EXPERIMENTAL

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Changes in regional blood flow and pCO$_2$ gradients during isolated abdominal aortic blood flow reduction

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Abstract *Objective:* pCO$_2$ gradients are used for the assessment of splanchnic regional and local mucosal blood flow changes in experimental and clinical research. pCO$_2$ gradients may not parallel blood flow changes because of concomitant changes in metabolism, hemoglobin, temperature, and the Haldane effect. *Design and setting:* A randomized, controlled animal experiment in a university experimental research laboratory. *Interventions:* An extracorporeal shunt with reservoir and roller pump was inserted between the proximal and the distal abdominal aorta in 16 pigs. In animals randomized to the low-flow group (*n*=8) splanchnic perfusion was reduced by running the roller pump. At baseline and after 45 min of stable shunt flow superior mesenteric artery, celiac trunk, spleen artery, and portal vein blood flows and regional venous-arterial and jejunal and gastric mucos-al-arterial pCO$_2$ gradients were measured, and the respective regional O$_2$ consumption rates (VO$_2$) calculated. *Measurements and results:* In the low-flow group all regional blood flows and the associated VO$_2$ decreased to roughly 50% of baseline values, and hemoglobin decreased from 7.3 (4.4–9.6) g/dl to 5.7 (4.1–8.9) g/dl. Decreasing regional blood flows were consistently associated with increasing regional and mucosal pCO$_2$ gradients. *Conclusions:* During isolated reduction in abdominal aortic blood flow there is no preferential distribution to any splanchnic vascular bed and changes in regional pCO$_2$ gradients reflect consistently the associated blood blow changes.

Keywords Low flow · Metabolism · pCO$_2$ gradients · Regional blood flow · Splanchnic perfusion

Introduction

The gastrointestinal tract seems to be a major contributor to the pathogenesis of the systemic inflammatory response syndrome, sepsis, and multiple organ failure in critical illness [1, 2, 3, 4]. The concept of an overproportional decrease in the perfusion of visceral organs in low-flow states to maintain central blood volume and cardiac output has been developed in earlier studies [5, 6, 7, 8, 9]. Hemodynamic variables and global parameters of tissue perfusion such as acid-base status, blood lactate concentration, and mixed venous oxygen saturation poorly reflect regional tissue perfusion [10, 11, 12, 13]. Monitoring of CO$_2$ tension in the arterial and venous blood has been suggested for detection of poor regional and local tissue perfusion and oxygenation [14, 15, 16, 17]. Under clinical conditions the direct determination of venous-arterial pCO$_2$ gradients in the splanchnic vascular beds is not feasible. Intramucosal pCO$_2$ can be measured indirectly within the lumen of the gastrointestinal tract by gastrointestinal tonometry [18]. However, the clinical utility of gastrointestinal tonometry remains controversial [19, 20, 21], and studies that evaluate the correlation between the intramucosal pH (calcu-

lated from intramucosal pCO_2 using the modified Henderson-Hasselbalch equation [22, 23]) and splanchnic blood flow demonstrate inconsistencies [24].

The cellular CO_2 level depends on the balance between (a) CO_2 production via both aerobic and anaerobic metabolism and (b) CO_2 removal by blood flow from the particular tissue bed and by alveolar ventilation from the systemic circulation [25]. The regional CO_2 production is expressed as the product of the respective blood flow and the venous-arterial CO_2 content difference. The rationale behind the use of mucosal-arterial (venous-arterial) pCO_2 gradients as a measure of the adequacy of mucosal (regional) blood flow is the almost linear relationship between the blood carbon dioxide content and the corresponding pCO_2 under stable conditions. However, there are potential confounding factors such as concomitant changes in hemoglobin, pH, temperature, and the Haldane effect: increasing oxygen saturation increases the pCO_2 for any given carbon dioxide content and vice versa [26]. Furthermore, changes in the local or regional CO_2 content can occur as a result of phenomena entirely related to metabolism. Hence pCO_2 gradients may not parallel blood flow changes.

Due to the complex anatomy and physiology of the splanchnic circulation and the limited access to these vascular beds in patients the interaction between changes in metabolism and blood flow and their effect on the measured regional and mucosal pCO_2 gradients cannot be tested in the clinical setting. We therefore developed an experimental porcine model to selectively reduce the abdominal blood flow with limited systemic side effects. The aims of this study were the following: (a) to assess the regional blood flow distribution within the splanchnic circulation, (b) to compare regional perfusion, oxygen consumption, and pCO_2 gradients during isolated reduction in visceral blood flow, and (c) to assess whether confounding factors such as changes in temperature, hemoglobin, and the Haldane effect affect the relationship between regional venous-arterial pCO_2 gradients and blood flow in a low visceral perfusion state.

