the improvements in liver function. Expression of $ETR_A$ and $ETR_B$ was enhanced on hepatic stellate cells of patients with liver cirrhosis, with the levels of expression of these receptors correlating with the degree of portal hypertension[24]. Therefore, the same decrease in ET-1 concentration may result in a greater reduction of intrahepatic portal vascular resistance and a greater increase in resulting NO production in patients with Child–Pugh B/C than in those with Child–Pugh A. In the peripheral circulation, ET-1 in advanced liver cirrhosis is associated with reduced vascular tone, owing to increased $ETR_B$-mediated NO production in endothelial cells, whereas ET-1 in early liver cirrhosis still contributes to the maintenance of vascular tone[25]. Therefore, the same reduction of ET-1 by splenectomy may result in a greater decrease in $ETR_B$-mediated NO production in endothelial cells in patients with Child–Pugh B/C than in those with Child–Pugh A.

The portal decompressing effect was significantly greater in severe (splenic weight 500 g or more) than in mild splenomegaly. This effect was also greater in patients with Child–Pugh B/C than in those with Child–Pugh A. Splenectomy may therefore have fewer benefits, except for prolonged haematological effects, in patients with Child–Pugh A and mild splenomegaly, where the aim is often induction of IFN therapy, than in those with Child–Pugh B/C or severe splenomegaly. If patients with Child–Pugh A require short-term improvements in thrombocytopenia during IFN therapy, partial splenic artery embolization or the use of eltrombopag, a new oral platelet growth factor, may be a better choice than laparoscopic splenectomy[8,26]. In patients with a Child–Pugh score of 7–10 or severe splenomegaly, many of whom have severe portal hypertension, laparoscopic splenectomy may be optimal, although long-term follow-up is still necessary. Laparoscopic splenectomy may provide clinically meaningful outcomes in patients who otherwise would be marginal candidates for liver transplantation. Moreover, it may be useful as a bridging therapy to liver transplantation.

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*Disclosure:* The authors declare no conflict of interest.

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**Supporting information**

Additional supporting information may be found in the online version of this article:

**Fig. S1** Correlation between splenic size and portal venous (PV) velocity, PV blood flow, PV cross-sectional area, PV congestion index and hepatic venous pressure gradient (HVPG) (TIFF file)

# Preemptive Thoracic Drainage to Eradicate Postoperative Pulmonary Complications after Living Donor Liver Transplantation

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**BACKGROUND:** Thoracic fluid retention after living donor liver transplantation (LDLT) has various negative consequences, including atelectasis, pneumonia, and respiratory distress or failure.

**STUDY DESIGN:** We analyzed the clinical impact of preemptive thoracic drainage in 177 patients undergoing adult-to-adult LDLT for chronic liver diseases at a single center. Recipients were divided into 2 time periods. The earlier cohort (n = 120) was analyzed for risk factors for postoperative atelectasis retrospectively; the later cohort (n = 57), with a risk factor for postoperative atelectasis, underwent preemptive thoracic drainage prospectively. The incidence of postoperative pulmonary complications was compared between these 2 cohorts.

**RESULTS:** Independent risk factors for atelectasis in earlier cohort were body mass index $\geq$27 kg/m$^2$ ($p < 0.001$), performance status $\geq$3 ($p = 0.003$) and model for end-stage liver disease score $\geq$23 ($p = 0.005$). The rates of atelectasis (21.1% vs 42.5%, $p = 0.005$) and pneumonia (1.8% vs 10.0%, $p = 0.049$) were significantly lower in later than in earlier cohort. Moreover, the mean durations of ICU stay (3.6 $\pm$ 0.2 days vs 5.7 $\pm$ 0.6 days, $p = 0.038$) and postoperative oxygen support (5.1 $\pm$ 0.8 days vs 7.1 $\pm$ 0.5 days, $p = 0.037$) were significantly shorter in the later than in the earlier cohort. There were no significant differences in the incidence of adverse events associated with thoracic drainages between these 2 cohorts.

