Evaluation of the use of lower body perfusion at 28°C in aortic arch surgery†

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INTRODUCTION

During complex repair of the aortic arch, hypothermic circulatory arrest (HCA) and selective cerebral perfusion (SCP) are widely accepted as standard neuroprotective procedures, when performed at hypothermic temperatures of 18–25°C [1, 2]. Although there is no lower body perfusion under these conditions—referring to the distal spinal cord, the mesenteric tissue and the lower extremities—significant postoperative end-organ injuries were reported only in the case of a preoperatively existing malperfusion syndrome [3]. More frequently, mesenteric malperfusion was observed after surgery of the descending aorta or the thoracic endovascular aortic repair in complicated type B dissections [4].

In the past 10 years, a clinical trend towards a less aggressive cooling policy for acute type A aortic dissection (ATAAD) repair could be observed [5]. Some European centres adopted a gradual increase in body temperature towards 30°C during SCP, in order to reduce the CPB-related complications like persistent bleeding and enhanced inflammatory reaction postoperatively [6]. Although the Hannover group reported a larger series of...
patients with AATAD, where prolonged SCP (>60 min) at moderate hypothermia (25–28°C) resulted in a significant higher mortality (27%), the subanalysis of complications following prolonged SCP did not include investigations regarding mesenteric ischaemia [7].

Since 2006, we have adopted the moderate cooling strategy for aortic arch surgery in our centre, raising the cooling temperature from deep hypothermia 18–24°C to a moderate 28°C. In a series of 122 consecutive patients with ATAAD and an overall mortality rate of 16.4%, we observed three cases of post-ischaemic multiple organ failure—including, finally, intestinal ischaemia (2.5%) and one case of isolated mesenteric ischaemia (0.8%). In all four cases, the SCP time exceeded 60 min. Our concern, supported also by others [8], that moderate hypothermic HCA and SCP may lead to an increased mesenteric vulnerability to prolonged ischaemia times of the lower body led us to the setting of the present study.

For this reason, we investigated, in an acute porcine model, if isolated SCP performed at 28°C for 60 min offers a sufficient mesenteric residual blood flow through thoraco-abdominal collateral vessels and if a concomitant low-flow perfusion of the lower body (CLBP) has a beneficial effect on the mesenteric oxidative stress level and the early inflammatory response in the jejunal and colonic tissue.

**MATERIALS AND METHODS**

Fourteen female pigs, ~4 months of age, with a weight of 35–45 kg, received preoperative humane care in compliance with the guidelines of the European Convention on Animal Care. After induction of anaesthesia, connecting to the CPB and systemic cooling to 28°C, the animals were randomly divided in two groups with different perfusion protocols:

(i) SCP group: 10 min of HCA (28°C), followed by 60 min of SCP at 10 ml/kg/min, with lower body ischaemia (n = 7);  
(ii) CLBP group: 10 min of HCA (28°C), followed by 60 min of SCP at 10 ml/kg/min and lower body perfusion (LBP) at 20 ml/kg/min (n = 7).

After systemic rewarming to 37°C, all animals were weaned from CPB. Measurement of mesenteric blood flow, metabolic parameters, early inflammatory activity and oxidative stress level into the mesenteric organs were made before, during and after SCP and CLBP.

**Perioperative management and anaesthesia**

After pre-treatment with intramuscular azaperon (2 mg/kg) and ketamine (15–20 mg/kg), the animals were anaesthetized with propofol 1% (1–2 mg/h), fentanyl (25 μg/kg/h) and midazolam (0.2 mg/kg/h). Paralysis was achieved with intravenous pancuronium (0.2 mg/kg/h). After endotracheal intubation, mechanical ventilation (Fabius, Dräger, Germany) was performed with a 0.5 FiO₂, in order to maintain a pCO₂ between 30 and 50 mmHg, and a Pao₂ above 100 mmHg. A temperature probe was placed in the oesophagus. Two 14 G arterial lines were placed for upper and lower body pressure monitoring and arterial blood sampling (Blood Gas Analyzer, ABL Radiometer Copenhagen, DK, Denmark): one was placed in the right intern carotid artery, and the other via the right femoral artery into the abdominal aorta.