Materials and methods

Anesthesia and monitoring

This study was performed according to the National Institutes of Health guidelines for the use of experimental animals, and the protocol was approved by the Animal Care Committee of the Canton of Berne. Sixteen pigs of both genders (weight range 37–43 kg) were deprived of food but not water 24 h before the experiment. They were premedicated intramuscularly with 0.05 mg/kg atropine, 4 mg/kg xylacine, and 15 mg/kg ketamine followed by cannulation of an ear vein and intravenous administration of 5–10 mg/kg thiopental sodium for endotracheal intubation. As described previously, anesthesia was maintained with 5 mg/kg thiopental sodium and 30 µg/kg fentanyl per hour during the operation [27, 28]. After completion of surgery the hourly fentanyl administration was reduced to 5 µg/kg and muscle relaxation with 1 mg/kg

pancuronium per hour was started. The animals were mechanically ventilated with a volume-controlled ventilator (Servo 900C, Siemens, Solna, Sweden) with 5 cmH2O end-expiratory pressure. Fractional inspired oxygen concentration was adjusted to keep paO_2 levels between 100 and 150 mmHg. Tidal volume was kept at 10 ml/kg and the minute ventilation was adjusted to maintain $paCO_2$ levels between 34 and 41 mmHg. A large-bore gastric tube was introduced into the stomach via the mouth. A right-sided carotid artery catheter, a pulmonary artery catheter (via the right submandibular vein), a catheter for later placement in a hepatic vein (via the right internal jugular vein,) and a right-sided femoral artery catheter were inserted. During surgery and the experiment the animals received per hour 10 ml/kg Ringer's lactate, 10 ml/kg gelatin solution (Physiogel), and 5 ml/kg 5% glucose. Additional fluid was administered if necessary to keep the pulmonary artery occluded pressure between 5 and 8 mmHg. The goal was to keep the body temperature of the animals at 39°±0.5°C by using an operating table heater, warmed fluids, and a blanket.

Animal preparation

The abdominal cavity was exposed by a midline abdominal incision. A drainage catheter was inserted into the urinary bladder. The superior mesenteric, splenic, hepatic, and femoral arteries, the celiac trunk, and the portal vein were exposed, and ultrasound transit time flow probes (Transonic Systems, Ithaca, N.Y., USA) were placed around the vessels. Two fluid-filled catheters were inserted proximally and distally into a mesenteric vein. The tip of the first catheter was placed into the portal vein, while the tip of the second catheter remained in the mesenteric vein. A third catheter was inserted into the splenic vein. The abdominal aorta was exposed through the left retroperitoneal space, and an ultrasound flow probe was placed around the aorta proximally to the origin of the celiac trunk. A 20-F curved venous cardiopulmonary bypass cannula (Medtronic Cardiopulmonary, Anaheim, Calif., USA) was inserted into the abdominal aorta proximal to the aortic flow probe, and a second 20-F cannula was inserted into the distal aorta with the tip located proximal to the aortic bifurcation. The cannulas were connected to each other using silicon cardiopulmonary bypass tubes, a cardiotomy reservoir (Jostra, Irvine, Calif., USA) and a precision blood roller pump (Cobe, Denver, Colo., USA). A vascular occluder was placed around the aorta proximal to the distal cannula, and an aortic ultrasound flow probe was placed distal to the cannula (a schematic illustration of the shunt system is shown in Fig. 1). The pig received boli of 1000–2500 IU heparin to keep the activated clotting time at a level of 400 s minimum.
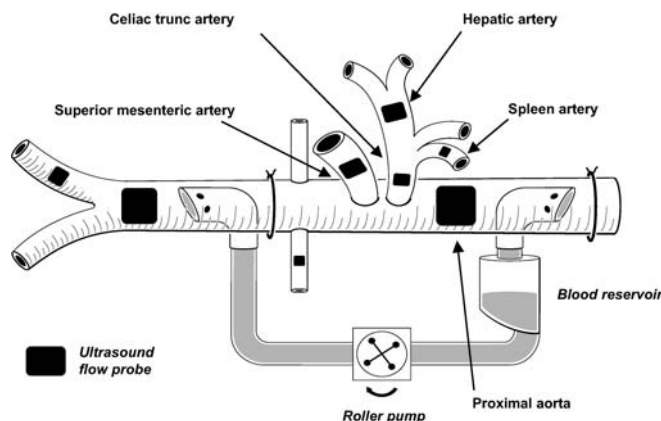

Fig. 1 Shunt system

The measurements were performed using high-range activated clotting time cartridges (Medtronic, Minneapolis, Minn., USA) and an automatized activated clotting time analyzer (Medtronic HemoTec, Parker, Colo., USA). The catheter within the right internal jugular vein was further inserted and the location of the tip within a hepatic vein was confirmed by direct manual palpation. Thereafter the catheter was withdrawn 0.5–1 cm from the wedged position to allow blood sampling. A jejunal and a gastric air tonometry catheter (Tonometrics, TRIP NGS Catheter, Datex-Ohmeda, Helsinki, Finland) were inserted through an incision in the jejunal wall and orally, respectively, and connected to a Tonocap system (Datex-Ohmeda), after which the incision in the intestinal wall was closed with sutures. When all surgical procedures were completed, the abdominal wall was reapproximated gently, and towels were placed on the surface to minimize heat loss.

