Eur J Vasc Endovasc Surg (2019) 58, 891–901

Cold Renal Perfusion During Simulation of Juxtarenal Aortic Aneurysm Repair Reduces Systemic Oxidative Stress and Sigmoid Damage in Rats

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WHAT THIS PAPER ADDS

Complications of juxtarenal aortic aneurysm repair include renal ischaemia reperfusion injury and post-operative colonic ischaemia. Renal ischaemia reperfusion injury results in the production of reactive oxygen species during oxidative stress. In the present study renal cooling preserved renal function. Above all, renal cooling was able to reduce the detrimental remote effects on sigmoid integrity of renal ischaemia reperfusion caused by suprarenal clamping; by preserving microcirculatory flow, attenuating circulating leukocyte ROS production, and decreasing leukocyte infiltration in the sigmoid.

Objectives: Juxtarenal aortic surgery induces renal ischaemia reperfusion, which contributes to systemic inflammatory tissue injury and remote organ damage. Renal cooling during suprarenal cross clamping has been shown to reduce renal damage. It is hypothesised that renal cooling during suprarenal cross clamping also has systemic effects and could decrease damage to other organs, like the sigmoid colon.

Methods: Open juxtarenal aortic aneurysm repair was simulated in 28 male Wistar rats with suprarenal cross clamping for 45 min, followed by 20 min of infrarenal aortic clamping. Four groups were created: sham, no, warm (37 °C saline), and cold (4 °C saline) renal perfusion during suprarenal cross clamping. Primary outcomes were renal damage and sigmoid damage. To assess renal damage, procedure completion serum creatinine rises were measured. Peri-operative microcirculatory flow ratios were determined in the sigmoid using laser Doppler flux. Semi-quantitative immunofluorescence microscopy was used to measure alterations in systemic inflammation parameters, including reactive oxygen species (ROS) production in circulating leukocytes and leukocyte infiltration in the sigmoid. Sigmoid damage was assessed using digestive enzyme (intestinal fatty acid binding protein - I-FABP) leakage, a marker of intestinal integrity.

Results: Suprarenal cross clamping caused deterioration of all systemic parameters. Only cold renal perfusion protected against serum creatinine rise: 0.45 mg/dL without renal perfusion, 0.33 mg/dL, and 0.14 mg/dL ($p = .009$) with warm and cold perfusion, respectively. Microcirculation in the sigmoid was attenuated with warm ($p = .002$) and cold renal perfusion ($p = .002$). A smaller increase of ROS production ($p = .034$) was seen only after cold perfusion, while leukocyte infiltration in the sigmoid colon decreased after warm ($p = .006$) and cold perfusion ($p = .018$). Finally, digestive enzyme leakage increased more without (1.5AU) than with warm (1.3AU; $p = .007$) and cold renal perfusion (1.2AU; $p = .002$).

Conclusions: Renal ischaemia/reperfusion injury after suprarenal cross clamping decreased microcirculatory flow, increased systemic ROS production, leukocyte infiltration, and I-FABP leakage in the sigmoid colon. Cold renal perfusion was superior to warm perfusion and reduced renal damage and had beneficial systemic effects, reducing sigmoid damage in this experimental study.

Keywords: Animal experimental study, Cold renal perfusion, Renal ischaemia reperfusion, Sigmoid damage, Suprarenal aortic cross clamping, Systemic oxidative stress damage

Article history: Received 25 February 2019, Accepted 29 May 2019,

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1078-5884/

https://doi.org/10.1016/j.ejvs.2019.05.021

INTRODUCTION

Open repair is the gold standard for fit patients with juxtarenal aortic aneurysms (JAA). Open surgery has good long term results but has a high impact on the patient recovery capacity and is associated with substantial peri-operative

