Portal hyperperfusion after major liver resection and associated sinusoidal damage is a therapeutic target to protect the remnant liver

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Running title: Hepatic circulatory alterations after major liver resection

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Abstract

Extended liver resection results in the loss of a large fraction of the hepatic vascular bed and thereby abrupt alterations in the perfusion of the remnant liver. Mechanisms of hemodynamic adaptation and associated changes in oxygen metabolism after liver resection and the effect of mechanical portal blood flow reduction were assessed.

A pig model (n=16) of extended partial hepatectomy that included continuous observation for 24 hours under general anesthesia was established. Pigs were randomly separated into 2 groups, one group with a portal flow reduction of 70% compared to the preoperative values, and the other group as a control (n=8, each).

In controls, portal flow [mean (SD)] increased from 74 (8) (ml/min)/100g preoperatively to 240 (48) (ml/min)/100g at 6 hours after resection ($p<0.001$). Hepatic arterial buffer response was abolished after resection. Oxygen uptake per unit liver mass increased from 4.0 (1.1) (ml/min)/100g preoperatively to 7.7 (1.7) (ml/min)/100g 8 hours after resection ($p=0.004$). Despite this increase in relative oxygen uptake, total hepatic oxygen consumption was not maintained and markers of hypoxia and anaerobic metabolism were significantly increased in hepatocytes after resection. Reduced postoperative portal flow was associated with significantly decreased levels of aspartate aminotransferase and bilirubin and increased hepatic clearance of indocyanine green.

In conclusion, major liver resection was associated with persistent portal hyperperfusion, loss of the hepatic arterial buffer response, decreased total hepatic vO_2 and with increased anaerobic metabolism. Portal flow modulation by partial portal vein occlusion attenuated liver injury after extended liver resection.

Keywords

Liver resection, liver injury, hepatic hemodynamics, portal flow modulation, hepatic arterial buffer response

New & Noteworthy

Because of the continuous monitoring, the experiments allow to precisely observe the influence of liver resection on systemic and local abdominal hemodynamic alterations and oxygen metabolism. Major liver resection is associated with significant and persistent portal hyperperfusion and loss of hepatic arterial buffer response. The correlation of portal hyperperfusion and parameters of liver injury and dysfunction offers a novel therapeutic option to attenuate liver injury after extended liver resection.

Introduction

Liver resection is currently the only curative treatment for primary and secondary liver tumors. The regenerative capacity of the liver allows full restoration of liver weight and function after resection of a majority of the liver mass. Nevertheless, hepatocellular regeneration may fail after extended liver resection, resulting in small for size syndrome (SFSS) (11). This state is defined as a reduced liver mass without appropriate regeneration leading to persistent liver insufficiency, which is associated with high mortality (30).

A possible cause for the development of SFSS may be alterations in the perfusion of the remnant liver after resection (8, 9, 27). Removal of a large fraction of the liver results in a reduction in the total hepatic sinusoidal cross-sectional area and thereby to portal hyperperfusion and an increase in portal pressure (22, 25). These circulatory alterations depend on the degree of resection and have been described as critical for the initiation of liver mass restoration, as shown by the lack of liver mass restoration in models of porto-systemic shunting (22, 25, 31). However, despite being a potential trigger for the initiation of regeneration, associated perfusion alterations may be detrimental to the hepatic tissue if they become too extreme as in the case of extended resection (1, 27) and may disturb regeneration and metabolic function of the liver at later stages of regeneration (17, 23). Recent research highlighted that in particular the degree of arterial perfusion is important for liver remnant integrity (7) and that sinusoidal damage is associated with elevated portal perfusion pressure (20).

Several surgical and interventional techniques are currently employed to protect the remnant liver from SFSS and to increase resectability of advanced hepatic tumors. Such techniques include portal ligation or embolization or more recently in situ split procedures (12, 26). The observation that portal vein occlusion increases the volume of the remaining liver lobe reveals that modulation of flow and pressure of the blood entering the liver may critically influence hepatic regeneration (21). We hypothesized that extensive portal hyperperfusion after liver resection is harmful to the remnant liver and reduction of portal blood flow could attenuate tissue injury and improve liver function.

The current study was designed to address the following specific issues in a novel model of extended liver resection with continuous invasive monitoring for 24 hours: First, to describe the kinetics of absolute and relative liver blood flow (per unit liver mass) and pressure patterns in the first 24 hours

after resection, including the relative contribution of portal vein and hepatic artery flow. Second, to understand the dynamic interaction between portal and arterial blood flow and pressure by exploring the hepatic arterial buffer response, which is defined as the compensatory increase in hepatic artery flow when portal flow is acutely reduced (15). Third, to investigate hepatic oxygen metabolism in response to changing portal and arterial flow patterns after resection. Fourth, to evaluate the effect of controlled reduction of postoperative portal vein blood flow on damage to the remnant liver after extended liver resection.