Experimental protocol

After preparation 180 min was allowed for hemodynamic stabilization before baseline measurements were made and samples were taken. The animals were randomized into two groups: a control group (n=8) and a low-flow group (n=8). In the low-flow group the distal abdominal aorta was occluded to prevent backflow from the distal cannula, and the abdominal aortic flow was reduced to a target total hepatosplanchnic blood flow (=celiac trunk + superior mesenteric arterial blood flow) of 50% of the baseline value by running the roller pump. Once the target abdominal aortic blood flow was reached, the shunt blood flow was kept constant for 45 min. In four randomly allocated animals in the control group ("control/low shunt flow"), the shunt was activated with a low shunt flow of 250 ml/min to control for potential effects of the shunt per se. In the other four animals of the control group the shunt remained closed. At the end of the experiment the animals were killed by an overdose of intravenous potassium. Afterwards the correct position of the hepatic vein catheter was confirmed by anatomical dissection of the liver.

The protocol was carried out in 18 animals. Two animals were excluded post hoc because of discrepancies between size of aorta and aortic cannula with subsequent hypoperfusion of the legs in one animal and bleeding with severe anemia (baseline hemoglobin level less than 4 g/100 ml) in another animal. Measurements after 45 min are labeled as "second measurement". There were no differences between groups in systemic hemodynamics and regional blood flow measurements at baseline. In one animal in the low-flow group the spleen flow probe was not properly working, and in one animal in the low-flow group and two in the control group it was not possible to obtain blood samples from the mesenteric vein. The corresponding results are therefore missing in the respective animals.

Hemodynamic monitoring

Aortic, carotid, pulmonary, and femoral arterial blood pressures, pulmonary artery occluded pressure, and central venous pressure were recorded with quartz pressure transducers and displayed continuously on a multimodular monitor and recorder (S/5 Critical Care Monitor, Datex-Ohmeda). All pressure transducers were simultaneously zeroed to the level of the heart. Cardiac output was measured by a thermodilution technique (mean value of four measurements). Central venous blood temperature was recorded from the thermistor in the pulmonary artery catheter. Heart rate was measured by electrocardiography, which was also continuously monitored.

Blood flow measurements

Abdominal aortic and regional blood flows were measured by ultrasound transit time flow probes (Transonic Systems). The transit time ultrasound volume flowmeter has been demonstrated to provide adequate measures of arterial and venous flows in experimental animals if carefully positioned and aligned with respect to the vessel [29, 30]. The perivascular ultrasound flow probes were calibrated in vitro and the signals were visualized and recorded continuously (Flowmeters T108 and T208, Transonic Systems).

Blood gas and lactate measurements

Blood samples for the measurement of hemoglobin, blood gas analysis, and lactate were taken at baseline and after 45 min of stable shunt flow from carotid and pulmonary arteries and from portal, hepatic, mesenteric, and splenic veins and were analyzed immediately after withdrawal. Hemoglobin concentrations and oxygen saturations were measured by an analyzer designed for porcine blood (OSM 3, Radiometer, Copenhagen, Denmark), and lactate and blood gases were analyzed at 37°C in a blood gas, electrolyte and lactate analyzer (ABL 520, Radiometer).

Calculations

Systemic and regional venous-arterial carbon dioxide content differences were calculated using an iterative procedure as proposed by Giovannini and colleagues [31] (see Appendix). Since the accuracy of this method may be reduced if the metabolic acid-base status in venous and arterial blood largely differs [31], calculations at 45 min were performed only in control animals. The Haldane effect and changes in blood pCO_2 mediated by changes in hemoglobin-O_2 saturation and O_2-linked CO_2 binding was calculated as [32]:

$$(v\text{-}a)pCO_{2HE} = 0.460 \ [(a\text{-}v)HbO_2]^{0.999} e^{0.015 \ (pvCO2)-0.852 \ (Hct)}$$

Regional carbon dioxide production was calculated as the product of venous-arterial carbon dioxide content difference and regional blood flow. Regional oxygen consumption was calculated as the product of regional blood flow and arterial-venous oxygen content difference.

Statistics

Statistical analysis was performed with the SPSS software (version 10.0, SPSS, Chicago, Ill., USA). Because the number of animals per group was small, nonparametric tests were used. Visualization of blood flow and metabolic changes within controls and "control/low shunt flow" animals demonstrated no differences. These groups were therefore combined for the statistical analysis ("control group"). Groups at baseline were compared using the Mann-Whitney U test; changes over time in each group were separately assessed by the Wilcoxon test. For the relative flow changes in the shunt group we used Friedman's test. Statistical significance was set at $p<0.05$. All results are presented as median (range) unless stated otherwise.

Results

Systemic hemodynamics and central temperature are shown in Table 1. At baseline there were no difference between the two groups. At the second measurement heart rate and cardiac output were increased in the control group while mean arterial pressure did not change in

Table 1 Hemodynamics, temperature, arterial blood gas measurements, and hemoglobin