morbidity and mortality.[1–7] Temporary aortic cross clamping proximal to the renal arteries induces renal ischaemia and reperfusion (I/R) and causes acute kidney injury (AKI) with deterioration in renal function in up to 40% of patients.[2] A previous observational study described post-operative serum creatinine increases in the majority of patients undergoing elective JAA repair with a mean clamp time of nearly 40 min.[8] The extent of AKI increases with suprarenal clamp time and might necessitate (temporary) dialysis in some patients.[1–4,9–12] Furthermore, damage to other organs and even multiple organ failure (MOF) may occur.[1–3,5–7,9] The sigmoid colon is particularly vulnerable to I/R during suprarenal cross clamping (SRC) because in patients with aortic aneurysm there often is a delicate circulatory equilibrium in an atherosclerotic splanchnic vascular system.[13] Changes in systemic circulation during open aortic surgery and temporary or permanent occlusion of the inferior mesenteric artery (IMA) may cause an imbalance in this equilibrium, leading to sigmoid ischaemia in up to 5% of patients undergoing elective JAA repair,[1–3,5–7,9–17] compared with more than 10% of the patients undergoing repair for ruptured abdominal aneurysms.[16,18]

Post-operative renal failure may have a deleterious effect on remote organs. During AKI, tubular and epithelial cell necrosis occurs thereby reducing renal elimination of asymmetrical dimethylarginine (ADMA) and production of arginine (an endogenous inhibitor of nitric oxide (NO) synthase and a NO precursor respectively).[19–21] As a result, NO bioavailability decreases leading to a dysregulation of haemodynamics and a systemic vasoconstrictive effect.[19–21] Moreover, it was demonstrated previously in an animal model that suprarenal clamping followed by infrarenal clamping, as performed during JAA repair, leads to increased renal damage and oxidative stress caused by I/R of the lower limbs.[22] Cold renal perfusion during the suprarenal clamping period could reduce kidney damage and preserve the bioavailability of NO.[23] Furthermore, renal tubular damage was reduced and renal extraction of dimethylarginines was preserved, leading to lower oxidative stress in the kidneys.[23] The same effect was also demonstrated in single centre observational cohort studies, as cold renal perfusion reduced the incidence of AKI after elective open JAA repair. Interestingly, renal cooling during JAA repair has a protective effect on the development of MOF after acute JAA repair.[8,24] Speculating on the mechanism, it could be that during I/R in general, oxidative stress occurs in circulating leukocytes. This results in the accumulation of intracellular reactive oxygen species (ROS), potentially exceeding the compensatory capacity of antioxidants, causing damage to DNA and membranes.[25–27] Finally, during AKI, leukocytes systemically infiltrate distant visceral organs.[5,6,14,28,29] These three parallel events might be the driving forces behind MOF.

Therefore, the present study aimed to prove the effects of (cold) renal perfusion on systemic oxidative stress, renal failure, and visceral organs and their interactions. Specifically, enterocyte necrosis was investigated in the sigmoid, because the occurrence of sigmoid ischaemia is significant after SRC. Therefore the primary outcomes measured were pre- and post-operative creatinine levels and renal tubular damage, as an indicator of renal I/R injury, and intestinal fatty acid binding protein (I-FABP) leakage as a marker of sigmoid damage. It is hypothesised that enterocyte necrosis increases as a result of I/R injury caused by JAA repair. By reducing AKI after JAA repair, cold renal perfusion may decrease the systemic effects of renal and lower limb I/R and thereby prevent sigmoid damage. To examine such an effect, enterocyte necrosis, tubular damage, microcirculation and parameters affecting systemic microcirculation and inflammation were determined. The aim was to examine the relationship between renal cooling and a reduction of the systemic oxidative effects.

