Monitoring of Splanchnic Regional Perfusion

An Experimental Study of New Application and Validation

BY

ITARU KOGA

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Abstract
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Systemic infection, major surgery, trauma and many other causes can lead to impaired organ
function. Compensated shock is not detected by global hemodynamic and oxygen
measurements, as they take no account for regional variations. Focus has therefore gradually
turned from looking at systemic changes to selective investigations of regional blood flow and
ischemia. This thesis presents a series of experiments evaluating new application and
validation of various monitoring techniques.

An experimental porcine model with anesthetized and invasively monitored animals was
used. The circulatory interventions included endotoxin infusion (septic shock), aortic
constriction and selective clamping of splanchnic arteries. The aim was to compare air with
saline tonometry, to validate the intraperitoneal use of tonometry and to reexamine the use of
endoluminal reflectance pulse oximetry. To investigate the relative contributions of regional
blood flow and detection of ischemia, measurements of hepatic venous oxygen saturation
(ShvO₂), lactate concentrations and PCO₂ gap were used.

Our findings support the use of air instead of saline as the preferred technique for
tonometric measurements. With the intraperitoneal application of tonometry we gain more
information on regional aspects of the splanchnic circulation, and it appears to be a reliable
monitoring option for early detection of ischemia in the small intestine. Measurements of
ShvO₂ will give an overall reflection of the intestinal circulation. The sigmoid colonic pulse
oximetry showed a non-linear response in relation to regional blood flow, and will therefore
not be able to detect gradual changes in oxygen saturation. Determination of the regional to
endtidal PCO₂ gap might prove valuable for monitoring of the intestinal circulation.

Because of sophisticated interactions between portal and hepatic arterial blood flow and
hepatic compensation for regional ischemia, a combination of monitoring techniques might be
needed. The results of this study will hopefully encourage clinical evaluation of
intraperitoneal tonometry and endtidal PCO₂ gap recordings for non-invasive,
semi-continuous, trend monitoring of the splanchnic circulation.

Keywords: Splanchnic circulation, Regional ischemia, Hepatic artery buffer response, Air
tonometry, Intraperitoneal, Reflectance pulse oximetry, Hepatic venous oxygen saturation,
Lactate, PCO₂ gap

Iitaru Koga, Department of Surgical Sciences, Akademiska sjukhuset, Uppsala University,
SE-75185 Uppsala, Sweden

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The Road

What waits ahead if I choose this road?  
Don't be afraid, or there will be no road.  
Take one step, for it will start a road,  
and it will become the road.  
Go on without hesitation,  
and you will find your destination.

Ikkyu Sojun, Zen master (1394-1481)
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


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ABBREVIATIONS

MOSF  Multiple organ system failure
pHi  Intramucosal pH
PrCO₂  Regional PCO₂
ShvO₂  Hepatic venous oxygen saturation
ScO₂  Colonic intraluminal oxygen saturation
PaCO₂  Arterial PCO₂
V/Q  Ventilation/Blood flow
PCWP  Pulmonary capillary wedge pressure
FiO₂  Inspired oxygen fraction
SvO₂  Mixed venous oxygen saturation
VO₂  Oxygen consumption
DO₂  Oxygen delivery
CVP  Central venous pressure
CA  Celiac (truncus) artery
SMA  Superior mesenteric artery
IMA  Inferior mesenteric artery
HABR  Hepatic artery buffer response
ssPrCO₂  Steady state adjusted regional PCO₂
aPrCO₂  Air tonometric regional PCO₂
r-aPCO₂ gap  Difference between arterial and regional PCO₂, PrCO₂ -
r-etPCO₂ gap  Difference between endtidal and regional PCO₂, PrCO₂ -
LPS  Lipopolysaccharide
INTRODUCTION

Shock is a condition in which the oxygen needs of the tissues are not met by the delivery of oxygen or its utilization. In the 1970’s, the clinical application of the pulmonary artery catheter resulted in a better understanding of the adequacy of systemic oxygenation. Measurements of mixed venous oxygen saturation, oxygen content and cardiac output gained popularity in intensive care. Derived data of systemic oxygen transport and oxygen uptake were increasingly used by many clinicians in the management of critically ill patients. However, compensated shock is not detected by global hemodynamic and oxygen measurements. Systemic measurements of the adequacy of tissue oxygenation take no account for regional variations in oxygen delivery and uptake. There may be significant regional ischemia present despite indications of traditional measurements that the patient is fully resuscitated. Regional ischemia may affect outcome by causing multiple organ system failure (MOSF). Thus, it is very important to evaluate quick, sensitive and valid monitoring methods of regional ischemia in the clinic. For this we need good understanding of the physiologic regulation of splanchnic circulation and its underlying pathophysiology. We have therefore set up a series of experiments to test the effects of different interventions on regional perfusion and the sensitivity of ischemia detection. We tested new application of already existing techniques and reexamined the validation of other monitoring available.
BACKGROUND

Systemic infection, major surgery, trauma and many other causes can lead to impaired organ function. MOSF is characterized by the failure of two or more vital organ systems, and this syndrome still has a high mortality rate. For decades the splanchnic organs have been identified as a possible origin of circulating endotoxins, cytokines and mediators contributing to the development of MOSF. Much research has been targeting the small intestine and its susceptibility to become ischemic in different kinds of shock or maldistribution of blood flow. Focus has gradually turned from looking at systemic changes to selective investigations of regional blood flow and ischemia.