**Operative technique**

With the pig in a supine position, a right-sided laparotomy was performed in the upper quadrant. The portal vein was identified and a venous catheter inserted and fixed on the portal vessel wall with 5–0 Prolene. The jejunum and the colon were mobilized and two jejunal and two colonic tissue samples (0.25 cm²), respectively, were taken for baseline measurement: one for immunohistochemical analysis and the other for real-time polymerase chain reaction (PCR). The lesions were closed with 5–0 Prolene, then the intestines gently repositioned and the laparotomy closed.

After turning the pig to a right-sided position, the chest was opened via a small left thoracotomy in the fourth intercostal space. The ascending and descending aorta were dissected and vessel loops were placed to define the future levels of clamping (Fig. 1). After heparinization (300 IU/kg), an 18 F arterial cannula (Fem-Flex II, Edwards Lifescience, Irvine, CA, USA) was inserted into the aortic arch, and a single 26 F venous cannula into the right atrium. Non-pulsatile CPB was initiated at a systemic flow rate of 80–100 ml/kg/min and then adjusted to maintain a minimum mean arterial pressure (MAP) of 50 mmHg. A 10 F vent catheter was inserted via the left atrium, and later used as an injection port for fluorescent microsphere injection. Systemic cooling was achieved using an heat exchanger (Biomedicus, Medtronic, MN, USA).

The cardiopulmonary bypass circuit included roller pumps (Stöckert Instr., München, Germany), cardiomyocyte reservoir and a membrane oxygenator (VPCML Plus, Cobe Cardiovascular Inc., Arvada, CO, USA) which was primed with saline solution, heparin (5000 IU) and KCl (1.5 mq/kg). The pH was maintained by means of alpha-stat principles, at 7.40 with an arterial pCO₂ of 30–50 mmHg and uncorrected for temperature. The haemoglobin level was maintained between 7 and 10 g/dl.

Once stable CPB was established, cooling to systemic temperature of 28°C was undertaken. Cardiac arrest was achieved by clamping the ascending aorta and adding blood cardioplegia (blood + 30 ml of 14.9% KCl + 10 ml of 50% Mg) with an infusion...
rate of 3.5 ml/min for 2 min (induction) and 2 ml/min for 2 min (maintenance, every 20 min during cross-clamp time) through a 4 F cannula into the ascending aorta. Additional myocardial protection was afforded by applying cold saline (~8°C) into the pericardium.

After 10 min of HCA, a second aortic clamp was set on the descending aorta, and low-flow SCP started at 28°C with a perfusion rate of 10 ml/kg/min (SCP group). For additional perfusion of the lower body (CLBP group), an 15 F self-inflate retrograde cardioplegia cannula (Terumo Cardiovascular Systems, MI, USA) was inserted into the descending aorta (Fig. 1). Via a separate arterial line (Fig. 2), low-flow perfusion with 28°C cold blood from the same CPB circuit was performed for the lower body (LBP: 20 ml/kg/min).

After 60 min of SCP or CLBP, systemic CPB was re instituted by releasing the clamp and removing the self-inflating catheter from the descending aorta. Systemic rewarming was begun and continued to 37°C. During weaning from CPB, administration of 3–5 mg/kg/min dobutamine was routinely utilized. When necessary, cardiac defibrillation was performed after administration of lidocaine (1 mg/kg).

Regional jejunal and colonic blood flow (RBF)

Regional mesenteric blood flow was measured in the jejenum and colon using fluorescent microspheres as described for other target organs in previous studies [9]. In brief, approximately 2 million microspheres (Ø = 15 µm), in six different colours, were injected and flushed with 5 ml of saline solution into a left ventricular catheter before CPB and into the aortic cannulae during SCP and CLBP, respectively.