MATERIALS AND METHODS

Experimental protocol

The present experimental protocol was based on previously published studies in which JAA repair was simulated in a rat model.[22,23] Animal surgery and care were performed according to established guidelines and approved by the Animal Ethical Commission VU University Medical Centre, Amsterdam, the Netherlands (protocol number FYS 07—08). Four groups of seven adult male Wistar rats were created (350 g; Harlan), every day a rat from another group was operated on sequentially. These rats have a vascular anatomy suitable to simulate the effects of suprarenal clamping, because it is comparable with the human anatomy. However, there are differences, as Wistar rats have an aortic trifurcation and a more distinct collateral circulation.[30] All rats received buprenorphine hydrochloride 0.03 mL intramuscularly and were anaesthetised with isoflurane 3%. The anaesthesia was maintained with continuous isoflurane 1.5—2.0%. Rats were then intubated and ventilated (Merlin Small Animal Ventilator, Ventronic services, Devon, UK) with a 50% oxygen and air mixture. Body temperature was continuously monitored by a rectal probe and maintained at around 36.0 °C. The right jugular vein was cannulated for intravenous Ringer's lactate infusion, and the left carotid artery to continuously monitor intra-arterial blood pressure and collect blood samples. Three blood samples (0.3 mL) were collected (start experiment, start renal perfusion, and end experiment), followed by infusion of 0.3 mL colloid suspension. Blood samples were centrifuged (7000 rpm) for 15 min and blood plasma was stored at −80 °C until analysis.

JAA repair and sample collection were performed by the same investigator in all experiments. Surgery was performed every day from another group sequentially. After JAA repair simulation as described below, all rats were euthanised by immediate excessive bleeding by harvesting the kidneys at the branch of the renal arteries while anaesthesia was maintained. Subsequently, autopsy was performed to detect potential macroscopic signs of systemic inflammatory tissue injury. Sigmoid and renal tissue was harvested from two different sections in the sigmoid and two different sections per kidney, and then stored

at −80 °C for microscopy and immunofluorescence semi-quantification analysis.

Semi-quantitative immunofluorescence microscopy

From each harvesting site three kidney and sigmoid cryosamples (thickness 5 µm) were fixed in 4% formaldehyde solution (Sigma—Aldrich, St. Louis, MO, USA) with phosphate buffered saline (PBS) for 10 min at 21 °C. Prior and subsequent to incubation with both primary and secondary antibodies (Alexa-488 labelled diluted in PBS 1:50), sections were washed three times in PBS with 0.05% between (PBST, Sigma—Aldrich) at 21 °C. Negative controls were not incubated with the primary antibody. All sections were stained for 20 min with Weath Germ Agglutinin (WGA) (rhodamine labelled diluted in PBS 1:50) and washed with PBST as described above.

Finally, the sections were stained and sealed with cover slips and Vectashield, Hard Set Mounting Medium with 4′,6-diamidino-2-phenylindole (DAPI, which stained nuclei), H-1500 (Vector Laboratories, Burlingame, CA, USA).

A Zeiss Axiovert 200M Marianas inverted microscope, with four epifluorescence channels (green: fluorescein isothiocyanate, secondary antibodies, red: cyanine dyes, rhodamine-WGA stained cell membranes, blue: aminomethylcoumarin acetate, cell nuclei, and a differential interference contrast), was used to examine cryosections after staining with secondary antibodies. Images were recorded with a 16 bit camera (Cooke Co., Tonawanda, NY, USA) and Slidebook 4.1.0.10 (Intelligent Imaging Innovations, Denver, CO, USA) was used to quantify fluorescent channels using 10 × or 40 × air objective lenses. The sum intensity immunofluorescence levels were corrected for area size and non specific background and autofluorescence (blank). Mean values per rat were used for statistical analysis.

Juxtarenal aortic aneurysm repair simulation groups

The surgical protocol has been published before, the clamping sequence and times were carefully chosen based on clinical studies.[8,22–24] In previous animal models, the effect of I/R injury in the kidneys could be seen clearly after 45 min of suprarenal clamping, 20 min of infrarenal clamping (simulating the mean time to perform the distal anastomosis), and 70 min of total body reperfusion (90 min of renal reperfusion). Moreover, the protective effect of renal cooling on renal tubular damage and renal function was shown. The present study used the same surgical protocol, but the systemic effects of renal cooling on sigmoid damage were investigated.

Group 0 (sham): after midline incision, the abdominal aorta, renal arteries, and kidneys were exposed. During a 30 min period, all rats were stabilised with no intervention or aortic cross clamping.