**CONCLUSIONS:** Preemptive thoracic drainage for transplant recipients at high risk of postoperative atelectasis could decrease morbidities after LDLT. (J Am Coll Surg 2014;219:1134–1142. © 2014 by the American College of Surgeons)

Owing to poor preoperative clinical conditions, the extensive surgical field, long operating times, and massive blood loss and blood transfusions, liver transplant recipients are susceptible to postoperative pulmonary complications.[1-3] The most frequent are immediate postoperative pulmonary complications, including pleural effusions and atelectasis.[1,2,4] However, infectious complications, which often complicate the former, are much more serious and are responsible for a significant part of the mortality.[3,5-7]

Atelectasis is an important predisposing factor for postoperative pneumonia.[8-10] In general, if a pulmonary segment remains atelectatic for longer than 72 hours, pneumonia is almost certain to develop.[11] Thoracic fluid retention increases the risk of atelectasis by compressing the lungs.[10,12] Postoperative thoracic fluid retention will usually clear with diuresis, but this process may take a considerable period of time.[13] Moreover, postoperative fluid control is difficult after living donor liver transplantation (LDLT) owing to the small graft volume.[14] Therefore, thoracic drainage of pleural effusions may be effective in preventing postoperative atelectasis after LDLT.

This study was designed to evaluate the impact of preemptive thoracic drainage on LDLT recipients at risk for postoperative atelectasis. Additionally, the clinical impact of and risk factors for postoperative atelectasis were analyzed.

## METHODS

### Patients

Between January 2008 and December 2013, 177 consecutive adult-to-adult LDLTs for chronic liver diseases were

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**Abbreviations and Acronyms**

AUC = area under the curve
DDLT = deceased donor liver transplantation
$FiO_2$ = fraction of inspired $O_2$
LDLT = living donor liver transplantation
MELD = Model for End-stage Liver Disease
OR = odds ratio
$PaO_2$ = partial pressure of arterial $O_2$
POD = postoperative day

performed at Kyushu University Hospital. All operations were performed after obtaining informed consent from the patients and approval from the Liver Transplantation Committee of Kyushu University.

## Groups and study design

### *Risk factors for and clinical impact of postoperative atelectasis*

The 177 recipients were divided into 2 groups based on the therapeutic strategy for postoperative pleural effusion adopted at Kyushu University Hospital. The earlier cohort, consisting of 120 LDLT recipients, underwent thoracic drainage when refractory pleural effusion occurred. The later cohort, consisting of 57 recipients, underwent preemptive thoracic drainage if they had at least 1 risk factor for postoperative atelectasis (Fig. 1). Recipients with preoperative pleural effusions of grade $\geq 2$[1] underwent preemptive thoracic drainage during both time periods. Thoracic drainage was performed by inserting a thoracic tube under mini-thoracotomy.