	Baseline		Second measurement		p	
	Median	Range	Median	Range	Baseline	Intervention
Heart rate (beats/min)					0.44	
Low flow	116	72–187	147	75–167		0.10
Control	103	73–156	129	81–178		0.03
Systemic mean arterial pressure (mmHg)					0.33	
Low flow	86	76–105	74	67–112		0.33
Control	94	66–118	90	75–114		0.89
Pulmonary artery occluded pressure (mmHg)					0.16	
Low flow	6	4–9	6	2–7		0.02
Control	7	5–9	6	5–9		0.07
Cardiac output (ml kg^{-1} min^{-1})					0.15	
Low flow	99	74–169	110	83–190		0.35
Control	118	94–160	115	107–210		0.07
Central temperature (°C)					0.65	
Low flow	39.8	37.3–41.3	38.7	34.4–39.9		0.01
Control	39.9	38.2–41.5	39.6	38.5–41.4		0.09
pH					0.33	
Low flow	7.41	7.34–7.47	7.37	7.30–7.43		0.09
Control	7.42	7.40–7.45	7.43	7.38–7.46		0.40
pCO_2 (mmHg)					0.96	
Low flow	40	35–48	38	34–44		0.02
Control	41	37–46	40	36–42		0.07
SaO_2 (%)					0.96	
Low flow	97	96–98	98	97–99		0.03
Control	98	95–98	98	95–98		0.12
Hemoglobin (g/dl)					0.65	
Low flow	7.3	4.4–9.6	5.7	4.1–8.9		0.02
Control	6.1	5.3–8.8	5.7	4.7–9.0		0.12

either group. In the low-flow group wedge pressure was decreased. Blood gas measurements and hemoglobin values are also shown in Table 1. Arterial pCO_2 and hemoglobin were decreased in the low-flow group, but remained unchanged in controls. Aortic and regional blood flows are shown in Table 2. Aortic and regional blood flows did not differ at baseline, and changes in controls were not significant. In the low-flow group abdominal aortic and all regional blood flows were decreased by 50%–80%. The relative flow changes did not differ in the individual vascular beds ($p=0.45$). There were no differences in blood lactate concentrations at baseline between animals in the two groups (Table 3). Lactate concentrations increased in the venous effluents of all vascular beds in animals from the low-flow group.

The regional venous-arterial pCO_2-gradients did not differ between groups at baseline (Table 4). In the low-flow group the splenic, mesenteric, and portal venous-arterial pCO_2 gradient was increased. In the control group overall venous-arterial CO_2 content differences remained unchanged. In general, small changes in CO_2 content differences corresponded well with the respective changes in pCO_2 differences (Fig. 2). Gastric and jejunal tonometric values, although highly variable in both groups, were increased in low flow animals (Table 4). Changes in blood pCO_2 mediated by changes in Hb-O2 saturation and O2-linked CO_2 binding provided a mean regional (v-a)pCO_2HE value of 4 mmHg (all animals and conditions). During low flow, values of up to 10 mmHg were obtained. The relative contribution to the measured regional pCO_2 gradients was 23%–48%, depending on the respective region. Results for regional oxygen consumption (VO_2) and oxygen extraction rates are shown in Table 5. Hepatic oxygen consumption was

Table 2 Aortic and regional blood flows

	Baseline		Second measurement		p	
	Median	Range	Median	Range	Baseline	Intervention
Aortic flow (ml kg^{-1} min^{-1})					1.00	
Low flow	71	30–103	16	2–38		0.01
Control	70	61–88	72	37–91		0.89
Superior mesenteric artery flow (ml kg^{-1} min^{-1})					0.06	
Low flow	18	12–31	11	5–23		0.01
Control	27	20–33	28	19–31		0.33
Celiac trunk artery flow (ml kg^{-1} min^{-1})					0.72	
Low flow	18	5–23	10	2–16		0.01
Control	16	11–22	19	14–21		0.07
Hepatic artery flow (ml kg^{-1} min^{-1})					0.57	
Low flow	8	4–18	2	1–14		0.01
Control	10	5–12	10	6–15		0.16
Spleen artery flow (ml kg^{-1} min^{-1})					0.69	
Low flow (n=7)	1.5	1.2–3.0	0.8	0.3–1.8		0.02
Control (n=7)	1.9	0.5–6.2	1.8	0.5–5.5		0.89
Portal vein flow (ml kg^{-1} min^{-1})					0.57	
Low flow	30	19–44	18	10–31		0.01
Control	33	25–41	34	20–40		0.78

Table 3 Systemic arterial and regional venous lactate

	Baseline		Second measurement		p	
	Median	Range	Median	Range	Baseline	Intervention
Arterial (mmol/l)					0.20	
Low flow	0.9	0.5–1.5	3.2	1.2–4.8		0.01
Control	0.7	0.8–2.1	0.8	0.5–1.4		0.50
Mesenteric vein (mmol/l)					0.30	
Low flow (n=7)	1.2	0.7–1.6	5.2	1.5–6.0		0.03
Control (n=6)	0.9	0.6–2.1	1.0	0.6–1.5		1.00
Hepatic vein (mmol/l)					0.23	
Low flow	0.7	0.3–1.1	3.1	1.1–5.5		0.01
Control	0.5	0.2–1.5	0.6	0.2–1.0		0.50
Splenic vein (mmol/l)					0.10	
Low flow (n=7)	1.1	0.6–1.7	3.1	1.4–5.6		0.01
Control (n=6)	0.7	0.6–2.2	0.9	0.7–1.7		0.34
Portal vein (mmol/l)					0.13	
Low flow	1.1	0.6–1.6	3.6	1.3–5.3		0.01
Control	0.8	0.6–1.8	0.9	0.5–1.5		0.43