Group 1 (no perfusion): JAA repair was simulated by suprarenal and distal aortic clamping just above the aortic bifurcation (i.e. above the aortic trifurcation) for 45 min, to mimic lower body ischaemia and proximal anastomosis of an aortic tube graft. Then, the suprarenal aortic clamp was replaced to the infrarenal aorta to start renal reperfusion. Twenty minutes later (simulating the mean time to perform the distal anastomosis), all clamps were removed and reperfusion was allowed over 70 min (Fig. 1).[22,23] The rats received6 mL saline intravenously, to compensate for the volume the perfused groups received by renal cooling.

Group 2 (warm renal perfusion): JAA repair was simulated as described above. Following suprarenal

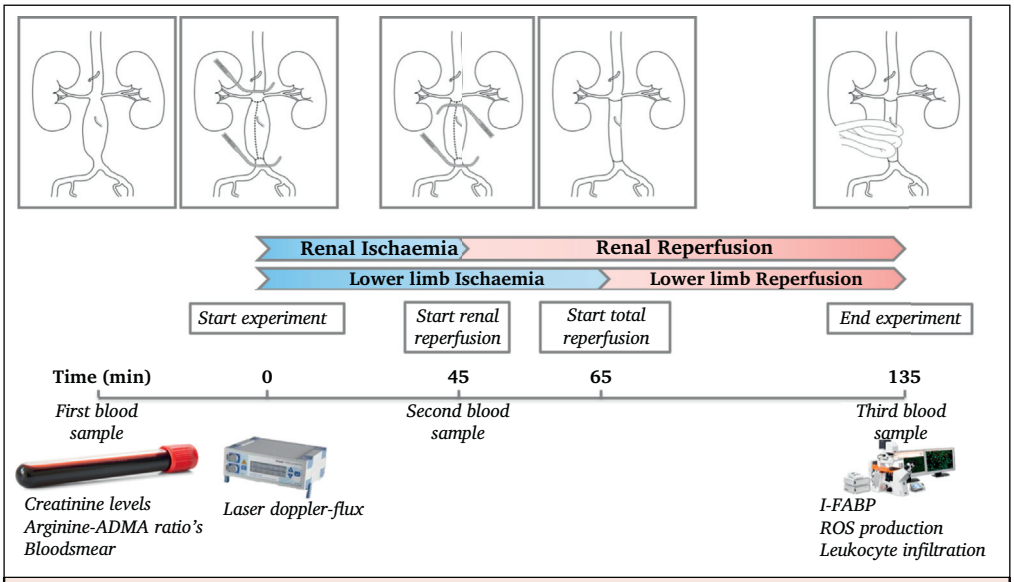

Figure 1. Flow chart showing the different stages, blood sample collections, and clamping positions during open aortic repair simulation. Left, the start of the experiment; right, the end of the experiment. ADMA = asymmetrical dimethylarginine; I-FABP = intestinal fatty acid binding protein; ROS = reactive oxygen species.

clamping, the infrarenal aorta was opened and a perfusion catheter was placed in front of the renal artery orifices and perfusion with saline at 37 °C was started with a 2 mL bolus followed by pump regulated continuous flow at 5 mL/h. Forty-five minutes later, the perfusion catheters were removed and antegrade blood flow to the kidneys was restored by placing the proximal clamp infrarenally. The aorta was closed, and 20 min later both clamps were removed followed by 70 min of total body reperfusion.

Group 3 (cold renal perfusion): The same procedure was performed as described in Group 2, but using saline at 4 °C.[22,23]

Endpoints

The outcomes were tubular necrosis and creatinine increase (to demonstrate renal damage), levels of serum arginine and ADMA, microcirculation, O^{2-} concentration in leukocytes, and expression of CD45 and ICAM-1 in the sigmoid mucosa (to characterise the systemic effects of renal I/R) and I-FABP leakage from enterocytes (to demonstrate sigmoid damage).

Renal damage. Renal tubular morphology was investigated in 40 round, perpendicularly sectioned renal tubules per slide. In total, 480 tubules were investigated, and brush border flattening and possible luminal obstruction were determined. Data are expressed in percentage of tubules with brush border damage

Serum creatinine in the blood was measured with high performance liquid chromatography (HPLC).[31] To detect AKI, absolute creatinine rise was calculated (pre-operative creatinine levels subtracted from end of procedure creatinine levels).