Presently, mainly gastric and sigmoid colonic tonometric measurements of intramucosal pH (pHi) are available for clinical monitoring of ischemia in the gastrointestinal tract. Several research groups have suggested that pHi measurements can be used to predict outcome and to guide therapy. PCO₂ is measured and pHi calculated using the Henderson-Hasselbalch equation with the approximation that the arterial HCO₃⁻ equals the intramucosal HCO₃⁻. There are several potential sources of error like local production of CO₂, difference in medium inside the balloon compared to the natural surrounding (gastric juice), difference in gas solubility, dilution by “washing out” and measurement errors in the blood gas machine. Saline tonometry is a tedious technique with a substantial time lag and it is not available for the small intestine. It has been repeatedly questioned if gastric pHi actually reflects overall intestinal perfusion. The search is therefore on for a technique that provides continuous data and that allows monitoring of regional splanchnic perfusion.

Tonometry (Paper I and II)

In our study tonometry refers to the measurement of partial pressure of carbon dioxide in the gut. The principle behind this technique is relatively simple. A silastic balloon filled with saline is inserted into the gastrointestinal tract and sufficient time is allowed for the CO₂ in the intestinal lumen to equilibrate with the CO₂ in the balloon. Intermittently,
saline is then withdrawn and the partial pressure of carbon dioxide measured in a blood gas machine. Some years ago, an automatic tonometry device was developed (TONOCAP™, Tonometrics Inc., Finland) in which air is used in the balloon instead of saline. The technical errors connected with sampling and transfer of the saline to a blood gas machine are thereby eliminated. Studies have shown good technical reliability and measurements correlated well with those of saline tonometry. However, clinical validation was still insufficient at the time of our study. This was the reason why we decided to look into this (Paper I). Simultaneously we chose to target another problem, namely the fact that tonometry was not available for monitoring of all splanchnic region. A great number of publications existed on gastric and sigmoid colonic tonometric measurements, but for practical reason the small intestine is not easily accessible for this kind of monitoring. We therefore investigated the possibility of applying tonometry catheters intraperitoneally to monitor intestinal perfusion (Paper I). Further studies were required to determine whether this application merely gave information on overall aspects of the splanchnic circulation or if it could be used to monitor regional ischemia (Paper II).

Reflectance pulse oximetry (Paper III)
Pulse oximetry is a widely used monitoring technique in anesthesia and perioperative care. It is non-invasive, easy to set up, continuous, and very reliable in the presence of a plethysmographic signal. As referenced in Paper III, the method had been applied to monitor viability of the stomach and various parts of the bowel during and after surgery. Measurements were reported both from the mucosal and the serosal side of the bowel. Most pulse oximeters are detecting transmitted light, their use being limited to external applications by the use of clips for fingers, toes or ears. The other option is pulse oximetry based on detection of reflected light. A commercially available application of this technique is the transcutaneous monitoring of brain surface oxygen saturation with both emitting and detecting device taped to the forehead. Another application has been the transanal positioning on the surface of the sigmoid colon mucosa. Two encouraging studies have been published (Vahl, Gardner) where a porcine shock model was used. We decided to reexamine the validation of this application.
Hepatic vein measurements (Paper IV)

Hepatic venous oxygen saturation (ShvO2) can be intermittently monitored by withdrawing blood from a catheter for analysis by the oximeter of a blood gas machine. In analogy with mixed venous oxygen saturation monitoring via pulmonary artery catheters this continuous technique is also applicable for the hepatic vein. The use of these options has been reported for detection of splanchnic ischemia and early graft malfunction after liver transplantation. However, we were not aware of any previous report experimentally investigating the validity of ShvO2 monitoring. Another means of indirectly monitoring splanchnic metabolism is by measuring lactate, a product of glycolysis in hypoxia, indicating mobilization of the anaerobic energy pathway. The interpretation of lactate concentration data is very complex with many factors involved. Conflicting data exist to where lactate is produced in septic patients and how the uptake and metabolism in the liver is regulated. It was therefore our intention to investigate if these measurements can predict or quantify changes in splanchnic circulation caused by endotoxin shock or selective obstruction of arterial flow.
AIMS OF THE STUDY

1. To assess the experimental accuracy of an air tonometric device \textit{in vivo} within a wide range of PrCO$_2$ values by using saline tonometry as the standard (Paper I).

2. To investigate the possibility of monitoring intestinal perfusion by applying tonometry in the intraperitoneal cavity (Paper I).

3. To experimentally validate the intraperitoneal use of tonometry for detection of regional intestinal ischemia (Paper II).

4. To determine if colonic endoluminal oxygen saturation, as determined by the reflectance pulse oximetric method, is an appropriate monitoring method of colonic ischemia produced by a gradual constriction of aorta (Paper III).

5. To experimentally validate hepatic venous oxygen saturation (ShvO$_2$) as a monitoring method for splanchnic ischemia (Paper IV).

6. To investigate the relative contributions of regional blood flow and oxygen consumption, where supplied by the celiac artery (CA) and the superior mesenteric artery (SMA), respectively (Paper II and Paper IV).
METHODS

Ethics
The study was approved by the Institutional Review Board for the Ethics of Research in Animals at the Uppsala University, the care and handling of animals being in accordance with legislation by the Swedish Board of Agriculture. Where possible, and when not affecting study results, we combined measurements for different studies to reduce the number of animals needed.

Animals
After one night without food but with full access to water 33 Swedish piglets of native breed were used. Thirteen were female and twenty were males. They were 11 –15 weeks of age at the time of the experiments. The mean weight was 23.2 kg with a range from 18 to 30 kg.