Material and Methods

Initial experiments were performed in a mouse model, revealing structural changes after partial hepatectomy. Since precise control of portal flow and monitoring of local and systemic hemodynamic parameters was not feasible in this model, further experiments were performed in a pig model.

The studies complied with the guide for the care and use of laboratory animals and Swiss national guidelines and were approved by the commission of animal experimentation of the canton of Bern, Switzerland, approval number BE 43/12 (mouse experiments) and BE 134/14 (pig experiments).

Liver resection experiments in mice

For partial hepatectomy experiments, 10 week old female C57/BL6 mice were anaesthetized using isoflurane followed by ligation and resection of the respective lobes as described previously (10). Briefly, animals were immobilized in a supine position and the abdomen entered through a midline laparotomy. After exposure of the liver, partial hepatectomy was performed by central ligature of the median and left lobe in order to achieve a standard 68% hepatectomy. The ligated liver lobes were surgically removed. The laparotomy was then closed with a two-layer running suture.

Electron microscopy of liver tissue

For analysis by electron microscopy, mice were euthanized 12 hours after partial hepatectomy. Tissue was immediately fixed by gently flushing of the remnant liver via the portal vein and thereafter immersed in a solution of 2.5% of glutaraldehyde and 2% of polymeric formaldehyde in 0.1M NA-cacodylate. Tissue blocks from the right superior lobe were cut, re-fixated and the tissue was then dehydrated in increasing concentrations of ethanol. Tissue was embedded in epoxy resin and subsequently imaged by transmission electron microscopy.

Liver resection experiments in pigs

Study design: Sixteen male domestic pigs (Sus scrofa domestica) ranging from 56 to 63 kg were used for the experiments. Animals were randomly allocated to the control (N=8) or the intervention group (N=8) during surgery, just prior to liver resection. In order to study the normal post-resection physiology, values from the animals in the control group were studied. Animals in the intervention group were compared to animals in the control group to test the effect of portal flow modulation.

Anesthesia: After premedication with ketamine 20 mg/kg and xylazine 2 mg/kg (i.m.), anesthesia was induced with 10 mg of midazolam and 1mg of atropine (i.v.). After endotracheal intubation, general anesthesia was maintained with continuous infusion of propofol (200-500 mg/h) and fentanyl (200-300 µg/h). The animals were mechanically ventilated in a volume-controlled mode (F_IO_2 0.3, positive end-expiratory pressure 5 cm H_2O, tidal volume 8 ml/kg and respiratory rate adjusted to keep end-expiratory CO_2 between 35 and 45 mmHg). Adequate depth of anesthesia was controlled by repeated nose pinch maneuvers and additionally documented by bispectral index monitoring. 10 ml/kg/h of Ringer's Lactate solution was administered during surgery and thereafter decreased to 2 ml/kg/h and adjusted according to urinary output. Before and after liver resection, lung recruitment maneuvers were performed in supine and Trendelenburg position in random order.

After performing the last set of measurements, the animals were euthanized in deep anesthesia by intravenous injection of 40 mmol of potassium chloride.

Installation of hemodynamic measurement equipment: Arterial blood pressure was measured through a sheath in the right carotid artery and central venous pressure through a catheter inserted through the right internal jugular vein. A flow probe (Transonic Systems Inc., Ithaca, NY) was positioned around the left common carotid artery. A pulmonary artery catheter was inserted through a jugular vein on the left side and its position confirmed using blood pressure tracing. A catheter was inserted into the bladder to measure urinary output via a midline laparotomy . The hepato-duodenal ligament was dissected, flow probes were positioned around the hepatic artery, the portal vein, the superior mesenteric artery and the inferior vena cava. A tourniquet was placed around the portal vein 1 cm proximal to the position of the flow probe. Two catheters for pressure measurement were inserted through a mesenteric vein and advanced into the portal vein to position one catheter tip proximal and the other distal to the tourniquet. Finally, a distally bent catheter (Infinit Diagnostic Catheter 5F, Cordis, Baar, Switzerland) was inserted through the right external jugular vein and positioned in the right hepatic vein under fluoroscopic guidance and its position was controlled by contrast injection. Pressures and flows were recorded using LabVIEW (National Instruments, Austin, TX) and processed offline using dedicated software (Soleasy, Alea Solutions, Zürich, Switzerland).

Vital parameters including ECG, arterial blood pressure and oxygen saturation were monitored continuously. Target for mean arterial blood pressure was 60 to 70 mmHg, target for heart rate 60 to 90 beats per minute. Further, S_vO_2 target was above 50%, blood lactate target was lower than 2

mmol/l and urine output was kept above 0.5 ml/kg/h. If these parameters were not met, an additional bolus of 100 ml of Ringer's lactate was given. If the animal did not respond to fluids, norepinephrine was administered continuously at a rate between 100 and 600 µg/h.