Systemic effects. Arginine and ADMA levels in the blood were measured together using HPLC.[31] To analyse the bioavailability of NO, the arginine/ADMA ratio was calculated (arginine divided by ADMA).[20,21]

At 10 strategic landmarks on the surface of the sigmoid not directly adjacent to major arteries or veins, the microcirculation was measured using a laser Doppler flux probe (periflux 4001 master, Perimed, Järfälla, Sweden). A 60 s normalisation period was applied. This probe measures the velocity and concentration of erythrocyte movement, expressed in arbitrary perfusion units. For analysis flow ratios were computed (mean end of procedure flow value divided by mean pre-operative flow value).

In a minimum of 100 circulating live leukocytes per blood sample, mitochondrial ROS production was studied. Leukocytes were acquired from the buffy coat of blood samples, after centrifugation (6000 rpm) at 4 °C, and were incubated with 900 µL of ADS (a HEPES buffering solution). Then stained sections were incubated with secondary antibodies; MitoSOX (red mitochondrial superoxide indicator diluted in PBS 1:50).

In sections from the sigmoid mucosa leukocyte infiltration was investigated. The sections were incubated with secondary antibodies; CD45 antibodies (Santa Cruz Biotechnology, diluted 1:50) and ICAM-1 antibody (GeneTex Inc, diluted in PBS 1:50) to study leukocyte infiltration and ICAM-1 expression.

Sigmoid damage. Leakage of I-FABP was measured from mature enterocytes in the sigmoid crypts, using rabbit polyclonal immunoglobulin G (IgG) antibody (Hycult biotechnology, diluted in PBS 1:25).

Statistical analysis

SPSS-23.0 (SPSS Inc, Chicago, IL, USA) was used for statistical data analysis. For normally distributed data, mean ± SD are provided and one way ANOVA with post-hoc Bonferroni were used to compare all groups and *t* tests to compare two groups. For non-normally distributed data,

Table 1. Outcomes after simulation of juxtarenal aortic aneurysm repair in 28 rats

Outcome	Sham (n=7)	No renal perfusion (n = 7)	p^a	Warm renal perfusion (n = 7)	p^b	Cold renal perfusion (n = 7)	p^b
End of procedure brush border damage percentage	0.0 (2.5)	27.5 (47.5)	.002	12.5 (30.0)	.05	10.0 (30.0)	.018
Absolute serum creatinine rise rise - mg/dL	0.3 (0.18)	0.46 (0.43)	.002	0.33 (0.29)	.096	0.14 (0.28)	.009
Arginine/ADMA ratio	122.2 ± 22.5	37.7 ± 10.1	.002	70.0 ± 26.6	.025	91.2 ± 19.1	.002
Microcirculatory flow ratio	0.9 ± 0.0	0.2 ± 0.0	.002	0.7 ± 0.1	.002	0.8 ± 0.1	.002
Mitochondrial ROS production	255.3 ± 80.9	806.1 ± 351.2	.034	429.5 ± 322.1	.16	275.8 ± 81.8	.034
End of procedure intestinal leukocyte infiltration[c]	20.0 (50.0)	90.0 (70.0)	.004	40.0 (50.0)	.006	40.0 (50.0)	.018
End of procedure ICAM-1 expression[d]	271.0 (122.0)	425.5 (246.0)	.019	356.0 (279.0)	.62	272.0 (106.0)	.019
End of procedure I-FABP expression[d]	1.2 ± 0.1	1.5 ± 0.1	.002	1.3 ± 0.3	.007	1.2 ± 0.1	.002

Data are presented as mean ± standard deviation or median (range). ROS production in circulating leukocytes was measured at three stages during juxtarenal aortic aneurysm repair simulation. All other variables were determined at the start and end of the experiment. ADMA = asymmetrical dimethylarginine; ICAM-1 = intercellular adhesion molecule 1; I-FABP = intestinal fatty acid binding protein; ROS = reactive oxygen species.

[a] *p* value reflects the difference between the group without renal perfusion and the sham group.