Before injection, the fluorescently labelled microspheres, suspended in 10% dextran with 0.05% polyoxymethylene sorbitan monoleate (Tween 80), were vortexted. To allow calculation of absolute blood flow rates, a reference blood sample was taken from the descending aorta (right femoral artery line) at a rate of 2.9 ml/min with a Harvard withdrawn pump (Harvard Bioscience, Inc, Holliston, MA, USA). The withdrawal of blood started 10 s prior to injection of the microspheres and continued for 110 s after the microsphere injection.

The animals were sacrificed 60 min after successful weaning of CPB with an intravenous injection of sodium pentobarbital (30 mg/kg) and saturated potassium chloride (6 mEq/kg).

Jejunal and colonic tissue samples (0.6–1.2 g) were taken for microscope count. Microspheres were recovered from the tissue and from the blood by using a commercial protocol (NuFlow™ Extraction protocol 9507.2, Interactive Medical Technologies Ltd., Irvine, CA, USA). Fluorescent analysis was carried out by the same company.

Regional jejunal and colonic blood flow (RBF) was then calculated from the intensity of fluorescence microspheres in blood and tissue samples using the following formula:

\[
\text{RBF} (\text{ml} \times 100g^{-1} \times \text{min}^{-1}) = \frac{(R \times I_F)}{(I_R \times W_t)}
\]

Where R was the rate at which the reference blood sample was withdrawn (2.9 ml/min); \( I_F \) was the fluorescence intensity of the tissue sample; \( I_R \) was the fluorescence intensity of the blood sample and \( W_t \) was the weight of the tissue sample (g).

Study protocol

Haemodynamics such as heart rate (HR), MAP as well as systemic temperature were monitored continuously (Omnicare 24C, Hewlett Packard, Böblingen, Germany). Additionally, arterial and portal venous blood gases, haemoglobin (Hb) and lactate levels were recorded and both colonic and jejunal RBF were calculated at six time points:

- at baseline;
- at 5 min of SCP/CLBP;
- at 60 min of SCP/CLBP;
- 5 min after weaning from CPB (off CPB);
- 30 min after weaning from CPB (off CPB);
- 60 min after weaning from CPB (off CPB).

Tissue and portal blood samples

Transmural jejunal and colonic tissue samples were taken at four time points: at baseline, at 60 min of SCP/CLBP, at 5 and 60 min after weaning from CPB. Prior to the sample harvesting, the laparatomy was opened and the intestines were exposed. Purse string sutures (5-0 Prolene) were placed around previously marked jejunal and colonic target areas. After harvesting of transmural tissue samples, 0.25 cm² in size, the intestinal lesions were closed and the areas treated with an antiseptic solution in order to prevent peritoneal contamination. At each time point, two jejunal and two colonic samples were taken. One of each was embedded in Tissue Tek O.C.T. (Sakura Finetek, Staufen,
Germany) and then shock frosted at −80°C in liquid nitrogen. Later on, these samples were used for RT-PCR. The second probe was immediately paraffin-embedded, to be later used for immunohistochemical staining.

At the same four time points, portal venous blood samples were taken via the previously inserted catheter. After citration, the blood was treated following a commercial protocol and analysed 24 h later using the FACS method for Oxy-DNA quantification.

Oxidative stress quantification

Oxidative DNA damage in portal venous blood cells was determined using a commercial fluorescent protein-binding method (Biotrin Oxy-DNA Test, Biotrin Biomarkers, USA; No: BIO81DNA) with a high specificity and avidity for 8-oxoguanine. The blood cells were fixed and permeabilized before being incubated with Oxy-DNA Test probe. After washing, the cells were studied using FACS.