Liver surgery: Anatomical resection was performed by removing the whole left and the right medial lobe, leaving behind segment I, VI and VII as previously described (5). After selective ligation of the arterial and portal branches supplying the tissue defined for resection, liver tissue including the hepatic veins were transected using a stapling device (Endo GIA, Johnson&Johnson, New Brunswick, NJ). The mass of the resected tissue was measured.

In the intervention group, a tourniquet consisting of a Teflon band and a plastic tube was positioned around the portal vein. In a series of pilot experiments, we found that after 70% hepatic resection, portal flow could maximally be reduced to 70% of baseline flow before hemodynamic instability occurred. Therefore, portal flow was reduced just prior to resection to 70% of the baseline portal flow. Portal flow was re-adjusted to the target value at hourly intervals, if it had varied by more than 10%.

Main measurement periods: A complete set of measurements was taken before resection (baseline measurement), directly after resection (post resection measurement) and 24 hours after resection (24h measurement). These measurements followed a standardized sequence:

i. Stabilization phase: no manipulation on the animal for 30 minutes.
ii. Establishment of euvolemia: stroke volume was measured (cold bolus thermodilution method, valid if 3 measurements with less than 10% deviation) before and after a volume challenge of 150 ml of Ringer's lactate. If stroke volume increased >10%, additional boluses were given until the animal was no longer volume responsive.
iii. Blood sampling: arterial, portal venous, hepatic venous and mixed venous blood gas sample were taken and analyzed immediately (ABL 90 FLEX Analyzer). Further, blood for analysis of liver parameters including prothrombin time was drawn, centrifuged and analyzed in a routine laboratory.
iv. Measurement of indocyanine green (ICG) disappearance rate: a bolus of 0.25mg/kg of ICG was injected and blood samples were taken after 1, 15 and 30 minutes. Plasma ICG concentration was measured by spectrophotometry at a wavelength of 805 nm. The resulting concentrations were used to generate an elimination curve.

v. Hepatic arterial buffer response measurements: hepatic arterial flow was measured before and after (partial) occlusion of the portal vein with a tourniquet to reduce the portal flow to 50%, 25% and 0% of the pre-resection flow. The occlusion was maintained for a period of 60 seconds. Then the portal vein was left open for 60 seconds, before the next occlusion was applied.

vi. Transit time measurements: we developed a new method for estimating blood flow velocity in the liver. A bolus of ICG was injected through a catheter into the portal vein, and time between injection and detection by the spectrophotometer at the tip of the pulmonary artery catheter was measured by a stop watch.

Periodic measurements: In addition to the main measurement cycles, all hemodynamic parameters were recorded every hour for a period of 5 minutes. Transhepatic venous resistance was calculated by division of transhepatic pressure by the corresponding portal flow. Every 8 hours, a complete set of blood gas analysis was performed as mentioned above.

Oxygen delivery/consumption: hepatic oxygen delivery was calculated by multiplication of flow values measured by probes on the hepatic artery and portal vein with the oxygen content measured in the blood samples of the respective vessels. Oxygen content (ml/l) was calculated by addition of bound oxygen [calculated by 1.34 (Hüfner's constant) × hemoglobin level (g/l) × oxygen saturation (%) × 0.01] and dissolved oxygen [oxygen partial pressure (mmHg) × 0.03 (solubility coefficient of oxygen at body temperature)]. Oxygen consumption was calculated by subtraction of oxygen transported by the liver vein from the sum of oxygen transported in the hepatic artery and portal vein.

Western blot analysis of HIF-1α protein levels, determination of lactate dehydrogenase (LDH) and pyruvate dehydrogenase (PDH) activities, measurement of hepatic ATP content: A small biopsy of liver tissue (2g) was taken at the beginning of liver resection as well as immediately before euthanasia and processed immediately. Western blot analysis of HIF-1α protein from tissue extracts was performed as described previously (24). Pyruvate Dehydrogenase Activity Assay Kit (Catalog Number MAK183) and Lactate Dehydrogenase Activity Assay Kit (Catalog Number MAK066) were obtained from Sigma-Aldrich (Buchs, Switzerland) and activities were measured according to manufacturer's instructions. To determine hepatic tissue ATP content tissue lysates were deproteinized using the

Deproteinizing Sample Preparation Kit (BioVision, Milpitas, CA, USA) and ATP content was measured using the Molecular Probes' ATP Determination Kit (Invitrogen, Life Technologies, Zug, Switzerland) according to the manufacturer's instructions. The assay kit is based on firefly luciferase and the production of light caused by the reaction of ATP with luciferase and D-luciferin. The assays were performed as described previously (4).