[b] *p* value reflects the difference between the group with warm or cold renal perfusion and the group without renal perfusion.

[c] Number per 10 000 microns^2 sigmoid mucosa.

[d] Relative expression represented in arbitrary units based on area and fluorescence intensity and corrected for background fluorescence.

median values are provided with ranges and Kruskal—Wallis tests were used to compare all groups and Mann—Whitney *U* tests to compare two groups. Wilcoxon tests were used to compare paired data and Spearman's rank correlation (R^2) was used for correlations. Tests were considered statistically significant at $p \leq .05$.

RESULTS

Open JAA repair was successfully simulated in all rats and the measurements were performed in each rat, except for the measurement of ROS production in circulating leukocytes. This took place in four rats in the sham group, the group without and with warm renal perfusion, but was only measured in three rats in the group receiving cold renal perfusion.

Effects of suprarenal clamping

The sham group did not suffer any damage to renal tubules or alterations in systemic parameters, while SRC led to significant changes in all outcomes, as listed in Table 1.

After SRC, brush border damage increased to 27.5% (12.5—60.0; $p = .002$) of the renal tubules compared with 0.0% (0.0—2.5) in the sham group. Absolute serum creatinine increased to 0.45 mg/dL (0.16—0.60; $p = .002$) compared with 0.25 mg/dL (0—0.88) in the sham group, while baseline creatinine levels were equivalent between groups.

The arginine/ADMA ratios decreased compared with the sham group, as did the microcirculatory flow ratio in the sigmoid wall. Baseline arginine/ADMA levels and microcirculatory flow were identical to those of the sham group.