Quantitative real-time polymerase chain reaction

Total RNA was prepared from snap-frozen colonic biopsies using Trizol reagent (Invitrogen, Darmstadt, Germany) and 1 µg total RNA was reverse transcribed to cDNA using the high-capacity cDNA reverse transcription kit (Invitrogen, Darmstadt, Germany), according to the manufacturer’s instructions. The expression levels of marker genes and the endogenous control (β-actin) were quantified by RT-PCR using Power SYBR green master mix with 10 ng cDNA and 2 nM target gene-specific primers per reaction on a Step-One Plus system (Applied Biosystems, Darmstadt, Germany). Cycling conditions were 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min and final extension at 60°C for 5 min. Each reaction was carried out in triplicate. Data analysis and calculation of relative expression levels were performed using Step-One software 2.2 and DataAssist software 2.0 (Applied Biosystems).

Immunohistochemical analysis

For immunohistochemical staining, freshly isolated jejunal and colonic tissue samples were paraffin embedded. The sections were then incubated with an appropriate concentration of primary antibody tumour necrosis factor (TNF-α), interleukin (IL) -6 or p38 followed by detection using the Alkaline Anti-Alkaline Phosphatase (APAPP) method (DCS Polymer System) according to the manufacturer’s instructions. Semi-quantitative assessment of immunoreactive signals was performed, using the following scoring system: + for no/occasional immunoreactive signal (<10 positive cells/high power field), ++ for a moderate immunoreactive signal (10–50 positive cells/high power field) and +++ for an intense immunoreactive signal (>50 positive cells/high power field).

Statistical methods

Randomization was carried out by the surgeon. The distribution of values was assured by a Shapiro–Wilk test. When the data were consistent with normality and equal variance assumptions, the groups were compared at different time points using repeated measures ANOVA, with tests for average differences between groups and for time-by-group interactions (change in the difference between groups over time). Otherwise, the groups were compared separately at each point in time using the Mann–Whitney U-test (between groups testing). Pairwise comparisons between groups (time point analyses) were conducted if the corresponding average difference or time-by-group interaction was significant. Hostelling–Spur and Friedman’s tests were used, as appropriate, for analysing changes over time for one group (within group testing). We report P-values unadjusted for multiple testing: their purpose is not meant as an exact global assessment but rather as a guide to help interpret the pattern of differences between groups at different times. Analyses were implemented by a certified biostatistician with SPSS software.

RESULTS

Comparability of experimental groups

All animals reached the final measurement time point of 60 min weaning from CPB. Preoperative animal weights were similar (SCP group: 37.1 ± 6 kg vs. CLBP group: 41.2 ± 4 kg) with no significant differences between the groups. As intended by the design of the study, blood gas analysis showed no significantly relevant differences between groups in arterial pH, partial oxygen and carbon dioxide pressure and haemoglobin value (Table 1). Systemic temperatures and HR did also not differ between groups.

Regional jejunal and colonic blood flow (RBF)

Baseline measurement showed a higher mean jejunal blood flow in the SCP group (62.5 ± 29 ml/min/100 g), when compared with the CLBP group (43.5 ± 27 ml/min/100 g). Nevertheless, this difference did not reach statistical significance (P = 0.075). During isolated SCP at 28°C, jejunal RBF decreased to 2.7% (5 min SCP) and 3.7% (60 min SCP) of the baseline value (SCP group), whereas combined SCP and LBP resulted in a significant higher residual flow of 46.6–54.3% (P = 0.001, CLBP group). After rewarming and going off CPB, it came to a significant increase in jejunal perfusion in the SCP group, with flow values up to 162.7% of the initial baseline value (P = 0.002), whereas the CLBP group showed almost physiological jejunal flow values (Table 2, Fig. 3).

Colonic blood flow baseline measurement presented, similar to the jejunal flow baseline values, a higher RBF in the SCP group (83.1 ± 45 vs. 67.9 ± 37 ml/min/100 g; P = 0.541). During SCP, the colonic RBF decreased significantly to 2% (5 min SCP, P = 0.012) and 2.7% (60 min SCP) of the baseline. At the same measurement time points, it showed a significantly higher residual colonic flow of 42.3 and 43.6% in the CLBP group.