Statistical Analysis: Unless stated otherwise, data is presented as mean (standard deviation). Differences between groups were compared using a two-tailed student's t-test. For repeated measurements, ANOVA or two-way ANOVA was used. Linear regression was performed to analyze correlation between portal flow and parameters for hepatic function, Pearson's r and respective p-values were calculated. For comparison of flow values between control and intervention group, four-hour means were calculated. Statistical significance was defined at the .05 level. Statistical analysis was performed using Prism 7 (Graph Pad, La Jolla, CA, USA) software.

Results

1. PHYSIOLOGICAL CHANGES AFTER EXTENDED LIVER RESECTION

Disruption of sinusoidal endothelium after extended liver resection in mice

Major liver resection was associated with a pronounced destruction of the liver sinusoids (representative EM picture before and after surgery: Fig 1 A, B). Endothelial cells were disrupted, large parts of the hepatic microvilli were no longer covered by an endothelium and underlying microvilli were partially diminished 12 hours after major hepatectomy (Fig 1 C, D). The percentage of non-covered endothelial surface (fenestrations) was 9.4 (5.1)% in control animals and 27.8 (5.7)% 24 hours after liver resection in the intervention group ($p<0.0001$).

Hemodynamic studies in pigs

Postoperative hemodynamic changes after extended liver resection were studied in eight animals of the control group.

Mean systemic arterial pressure dropped by 15 (8) mmHg during surgery ($p=0.09$) and continued to decrease during the rest of the observation period, while cardiac output and flow in the inferior vena cava increased over the postoperative course of 24 hours (Tab 1). Superior mesenteric artery flow dropped from 0.93 (0.20) l/min preoperatively to 0.63 (0.18)l/min after resection ($p=0.005$) and thereafter gradually returned to the initial flow rate 16 hours after surgery.

Absolute portal blood flow decreased from 1.14 (0.15) l/min preoperatively to 0.72 (0.06) l/min after resection ($p=0.004$) and recovered to baseline values after 6 hours (Fig 2A). Relative portal flow (flow per unit liver mass) significantly increased from 74 (8) ml/min/100g to 240 (48) ml/min/100g at 6 hours after resection ($p<0.001$). This portal hyperperfusion persisted for the entire length of the experiment (Fig 2A).

Absolute hepatic arterial flow decreased from 0.19 (0.9) l/min preoperatively to 0.03 (0.02) l/min postoperatively and recovered to 0.05 (0.03) l/min after 24 hours (Fig 2B). Relative hepatic arterial flow per unit liver mass was reduced initially after resection, returning close to the preoperative values within 24 hours after surgery (Fig 2B).

As a consequence of the described changes in hepatic arterial and portal venous flow, the fraction of arterial perfusion on total liver perfusion decreased from 13.6% (4.9) to 4.4% (2.9) directly after resection and transit time of portal blood passing the liver decreased considerably by 66.6% immediately after surgery (Fig 2C).

Transhepatic venous blood pressure increased from 1.5 mmHg (0.8) preoperatively to 3.9 mmHg (0.9) after resection (Fig 2E), while liver venous pressure remained unchanged.

Transhepatic venous resistance increased from 1.3 (0.8) mmHg*min/l preoperatively to 4.6 (1.9) mmHg*min/l after resection. During the following hours, transhepatic resistance gradually decreased to 3.0 (1.5) mmHg*min/l at 16 hours (Fig 2D).

2. HEPATIC ARTERY BUFFER RESPONSE IN THE CONTEXT OF LIVER RESECTION

An immediate increase of hepatic arterial blood flow was seen in response to the restriction of portal blood flow with a tourniquet prior to resection (Fig 3A). In response to a reduction of portal flow of 50%, hepatic arterial flow increased from 0.14 (0.053) l/min at baseline to 0.20 (0.067) l/min (mean difference 0.059 l/min, p=0.015), with no further increase when portal flow was further reduced. Immediately after, as well as 24 hours after resection, the hepatic artery did not show a reaction to portal flow restriction, independent of the degree of flow reduction (Fig 3B).

3. OXYGEN CONSUMPTION AND CELLULAR ENERGY PRODUCTION AFTER LIVER RESECTION

Liver dO_2 decreased proportionally to resected tissue, but gradually recovered to preoperative values, although with a higher contribution of portal and a lower contribution of arterial to total oxygen delivery as compared to the preoperative values (Fig 4A).

Liver oxygen extraction decreased postoperatively and remained on this lower level during the whole perioperative phase. Consequently, liver vO_2 decreased more than dO_2 post-resection (Fig 4B, C). However, vO_2 relative to unit liver mass increased gradually to higher values than before resection (Fig 4D).

Despite increased oxygen uptake per gram of liver tissue, expression of HIF1alpha as a marker of hypoxia was significantly increased in biopsies from the remaining liver tissue 24 hours after liver resection (Fig 4E). According to the described function of HIF1 alpha, LDH activity was increased in biopsies, in parallel a decreased activity of PDH was observed (Fig 4 F, G). Hepatic ATP concentration increased significantly 24 hours after hepatectomy (Fig 4H).