Table 4 Regional and mucosal pCO_2 gradients and tonometry data

	Baseline		Second measurement		p	
	Median	Range	Median	Range	Baseline	Intervention
Mesenteric pCO_2 gradient (mmHg)					0.18	
Low flow ($n=7$)	15	6–19	28	8–54		0.04
Control ($n=6$)	11	8–16	11	5–17		0.89
Hepatic pCO_2 gradient (mmHg)					0.65	
Low flow	11	6–18	15	7–44		0.11
Control	10	8–13	10	7–12		0.64
Splenic pCO_2 gradient (mmHg)					0.61	
Low flow	7	5–12	15	8–31		0.01
Control	8	3–14	6	1–12		0.74
Portal pCO_2 gradient (mmHg)					0.23	
Low flow	13	7–19	22	10–39		0.03
Control	10	8–13	9	7–15		0.89
Gastric tonometric-arterial pCO_2 gradient (mmHg)					0.96	
Low flow	33	6–84	45	6–82		0.05
Control	36	14–79	39	14–85		0.31
Jejunal tonometric-arterial pCO_2 gradient (mmHg)					0.52	
Low flow	9	1–24	44	10–101		0.04
Control	7	1–10	6	2–10		1.00

Table 5 Regional oxygen consumption and oxygen extraction rates

	Baseline		Second measurement		p	
	Median	Range	Median	Range	Baseline	Intervention
Mesenteric VO_2 (ml kg^{-1} min^{-1})					0.10	
Low flow ($n=7$)	0.8	0.4–1.3	0.5	0.3–1.2		0.02
Control ($n=6$)	1.1	0.8–2.3	1.1	0.7–2.0		0.31
Hepatic VO_2 (ml kg^{-1} min^{-1})					0.04	
Low flow	0.8	0.4–1.6	0.4	0.2–1.8		0.26
Control	1.2	0.8–2.5	1.4	0.7–2.7		0.89
Splenic VO_2 (ml kg^{-1} min^{-1})					0.38	
Low flow ($n=7$)	0.06	0.02–0.12	0.04	0.01–0.07		0.04
Control ($n=6$)	0.03	0.02–0.08	0.03	0.02–0.09		0.50
Portal VO_2 (ml kg^{-1} min^{-1})					0.38	
Low flow	1.4	0.7–2.1	0.8	0.5–1.6		0.01
Control	1.2	0.8–1.6	1.0	0.4–1.4		0.12
Mesenteric oxygen extraction (%)					0.14	
Low flow ($n=7$)	49	20–75	77	30–83		0.09
Control ($n=6$)	41	34–44	40	27–72		0.75
Hepatic oxygen extraction (%)					0.13	
Low flow	38	19–68	59	21–90		0.05
Control	51	32–68	51	41–62		0.67
Splenic oxygen extraction (%)					0.54	
Low flow ($n=7$)	37	13–63	76	26–92		0.01
Control ($n=6$)	38	11–44	27	10–64		1.00
Portal oxygen extraction (%)					0.13	
Low flow	53	24–61	70	40–81		0.07
Control	42	23–51	39	13–56		0.40
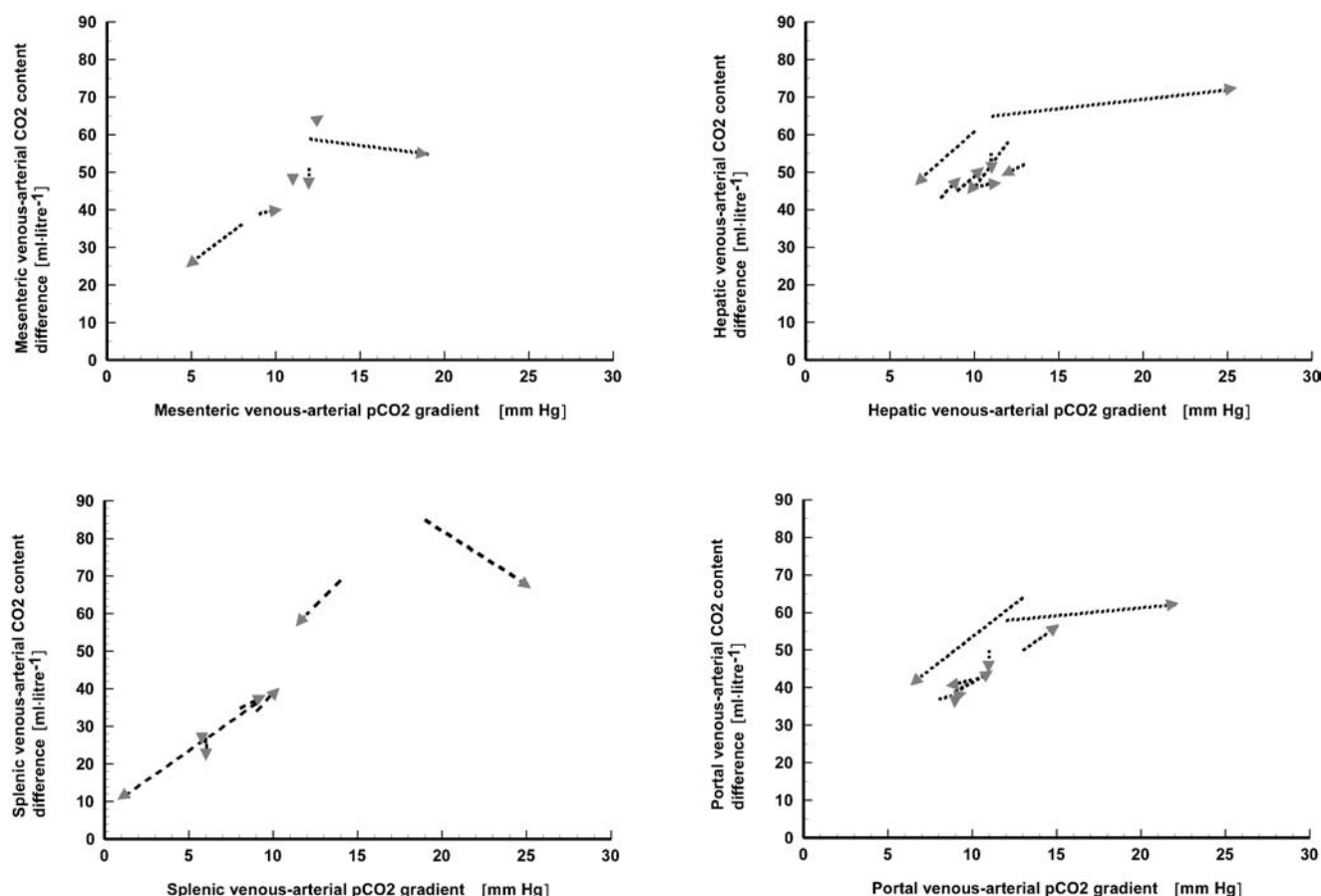
Fig. 2 Splenic, mesenteric, hepatic, and portal venous-arterial pCO_2 gradient and venous-arterial pCO_2 content difference

