FLOW COMPETITION BETWEEN HEPATIC ARTERIAL AND PORTAL VENOUS FLOW DURING HYPOTHERMIC MACHINE PERFUSION PRESERVATION OF PORCINE LIVERS

Running head: ex vivo competition between hepatic arterial and portal venous flow

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ABSTRACT

Hypothermic machine perfusion (HMP) is regarded a better preservation method for donor livers than cold storage. During HMP, livers are perfused through the inlet blood vessels, being the hepatic artery (HA) and the portal vein (PV). In previous HMP feasibility studies of porcine and human livers, we observed that the PV flow decreased while the HA flow increased. This flow competition restored either spontaneously or by lowering the HA pressure (P_{HA}). Since this phenomenon was never observed before and affects the HMP stability, it is essential to gain more insight into the determinants of flow competition. To this end, we investigated during controlled experiments the influence of the HMP boundary conditions on liver flows. This paper presents the flow effects induced by increasing P_{HA} and by obstructing the outlet blood vessel, being the vena cava inferior (VCI).

Flow competition was evoked by increasing P_{HA} to 55-70 mmHg, as well as by obstructing the VCI. Remarkably, a severe obstruction resulted in a repetitive and alternating tradeoff between the HA and PV flow. These phenomena could be related to intra-sinusoidal pressure alterations. Consequently, a higher P_{HA} is most likely transmitted to the sinusoidal level. This increased sinusoidal pressure reduces the pressure drop between the PV and the sinusoids, leading to a decreased PV perfusion. Flow competition has not been encountered nor evoked under physiological conditions and should be taken into account for the design of liver HMP protocols. Nevertheless, more research is necessary to determine the optimal parameters for stable HMP.

KEYWORDS

Organ preservation, pump perfusion, hypothermic machine perfusion, hepatic flow.

INTRODUCTION

Hypothermic machine perfusion (HMP) is an alternative method to preserve organs prior to transplantation. Currently, there is a renewed interest in this preservation method given the increasing application of so-called “expanded criteria donors” as a countermeasure for the growing shortage of organs suitable for transplantation (1, 2).

In kidney transplantation, HMP optimizes the preservation of kidneys from “expanded criteria donors”, assesses their quality prior to transplantation, improves the outcome, and eventually increases the number of transplantations (3, 4). In addition, fluid-dynamical and biochemical parameters can be monitored during HMP to assess the organ’s viability. Such parameters could help transplant surgeons to discard grafts destined to fail after transplantation. HMP has also been suggested to better preserve livers from “expanded criteria donors” (5). These livers convey a higher risk of early graft dysfunction and primary graft non-function, jeopardizing the outcome for the recipient, especially in the absence of liver dialysis and/or an emergency re-transplantation.

Contrary to the kidney, HMP has not yet become common clinical practice in liver transplantation. Several factors such as the complex anatomical and functional features of the liver - compared to the kidney - certainly contribute to this. At present, ideal HMP settings for livers are still to be defined. These optimal settings should consist of an optimal combination of parameters such as the route of perfusion {hepatic artery (HA), portal vein (PV), or both}, the concomitant fluid dynamics (continuous or pulsatile perfusion and the optimal pressure/flow rates), the perfusion solution, the applied temperature, and whether or not to administer oxygen (6).

Previously, we studied hepatic flow characteristics during HMP feasibility studies on porcine livers (7-9) and human livers discarded for clinical transplantation. The default temperature and perfusion solution settings were initially similar to those commonly applied for renal HMP. Both the HA and PV were continuously perfused with a higher

perfusion pressure (P) at the HA compared to the PV. In line with the Lifeport™ technology - currently applied for clinical renal HMP and for the liver HMP prototype used in our centre - the perfusion flow (Q) through the organ is secondary to the pressure, which is set by the operator. As such, in this case, HMP is pressure-driven.

During our initial experience with liver HMP using Lifeport™ technology, vascular resistance (VR) was found consistently higher in the HA compared to the PV. During perfusion, VR_{HA} gradually decreased (similar to observations in the kidney), resulting in an increasing Q since P was kept constant (design and operator controlled). This decrease of VR during HMP is thought to reflect the relaxation of the peri-vascular smooth muscle cells present in the vessels of the HA vascular bed which are much less developed in the PV. To limit the potential shear stresses exerted on the endothelium by a high flow of a cold (4-6°C) viscous perfusion fluid in the PV (Q_{PV}), a flow limitation feature was added to the operating facilities of the HMP liver device.

During some HMP feasibility machine perfusion experiments, a progressive decrease and/or a complete cessation of the Q_{PV} was observed whilst Q_{HA} was increasing as a result of the decreasing VR_{HA}. Q_{PV} restored either spontaneously or by lowering P_{HA}. This phenomenon was defined as flow competition evoked by an increasing Q_{HA} and observed during HMP of porcine livers as well as a human liver discarded for clinical transplantation, which illustrates that the observation was not species-specific. This increasing Q_{HA} was associated with a progressive decrease of Q_{PV}. Hereby, the inflow P_{HA} was remarkably higher compared to the P_{PV}. The flow competition was completely reversible when P_{HA} was reduced.

The aim of this study was to evaluate whether this phenomenon of flow competition could be reproduced in an animal model. Hereby, two hypotheses concerning the induction of

flow competition between the HA and PV during liver HMP were tested. Since an elevated pressure in the sinusoids reduces the pressure difference between the PV and the sinusoids, and consequently reduces Q_{PV}, we hypothesized that Q_{PV} is hampered as a consequence of an elevated sinusoidal pressure. To test this hypothesis, we evaluated whether an increased pressure at the sinusoidal level could evoke the previously observed flow competition during HMP. First, an increased intra-sinusoidal pressure was evoked by an increased P_{HA} (livers 1 – 5). Secondly, as a proof of concept that an increased sinusoidal pressure might indeed affect the flow behavior, a moderate or severe obstructed hepatic outflow at the level of the supra-hepatic vena cava inferior (VCI) was applied to evoke flow competition (liver 3 – 5). In addition, the reversibility of the flow competition was investigated. In contrast, the opposite flow competition (by increasing P_{PV}) was not examined in this study. Due to the particular compliance (high flow, low resistance) of the portal vasculature, a rise in P_{PV}, would not immediately result in a substantial increase of the Q_{PV}. Furthermore, a substantial increase of P_{PV} to overcome the P_{HA} would result in an extremely high Q_{PV} way out of the physiological range.