4. EFFECT OF PORTAL FLOW MODULATION AFTER EXTENDED LIVER RESECTION

With portal flow modulation (reduced just prior to resection to 70% baseline), postoperative portal flow per unit liver mass could be reduced to twofold the preoperative value in the intervention group compared to fourfold in the control group. This was not associated with an increase in hepatic arterial flow (p=0.43, no time-group interaction). In both groups, the hepatic artery showed a flow depression early after resection with subsequent recovery close to the preoperative value (Fig 5 A). Short-term reduction of portal flow that triggered the hepatic arterial buffer response preoperatively, did not show increased hepatic arterial flow postoperatively in the intervention group or in the control group (Fig 5 B).

In the intervention group, the reduced portal venous blood-flow was compensated by an increased extraction of oxygen in the liver, with a maximum effect at 16 hours after surgery (p=0.005, Fig 5C). Portal flow reduction did not lead to a decrease in oxygen uptake of the remnant liver (36.2 (11.9) vs. 34.3 (8.9) mlO_2/min, p=0.76).

Comparison between intervention and control group did not show significant differences (all p-values >0.05) in parameters reflecting liver damage (aspartate aminotransferase, alanine aminotransferase) and function (Bilirubin, Prothrombin, ICG Clearance). However, since some animals in the control group showed a lower than expected portal flow, probably as a consequence of portal vein narrowing during hepatic resection, there was a considerable overlap in mean portal flow post resection (Fig 5 D-H). Therefore, a post hoc analysis was performed where mean postoperative portal flow of animals from both groups was correlated with parameters reflecting liver damage and function.
Bilirubin levels at 24 hours showed a significant positive correlation with mean portal venous flow (p=0.02, Fig 5D). Similarly, AST and ALT as parameters of liver injury showed a positive correlation with portal venous flow (p=0.005 and 0.1 respectively, Figure 5E, F). ICG clearance, a measure of liver function, showed a significant negative correlation with portal venous flow, indicating a worsening function with increasing post-operative hyperperfusion (p=0.01, Fig 5G), a similar trend was seen for prothrombin time as a measure of the synthetic function of the liver (p=0.17, Fig 5H).

Discussion

Our data reveal a sustained relative portal venous hyperperfusion after major liver resection during the whole observation period of 24 hours that is accompanied by an arterial hypoperfusion. Portal hyperperfusion was associated with extensive destruction of the sinusoidal structure in our mouse model of major liver resection. An elevated oxygen demand has been observed in the postoperative phase, given increased oxygen consumption per unit liver mass after resection and upregulation of HIF-1 alpha and LDH in hepatocytes, indicating increased anaerobic glycolysis. The hepatic arterial buffer response was absent at all observed time points after hepatic resection. A relative decrease in portal blood flow was associated with reduced liver injury 24 hours after resection. However, portal flow modulation did not increase postoperative hepatic arterial flow and could not prevent the loss of the hepatic arterial buffer response postoperatively. Hepatic oxygen consumption was maintained during portal flow modulation via a higher hepatic oxygen extraction.

The reason for the observed flow behavior is the dual blood supply of the liver. The arterial branch has an overall layout as in most other organs, with blood inflow from the aorta and drainage into the caval vein. The portal branch however, is connected in series with the vasculature of the intestine and spleen. The resistance of the entire intestinal-liver vasculature system is dominated by the intestinal resistance while the portal vasculature of the liver has a relatively low resistance. Therefore, pressure drop over the liver is relatively low. As a consequence, resection of a considerable part of the liver leads only to a small change in the total intestinal-liver resistance and thereby to a small change in absolute portal flow.

Sinusoidal pressure, and as a consequence portal venous pressure rise because of the increased resistance after resection. This may lead to a widening of the hepatic sinusoids and portal venous branches. The measured decrease in transhepatic resistance in the first 16 hours after resection may be the consequence of the sinusoidal widening over time, such that their hemodynamic resistance is reduced.

We interpret the decrease in systemic mean arterial pressure and gradual increase in cardiac output during the experiment to reflect a temporary vasoplegia most likely caused by extensive surgical trauma, prolonged anesthesia and probably also intestinal bacterial translocation due to elevated portal pressure after hepatectomy.

The early postoperative arterial hypo-perfusion represents a disproportionate hepatic artery flow reduction compared to the amount of resection. Considering the simultaneous relative portal hyperperfusion, this could be interpreted as a reversal of the hepatic arterial buffer response mechanism with increased portal flow leading to decreased arterial flow. However, several observations refute this theory: First, animals in the intervention group show a similar decrease in hepatic arterial flow, even though portal flow was considerably reduced in these animals. Second, relative hepatic arterial flow recovered to values similar to preoperative ones at 24 hours after resection, whereas the portal hyperperfusion persisted, meaning there was no temporal connection. Third, an acute and strong reduction of portal flow post resection did not lead to an increase in hepatic arterial flow, indicating that the hepatic arterial buffer response was absent. Therefore, it is likely that the observed initial relative arterial hypoperfusion was rather the result of hepatic arterial vasospasms associated with surgical trauma or intrahepatic injury, than a response to an abrupt increase in portal perfusion.