somewhat higher in control animals at baseline. Regional VO_2 remained stable in control animals. In the low-flow group mesenteric, splenic and portal oxygen consumption was decreased, while only the increase in hepatic and splenic oxygen extraction rate reached statistical significance.

Discussion

The main findings were as follows: (a) In the low-flow group all regional flows decreased proportionally, no vascular bed was preferentially perfused, and the perfusion was not preferentially reduced to any vascular bed. (b) Low abdominal blood flow was associated with a substantial reduction in O_2 consumption in several splanchnic tissues. (c) In most instances small changes in pCO_2 gradients reflected the respective changes in CO_2 content differences. (d) Despite major changes in oxygen extraction, hemoglobin, and temperature the pCO_2 gradients reflected changes in corresponding regional blood flows in most of the cases (Fig. 3).

This study describes a new model for the assessment of hemodynamic and metabolic effects of low splanchnic blood flow. The model excludes to a great extent effects of hemodynamic and metabolic changes in other vascular beds. The slight increase in cardiac output and heart rate in the control group may reflect a systemic inflammatory reaction to surgery and indwelling catheters. The lack of such a circulatory response in the low-flow group may be explained by the slight reduction in left ventricular filling, as suggested by a reduction in pulmonary capillary occlusion pressures in these animals. Both a reduction in systemic vascular resistance due to hemodilution and the resulting reduction in blood viscosity as well as potential mechanic effects of the running roller pump may contribute [33, 34]. Another effect of the extracorporeal circulation was a reduction in temperature [35, 36, 37], which we were not able to prevent completely by using warmed fluids and heating blankets. In future experiments an in-line blood heater can be integrated in the extracorporeal shunt system to prevent this problem.