MATERIALS AND METHODS

Animal model and liver procurement:

This study was approved by the local animal care committee of the University Hospitals Leuven (Belgium). All experiments were carried out in accordance with the federal guidelines (10). Five porcine livers (average weight 600 g, from 30-35 kg pigs) were procured according to the standard methods of liver retrieval which are routinely applied in our lab (11). Pigs were fastened during 12 hours prior to surgery with unlimited access to water. Under general anaesthesia, livers were freed from their peritoneal attachments, the common bile duct was ligated and the PV, the infra-renal aorta and VCI were prepared for

cannulation. Livers were washed-out with 5 litres of 4°C Histidine-Tryptophane-Ketoglutarate solution retrogradely via the aorta into the HA, and antegradely via the PV. The supra-diaphragmatic aorta and supra-hepatic VCI were clamped. The insertion of a large cannula in the infra-renal VCI allowed the removal of all blood and Histidine-Tryptophane-Ketoglutarate solution after hepatic wash-out. Afterwards, the liver was removed and prepared for HMP.

Hypothermic machine perfusion:

Livers were prepared for HMP by cannulation of the PV and the HA with an atraumatic "straight-in" cannula. The HA was left in continuity with an aortic conduit. Subsequently, a large diameter outflow drain was positioned in the infra-hepatic VCI, whilst the supra-hepatic VCI was clamped.

All livers were then perfused using the default settings for clinical HMP of kidneys, as adapted for livers in our department (11). Livers were perfused with 2 liters of non-oxygenated 4-6°C KPS-1™. This perfusion solution is routinely used for renal HMP and is similar to the solution originally designed by Belzer (12). A specially designed prototype device for liver HMP, the Liver Workstation™ (Organ Recovery Systems, Zaventem, Belgium), allowed for a pressure-controlled, flow-unlimited continuous perfusion through the HA and a pressure-controlled, flow-limited continuous perfusion through the PV (Figure 1). Q_{PV} was limited to 0.5 ml/g/min (resulting in approximately 300 ml/min for an average liver weight of 600 g). This flow limitation feature was implemented in our setting because it was previously observed that P_{PV} control alone - even at low temperatures - gave rise to very high flow rates {e.g. >2 ml/g/min, (7)}. Such high flows could induce an important shear stress during HMP, damaging the sinusoidal endothelial cell lining. Similarly, in porcine HMP protocols, flows between 0.4-0.6 ml/g/min have been reported (13,14). During HMP, livers were placed in a dome-shaped cassette, and partially immersed in

KPS-1™ (Figure 1). Moreover, the device regularly (every 10 minutes) foresees – by its design – a short period (10 seconds) to wash-out the tubing. During these periods, the organ is not perfused and the pressure drops. After this so-called wash-out, the perfusion restarts and the pressure builds up again.

Pressure was measured at the inflow of the HA (P_{HA}) and PV (P_{PV}), immediately proximal to the vessels using a pressure sensor and transducer integrated in the HMP device (Honeywell, Ohio, USA). The accuracy of these pressure measurements was acquired before every experiment through a second measurement using an external pressure monitor (Siemens, SC 7000, Dräger Medical, USA) which was calibrated before use. Moreover, pressure measurements were first zeroed corresponding to the ambient air pressure. Pressure at the supra-hepatic VCI (P_{VCI}) was measured using the external pressure sensor, connected to the standard external monitor (Siemens, SC 7000, Dräger Medical, USA). Flow rates in the HA and PV were measured separately and expressed as ml/min. These measurements were obtained by multiplying the rotations per minute of the roller pump by the volume delivered per revolution. Previously, it was found that the volume delivered per revolution is constant as long as the pump tubing is not used more than 48 hours. The flow rate was measured by a tachometer on the pump motor using a digital pulse-counting method to record angular position and speed of the pump. The tachometers were integral parts of the applied roller pumps (Alitea, Sweden) on the HMP machine. The accuracy of the flow measurements was checked by calibration of the flow prior to every experiment. This was done by comparing the flows calculated based on the rotation speed of the pumps with the flows acquired by physically collecting the volume of perfusate pumped per unit of time prior to the measurements.

Data acquisition was done continuously throughout the experiment, with a sampling frequency of 1 Hz. Based on P_{HA}, P_{PV} and the flow rates of the rotor pumps, VR was

calculated as P/Q and expressed as mmHg*min/ml for the HA and PV. Post-processing of the acquired data was done using the software packages “Excel” (Microsoft, USA) and “Sigmaplot” (Systat Software Inc, USA). The data presented in the figures and tables of this paper, are the raw and unfiltered data.

Experimental design:

Following appropriate insertion of the cannulas in the vessels and positioning of the porcine livers (n=5) in the organ cassette, HMP was initiated applying the following conditions: P_{PV}≤7 mmHg and Q_{PV}≤300 ml/min for the PV inlet, and P_{HA}=25 mmHg with unlimited Q_{HA} for the HA inlet. After the initiation of HMP perfusion, a stable perfusion regime was awaited (approximately 1 hr) before starting the experimental measurements until the arterial vascular resistance no longer decreased over time.

Two experimental settings were designed to increase the sinusoidal pressure and to study whether flow competition could be evoked. After HMP initiation, a stable hepatic flow was established to illustrate the competition in each of the five porcine livers either (a) by increasing P_{HA} or (b) by obstructing the VCI outflow.

a) Incremental increase of HA inflow pressure/flow

The initial P_{HA} was set at 25 mmHg and incrementally increased (steps of 10 mmHg) once a stable Q was achieved following a given change in P_{HA}. The P_{HA} was increased incrementally until a significant decrease in Q_{PV} was observed, resulting in a flow competition (Table 1, Figure 2).

In case the maximal Q_{HA} (being 300 ml/min as determined by the design of the pumps) was reached during the incremental increase of P_{HA}, the P_{PV} was gradually decreased.