Anesthesia and peroperative care
All pigs were premedicated with the combined administration of tiletamin and zolazepam (each 3 mg/kg) (Zoletil 100®, Reading, France) and xylazine (2.2 mg/kg) (Rompun®, Bayer, Germany) intramuscularly. To avoid excess salivation intramuscular atropine sulfate (0.04 mg/kg) was also administered. Three to five minutes later the animals were placed in dorsal recumbent position and after introduction of an intravenous line 20 mg of morphine (Morfin, Pharmacia, Sweden) was given. Then followed a tracheotomy and connection to a ventilator (Servo 900C, Siemens-Elema, Sweden). A volume controlled ventilation mode was used and the minute volume was adjusted to keep the PaCO₂ in normal range. The respiratory rate was set to 25/min. The FiO₂ was 0.3 and a positive end-expiratory pressure (PEEP) of 4 cm H₂O was applied. After the stabilization period, the setting of the ventilation was
not changed. Anesthesia was maintained throughout the experiments with intravenous infusion of an anesthetic mixture (4 ml/kg/h), consisting of 5 mg/ml ketamine (Ketalar®, Parke-Davis, Spain), 0.12 mg/ml morphine and 0.06 mg/ml pancuronium bromide (Pavulon®, Organon, The Netherlands) in Rehydrex® (Pharmacia and Upjohn, Stockholm, Sweden). In addition, a continuous infusion of saline (20 ml/kg/h) was given. During the stabilization period dextran 70 (Macrodex®, Medisan, Sweden) was administrated to keep the PCWP 8 - 12 mm Hg. Where wedging was difficult fluid replacement was guided by the diastolic PA-pressure. To maintain body temperature, the animals received pre-warmed infusions. We also used a thermostatically controlled heating pad and a humidifier with a heated circuit. No catecholamines or alkaline buffers were administered. At conclusion of our experiments, the piglets were euthanized with intravenous injections of potassium chloride.

Catheterization and invasive monitoring

Bilateral neck incisions were performed. Polyethylene catheters were positioned via the carotid artery in the thoracic aorta for continuous blood pressure monitoring and arterial blood sampling. A Swan-Ganz catheter (7 Fr., OHMEDA, USA) was inserted via the right external jugular vein and positioned in the pulmonary artery, its positioning being guided by the typical pressure trace pattern. An additional separate double-lumen central venous catheter was positioned via the external jugular vein for bolus injection of cold saline when measuring cardiac output by the thermodilution method and for monitoring of CVP. Another catheter was introduced through the jugular vein and under fluoroscopic control positioned in the hepatic vein. Blood pressures (aorta, pulmonary artery, central vein) and cardiac output were continuously monitored and registered (1281, Siemens Medical Electronics Inc., USA). Urine was deviated through a transvesically placed catheter.

Surgical preparation

The celiac artery (CA), the superior mesenteric artery (SMA) and the inferior mesenteric artery (IMA) were identified using the extraperitoneal approach through a left paramedian incision. Special care was taken not to injure the peritoneum. Transit-time ultrasonic flow probes were positioned around the proximal end of the celiac artery (4-6 mm probe), the SMA (6-8 mm probe) and the abdominal aorta upstream of the celiac artery (12 mm
probe). The probes were connected to a blood flowmeter (T208, Transonic system, USA). A cotton ribbon was placed around the aorta, about 4 cm proximal to the aortic flowmetry probe, for partial occlusion of the vessel (Paper I, III and IV).

Saline tonometry catheters (TRIP® NGS catheter, Tonometrics, Inc., USA) were positioned intraperitoneally in the space between the hepatic hilus and the lesser curvature of the stomach (epigastric region, CA blood supply) (Paper II), on the serosal surface of the small intestine (intestinal region; SMA blood supply) (Paper I and II) and close to the sigmoid colon (colonic region; IMA blood supply) (Paper II). The incision in the peritoneum was closed with tight sutures. After a cleansing irrigation, a sigmoid tonometry catheter (TRIP® SIGMOID catheter, Tonometrics, Inc., USA) was inserted approximately 20 cm into the sigmoid colon (Paper I, II and III).

Interventions

**Hypoperfusion shock (n = 10)**
1. 60 min of stabilization after preparation.
2. The aorta, proximal to the celiac artery, was partially occluded in order to reduce the distal aortic blood flow to one third of baseline values for a 60-min period.
3. Occlusion was subsequently increased to reduce the aortic blood flow to two thirds of the baseline flow for another 60 min.
4. Complete release of the constriction, and reperfusion for 60 min.

**Endotoxin shock (n = 11)**
1. 60 min of stabilization after preparation.
2. Administration of LPS 10 µg/kg/h via the central venous line for 3 hours.

**Selective clamping (n = 6)**
1. 60 min of stabilization after preparation.
2. Complete occlusion of one of the splanchnic arteries for 20 min followed by complete release of occlusion for 40 min (Table 2).
3. Complete occlusion of one of the other two splanchnic arteries for 20 min followed by complete release of occlusion for 40 min.
4. Complete occlusion of the third splanchnic artery for 20 min followed by complete release of occlusion for 40 min.
Control group (n = 6)
1. 60 min of stabilization after preparation.
2. The same measurements as in the experimental groups but without any intervention.

Measurements

Tonometry (Paper I-III)
In the first paper we used for air tonometry (aPrCO₂) the automatic device (TONOCAP™, Tonometrics. Inc., Finland) with readings every 15 min intraperitoneally and every 20 min in the sigmoid colon. In our experiments, we had to switch the air connection of the TONOCAP™ device from the catheter placed in the intraperitoneal region to the sigmoid colon, and vice versa, since we had access to only one device. Saline tonometric measurements were carried out every 30 min. The balloon of the tonometry catheter was filled with 2.5 ml of saline. After equilibration, 1.0 ml of saline was slowly aspirated and discarded, and the remaining 1.5 ml was aspirated and analyzed in the blood gas analyzer (ABL 300, Radiometer, Copenhagen, Denmark). The amount of saline aspirated was immediately replaced. Steady state saline carbon dioxide pressure (ssPrCO₂) was calculated with a correction factor according to the manufacturer’s instructions. In the second paper saline tonometric measurements were made every 20 min and pH calculated by use of the Henderson-Hasselbalch equation. All measurements were done at baseline (just before the clamp), 20 min after clamping and immediately before declamping as well as 20 and 40 min after declamping in all three intraperitoneal sites and in the sigmoid colon. During the experiments for the third paper, saline tonometric measurements were performed in the sigmoid colon every 30 minutes. We calculated the PCO₂ gap, i.e. difference between arterial and regional PCO₂.