Earlier studies have demonstrated an overproportional decrease in the perfusion of visceral organs in experimental circulatory shock [5, 6, 7, 8, 9]. It has been suggested that contraction of the splanchnic, and specifically the splenic vascular bed, helps to maintain central blood volume and cardiac output in hypovolemic states,
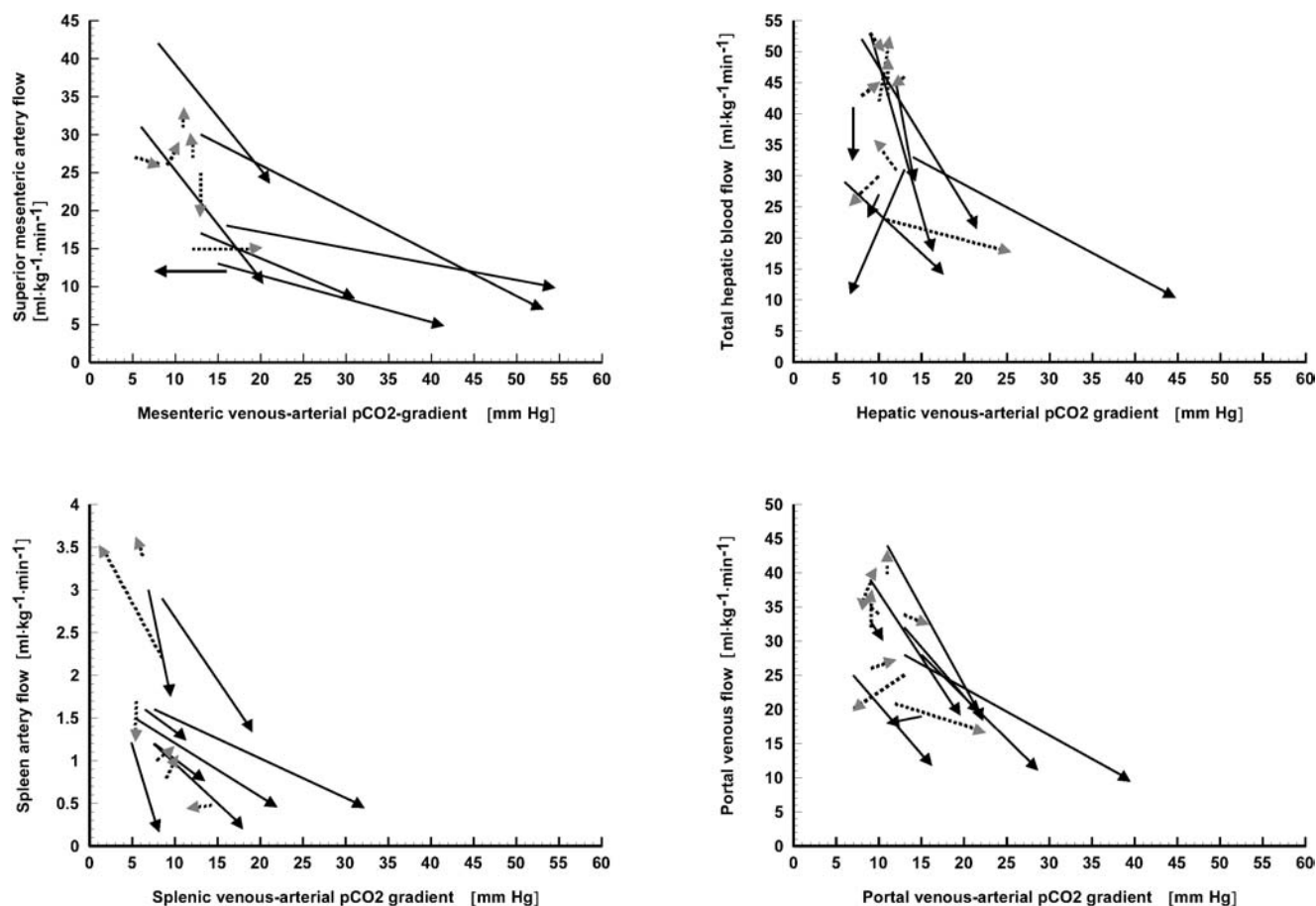
Fig. 3 Mesenteric venous-arterial pCO$_2$ gradient and superior mesenteric artery flow, splenic venous-arterial pCO$_2$ gradient and splenic artery flow, hepatic venous-arterial pCO$_2$ gradient and total hepatic blood flow, and portal venous-arterial pCO$_2$ gradient and portal venous flow

for instance, during fluid removal associated with hemodialysis [38]. In the present study all regional blood flows decreased in parallel by roughly 50% (Table 2); the more marked reduction in abdominal aortic flow may be explained by measuring errors due to turbulent blood flow. It is important to realize that in the experiments by Bailey and Bulkley and colleagues [7, 8, 9] low systemic blood flow was achieved by a combination of hemorrhage and cardiac tamponade. The authors combined hemorrhage and cardiac tamponade because they realized that prior hemorrhage sensitized the animals to subsequent tamponade. In our experiment hypovolemia was prevented, which could explain the discrepancies between our findings and those of their experiments.

The reduction in regional blood flows was associated with marked decreases in regional O$_2$ consumption and increases in oxygen extraction rates in individual animals, especially in the low-flow group (Table 5). While

some of the changes may be due to mathematical coupling associated with the use of shared variables in the calculations [39], and others may be explained by associated falls in temperature, we believe that the reduction in regional metabolism in animals in the low-flow group is due to a transition to flow-dependent metabolism [40]. The rising blood lactate concentrations from the different splanchnic tissues (Table 3) support this assumption. Our results and those of others [41] also demonstrate that the flow/metabolism relationship is not uniform in different splanchnic vascular regions. On the other hand, in this experimental setting gastric and jejunal pCO$_2$ gradients increased similarly, corresponding to a similar decrease in celiac trunk and superior mesenteric arterial blood flow. Accordingly, as far as our experimental design is comparable to a situation of low systemic blood flow in humans, it can be postulated that gastric tonometry detects substantial changes in mesenteric perfusion.

The rationale behind the use of pCO$_2$ gradients as an estimate of related blood flow changes is the almost linear relationship between blood carbon dioxide content and pCO$_2$, assuming *unchanged* oxygen saturation, hemoglobin, and pH. Although our results demonstrate such a linear relationship in the group as a whole, in individual animals there was a dissociation between changes in