During reperfusion and weaning from CPB, the colonic RBF recovered slower in the SCP group, showing only 61.1% of the baseline value at 5 min off CPB (P = 0.012). At 30 min off CPB, a short interval of post-ischaemic colonic ‘luxury perfusion’ could be observed in the SCP group (128.4% of baseline, P = 0.069), which turned to physiologic values 60 min after weaning from CPB. The CLBP group showed almost physiologic colonic flow.
values during reperfusion and after weaning from CPB (Table 2, Fig. 3).

### Lactate in the portal venous blood

As seen in Table 2 and Fig. 3, portal venous lactate levels were similar in both groups at control measurement. During SCP and CLBP, lactate values rose above baseline, reaching a maximum of $15.1 \pm 1.5$ mmol/l in the SCP group and of $11.9 \pm 2.8$ mmol in the CLBP group ($P_{60 \text{ min}, \text{SCP}} = 0.01$). Portal blood lactate levels remained elevated throughout the recovery interval in both groups, showing significant higher values in the SCP group ($P = 0.017$).

### Oxydative stress (Oxy-DNA)

Due to a high variance of baseline values, Oxy-DNA levels were compared as absolute values, as well as a procentual change of baseline at a certain time point. Nevertheless, there was no difference of mean Oxy-DNA levels at baseline measurement.
between groups. During HCA and SCP/CLBP, the oxidative stress increased (Fig. 4: shift to the right on OX axes), reaching 137% of baseline (SCP) and 129% (CLBP). After reperfusion, pH-buffering and CPB-weaning the Oxy-DNA levels decreased in both groups (Fig. 4: shift to the left on OX axes), reaching baseline levels at 60 min after weaning from CPB. Differences between the groups showed no significance over time (Table 3).

**Unspecific inflammatory reaction (TNF-α, IL-6, COX, p38)**

Quantitative real-time PCR analysis of mucosal cytokines revealed a poor dynamic of TNF-α changes over time, with no differences between groups, reaching 0.9 ± 0.3 (SCP) and 0.7 ± 0.3 (CLBP) of the initial baseline at 60 min off CPB. In contradiction to this, IL-6 and COX expression increased significantly over time, reaching significantly higher levels in the SCP group (29.3 ± 0.3 vs. 1.8 ± 1.6 for IL-6, P = 0.027; 7.4 ± 2.6 vs. 2.9 ± 2.0 for COX, P = 0.016) (Table 3).

An increase in unspecific inflammatory markers could be also observed using semi-quantitative assessment of immunoreactive signals for TNF-α, IL-6 and p38 in the colonic mucosa.

Immunohistochemical analysis revealed a more intensive signal for TNF-α and IL-6 in the SCP group (+++) vs. (+) in the CLBP group. Additional marking of p38 showed a more intense immunoreactive induction in the SCP group (+++) vs. the CLBP group (++) as seen in Fig. 5.

**DISCUSSION**

Visceral organ dysfunction due to mesenteric ischaemia and reperfusion injury following cardiac surgery is a rare (<1%) but serious complication, with an increased mortality (>50%) [10]. Especially, in aortic arch surgery, visceral malperfusion and ischaemia dramatically reduce the chance of a successful outcome [11]. For treatment of ATAAD, two possible patho-mechanisms for mesenteric ischaemia can be distinguished: a preoperative existent visceral malperfusion syndrome, due to arising of the mesenteric arterial branches from the false lumen (16–33% of all type A dissections) [12], and a non-occlusive mesenteric injury caused by prolonged ischaemia of the lower body and inadequate hypothermic protection. Both patho-mechanisms may coincide, being often undetected until clinical signs of an acute bowel syndrome appear.