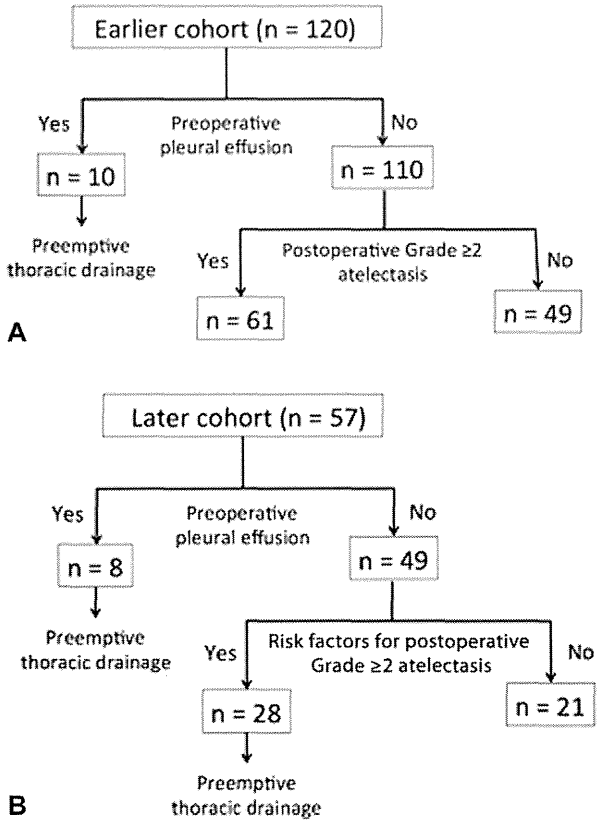


**Figure 1.** Schematic presentation of the 2 recipient groups of living donor liver transplant recipients. (A) Earlier cohort; (B) later cohort.

Risk factors for and the clinical sequelae of grade $\geq 2$ postoperative atelectasis were examined retrospectively in the earlier cohort. Of these 120 patients, 10 had grade $\geq 2$ preoperative pleural effusion; these 10 patients were excluded from analysis of risk factors and clinical effects of postoperative pleural effusion.

### *Validation of preemptive drainage*

The incidence of postoperative pulmonary complications was compared in the 2 cohorts to validate our policy of preemptive thoracic drainage in the later cohort. Subgroup analysis was performed to assess characteristics that influenced between-group differences in clinical outcomes. Patients in each cohort were divided into 3 subgroups: those with preoperative pleural effusion, and those with and without risk factors for postoperative atelectasis. Performance status was determined using the Eastern Cooperative Oncology Group performance status scale.[15]

### *Preemptive thoracic drainage*

Between January 2008 and April 2012, only recipients with pleural effusions of grade $\geq 2$, detectable before LDLT, underwent preemptive thoracic drainage. Since May 2012, however, preemptive thoracic drainage has been performed in patients with a risk factor for postoperative atelectasis grade $\geq 2$, as well as in patients with preoperative pleural effusions. All thoracic drainages in both cohorts were performed under mini-thoracotomy, in which we coagulated and divided intercostal muscles and parietal pleura along the superior edge of the rib using an electric scalpel to prevent unexpected bleeding (Supplementary video, online only). A 12-Fr catheter (Covidien Japan) was placed bilaterally under sterile aseptic conditions, with full barrier precautions. The tubes were placed in the anterior axillary line, and the catheter was attached to a closed drainage system with $-10$ cm water pressure suction. Chest radiography was performed after the procedure. Thoracic tubes remained in place until fluid removal over 24 hours was less than 100 mL.

### *Graft selection criteria and surgical procedures*

The graft selection criteria for adult-to-adult LDLT[16] and the surgical procedures in both donors and recipients[17]

have been described. Splenectomy was routinely performed in patients with hepatitis C virus infection or portal hypertension.[18]

### Postoperative management

All LDLT recipients were transferred to the ICU and mechanically ventilated postoperatively. The respirator was set to provide pressure-controlled ventilation with a positive end-expiratory pressure of 5 cm $H_2O$. To correct intraoperative fluid overload, a sufficient amount of diuretic was administered intravenously. Extubation was indicted within 24 hours after LDLT, when the ratio of the partial pressure of arterial $O_2$ ($PaO_2$) to the fraction of inspired $O_2$ ($FiO_2$) was >250 and when the patient's cardiovascular, graft, and renal conditions were stable.[6] After extubation, oxygen support was maintained until oxygen saturation of the peripheral arteries remained greater than 97% under room air. Patients were administered early enteral nutrition because of its impact on postoperative bacterial sepsis after LDLT.[19] Routine postoperative investigations included arterial blood gas tests ($PaO_2$, $PaCO_2$, pH, $HCO^{3-}$, and base excess) every 4 hours, and blood tests (complete blood count, coagulation profile, and serum liver enzymes), Doppler ultrasonography to examine blood flow in the graft vessels, and portable chest and abdominal radiographs twice daily. Patients with an uneventful course of recovery were transferred to the surgical ward on postoperative day (POD) 3. All patients underwent routine chest and abdominal CT on POD 7; if any clinical data were abnormal, roentgenographic examinations were performed.