Using defined short portal vein occlusion steps with simultaneous measurement of hepatic arterial flow we could show that arterial perfusion was fully decoupled from portal perfusion after resection and consequently the hepatic arterial buffer response is absent in the postoperative period. This disagrees with the proposed mechanism that portal hyperperfusion triggers small for size syndrome by the mechanism of a reversed hepatic arterial buffer response (1, 19). The “adenosine-washout-hypothesis” introduced by Lautt (16) attributes the increased arterial blood flow to the vasodilator effect of adenosine in the space of Mall, because adenosine is removed at a lower rate if portal flow is low. Given the fact that the levels of both ATP and its hydrolyzed form adenosine are highly elevated in the regenerating liver (2, 14), it is likely that adenosine washout does not impact on vasodilatation in this situation.

More likely, portal hyperperfusion may be the cause for hepatic dysfunction after extended liver resection leading to extensive sinusoidal damage after resection. This goes in line with recent research on perfused human liver monosegments that showed progressive loss of sinusoidal integrity with increasing portal pressure and flow (20). This sinusoidal destruction does not only involve the endothelial cells but also shows a loss of hepatocyte microvilli lining the liver sinusoids. Whereas slight portal hyperperfusion may stimulate liver regeneration, excessive hyperperfusion could create

endothelial and hepatocyte damage by flow-induced viscous shear stress and by extensive pressure-induced widening of the sinusoids, leading to a liver dysfunction proportionally to the degree of resection (22, 25). This is confirmed by recently published human data that show that portal vein pressure above 20mmHg or hepatic to portal vein gradient above 15 mmHg after in situ split procedures are associated with decreased liver regeneration and function (29).

AST and ALT levels were lower in animals with reduced portal flow after hepatic resection. Since those parameters are a direct measure for hepatocyte damage, this finding supports the thesis of portal hyperperfusion mediated sinusoidal and hepatocyte damage that can be decreased by portal flow modulation. The same animals showed better metabolism of bilirubin and ICG clearance. These findings agree with the current literature (3) and our in-depth measurements of perioperative hemodynamics allowed novel mechanistic insight into the development of small for size syndrome after liver resection.

Alterations of hepatic oxygen metabolism reveal Warburg-like changes in the post-resection period that results in elevated hepatic ATP-generation during regeneration (6, 13). This strong increase in ATP generation is achieved by an increase in glycolytic ATP production similar to tumor cells (18, 28). The reason for increased glycolytic ATP production is not explained by a hyperperfusion-related lack of oxygen, since relative oxygen consumption even increased after resection, but rather by the increased need of energy for liver regeneration and increased metabolic demand per liver cell as compared to baseline. We suggest that decreased hepatic oxygen extraction compared to baseline was an effect of endothelial damage. Alternatively – or in combination – increased portal blood flow velocity coupled with an increased fraction of oxygen delivered by the portal vein, where oxygen content per ml of blood is lower compared to hepatic artery, may have led to a decreased maximal oxygen extraction. The measurements in the intervention group assure that portal flow modulation does not limit hepatic oxygen uptake and accordingly does not jeopardize oxygen delivery to the regenerating liver tissue. This behavior adds evidence to the concept that oxygen-extraction from portal venous blood may be flow dependent after liver resection.

Reduction of portal flow is associated with reduced liver injury and increased hepatic synthetic capacity. The translation of these experiments is limited because only pigs with healthy livers were

used. It is possible that portal blood flow modulation would be even more effective in preexisting conditions such as hepatic steatosis or cirrhosis. Furthermore, because of technical aspects, continuous observation was only possible for 24 hours. It is not unlikely that the differences between intervention and control would be more pronounced in experiments with a longer duration.

In conclusion, our novel model of continuous assessment of outcomes after major liver resection reveals significant and persistent portal hyperperfusion and the loss of the hepatic arterial buffer response. Despite a major increase in oxygen consumption per unit liver mass in the regenerating liver, there is a simultaneous increase in anaerobic metabolism. Portal flow modulation by partial portal vein occlusion offers the potential to attenuate liver injury after extended liver resection.