The use of an antegrade perfusion of the lower body during distal open anastomosis is not new. Hirose et al. [13] used a balloon catheter for the same purpose. In a clinical study published in 2002, Takagi et al. [14] described the successful use of an aortic balloon occlusion catheter for perfusion of the lower body during distal anastomosis in aortic arch surgery. With this technique, they performed a lower body perfusion for 30 ± 17 min, with a flow rate of 33 ± 8 ml/kg/min, reaching a pressure of 44 ± 12 mmHg in the lower extremity. Nevertheless, these studies were performed in an era in which deep (below 20°C) or moderate hypothermia (25°C) was used for organ, and especially cerebral, protection. At these degrees of cooling, the abdominal organs were well protected, so that an additional blood supply to the lower body could be avoided for the duration of 30–45 min. Since, as mentioned in our paper and published in larger clinical series by others, the trend in recent years goes toward a less aggressive cooling policy, the topic regarding protection of the lower body during prolonged SCP gains in our opinion new importance.

Clinical studies have identified the duration of CPB, need of post-operative blood perfusion and significant use of vasopres-sor inotropes as risk factors for a decrease in mesenteric blood flow, leading finally to mesenteric non-occlusive ischaemia [15].

The aim of this study was to determine the benefit potential of a low-flow lower body perfusion during SCP in complex aortic arch surgery, looking at three different levels:

1. Haemodynamic/metabolic clinical data (RBF/portal venous lactate levels);
2. Levels of Oxy-DNA determined by FACS analysis for oxidative stress quantification;
3. Semi-quantitative evaluation of cytokine expression (real-time PCR) and immunoreactive signals (immunohistochemical analysis) in the jejunal and colonic mucosa.

Haemodynamic data showed that the residual jejunal and colonic blood flow during isolated SCP—with no direct perfusion of the descending aorta—reaches only 2–3.7% of the normal mesenteric blood flow. These conditions were unphysiological enough, to let the portal lactate level increase, exceeding 15 mmol/l in the SCP group. Earlier, serum lactate was identified...
to be the most useful and sensitive early diagnosis criteria and follow-up control tool for mesenteric ischaemia [16]. During reperfusion, the RBF showed a sudden increase, leading to ‘excess of perfusion’ in the early post-ischaemic period. Combined cerebral and lower body perfusion resulted, even at a low-flow perfusion rate, in a significant higher mesenteric blood flow (during CLBP) and almost physiologic post-ischaemic RBF and lower lactate levels, respectively. These findings suggest that low-flow perfusion with a pump-flow rate of 20 ml/kg/min could be benificial for more extended no-flow intervals in the descending aorta.

FACS analysis of Oxy-DNA showed an increase in oxidative stress during SCP/CLBP in both groups, with a slightly more pronounced character in the SCP group. Nevertheless, these changes were observed in blood cells. Especially, the leukocytes are known to be extremely stress-resistant, so that the prompt recovery of stress levels to baseline values during reperfusion and pH-buffering may be explained by this.

Figure 4: Time course of Oxy-DNA levels in the portal venous blood cells of one animal at four different time points: at baseline, 60 min SCP, 5 and 60 min off CPB. The FACS (right sided) were converted for quantification in Histograms (left sided). OX-axes: fluorescence intensity; OY-axes: cell number. A shift to the right represents an increase of Oxy-DNA.
Previous studies have demonstrated that pro-inflammatory cytokines, such as IL-6 and TNF-α, activate the p38 mitogen-activated protein kinase (MAPK) pathway [17, 18] and suggested that the p38 MAPK family plays important roles in pathophysiology of inflammatory mesenteric diseases [19, 20].