This was done to evoke flow competition when the limits of the perfusion device were encountered. Whenever flow competition was observed, the reversibility was consequently investigated by a step-wise decrease of the P_{HA} (Figure 2).

b) Increased outflow resistance at the level of the infra-hepatic VCI

An outflow obstruction was evoked by clamping the outflow cannula inserted into the VCI. This outflow obstruction was done in a controlled way whilst monitoring the pressure at the VCI. Two different situations were created (3 experiments for each situation). In a first set of experiments, the outflow cannula was clamped to obtain a "moderate" obstruction aiming to increase the pressure from a normal value of <1 mmHg to 7-9 mmHg. In a second experimental setting, a higher resistance at the outflow was created by a subtotal clamp on the outflow tube. This "severe" obstruction resulted in a rise of the pressure at the VCI of >9 mmHg whilst complete cessation of the hepatic flow was avoided.

RESULTS

a) Incremental increase of HA inflow pressure/flow

In 2/5 experiments (Table 1: livers 1 and 3), increasing the P_{HA} from 25 to 55 mmHg and from 25 to 70 mmHg resulted in an increase of the Q_{HA} (from 91 to 231 ml/min and from 78 to 281 ml/min, respectively), evoking a gradual flow competition resulting in a Q_{PV} cessation at the highest P_{HA}. In 2 other experiments (Table 1: livers 4 and 5; Figure 2), a Q_{HA} of approximately 300 ml/min was reached (P_{HA} of 49 and 51 mmHg, respectively) without a significant decrease in Q_{PV}. This is the maximal Q that could be generated by the applied pump. Subsequently, flow competition with Q_{PV} cessation could then be evoked by lowering the inflow P_{PV} to 1 mmHg. Of note, only a substantial decrease of P_{PV} resulted in a significant decrease of Q_{PV}, since a P_{PV} of 7 mmHg is never reached due to the flow limitation feature added.

Flow competition could be reversed by lowering P_{HA} (livers 1 and 3) or additionally increasing P_{PV} (livers 4 and 5).

During the experiment with liver 2, the evoked flow competition was not fully reversible (Table 1) since it occurred intermittently and continuously after a spontaneous restoration. As such, the Q_{PV} never stopped completely (indicated in Table 1 by a mean value >0 ml/min).

b) Increased outflow/back pressure at the level of the infra-hepatic VCI

Subsequent to the flow competition experiments, a stable P/Q condition - similar to the start conditions - was awaited and ensured (P_{PV}≤7 mmHg, Q_{PV}≤300 ml/min, P_{HA}=25 mmHg and Q_{HA} unlimited). This resulted in a P_{VCI}≤1 mmHg.

A *moderate outflow obstruction* first resulted in a gradual decrease of Q_{HA} until a constant flow was reached whilst Q_{PV} remained stable (Figure 3A and 3B, Table 2). After removal of the moderate outflow obstruction, Q_{HA} restored to the (pre-outflow obstruction) start values.

During *severe outflow obstruction*, a distinctive pattern, defined as a repetitive and alternating increase/decrease between Q_{HA} and Q_{PV} was observed (Table 2, Figure 3C and D). First, a decreasing Q_{PV} was observed, whilst Q_{HA} increased, then followed by a spontaneous restoration of Q_{PV} with a decreasing Q_{HA}. This flow competition pattern or interaction repeated itself and was eventually observed to be self-limiting.

DISCUSSION

Under normal physiological conditions, the hepatic blood flow is well-characterized. Approximately 1 ml of blood passes through 1 g of liver every minute (approximately 25-30% of the cardiac output) (15). About 25% of the total hepatic blood flow is delivered by the HA, accounting for half of the livers' oxygen supply. The remaining total hepatic blood flow and oxygen supply is provided by the PV. Nevertheless, some significant

physiological decreases in hepatic blood flow (related to sleep, exercise and expiration) or increases (related to feeding, inspiration and aging) are well-described in literature (15,16,17).

Under in vivo conditions, intrinsic auto-regulation mechanisms guarantee a constant flow through the vascular bed by adaptations of the VR, which is normally lower at the PV and higher at the HA (pressure gradient of ±5/20 mmHg between the PV/HA and the hepatic veins). This auto-regulation takes place primarily in the HA vascular tree. The peri-vascular smooth muscle cell tone of the HA is mediated by stretching and adenosine concentrations. Under normal conditions, adenosine is washed away by the portal flow. A decreased PV flow, resulting in higher levels of adenosine, induces a vasodilatation of the HA, which is followed by an immediate increase of arterial blood flow. This is defined as the so-called hepatic arterial buffer response (18). An example of arterial flow decline in case of portal hyperperfusion is observed in small or partial liver grafts displaying small for size syndrome. Indeed, in partial liver transplantation, liver grafts are subjected to a portal flow normally destined for a whole liver. Consequently, portal flow is increased while arterial flow decreases (19).

In contrast to the previously described in vivo buffer response (where portal flow directly influences arterial flow), the ex vivo response observed in our study showed that arterial pressure/flow directly determines the portal flow. To the author's knowledge this has never been observed.

Neural regulation is suggested to be of little importance for the hepatic blood flow contrary to the numerous effects of humoral regulation (e.g. NO, CO), and the effects of some drugs such as volatile anaesthetics, β-adrenergic stimulants, calcium antagonists (20).

On a morphological level, hepatic blood flow is mainly regulated at three different sites of resistance: at the terminal hepatic arterioles (21), at the arterioles of the splanchnic circulation (influencing the portal venous flow), and at the sinusoidal level, more specifically at the terminal hepatic venules (22).

Under unphysiological conditions such as isolated liver HMP, information on the function of these auto-regulation mechanisms and the interaction between the flow in the HA and PV vascular beds are scarce. During ex vivo liver HMP conditions, all hepatic nerves are transected and the livers are perfused with a hypothermic a-cellular solution at perfusion pressures lower than in physiological in vivo conditions. Previously, we characterized the vascular resistance under such conditions and observed a gradual but remarkable decrease in the arterial VR during the first 4-6 hours of HMP, while the portal VR remained constant (9,11).