Sigmoid colonic reflectance pulse oximetry (Paper III)
A Nellcore RS-10 reflectance pulse oximeter probe, designed to monitor transcutaneous oxygen saturation, was attached to a 10 ml balloon of a 15 French Foley catheter with an adhesive. The probe and the Foley catheter balloons were rendered waterproof with latex. The device was advanced via the rectal route about 20 cm into the sigmoid colon with the probe facing the posterior wall of the gut. The pulse oximeter device, Nellcore N-185 (Nellcore Inc. CA USA), was used to record the oxygen saturation readings.
When no plethysmographic signal was detected the device gave the reading “0”. Measurements were recorded every 5 min.

Hepatic venous oxygen saturation and blood lactate concentration (Paper IV)

Arterial, hepatic venous and pulmonary venous blood was sampled every 15 min in the hypoperfusion group and endotoxin group, every 20 min in the selective clamping group. Blood gases were analyzed, oxygen saturation measured in the blood-gas analyzer (ABL 300, Radiometer, Copenhagen, Denmark). For assay of lactate, these blood samples were immediately precipitated with ice-cold perchloric acid (3M). After centrifugation, the protein-free extracts were deep-frozen (-70 °C) pending analysis by microfluorometry. In the control group, data from two pigs were excluded because lactate concentrations were missing.

Data presentation and statistical analysis

Results are presented as the mean ± standard error of the mean (SEM). A statistical probability of < 0.05 was considered significant. Because of the limited number of animals we assumed the distribution of data to be non-parametric in the experimental groups. For most measurements, the possible differences at matched time points between the experimental group and the control group were determined using the Mann-Whitney U test. All measurements of the control group were examined by analysis of variance for repeated measurements. For comparisons between air and saline tonometry we used Pearson product-moment correlation (r), the p-value being calculated with Fisher’s r-z transformation. The limits of agreement were estimated using the 95% confidence interval for the difference of means (dPrCO₂ = aPrCO₂ - ssPrCO₂.)
RESULTS

All animals were stable at the end of the stabilization period. The estimated average blood loss during surgical preparation was 40 ml. Mean hemoglobin reading was 83 grams per liter. Hourly urine output was 2 ml/kg during stabilization and the mean body temperature was kept at 37.7 °C. Other compiled data from baseline readings include pH 7.4, PaCO₂ 5.7 kPa, endtidal CO₂ 5.2, PaO₂ 19.3 kPa and base excess 2.5. In the hypoperfusion group we noted an expected increase in MAP as a result of aortic constriction and a slight decrease after its release. MPAP did not change significantly. In the endotoxin group the main finding was a significant increase in MPAP during the initial phase of LPS infusion. The measured variables of the systemic circulation (CO, MAP and SvO₂) showed a slower and more moderate decrease (Figure 1).

Figure 1 Hemodynamic changes in endotoxin group.
Relative changes (%) as compared to baseline. Abbreviations: HR = heart rate, MAP = mean arterial pressure, MPAP = mean pulmonary arterial pressure, CO = cardiac output, SvO₂ = mixed venous oxygen saturation.
In the selective clamping group all hemodynamic changes, with one exception, were modest. Upon clamping of the superior mesenteric artery, the MAP increase was significant and in accordance with the relative size of the portion of the vascular bed that was occluded (Figure 2).

Figure 2 Hemodynamic changes in selective clamping group.
Relative changes (%) as compared to baseline. Abbreviations: MAP-CA = mean arterial pressure during celiac artery (CA) clamping, MAP-SMA = mean arterial pressure during superior mesenteric artery (SMA) clamping, MAP-IMA = mean arterial pressure during inferior mesenteric artery (IMA) clamping, MPAP-CA = mean pulmonary arterial pressure during CA clamping, MPAP-SMA = mean pulmonary arterial pressure during SMA clamping, MPAP-IMA = mean pulmonary arterial pressure during IMA clamping.
Figure 3 Blood gas changes in selective clamping group. Relative changes (%) as compared to baseline. Abbreviations: PaCO$_2$-CA = Arterial PCO$_2$ during celiac artery (CA) clamping, PaCO$_2$-SMA = Arterial PCO$_2$ during superior mesenteric artery (SMA) clamping, PaCO$_2$-IMA = Arterial PCO$_2$ during inferior mesenteric artery clamping (IMA), PaO$_2$-CA = Arterial PO$_2$ during CA clamping, PaO$_2$-SMA = Arterial PO$_2$ during SMA clamping, PaO$_2$-IMA = Arterial PO$_2$ during IMA clamping.

For the different selective clamping sites only SMA revealed significant changes in blood gas parameters. During SMA clamping PCO$_2$ showed a sudden decrease with slow normalization during reperfusion. Concomitantly PO$_2$ exposed a reverse pattern with a mild increase during clamping and a moderate decrease upon reperfusion. This pattern was expected and probably reflects changes in the metabolism of the small intestine (Figure 3).

Paper I

Tonometry

In hypoperfusion shock there was a stepwise reduction in blood flow, and in accordance to that a distinct pattern in response regarding hemodynamic and blood gas data. Interestingly, during reperfusion we noted a selective increase in CA flow whereas aortic and SMA flow barely reached baseline levels again (Figure 4a). We were also noted a two-phase hemodynamic
response in the endotoxin shock group, and again we saw a selective increase in CA flow after the initial hypodynamic phase (Figure 4b).