We used PCR and immunohistochemical analyses as two different measurement methods for detection of inflammatory activity: whereas PCR identifies the genetic expression of TNF at a very early stage, the immunohistochemical visualizes the already present factor in the mucosa. Evaluating the effect of the two different perfusion strategies on the level of the major inflammatory cytokines using PCR, we found significantly higher IL-6 and COX expression in the SCP group. The same findings were seen using a different method, the semi-quantitative

### Table 3: Oxydative stress (FACS) and inflammatory reaction levels (real-time PCR)

<table>
<thead>
<tr>
<th>Variable/analysis</th>
<th>Baseline</th>
<th>60 min SCP</th>
<th>5 min off CPB</th>
<th>60 min off CPB</th>
<th>Over time within one group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxy-DNA/FACS (fluorescence intensity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SCP group</td>
<td>5594 ± 3873</td>
<td>9107 ± 10410</td>
<td>8745 ± 10498</td>
<td>7151 ± 7693</td>
<td>P = 0.528</td>
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<tr>
<td>CLBP group</td>
<td>5464 ± 1915</td>
<td>5445 ± 11123</td>
<td>6323 ± 2105</td>
<td>6259 ± 1886</td>
<td>P = 0.633</td>
</tr>
<tr>
<td>Between groups</td>
<td>P = 0.087</td>
<td></td>
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<tr>
<td>TNF-α/quantitative RT-PCR (relative expression vs. baseline)</td>
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</tr>
<tr>
<td>SCP group</td>
<td>1.0 ± 0</td>
<td>1.1 ± 0.6</td>
<td>0.9 ± 0.3</td>
<td>P = 0.368</td>
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</tr>
<tr>
<td>CLBP group</td>
<td>1.0 ± 0</td>
<td>1.1 ± 0.6</td>
<td>0.7 ± 0.3</td>
<td>P = 0.504</td>
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<tr>
<td>Between groups</td>
<td>P = 0.900</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>COX/quantitative RT-PCR (relative expression vs. baseline)</td>
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<tr>
<td>SCP group</td>
<td>1.00 ± 0</td>
<td>6.0 ± 4.7</td>
<td>7.4 ± 2.9</td>
<td>P = 0.022</td>
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<tr>
<td>CLBP group</td>
<td>1.00 ± 0</td>
<td>1.4 ± 0.8</td>
<td>2.6 ± 2.0</td>
<td>P = 0.449</td>
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<tr>
<td>Time point analysis</td>
<td>P = 1.000</td>
<td>P = 0.062</td>
<td>P = 0.016</td>
<td></td>
<td></td>
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<tr>
<td>Between groups</td>
<td>P = 0.008</td>
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<tr>
<td>IL-6/quantitative RT-PCR (relative expression vs. baseline)</td>
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</tr>
<tr>
<td>SCP group</td>
<td>1.00 ± 0</td>
<td>23.3 ± 41.0</td>
<td>n.a.</td>
<td>29.3 ± 22.7</td>
<td>P = 0.165</td>
</tr>
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<td>CLBP group</td>
<td>1.00 ± 0</td>
<td>1.6 ± 2.2</td>
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<td>P = 0.623</td>
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<td>P = 0.222</td>
<td>n.a.</td>
<td>P = 0.008</td>
<td></td>
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<tr>
<td>Between groups</td>
<td>P = 0.008</td>
<td></td>
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</table>

Values are shown as mean ± standard deviation. Between group: P-values for two way ANOVA; time point analysis was done if P < 0.05 in ANOVA testing; within group: changes over time within groups (Friedman’s test).

![Figure 5: Immunohistological analysis for semi-quantitative assessment of immunoreactive TNF-α, IL-6 and p38 signals in the colonic mucosa. +, no/occasional (<10 positive cells/HPF); ++, moderate (10–50 positive cells/HPF) and ++++, intense immunoreactive signal (>50 positive cells/HPF).](image)
immunohistochemical analyses, which indicated a markedly increased expression of TNF-α, IL-6 and p38 at the end of the experiment in the SCP group, and almost physiological levels in the CLBP group. Similar dynamics observed with two different methods led us to the conclusion that lower body ischaemia during SCP—if performed for more than 60 min—leads to a pronounced activation of immunological pathways, which may produce further mesenteric complications in the early operative period.