The observation of flow competition, defined as a hampered or obstructed flow into the PV during HMP, was first occasionally observed during feasibility experiments, but could now be consistently evoked when a critical flow into the HA was reached. We hypothesized that this results from an increased Q_{HA}. Under the HMP settings as applied in our laboratory, the Liver Workstation™ for HMP is set to provide arterial perfusion at a constant pressure. Normally, during perfusion at hypothermia, HA relaxation can be expected, resulting in an increased Q_{HA}. An increased sinusoidal pressure can be a consequence of this vasorelaxation, despite the higher pressure losses along the HA tree due to the higher blood flow. Such an increased sinusoidal pressure could reduce the Q_{PV} by decreasing the pressure drop between the PV and the sinusoids, thereby reducing the driving force for PV perfusion. This seems to make sense, as P_{HA} is higher compared to the P_{PV}, and the hepatic natural flow regulation mechanisms, as described above, might be impaired or

deficient during the reduced metabolic state of hypothermia. In another study, the blood circulation through a human liver was mathematically simulated using an electrical model (23). The results of this model illustrated the appearance of flow competition when using an increased P_{HA} or obstructed VCI as boundary conditions. These results support our hypothesis that when changing the arterial flow, portal flow changes through an altered sinusoidal perfusion.

Similar observations of flow competition - albeit in a different isolated perfusion model using cirrhotic rat livers - were made by Zipprich et al. (24). However, most studies concerning liver perfusion did not report any occurrence of flow competition between the HA and PV. Table 3 shows an overview of several liver perfusion studies and the corresponding perfusion conditions (species, HA and PV perfusion settings, temperature, perfusion period, pressure or flow control). To the best of the authors' knowledge, and in contrast to our experience, none of these studies describes the exact flow and pressure profiles over time. Comparing the perfusion setups of Table 3 (26-46), it becomes clear that the hydrodynamics and settings are very different. For instance, no flow competition will be possible when using flow controlled perfusion, since using those settings the flow is prescribed and not allowed to compete between the HA and PV. In addition, other parameters (such as the species studied, the application of high/low pressure/flow, different routes of perfusion (HA and/or PV), relatively short versus longer periods of HMP studied, pulsatile or continuous perfusion…) might also influence the occurrence of this phenomenon. Each of these parameters might play a role in whether or not triggering the initiation of flow competition, and consequently might be the reason why other groups possibly did not observe a similar competition phenomenon.

To our knowledge, this ex vivo flow competition interaction was never reported before. We speculate that this phenomenon results from an altered function of the pre-sinusoidal sphincters at the level of the terminal hepatic arterioles. Such an alteration could be multi-factorial and induced by the denervation, the use of an a-cellular perfusion solution (essentially different from plasma, absence of humoral factors), the hypothermic conditions leading to a decreased metabolism and enzymatic activity, the lack of oxygen and essential nutrients. Denervation can probably be neglected as a possible cause, since hepatic denervation has been shown to have no major deleterious effects on hepatic blood flow after liver transplantation (20). A factor which is more likely to contribute is the negative influence of the cold temperature (and concomitant intracellular energy depletion) on the pre-sinusoidal sphincters. This may eliminate the normal pressure drop between these terminal hepatic arterioles and the sinusoids. Consequently, the arterial flow will reach the sinusoids at a higher pressure. Other possible explanations for our observations can be found in an increased flow through the so-called arteriosinus twigs or in an increased resistance of the venous inlet sphincters.

Comparison of the flow competition evoked by P_{HA} alterations and VCI obstructions reveals a clear difference. P_{HA} increases led to competition in one direction: Q_{HA} increased while Q_{PV} decreased (Figure 2). In contrast, VCI obstructions affected both Q_{HA} and Q_{PV} in an alternating and repetitive way (Figure 3).

When theoretically interpreting the hepatic circulation as a passive system, the observation of flow competition evoked by an increased P_{HA} would analogously lead to flow competition evoked by an increased P_{PV} leading to a decreased Q_{HA}. However, we did not manage to evoke flow competition by increasing P_{PV}. This can be explained by the fact that theoretically {as illustrated by the electrical model of the liver blood flow (23)}, flow

competition cannot be reached within the physiological ranges of P_{PV} and Q_{PV} by increasing the P_{PV}.

However, due to the absence of equipment to directly measure sinusoidal pressure and flow in an intact organ, our data do not allow analyzing the mechanisms of the observed flow competition more profoundly. As such, the roles of the above discussed potential factors contributing to flow competition, are of a speculative nature. We emphasize that the flow competition phenomenon was also observed during HMP of human livers in our laboratory, supporting the clinical relevance of the observations made in the porcine model. The authors acknowledge that the model in its current format has some flaws (such as the lack of direct measurement of the sinusoidal pressure), but the observations made in this study showed that the flow competition could be consistently reproduced, justifying the nature of our hypothesis.

These observations might have some important implications on the design and flow control for clinical ex vivo machine perfusion devices. Within this study, we were not able to deduce a clear pressure/flow threshold that consistently triggers the flow competition in all porcine livers. This can be explained by the fact that every porcine liver has its individual anatomical features, and consequently, its unique physiological behavior. However, given the possibility of flow competition during HMP, we propose general guidelines to achieve a fixed arterial inflow limitation as follows. To avoid the occurrence of flow competition during HMP, the pressure at the HA inflow should be as low as possible, albeit allowing sufficient perfusion of the arterial microvasculature. We would, however, not recommend omitting perfusion of the arterial vasculature since it supplies the capillary plexus around the bile ducts. Preservation of this bile duct capillary plexus is regarded essential to prevent

ischemic bile duct strictures after liver transplantation. Consequently, to exclude the occurrence of flow competition, we would recommend the use of pressure driven perfusion at both the HA and PV side in combination with a flow limitation not only at the PV side, but also at the HA side. This flow limitation feature could prevent the onset of flow competition by restricting the HA flow.

In conclusion, we observed flow competition between the HA and PV flow during liver HMP. This resulted in an impaired and/or inhibited PV flow. Flow competition could be evoked by increasing the HA pressure or severe clamping of the VCI, suggesting that this is related to alterations in pressure differences between PV and HA, the sinusoids, and the HV. This phenomenon should be taken into account when designing appropriate dual perfusion protocols for HMP control.