*Figure 4a. Blood flow distribution in the hypoperfusion group. Transonic flow measurements for aorta (Ao), celiac artery (CA) and superior mesenteric artery (SMA) in the hypoperfusion group. Relative changes (%) as compared to baseline.*
Figure 4b. Blood flow distribution in the endotoxin group. Transonic flow measurements for aorta (Ao), celiac artery (CA) and superior mesenteric artery (SMA) in the endotoxin group. Relative changes (%) as compared to baseline.

The tonometry data collected (from both intraperitoneal and intraluminal locations) showed a strong positive linear relationship between aPrCO₂ and ssPrCO₂ ($r = 0.959$, $p < 0.0001$, $r^2 = 0.919$). The results also demonstrated that aPrCO₂ tended to overestimate ssPrCO₂ on average by 0.384 kPa throughout the range of measurements. The 95% confidence interval of the difference of means (dPrCO₂) was between 0.31 kPa and 0.46 kPa ($t$-value $= 1.972$, df $= 218$) and thus, the two methods of measuring regional CO₂ were not significantly different. Nor did we see any difference between the hypoperfusion and the endotoxin shock group regarding dPrCO₂.
Paper II

Tonometry
In the epigastric region, 20 min of CA clamping significantly increased PrCO₂, but the increase was confined to this region. The same period of SMA clamping led to a substantial PrCO₂ and PCO₂ gap (= PrCO₂ - PaCO₂) increase in the intestinal region and a notable PrCO₂ decrease in the sigmoid colon region. The latter was attenuated when the calculated PCO₂ gap was displayed (Figure 5). There was no correlation between PrCO₂ values inside sigmoid colon and the sigmoid colon region (r² = 0.036, P = 0.337). In the epigastric region, 20 min of clamping increased the PCO₂ gap significantly. However, these changes in the gap were much less pronounced than in the intestinal region. Clamping of IMA did not elicit any significant changes.

Paper III

Sigmoid colonic reflectance pulse oximetry
Colonic mucosal oxygen saturation (ScO₂) measurements showed values in the range of 90 - 100 % throughout the stabilization period in all pigs. During one-third flow reduction the plethysmographic signal disappeared suddenly in seven out of the eight pigs as soon as the partial aortic constriction was applied. With the exception of the one pig, in which the plethysmographic signal did not disappear, the oxygen saturation readings showed zero. During two-thirds flow reduction plethysmographic signals were not observed in any of the pigs. In all pigs, saturation readings regained its 90 - 100 % range immediately after complete release of the constriction. Simultaneous tonometric measurements and calculations of the PrCO₂ gap (each pHi and PCO₂ gap measurement reflecting the average value of a 30 min sampling period), showed the assumed ischemic changes when two-thirds flow reduction was applied. A rather slow recovery of the PCO₂ gap was observed during the reperfusion period.
Paper IV

Hepatic venous oxygen saturation ShvO₂

Two levels of splanchnic hypoperfusion were used in the hypoperfusion group. ShvO₂ values showed a distinct stepwise decrease in accordance with the flow reduction. Interestingly the mixed venous oxygen saturation did not change significantly. In the reperfusion period, ShvO₂ values returned to baseline, however, CA flow increased about 150 % of the baseline. We noted two phases of shock during endotoxin (LPS) infusion. Thirty minutes after the initiation of the LPS infusion, blood flow decreased by nearly 50 % of baseline. Upon reperfusion, the CA flow increased approximately 150 % of the baseline as it did in the hypoperfusion group (Figure 4a and 4b). ShvO₂ showed a marked drop during the first 30 minutes of LPS infusion. The readings returned to baseline within the next 30 minutes but during the following two hours ShvO₂ progressively decreased again (Figure 6). Only CA clamping significantly affected the ShvO₂ in the selective clamping group. SMA clamping increased ShvO₂ and pulmonary venous oxygen saturation (SvO₂), but not sufficient to reach significance.
Figure 5 PrCO$_2$ gap in the selective clamping group. The three panels illustrate the positioning of intraperitoneal tonometry in the abdomen and the response to different clamping as compared to the control group. Top = epigastric region, Middle = SMA region (small intestine), Bottom = IMA region (sigmoid colon). *: $p < 0.05$ and **: $p < 0.01$ depict significant difference from the matched time point in the control group.
Figure 6 Hepatic venous oxygen saturation in the endotoxin group. The graphs illustrate changes in hepatic venous oxygen saturation (ShvO2) during LPS infusion. Significant difference from the matched time point in the control group is depicted by *: p < 0.05 or **: p < 0.01.

Lactate

In the hypoperfusion group, arterial and hepatic venous lactate showed simultaneous changes at any given measuring point with significant increases during 2/3 flow reduction. There was no significant divergence between arterial and hepatic venous lactate concentrations at any measuring point in the endotoxin group or the selective clamping group. After SMA clamping however, the arterial and hepatic venous lactate concentrations never returned to baseline, thus invalidating this part of the study.
GENERAL DISCUSSION

Experimental investigations by Rozenfeld and the excellent review by Tønnessen provide important information about tissue and venous PCO₂ measurements in relation to the anaerobic threshold and the consequences of changes in capillary flow. The importance of increased CO₂ production as opposed to accumulation in stagnant flow is also addressed in an experimental study by Rémi. I will discuss the results of our series of experiments and try to put our findings into context of what is currently considered evidence based.