**Conclusion**

Using an acute large animal model, we demonstrate that a separate perfusion of the upper and lower body is feasible, without major complications. This strategy, using a self-inflating balloon-tip cannula for the descending aorta, may be transposed—with some sizing adaptations—into the human operating room, although we admit that it makes the procedure cumbersome. For this reason, it may have its justification during prolonged aortic arch operations at moderate or mild hypothermia, and in complex aortic arch repair combined with endovascular treatment of the descending aorta [21]. These findings should be considered for development of further perfusion strategies in aortic arch surgery.

**Limitations of the study**

In a series of pilot animals, we reached pressure levels of over 40 mmHg in the lower body at a flow rate of 30 ml/kg/min. This coincides with the findings of other groups [13]. Unfortunately, the tightness of our self-inflating catheter (retrograde cardioplegia catheter) was not given at higher pressure levels and we had backflow leaking between the balloon and the aortic wall. At a flow rate of 20 ml/kg/min, these problems did not occur anymore. We admit that the optimal perfusion flow rate for lower body perfusion should be higher than that used in our study. But as we could show, this low-flow perfusion was enough to produce less lactate elevation providing a considerable mesenteric flow level.

Regarding the detection of inflammatory reaction, we used two different methods which allow only semi-quantitative evaluations. The fact that the ‘earlier stage’ detecting method (PCR) showed no significant difference between the groups may be caused by the small number of animals, and by the high variability of inflammatory reaction produced by extracorporeal circulation.

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**Conflict of interest:** none declared.

**REFERENCES**


**APPENDIX. CONFERENCE DISCUSSION**

Dr G. Bol Raap (Rotterdam, The Netherlands): I have two questions. As we know, the gold standard for repair of the aortic arch is deep hypothermic arrest at temperatures of 18–25°C and selective cerebral perfusion. Did you consider measuring the baseline for lower temperatures? That is the first question.

And the second question, you showed that CLBP causes higher mesenteric flow than SCP, and both groups show a huge rise in lactate. And also at a point of 60 min off pump, there are still high levels of lactate. Do you think that will have consequences in the clinic?
Dr Haldenwang: Coming to the first question, the trend in the last five years, at least in Germany, is towards less deep hyperthermia. The groups from Frankfurt and Cologne, as well as our group from Bochum, perform aortic arch repair, even in an acute situation, at temperatures of 27, 28°C. In Bochum we use cannulation of the right subclavian artery for going on bypass. Clamping the brachiocephalic trunk, we switch from systemic perfusion to a unilateral flow to the brain, so that we do not actually have a complete hyperthermic circulatory arrest. Then when we start SCP, actually we open the arch, inspect its pathology and put the second selective cannula in the left carotid artery.

With this strategy we do not need 18 or 20°C Celsius which takes a long time to lower the temperature and a much longer time to recover to normality.

Dr Bol Raap: I know, yes.

Dr Haldenwang: That is the reason why we looked at this temperature and not at other temperatures because at 20°C, of course, we suppose that the mesenteric tissue is better protected. At 28°C this certainty is not given any more.

Dr Bol Raap: I know. But you did a very nice study, and it would be nice also to have measurements at temperatures lower than 28°C Celsius.

Dr Haldenwang: Okay. And the second questions about the lactate. Of course, lactate is just one measured parameter. We know that a level of 15 mmol/l is high, especially in pigs. High lactate levels of 11 and 15 mmol/l were seen in all seven animals of the isolated SCP group; all 7 animals had the same trend. Of course, 7 animals is a very low number. Maybe we have to increase the number of investigated animals in subsequent experiments. But what we saw here was a certain trend, and that is what we reported.

Dr Bol Raap: Yes. But the question was, do you think it will have clinical relevance?

Dr Haldenwang: Yes, it may have.

Dr Bol Raap: Okay.