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ABBREVIATIONS

HA	Hepatic Artery
HMP	Hypothermic Machine Perfusion
P	Pressure
PV	Portal Vein
Q	Flow
VCI	Vena Cava Inferior
VR	Vascular Resistance

LEGENDS

Figure 1

Schematic overview of the liver vasculature and the experimental HMP setup.

Hypothermic Machine Perfusion (HMP) preservation of the livers is achieved through a separate perfusion of the arterial (red) and portal (purple) circulation. Two roller pumps provide the inflow of perfusion fluid into the HA and PV via the cannulas and tubing. The vascular trees of the HA and PV have a similar topology and branch inside the liver in several orders of generations until they finally reach the microvasculature as hepatic arterioles and portal venules (see right panel). At this level, perfusion fluid supplied by the hepatic arterioles and portal venules enters the periphery of hepatic functional units (the lobules) and mixes in the hepatic-specific capillaries (the sinusoids). Consequently, the mixture is drained radially until it reaches the central vein. Afterwards, the central venules cluster until the fluid reaches the hepatic veins at the outflow of the liver [24]. A large drain is placed in the infra-hepatic vena cava inferior which drains the outflow of the liver (blue). Once the fluid left the liver, it is recirculated.

Throughout the HMP experiments, pressures and flows are measured at the level of the HA and PV cannulas. In normal conditions, the HA is typically characterized by a high pressure and low flow, while the PV is characterized by a low pressure and high flow.

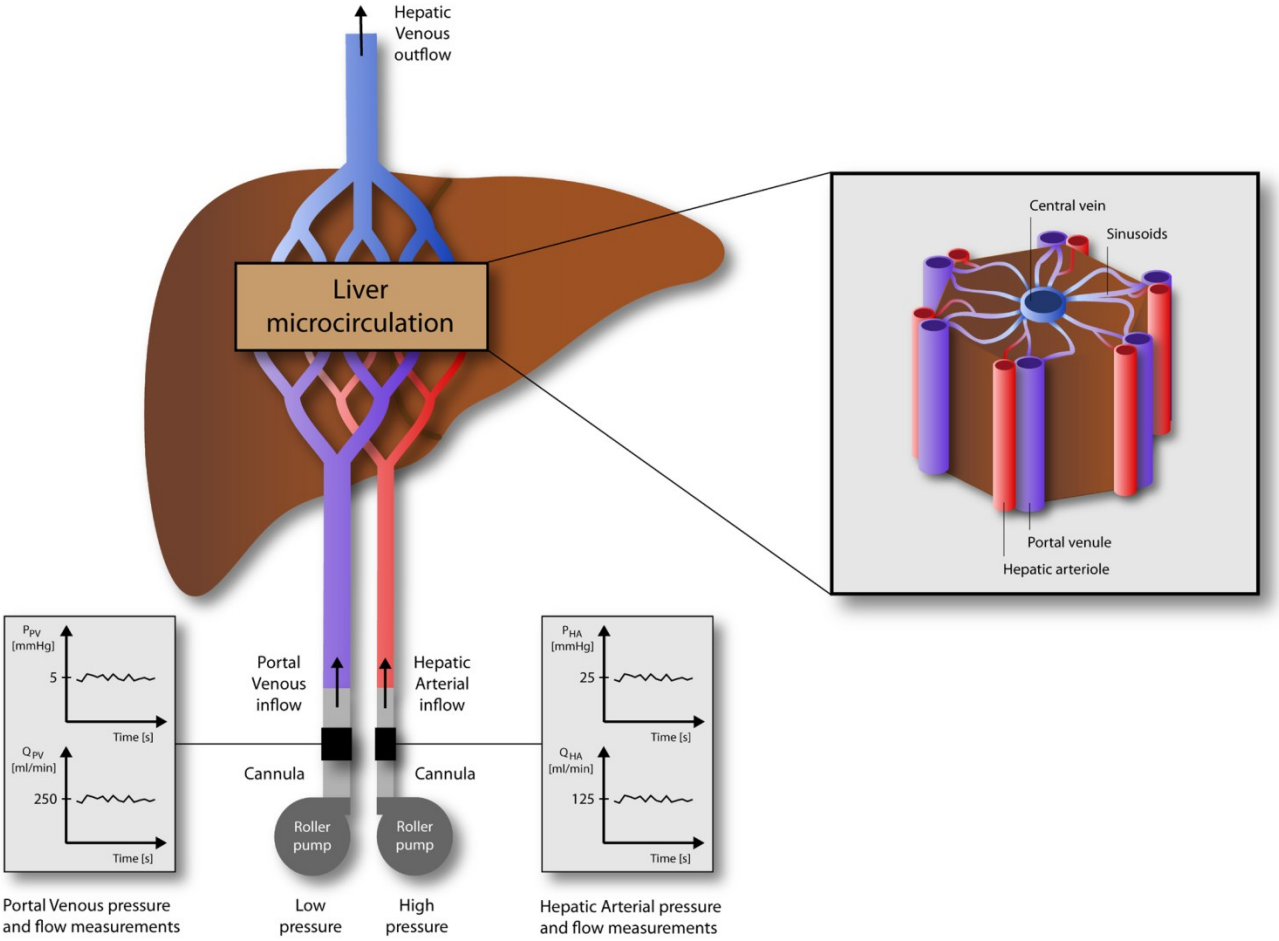
Hepatic
Venous
outflow
Liver
microcirculation
Central vein
Sinusoids
Portal venule
Hepatic arteriole
P_{PV}
[mmHg]
5
Time [s]
Q_{PV}
[ml/min]
250
Time [s]
Portal
Venous
inflow
Hepatic
Arterial
inflow
Cannula
Cannula
Roller
pump
Roller
pump
P_{HA}
[mmHg]
25
Time [s]
Q_{HA}
[ml/min]
125
Time [s]
Portal Venous pressure
and flow measurements
Low
pressure
High
pressure
Hepatic Arterial pressure
and flow measurements

Figure 2

Time-dependent measurements of flow competition as evoked by using liver 5. Panel A depicts P_{HA} and panel B depicts P_{PV}, while panel C depicts Q_{HA} and Q_{PV}. P_{HA} was incrementally increased every 10 minutes starting from 25 mmHg. This resulted in an incremental Q_{HA} increase. Meanwhile, a Q_{PV} flow limit (±330 ml/min) was set by the operators while the maximal P_{PV} was set at 7 mmHg. A P_{HA} of 50 mmHg (arrow at t=37 min) evoked a series of decreasing but initially self-restoring Q_{PV}. These flow oscillations occur as the precursors of a flow instability. Eventually, Q_{PV} was hampered indicating the initiation of the flow competition (arrow at t=75 min), resulting in a complete cessation of Q_{PV}. To reverse the flow competition P_{HA} was lowered to 45 mmHg (arrow at t=95 min). Portal flow was gradually restored by also increasing the P_{PV} (arrow at t=103 min). Further decreasing the P_{HA} (arrow at t=135 min), resulted in a decreased Q_{HA}, allowing the full restoration of Q_{PV}.