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Table 1 Systemic hemodynamic parameters

	Baseline	After surgery	8 hours after surgery	16 hours after surgery	24 hours after surgery
Mean arterial pressure (mmHg)	85 (16)	70 (11)	67 (7)	61 (6)	57 (8)
Central venous pressure (mmHg)	7.7 (2.8)	7.7 (1.9)	7.7 (3.1)	7.6 (3.2)	7.5 (2.4)
Cardiac output (l/min)	6.1 (1.0)	5.6 (1.0)	5.6 (1.1)	5.9 (0.7)	6.8 (1.1)
Superior mesentery artery flow (l/min)	0.93 (0.20)	0.63 (0.18)	0.85 (0.21)	0.91 (0.30)	0.86 (0.25)
Vena cava inferior flow (l/min)	1.70 (0.27)	1.55 (0.57)	1.91 (0.41)	2.12 (0.59)	1.94 (0.64)
Carotid artery flow (l/min)	0.26 (0.08)	0.19 (0.03)	0.26 (0.06)	0.26 (0.05)	0.23 (0.08)

Data is displayed as mean (standard deviation).

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Figure Legends

Figure 1. Major liver resection is associated with structural changes of the sinusoids. (A, B) Electron microscopy images of murine liver sinusoids. Twelve hours after partial hepatectomy, sinusoidal endothelial cells are disrupted from the parenchymal cells, thereby leaving large fenestrations in the sinusoidal wall (white arrows). Microvilli of the hepatocytes are reduced after partial hepatectomy (black arrows). **(C)** Size of fenestration of 5 randomly chosen electron microscopy pictures per animal (2 animals in each group), showing a significant increase in fenestration size in the sinusoidal wall after partial hepatectomy. **(D)** Percentage of fenestrated lining in relation to total endothelial surface in 5 randomly chosen electron microscopy pictures per animal (two animals in each group) showing a clear increase in uncovered sinusoidal wall. Horizontal lines represent means, comparisons by student's t-test.

Figure 2. Major liver resection results in persistent portal hyperperfusion and elevated portal pressure. Displayed values represent mean with standard deviation from 8 animals in the control group.**(A)** Absolute and relative portal flow after major liver resection. After a short-term decrease, absolute portal flow returns to preoperative values after 6 hours. The nearly unchanged absolute flow in the portal vein leads to a hyperperfusion of the remaining liver tissue. **(B)** Absolute hepatic arterial flow decreases strongly after resection and recovers only minimally. Relative arterial perfusion (flow per tissue mass) returns close to preoperative values 24 hours after surgery. **(C)** Portal blood flow velocity in the liver strongly increases as shown by the decreased liver transit time of ICG. **(D)** Portal venous resistance increases inversely proportional to the remaining liver tissue directly after resection and then decreases during the following hours. **(E)** Portal pressure is the sum of liver vein pressure and transhepatic venous pressure. Liver vein pressure was stable after resection, while transhepatic venous pressure increased inversed proportionally to the amount of resection with a maximum at 8 hours postoperatively.

Figure 3. Hepatic artery buffer response is absent after major liver resection. (A) Representative example of the hepatic artery buffer response before and after liver resection. Reduction of portal flow (blue curve) is associated with an imediate and strong increase in hepatic arterial flow (red curve) before resection. Corresponding pressure changes are shown below, pre-occluder portal pressure (purple) rises strongly upon occlusion, post occluder portal pressure (mangenta) and hepatic venous pressure (green) decrease slightly. **(B)** Summarized data showing hepatic arterial flow after reduction of portal flow to 50%, 25% and 0% respectively. The hepatic artery buffer response can be triggered preoperatively but not postoperatively directly or 24 hours after resection, N=8.

Figure 4. Hepatic oxygen consumption per liver mass unit increases after liver resection, enzymes for anaerobic energy production are activated simultaneously. (A) Arterial oxygen delivery to the liver by the hepatic artery decreases after resection due to distinct reduction in hepatic arterial flow. Oxygen delivery by the portal vein drops initially but returns to preoperative values shortly after resection. **(B)** Absolute oxygen consumption of the liver decreases proportionally to the amount of resection directly postoperatively and rises gradually thereafter. **(C)** Oxygen extraction is lower after resection, consistent with the increase in portal blood flow velocity after resection. **(D)** Oxygen consumption per liver mass unit increases stepwise during the postoperative phase. **(E-G)** HIF1alpha concentration in the liver increases significantly 24 hours after resection. Consistent with this observation, LDH activity is increased and PDH activity is decreased in order to stimulate anaerobic energy production. N=8, comparison by student's t-test. **(H)** Hepatic ATP content in the regenerating liver is strongly increased compared to preoperative ATP levels. N=8, comparison by student's t-test. **(I)** Schematic representation of the analyzed regulation of anaerobic energy production on the cellular level.