Tonometry

In the first paper, CO₂ tonometric data obtained from catheters positioned in the peritoneal cavity disclosed lower readings than those obtained from the mucosal side of the sigmoid colon. However, the changes were in parallel. The reason for the higher PrCO₂ values in the colon may be the metabolism by the bacterial flora or a pressure gradient for CO₂ within the layers of the bowel wall. On the other hand data obtained from the intraperitoneal catheters showed, that during reduced intestinal blood flow this position gave a faster response to changes than the sigmoid catheter system. The intraperitoneal application encouraged us to undertake further studies. Another aim of the first paper was to compare the air and saline methods of obtaining PrCO₂ values from the intestine within a range of 5-15 kPa. Other investigations have shown that values from the saline catheter may be lower than for air tonometry, especially so with high PrCO₂ values. This difference was confirmed in our study, the difference being constant throughout the measurement range and not related to the level of PrCO₂ (range 5.1-14.7 kPa). The main reason why conventional saline tonometry underestimates PrCO₂ compared to air tonometry is the fact that saline has little buffering function (Knichwitz 1995, Rozenfeld).

In Paper II, we measured regional PCO₂ intraperitoneally at three different locations. Because the splanchnic organs are in close contact with each other within the peritoneal cavity, we assumed that the measurements would show the same tendency regardless of the location of the tonometry
catheters and the location of the arterial clamping as CO₂ is a highly diffusible gas. In contrast to our hypothesis, the measurements showed considerable regional differences. Knichwitz (1998) investigated the effects of controlled SMA flow reduction by using an intraluminal application of a multi-parameter sensor. In their pig experiments, intramucosal PCO₂ proved to be superior to tonometry in detecting intestinal malperfusion. Knichwitz (2000) later used the same device when investigating PCO₂ changes on the serosal side of ileum in the peritoneal cavity. This application compared well with intraluminal measurements for detection of regional intestinal ischemia, a conclusion strongly supporting our own original description of intraperitoneal monitoring.

Our measurements in the intestinal region showed increased PCO₂ only during SMA clamping, indicating that intraperitoneal tonometry in the intestinal region can be a specific monitor of ischemia in the corresponding part of the bowel. In the epigastric region, signs of ischemia were accordingly seen only during CA clamping, although the change was not as great as in the intestinal region during SMA clamping. This difference can conceivably be explained by the position of the catheter and possible collateral blood flow in adjacent organs or the interplay between the portal venous and hepatic arterial blood flow. This will be referred to as the hepatic artery buffer response, HABR. In the sigmoid colon region, IMA clamping did not elicit signs of ischemia as measured by PCO₂-tonometer. The explanation can be that the sigmoid colon receives collateral blood flow from both the SMA and the internal iliac artery. In the sigmoid colon region, regional to arterial PCO₂ gap increased during SMA clamping although PrCO₂ decreased.
Relative changes (%) as compared to baseline. Abbreviations: cPrCO₂ = regional PCO₂ in control group, cPr-EtCO₂ gap = regional PCO₂ - endtidal CO₂ in control group, PrCO₂-endo = regional PCO₂ in endotoxin group, Pr-EtCO₂ gap-endo = regional PCO₂ - endtidal CO₂ in endotoxin group.

We have data to be published, where we look at the regional to endtidal PCO₂ gap. Compared to the selective clamping group the changes for regional PCO₂ in the endotoxin and hypoperfusion groups were less impressive. In the hypoperfusion group regional PCO₂ notably increased during 2/3 flow reduction. For both groups though, we noted much more pronounced increases for PCO₂ gap when compared to the control group. This was the case for all measuring points during LPS infusion and during 2/3 flow reduction in the hypoperfusion group. (Figure 7 and 8).
Figure 8. PCO₂ and PCO₂ gap in the hypoperfusion group.
Relative changes (%) as compared to baseline. Abbreviations: cPrCO₂ = regional PCO₂ in control group, cPr-EtCO₂ gap = regional PCO₂ - endtidal CO₂ in control group, PrCO₂-hypo = regional PCO₂ in hypoperfusion group, Pr-EtCO₂ gap-hypo = regional PCO₂ - endtidal CO₂ in hypoperfusion group.

Continuous or semi-continuous non-invasive monitoring would of course be ideal but this PCO₂ gap has its limitations in patients with lung injury and V/Q-mismatch. Tenhunen (2001b) studied septic pigs and found that PCO₂ gap can be useful as semi-quantitative trend monitoring, bearing in mind the importance of marked changes in dead-space ventilation. In a pig hemorrhagic model, Kvarstein noted concomitant changes in intestinal and muscle tissue PCO₂ with decreasing cardiac output. They suggested that the easily accessible muscle measurements of PCO₂ could have potential for routine online monitoring in unstable patients. In a similar model Puyana measured microelectrode tissue pH and compared with gastric tonometry. Of the three locations tested small bowel pH reacted faster than that of gastric and abdominal wall muscle. The magnitude of change was superior for jejunal and muscle pH compared with tonometry. In a clinical study Levy examined the correlation between gastric tonometry PCO₂ gap and outcome. Of all clinical and physiological parameters available only three remained independently associated with mortality in a univariate analysis. Those were organ system failure, arterial lactate and PCO₂ gap at 24 hours.
Colonic pulse oximetry