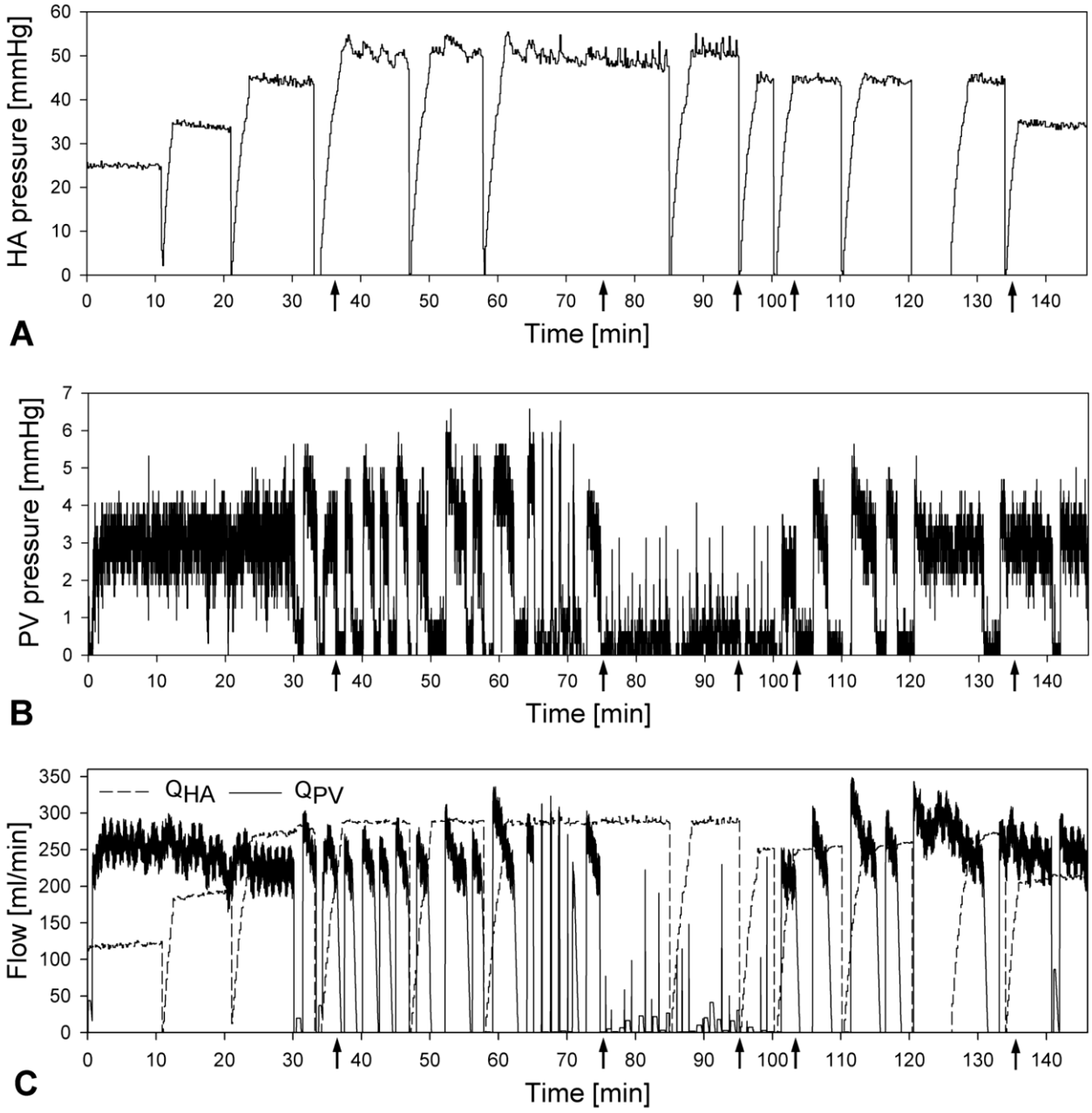
HA pressure [mmHg]
Time [min]
A
PV pressure [mmHg]
Time [min]
B
Flow [ml/min]
Q_{HA}
Q_{PV}
Time [min]
C

Figure 3

Time-dependent measurements of flow competition evoked by the incremental increase of sinusoidal pressure caused by a moderate or severe outflow obstruction at the VCI (liver 4). Panel A and B display the HA and PV pressure and flow measurements, respectively. When initiating a moderate obstruction (arrow at t=3 min), Q_{HA} decreased until the outflow obstruction was stopped (arrow at t=17 min). Meanwhile, Q_{PV} as well as P_{HA} and P_{PV} remained approximately constant. After the initiation of a severe obstruction (arrow at t=68 min), an alternating increase/decrease between Q_{HA} and Q_{PV} was evoked, until the outflow obstruction was removed (arrow at t=87 min). Panel C and D are close-ups (t=78-90 min) of a part of the severe obstruction. The results showed a repetitive pattern of alternating increases and decreases between Q_{HA} and Q_{PV}. After the initiation of severe outflow obstruction, a decrease in Q_{PV} was observed with a concomitant Q_{HA} increase. Subsequently, the Q_{PV} recovered spontaneously and Q_{HA} decreased. This pattern of mutual flow competition (illustrated by the arrows in panel D) was repeated until the severe obstruction was removed. The fluctuations were also detectable in the P_{HA} and P_{PV} measurements.