Figure 5. Portal flow modulation after major liver resection does not increase hepatic arterial perfusion nor preserve the hepatic artery buffer response. However, reduced portal flow correlates with decreased markers of hepatic dysfunction and cell damage. Displayed values represent mean, standard devation where appropriate. **(A)** Decreased postoperative portal flow by

artificial flow modulation does not change blood flow in the hepatic artery. **(B)** The hepatic artery buffer response is not observed immediately and 24 hours after resection in both groups. Portal flow modulation does not preserve postoperative function of the hepatic artery buffer response. **(C)** Oxygen extraction is significantly increased in the intervention group compared to the control group (two-way ANOVA, p=0.005 for group comparison, no interaction). This may be the consequence of a decreased portal blood flow velocity compared to the control group. **(D)** Bilirubin levels 24 hours after resection showed a significant positive correlation with mean postoperative portal flow. **(E-F)** AST and ALT levels showed a positive correlation with postoperative portal flow (p=0.006 for AST, p=0.112 for ALT). **(G-H)** ICG clearance, as a marker of liver function showed a significant negative correlation with portal flow (p= 0.014). Similarly, measurement of prothrombin time showed a trend to lower values if mean postoperative flow was high (p=0.170).

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Previous Communication

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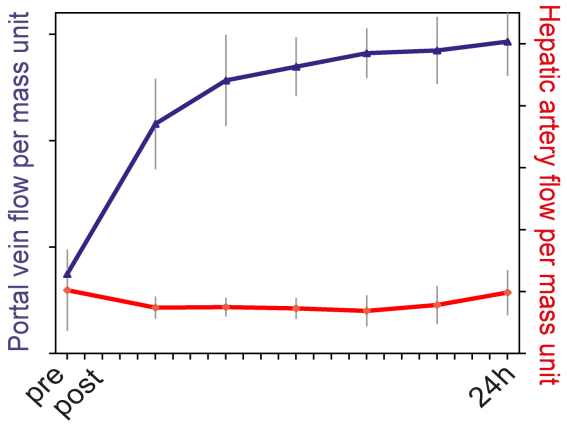
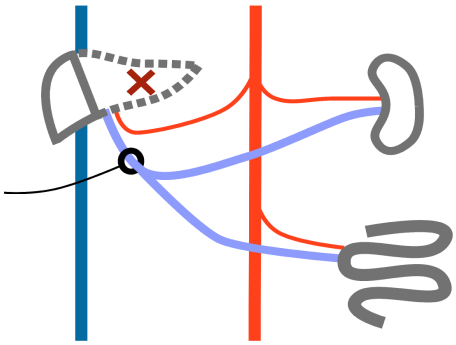
Glossary

ALT	alanine aminotransferase
AST	aspartate aminotransferase
ICG	Indocyanine green
LDH	lactate dehydrogenase
PDH	pyruvate dehydrogenase
SFSS	Small for size syndrome

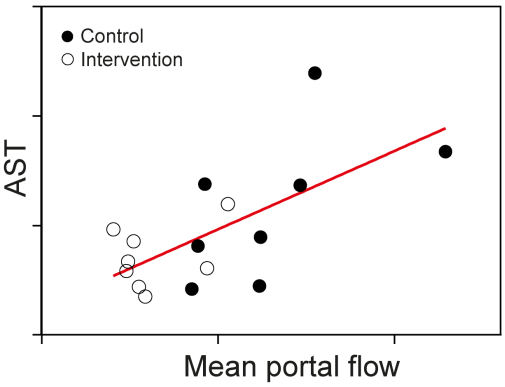
Extended liver resection leads to pronounced **portal hyperperfusion**



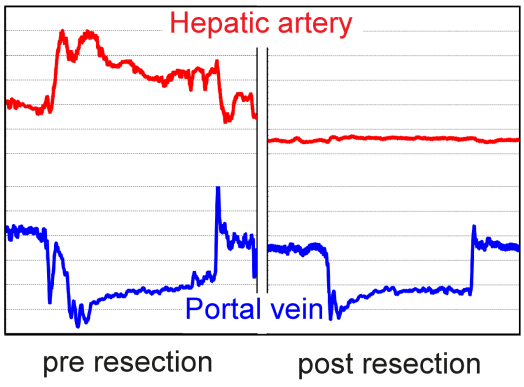
A model of perioperative mechanical **portal flow restriction** was tested



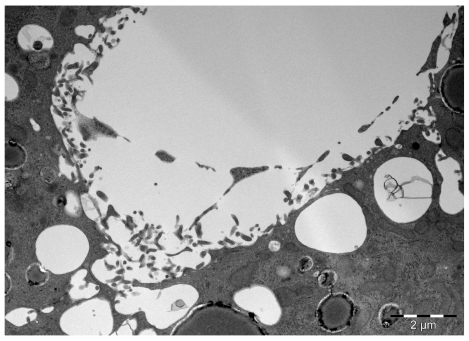
We found following flow related behaviour:



High postoperative portal flow is associated with **liver injury**



The **hepatic arterial buffer response** is inactivated after resection



Sinusoids show extensive damage after resection