In paper III we used intraluminal tonometry in the sigmoid colon to verify the ischemia instead of histological investigations. We did not measure the ultrasonic IMA blood flow because, in the pig IMA blood flow interruption alone cannot produce ischemia in the sigmoid colon. The \( \text{ScO}_2 \) measurements showed an “all or nothing” pattern, not proportional to the aortic blood flow. Similar studies to ours have been performed. Avino examined colonic ischemia in dogs by sampling and analyzing ABG from inferior mesenteric vein (IMV) comparing it with laser Doppler flowmetry, transmission plethysmography and acquired histological confirmation of ischemia. A combination of ligation created no ischemia, partial ischemia or complete ischemia. The most reliable predictor of complete ischemia was found to be the increase in \( \text{PCO}_2 \) and the decrease in \( \text{pHi} \) and oxygen saturation of IMV blood. Transcolonic pulse oximetry proved to be sensitive to changes in saturation as long as the signal was pulsatile but could not differentiate quality of perfusion once the pulsatility was lost. The use of transmission oximetry is restricted to the peroperative situation. Gardner studied a pig model where the flow of the caudal mesenteric artery was stepwise obstructed and the intracolonic oxygen saturation measured with reflectance oximetry. In sharp contrast to the findings in Avino's and our studies, Gardner could demonstrate a gradual decrease in oxygen saturation down to 20 % of baseline blood flow. A possible explanation for this could be collateral circulation with mixing of blood. Vahl used an experimental setup similar to that of Gardner but with histological examination of mucosal specimens. In cases where ischemia was evident oximetry was characterized by disappearance of the pulse waveform and saturation readings. In some animals the oximetric signal reappeared three hours after arterial clamping. The detected pulses had decreased amplitude and the readings were considerably lower. The authors conclusion was that colonic oximetry was reliable when a normal pulse waveform was displayed.

Hepatic venous oxygen saturation and lactate

In sepsis, intestinal ischemia and liver dysfunction are common. This will result in increased lactate production and decreased lactate clearance respectively. Both mechanisms may explain hyperlactatemia, either alone or in combination. On the other hand, the liver is often able to clear excess lactate and thus prevent systemic lactate increase. De Backer (1998) addresses the problem that liver metabolism in sepsis can increase out of proportion to blood flow, at least this is a common explanation for the
gradient between mixed venous and hepatic venous oxygen saturation. They studied 42 septic patients treated with varying dobutamine doses and levels of PEEP. Hemodynamic, metabolic and flow data (indocyanine green clearance) were collected and sophisticated calculations performed. They noted that a saturation difference (SvO₂-ShvO₂) of more than 10% could predict VO₂/DO₂ dependency. De Backer (2001) investigated the role of the splanchnic region in lactate kinetics in 90 septic patients. Again a similar measurement setup as in the 1998 paper was used, but with the addition of gastric PCO₂ gap determinations. In contrast to many other reports they could only find increased net splanchnic lactate release in a small number (6/90) of their patients. Lactate sources, other than splanchnic, are mentioned, such as extraabdominal inflammation and production in the lungs or circulating leukocytes. As in our study, the arteriovenous lactate difference was small. The authors discuss the relative importance of hepatic blood flow versus arterial lactate concentration in relation to the regulation of lactate consumption.

In many animal studies, hepatic lactate uptake increases with increasing influx, but this process is saturable. In our fourth paper, CA clamping significantly affected ShvO₂ due to interruption of arterial supply to the liver. In contrast, ShvO₂ and SvO₂, increased in SMA clamping. At baseline, the SMA flow was more than twice that of the CA. We therefore postulate that SMA clamping increases CA flow, and that this hyperperfusion can explain the increase in ShvO₂. In addition to the increased perfusion of the liver two further factors contributed. One was the fact that hepatic arterial blood has higher oxygen content than the portal blood, and the other that during SMA clamping oxygen consumption in the small intestine decreased. Accordingly, in CA clamping ShvO₂ decreased significantly due to interruption of the arterial supply to the liver. In the hypoperfusion and endotoxin shock groups, ShvO₂ showed a quick response to the changes in blood flow. Dahn adjusted flow rates in perfused rat liver and used ShvO₂ (range 10-75%) as indicator of change in oxygen transport. The authors found that hepatic vein oxygenation was delivery dependent when stress hormones or high lactate concentrations were present. Regarding synthetic functions of the liver (assessed by measuring albumin production), it was concluded that ShvO₂ monitoring could play an important role in detecting functional impairment.

Blood flow distribution

In a hypoperfusion (SMA occlusion) study in pigs Jakob (2000) tested if increased uptake could prevent systemic hyperlactatemia and thereby mask
splanchnic ischemia. This was found to be the case and the explanation was probably the combination of fluid administration and a very active hepatic arterial buffer response (HABR) with an impressive compensatory (up to five-fold) increase in hepatic arterial flow. In another similar study, Tenhunen (2001a) used jejunal tonometry and microdialysis to demonstrate a threshold in mesenteric flow below which regional lactate and PCO₂ gap increases. The necessary reduction was in the range of 80-90% but this varied and was dependent on the duration of occlusion as well. Schiffer had demonstrated the effect of endotoxemia on the HABR some years earlier in a sheep model. For critical analysis of publications on splanchnic circulation one must consider the fact that endotoxin affects vascular capacitance. In further studies Jakob (2001) investigated whether systemic hypoperfusion could attenuate the HABR. They used a cardiac tamponade porcine model. Their main finding was that the HABR was exhausted during low systemic perfusion, and so was the increase in lactate uptake. Interestingly, they could show a more reliable PCO₂ gap for the jejunal compared to the gastric mucosa. Effects on HABR in the tamponade model can be difficult to interpret, as the resulting impact on venous return and right heart filling pressure can cause obstruction of the hepatic outflow. The same author, Jakob (2002), published further data on SMA-occlusion and reperfusion. They could confirm that the HABR was exhausted in low perfusion and also that this was not completely reversible during reperfusion. Even more interestingly was the observation that, the distribution of blood flow within the celiac trunk in HABR was ranging from “steal” to “inverse steal” regarding hepatic blood flow. This finding implies that in the HABR not only changes in portal and hepatic arterial flows are involved. “Hepatic steal” in celiac circulation may have a major impact on ischemic risk in pancreas and stomach.