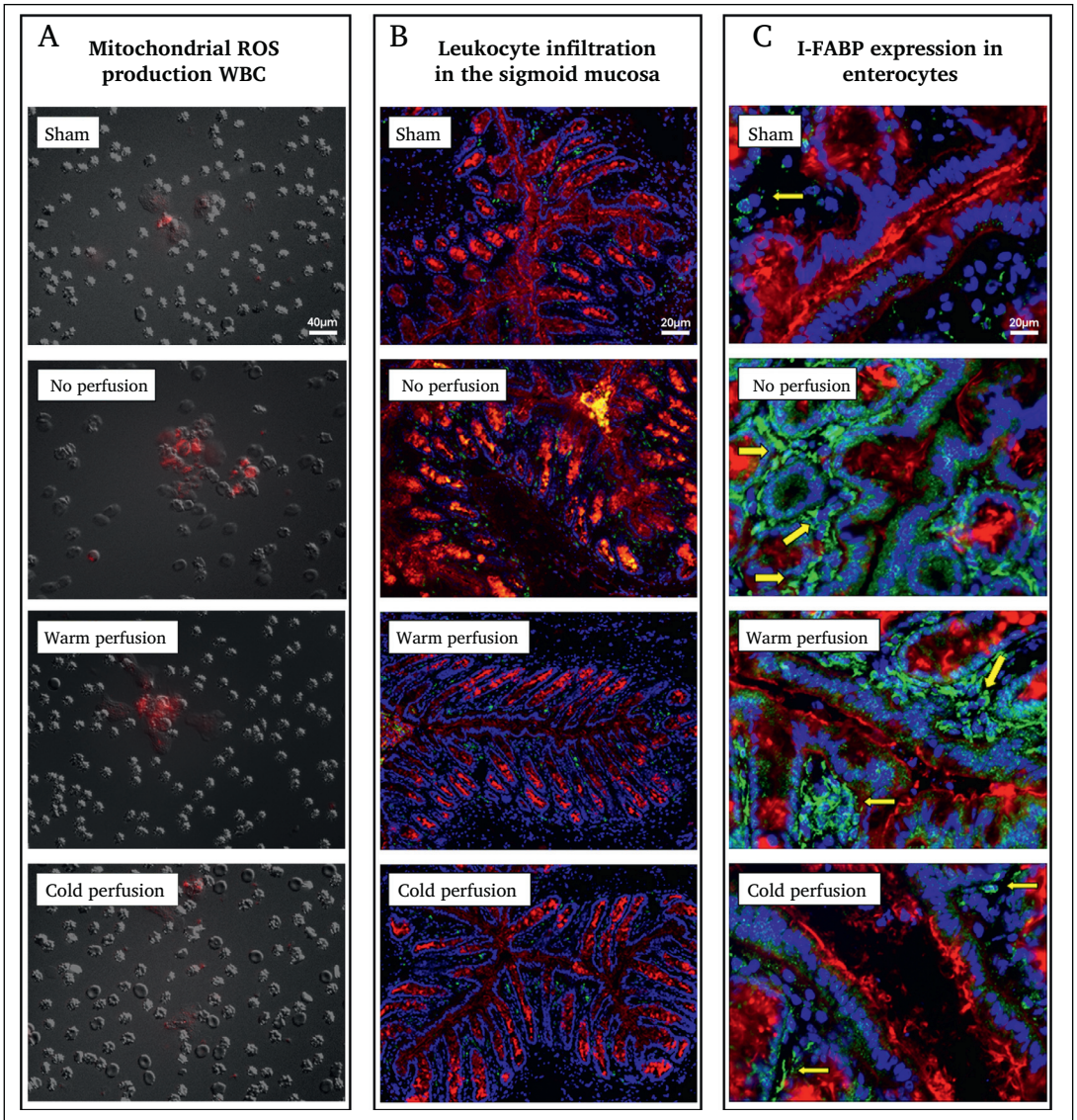

Figure 2. Representative images of immunofluorescence microscopy quantification, left box (A): mitochondrial ROS production in peripheral blood smears represented in red (n = 4 per group), middle box (B): leukocyte infiltration in the sigmoid mucosa (n = 7 per group), right box (C): intestinal fatty acid binding protein (IFABP) leakage from enterocytes in the sigmoid mucosa represented in green (n = 7 per group). ROS = reactive oxygen species; WBC = white blood cells.