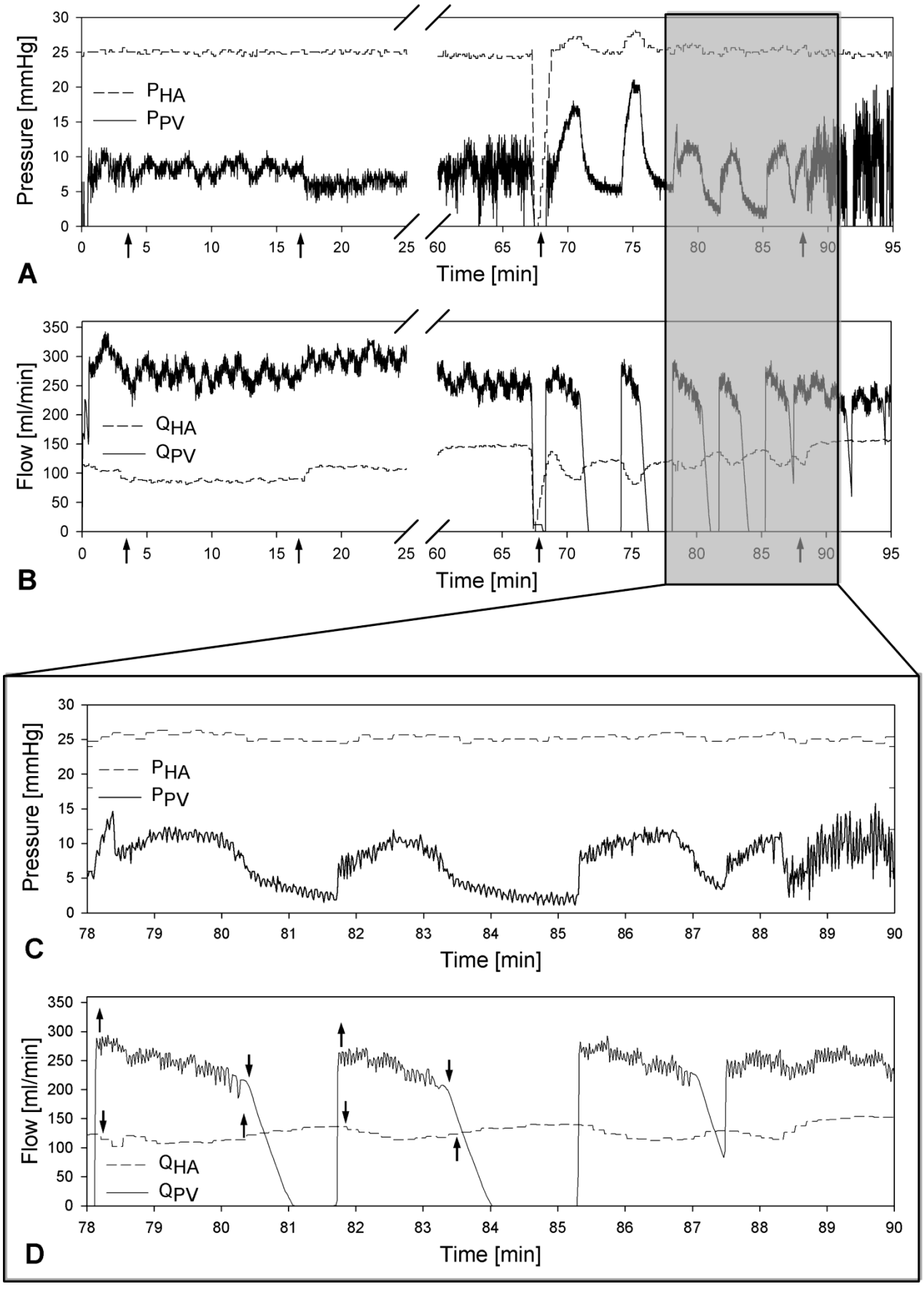
Pressure [mmHg]
P_{HA}
P_{PV}
A
Time [min]
Flow [ml/min]
Q_{HA}
Q_{PV}
B
Time [min]
Pressure [mmHg]
P_{HA}
P_{PV}
C
Time [min]
Flow [ml/min]
Q_{HA}
Q_{PV}
D
Time [min]

Table 1

Overview of the induction of flow competition evoked by increasing P_{HA} in each of the 5 porcine livers. Concomitant flows and vascular resistances at the HA and PV inlets were measured and calculated. P_{HA} was incrementally increased. When flow competition was evoked (indicated by *), P_{HA} was gradually decreased, which allowed reversal of the flow competition in all livers (except for liver 2). In livers 3, 4 and 5, the maximal Q_{HA} of ±300 ml/min (limitation of the HMP pump) was reached by increasing the P_{HA}. Subsequently, P_{PV} was incrementally decreased in those livers. Data are presented as mean values of P, Q and VR of the HA and PV.

	P_{HA} (mmHg)	**Q_{HA} (ml/min)**	**VR_{HA} (mmHg *min/ml)**	**P_{PV} (mmHg)**	**Q_{PV} (ml/min)**	**VR_{PV} (mmHg *min/ml)**
Liver 1	25	91	0.27	3	128	0.023
	35	143	0.24	3	166	0.018
	45	194	0.23	3	121	0.025
	55	231	0.24	3	14*	0.21
	45	197	0.23	3	10	0.3
	35	169	0.21	3	8	0.37
	25	149	0.17	4	91	0.044
Liver 2	25	37	0.68	5	174	0.029
	35	109	0.32	8	154	0.052
	45	152	0.29	8	184	0.043
	54	144	0.38	4	70*	0.057
Liver 3	25	78	0.32	3	228	0.013
	40	138	0.29	4	204	0.019
	59	232	0.25	3	97	0.031
	70	281	0.25	4	8*	0.5

	60	266	0.24	1	0	-
	50	211	0.24	4	135	0.029
	40	174	0.23	3	166	0.018
Liver 4	24	142	0.17	0.62	262	0.0038
	35	192	0.18	0.35	307	0
	45	249	0.18	0.43	295	0
	49	287	0.17	1.23	303	0.0033
	64	289	0.22	0.51	19*	0.053
	55	262	0.21	0.71	0	-
	45	244	0.18	0.93	0	-
	35	183	0.19	2.66	123	0.024
Liver 5	25	119	0.21	3	240	0.013
	35	188	0.19	3	242	0.012
	44	273	0.16	3	203	0.015
	51	288	0.18	2	168	0.012
	45	250	0.18	0	4*	0
	34	211	0.16	3	223	0.014