During the reperfusion period in our hypoperfusion group, ShvO₂ returned to the baseline. Interestingly the CA flow increased approximately 50% beyond baseline while SMA flow and cardiac output (and aortic flow) regained baseline values. We noted a similar “selective” increase in CA flow in our endotoxin shock group. Following an initial decrease, ShvO₂ returned to baseline 60 to 90 minutes after the initiation of LPS infusion. Despite a slight decrease in cardiac output we noted a 150% CA flow as compared to baseline. The explanation for this can be adaptation of hepatic artery flow to hepatic hypermetabolism caused by ischemic reperfusion injury. More interesting is the speculation that the HABR is activated by changes in oxygen saturation in portal venous and hepatic arterial blood, in addition to the importance of changes in flow.

In a porcine endotoxin model Tenhunen (2003) demonstrated a biphasic (hypodynamic followed by hyperdynamic) response to LPS infusion. We
saw the same response in our studies and it is typical for rapid, high-dose, LPS infusion. In slow-rate LPS infusion and in human sepsis, a hyperdynamic state with high cardiac output and low systemic vascular resistance (SVR) is more common. The authors concluded that redistribution occur between SMA and celiac blood flow, why markers of perfusion over one visceral region not with certainty reflects perfusion over other splanchnic areas.

Ujhelyi describes a moderate increase in lever size and simultaneously a marked decrease in splenic volume when LPS is rapidly infused without fluid resuscitation. Slow infusion induced only mild changes, most notably a decrease in liver volume. In a recent article Jakob (2003) summarizes current knowledge on hepato-splanchnic blood flow regulation and describes how the adenosine washout hypothesis can explain the HABR. Iwao made vascular ultrasound examinations (flow and pulsatility index) of the hepatic artery buffer response in individuals with liver cirrhosis and healthy controls. Heavy meals and vasopressin infusion was chosen as intervention. Both groups showed the expected changes in portal blood flow but the counteracting compensation in hepatic artery pulsatility was blunted in the group of patients with liver cirrhosis.

Hiltebrand measured regional blood flow (Transonic) and bowel microcirculation (Laser Doppler) in pigs with sepsis caused by fecal peritonitis. They observed that in hypodynamic sepsis intestinal blood flow decreased in parallel to systemic flow. Even if the muscularis perfusion was decreased the mucosal microcirculation was largely intact due to redistribution of flow within the intestinal wall. This compensation could not be proven sufficient as decreasing jejunal tonometric pH and increasing serum lactate concentrations indicated a hypoxic situation. Lundgren describes the myogenic and metabolic theories of intestinal blood flow autoregulation in greater detail in a review. Among other mechanisms, tonus changes in pre- and postcapillary sphincters are involved in the control of fluid shifts and the countercurrent exchange principle offers an explanation for the villous sensitivity to hypoxia.

**Clinical implications and future studies**

Our findings support the use of air instead of saline as the preferred technique for tonometric measurements. Compared to saline it is a user-friendly technique with a shorter calibration time and more frequent automated readings. It can be used for monitoring both during surgery and in the postoperative period as well as in intensive care. The potential for tonometry to become routine monitoring is even greater in its intraperitoneal
application. By this approach we can gain more information on regional aspects of the splanchnic circulation. The balloon of the monitoring catheter can be positioned close to the small intestine. This part of the bowel is supplied by SMA and more sensitive to ischemia than the stomach in most clinical situations. The routine administration of H₂ blockers, as recommended for gastric tonometry, can thereby be omitted. Hepatic venous oxygen saturation measurements may be useful for rapid and sensitive detection of splanchnic ischemia. This technique will give an overall reflection of the intestinal circulation. Because of the sophisticated interactions between portal and hepatic arterial blood flow, the combination with intraperitoneal tonometry might prove to be a valuable option.

To further strengthen the evidence for our clinical suggestions there are several studies we would like to undertake. In greater detail we need to look in to microvascular aspects by comparing air tonometry when applied both on the mucosa and the serosal side of the small bowel wall. Conflicting data exist to whether ischemic mucosal damage first appears in the tips of the villi, or if the mucosa and the serosa become dysoxic at the same time. Our tonometric measurements in the colonic lumen were not conclusive. This was possibly due to erroneously high PrCO₂ that we could ascribe local production of carbon dioxide in fecal contents. We would also like to make experimental and clinical studies of intraperitoneal tonometric monitoring and how it is affected by peritonitis or peritoneal effusions. This can be studied by intraperitoneal placement of air tonometry catheters during major abdominal surgery for postoperative monitoring of the splanchnic circulation. Another interesting study objective would be to compare intraperitoneal with intracolonic tonometry for early detection of bowel ischemia following major vascular surgery. In connection with these studies we would also make observations regarding the validity of PCO₂ gap monitoring. Lastly we would focus on regional blood flow distribution within the splanchnic area, with the aim to elucidate the mechanisms involved in the HABR and regulation of blood flow in the hepatic artery.
CONCLUSIONS

1. We have shown that air tonometry, in its regional application, is equivalent to the saline technique. (Paper I)

2. Intraperitoneally applied tonometry appears to be a reliable monitoring option for early detection of ischemia in the small intestine. (Paper I and II)

3. Pending further validation, the determination of the r-etPCO2 gap can be a valuable option for continuous noninvasive trend monitoring of the splanchnic circulation.

4. The sigmoid colonic pulse oximetry showed a non-linear response in relation to regional blood flow, and will therefore not be able to detect gradual changes in oxygen saturation. (Paper III)

5. Hepatic venous oxygen saturation measurements may be useful for detection of splanchnic ischemia, whereas central venous or arterial lactate concentration only reflects systemic changes in oxidative metabolism during ischemia. (Paper IV)

6. The understanding of the mechanisms behind of the hepatic artery buffer response is crucial to the interpretation of our work. This might help us explain the selective changes in hepatic arterial blood flow that we observed. (Paper I, II and IV)
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