The incidence of each grade of pleural effusion and atelectasis, as well as pneumonia, through POD 7 were assessed based on radiologic findings. To minimize the risk of bias, arterial blood gas tests, chest radiographs, and/or CT were performed at around the same time each day.

Perioperative antibacterial and immunosuppressive management have been described in detail.[6] The basic immunosuppressive regimen consisted of tacrolimus (Prograf; Astellas Pharma Inc) or cyclosporine A (Neoral; Novartis Pharma KK), and steroids, with or without mycophenolate mofetil (Cellcept; Chugai Pharmaceutical Co Ltd).

### Perioperative respiratory management

Preoperative respiratory management included smoking cessation and pulmonary rehabilitation, such as respiratory muscle training, especially in patients with abnormal spirometry results. Postoperative pulmonary management included good oral hygiene, intermittent suction, adequate positional changes, nebulized bronchodilators and mucolytics after extubation, and enforcement of early postoperative ambulation.

In managing pulmonary complications, diuretics were first administered to patients with pleural effusions. Atelectasis was first managed by a combination of positional changes, such as to the prone position, elevation of positive end-expiratory pressure, breathing exercises, and suction under bronchoscopy. If atelectasis was accompanied by pleural effusions, thoracic drainage under minithoracotomy was considered. Tracheotomies were performed in patients who could not be weaned from mechanical ventilation at POD 7.

### Radiologic findings

Atelectasis was classified into 3 grades, with grade 1 indicating the involvement of ≤1 subsegment or discoid atelectasis and grades 2 and 3 indicating the involvement of 2 and ≥3 subsegments, respectively (Fig. 2). Pleural effusion was also classified into 3 grades, with grade 1 indicating a loss of sharpness of the costophrenic angle and diaphragmatic profiles or subpulmonary effusion, grade 2 indicating effusion involving <25% of a hemithorax, and grade 3 indicating involvement of >25% of a hemithorax, including a massive effusion with mediastinal shift.[1]

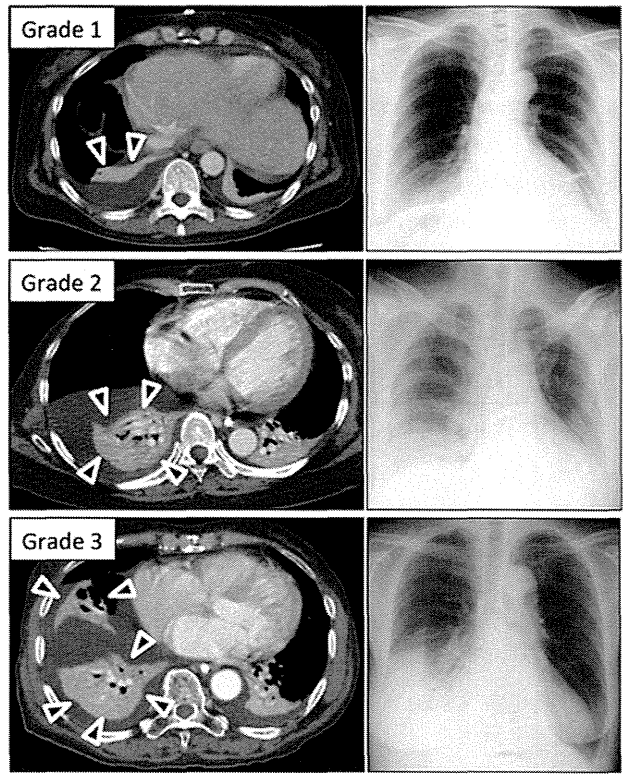


**Figure 2.** Chest CT and radiograph of each grade of postoperative atelectasis. Atelectasis is indicated by arrowheads. Grade 1 atelectasis is defined as involvement of ≤1 subsegment or discoid atelectasis; grades 2 and 3 indicate the involvement of 2 and ≥3 subsegments, respectively.