Table 2

Overview of the induction of flow competition evoked by hepatic outflow obstruction as observed during experiments with livers 3, 4 and 5. For moderate outflow obstruction, data are given at the moment just before, during and after the obstruction. In this case, Q_{HA} decreased whilst Q_{PV} remained stable despite the increased vascular resistance, indicated by a higher P_{PV}. After the obstruction, Q_{HA} returned to the initial values (see also Figure 3). In contrast, a repetitive and alternating increase/decrease between Q_{HA} and Q_{PV} was observed during the severe obstruction. First, a decreasing Q_{PV} was observed, whilst Q_{HA} increased, followed by a spontaneous restoration of Q_{PV} with a decreasing Q_{HA} (see Figure 3). Time stamp "1" corresponds with the moment where Q_{PV} starts decreasing while Q_{HA} is increasing, "2" corresponds with the moment when the reverse phenomenon is observed.

	Type obstruction	**Time stamp**	**P_{VCI} (mmHg)**	**P_{HA} (mmHg)**	**Q_{HA} (ml/min)**	**P_{PV} (mmHg)**	**Q_{PV} (ml/min)**
Liver 3	Moderate	before obstruction	-	25	67	3	297
		during obstruction	6	25	45	6	296
		after obstruction	-	25	60	4	293
	Severe	1	10	59	232	3	130
				59	230	4	135
				59	230	6	138
		2	10	58	238	3	0
				58	242	2	0
				58	245	2	0

Liver 4	Moderate	before obstruction	-	25	103	7	284
		during obstruction	5	25	86	7	266
		after obstruction	-	25	98	5	288
	Severe	1	13	27	89	14	240
				28	83	17	242
				26	113	7	217
				25	117	7	207
		2	13	25	120	5	0
				25	123	4	0
				25	136	2	0
				25	139	2	0
Liver 5	Moderate	before obstruction	-	25	119	4	296
		during obstruction	4	25	99	7	274
		after obstruction	-	24	125	4	303
	Severe	1	10	26	92	9	228
				25	94	11	246
				27	83	14	266
		2	10	25	120	3	0
				24	118	3	0
				24	143	1	0

Table 3

Overview of several liver perfusion studies (13,26-47) and the corresponding perfusion conditions (species, HA and PV perfusion settings, temperature, perfusion period, pressure or flow control) during experimental and clinical machine perfusion preservation of livers (C = Continuous, P = pulsatile).

Author and year of publication	Species	PV	HA	Temp (°C)	Perfusion period (hours)	Perfusion control
Slapak et al., 1969 (26)	Canine	0.23-0.59 ml/min/g (2:1 ratio PV flow over HA flow) 8.8-10.3 mmHg	76/23 mmHg (P)	11	6	Pressure (PV)
Brettschneider et al., 1968 (27)	Canine	4.8 ml/min/g	1.2 ml/min/g	4	8-12	Flow
Belzer et al., 1970 (28)	Porcine	5-8 mmHg	60/40 mmHg (P)	8-10	8-24	Pressure (HA) Flow (PV)
Gellert et al., 1985 (29)	Porcine	2 mmHg (C) 0.5 ml/min/g	80/40 mmHg (P) 0.125 ml/min/g	10-12	6	Flow
Pienaar et al., 1990 (30)	Canine	16-18 mmHg (P; 30 bpm)	-	5	72	Pressure
Boudjema et al., 1991 (31)	Rabbit	15-25 mmHg	-	5	24-48	Pressure
Yamamoto et al., 1991 (32)	Porcine	0.5-0.6 ml/min/g	-	7	72	Flow
Rossaro et al., 1992 (33)	Rat	0.5 ml/min/g	-	6-10	24	Flow
Kim et al., 1997 (34)	Rat	11 mmHg 0.5 ml/min/g	-	4	48	Flow
Kozaki et al., 1997 (35)	Porcine	7-8 mmHg	50-60 mmHg	8	2	Pressure
Iwamoto et al., 2000 (36)	Porcine	7-8 mmHg	50-60 mmHg 20-30 mmHg	8	2	Pressure
Southard et al., 2000 (37)	Rat	0.14 ml/min/g	-	4	24	Flow
Compagnon et al., 2001 (38)	Rat	0.4 ml/min/g	0.1 ml/min/g	4	24	Flow
Uchiyama et al., 2001 (39)	Porcine	-	30-60 mmHg (P or not)	8	2	Pressure
Dutkowski et al., 1998 (40)	Rat	4.48 ± 0.54 mmHg 0.44 ml/min/g	-	3-6	10	
Lauschke et al., 2003 (41)	Rat	0.5 ml/min/g	-	4	0.75	Flow
Guarrera et al., 2005 (14)	Human/	3-5 mmHg	12-18 mmHg	3-5	5-10	Flow
	Porcine	0.7 ml/min/g			12	Flow
Jain et al., 2005 (42)	Porcine	10-12 mmHg 0.3 mL/min/g	30 mmHg 0.1 ml/min/g	Hypothermic	24	Pressure
't Hart et al., 2007(43)	Rat	4/8 mmHg 350 ml/min	25/50 mmHg (P) 80 ml/min	1-3	24	Pressure
Vekemans et al., 2007 (8)	Porcine	3-5/7 mmHg 0.5-1 ml/min/g	20/25 mmHg	4-7	24	Pressure
De Rougemont et al., 2009 (13)	Porcine	Unknown	-	4-8	1	
Guarrera et al., 2010 (44)	Human	2.9 mmHg	5.5 mmHg	4-6	3-7	Flow
Butler et al., 2002 (45)	Porcine	7.22 mmHg 1.75 l/min	90.3 mmHg 240 ml/min	39 (Normothermic)	72	Pressure (PV) Flow (HA)
Schon et al., 2001 (46)	Porcine	11 mmHg 250 ml/min	100 mmHg 150 ml/min	37 (Normothermic)	4	Pressure