pCO_2 gradients and the respective CO_2 content differences (Fig. 2). Several methods exist to calculate regional venous-arterial CO_2 content differences [42, 43]. Unfortunately their accuracy is limited in low flow states. The method proposed by Giovannini et al. [31], for instance, may not be applied in situations with massive release of acid ions from anaerobic tissue metabolism into slowly flowing venous blood in shock states because of the discrepancy between CO_2-binding properties of venous and arterial blood. Therefore calculation of regional venous-arterial CO_2 content differences and CO_2 production is difficult. Major changes in (venous) oxygen saturation, hemoglobin, and the venous-arterial pH difference occurred in the present experiment, and such changes also occur during surgery and intensive care in patients [26]. Among these effects the Haldane effect is quantitatively the most important one. The Haldane effect describes the phenomenon of a leftward shift of the CO_2 dissociation curve with decreasing oxygen saturation [44, 45]. The Haldane effect plays an important role for the magnitude of changes in pCO_2 gradients for a given change in blood flow as long as the oxygen extraction increases with decreasing blood flow, and hence the venous oxygen saturation decreases. In our experiment the mean Haldane effect on venous-arterial pCO_2 gradients was relatively small (4 mmHg) although maximal values of 10 mmHg were calculated. These values correspond to 23–48% of the total pCO_2 gradients. Despite this decreases in regional blood flows were almost always associated with corresponding increases in the respective pCO_2 gradients. Errors in measurements of pCO_2, hematocrit, and pH are further explanations for the exceptional discrepancies between changes in CO_2 content and pCO_2 differences. The present model produces regional ischemic hypoxia and obviously lacks the ability to limit the increasing pCO_2 gradients associated with major CO_2 stagnation. In models in which the oxygen content is decreased in the presence of maintained blood flow (hypoxic hypoxia [46, 47]), the Haldane effect would become a principal process in eliminating CO_2.

Progressive anemia during the experiment is another potential confounding factor. During isovolemic anemia down to a hematocrit of 12%, CO_2 transport is maintained mainly by an increased blood flow and an augmented Haldane effect induced by the increased oxygen extraction [48]. Arterial, venous, and mucosal pCO_2 are maintained [48, 49, 50]. In our experiment blood flow decreased, and anemia progressed at the same time. We believe that under these circumstances anaerobic metabolism develops earlier than reduction in blood flow without anemia. The combined effect of low blood flow, anaerobic CO_2 production, and an augmented Haldane effect on the course of the pCO_2 gradients is difficult to predict. Furthermore, changing body temperature in individual animals may also have had an impact on the relationship between metabolism and blood flow.

We conclude that this new experimental model allows a selective reduction in visceral blood flows to clinically relevant levels while systemic perfusion is maintained. The limitations are extensive surgery, difficulty in obtaining stable blood flow conditions and temperature, and substantial hemodilution. Under the experimental conditions, with prevention of hypovolemia, no preferential reduction or preservation of blood flows to the different visceral tissues occurred. In a setting of low blood flow, even with concomitant metabolic changes, and progressive anemia pCO_2 gradients can be used for the assessment of blood flow changes.

Appendix: calculation of the venous-arterial CO_2-content difference

Venous-arterial CO_2-content difference was calculated using the method developed by Giovannini et al. [31]. Briefly, the concentration of CO_2 in arterial blood is determined by the concentration of combined CO_2 in plasma ($CaCO_2p$) and red blood cells ($CaCO_2r$), dissolved CO_2 ($CaCO_2d$ ml/100 ml for all), and hematocrit. $CaCO_2$ =$CaCO_2p$. [1-Hct×(1−$CaCO_2r$/$CaCO_2p$)]+$CaCO_2d$. $CaCO_2p$ is calculated from the arterial pCO_2 and pH on the basis of the Henderson-Hasselbalch equation: $CaCO_2p$=0.06868× ($PaCO_2$)×{10[1.04214×(pHa)−6.41036]}. The value of ra=$CaCO_2r$/$CaCO_2p$ is obtained as a function of plasma pH and arterial O_2 saturation and by using a concentration fraction of water in plasma and red blood cells of 0.938 and 0.720, respectively: ra=2.5814−0.2640× (pHa)−[0.2203−0.0376×(pHa)]×(1−SaO_2). $CaCO_2d$ is finally calculated assuming a Bunsen coefficient for red blood cells 0.00947 ml/100 ml mmHg lower than that of plasma. $CaCO_2d$=$PaCO_2$×[0.06868−0.00947×(Hct)]. Any increase in blood CO_2 concentration related to the increase in pCO_2 and to the simultaneous decrease in SaO_2, which takes place when arterial blood becomes venous, is calculated by considering these two components separately and by using the mathematical model developed and described in detail by Giovannini et al. [31]. This model simulates the equilibration of whole blood at increasing pCO_2 to determine the virtual values of combined CO_2 concentration in plasma and of plasma pH, resulting from the increase in pCO_2 from arterial to venous value at constant SaO_2. In a further step the increase in blood CO_2 concentration that is related to the decrease in O_2 saturation from arterial to venous value at constant pCO_2 is then calculated. The total increment in CO_2 concentration from arterial to venous blood is finally calculated from the sum of the two changes.

We compared previously the calculated and measured systemic venous-arterial CO_2 content difference (measured as systemic arterial-venous O_2 content difference multiplied by RQ obtained from indirect calorimetry [51]). The coefficient of correlation was 0.772 ($p<0.001$).

The mean of the calculated venous-arterial CO_2 content difference was 3.52±0.95, and the mean of the measured venous-arterial CO_2 content difference was 3.97±1.06. The difference between the means was therefore 0.44±0.69, and the 95% confidence interval was −0.63 to −0.25, and significantly different from zero ($p<0.001$).

Although the standard deviation of the means was similar to the one reported by Giovannini et al. (0.69 vs. 0.59), we found a bias which was not reported in their investigation. Since we were looking at changes in venous-arterial CO_2 content difference rather than absolute values, this bias is unlikely to confound the results.

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