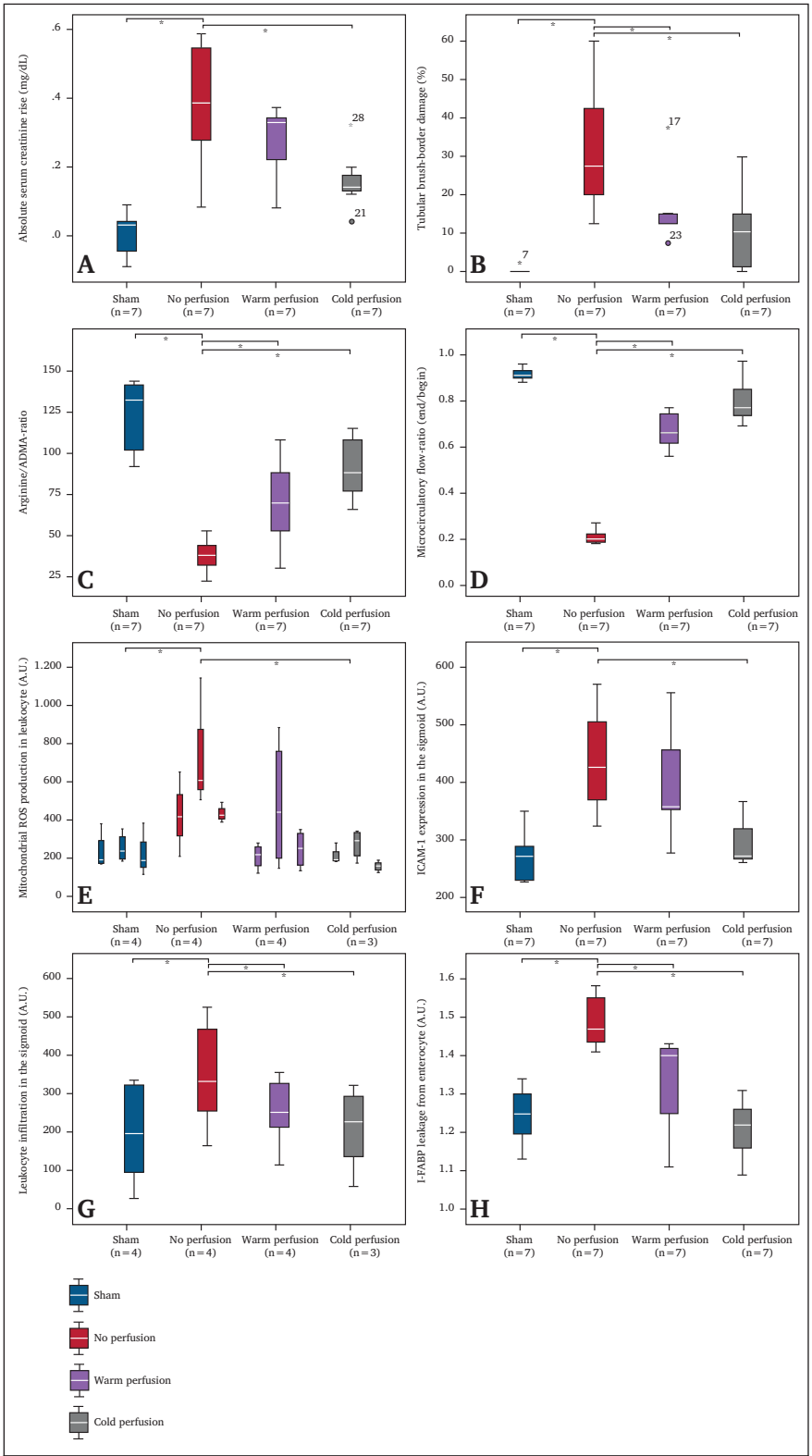

Figure 3. Representation of systemic variables in all groups. (A) absolute creatinine levels ($n = 7$ per group), (B) tubular brush border damage ($n = 7$ per group), (C) arginine/asymmetrical dimethylarginine (ADMA) ratio ($n = 7$ per group), (D) microcirculatory flow ($n = 7$ per group), (E) mitochondrial reactive oxygen species (ROS) production in circulating leukocyte ($n = 4$ per group presented in arbitrary units (A.U.)), (F) intracellular adhesion molecule 1 (ICAM-1) expression in the sigmoid wall ($n = 7$ per group presented in A.U.), (G) leukocyte infiltration in the sigmoid mucosa ($n = 7$ per group presented in A.U.), and (H) intestinal fatty acid binding protein (I-FABP) leakage from mature enterocytes ($n = 7$ per group presented in A.U.). * indicates statistically significant differences between groups.

Mitochondrial ROS production in circulating leukocytes, leukocyte infiltration, and ICAM-1 expression in the sigmoid all increased after SRC compared with the sham group (Fig. 2). Ultimately, I-FABP leakage from enterocytes increased from 1.2AU (95% CI 1.17—1.32) to 1.5AU (95% CI 1.42—1.55; $p = .002$)

Renal damage

Cold renal perfusion (4 °C) preserved renal function and led to a decrease of all systemic effect deteriorations (Fig. 3), compared with no renal perfusion. Warm perfusate (37 °C) did not decrease tubular lumen obstruction or creatinine rises, which were 12.5% (7.5—37.5; $p = .05$) and 0.33 mg/dL (0.08—0.37; $p = .096$), respectively, compared with 27.5% (12.5—60.0%) and 0.45 mg/dL (0.16—0.60) without renal perfusion. Whereas cold renal perfusion decreased tubular lumen obstruction to 10.0% (0.0—30.0%; $p = .018$) and absolute creatinine rises to 0.14 mg/dL (0.04—0.32; $p = .009$).

Systemic effects

Arginine levels increased and ADMA levels decreased, leading to an increased arginine/ADMA ratio after warm and cold perfusion, compared with no perfusion. Meanwhile, the microcirculation in the sigmoid wall increased after both warm and cold perfusion compared with no perfusion. Cold perfusion had a stronger effect in maintaining arginine/ADMA ratios and microcirculatory flow. A high correlation was found between arginine/ADMA ratios and microcirculatory flow ratios, $R^2 = 0.85$; $p < .001$ (Fig. 4).

Warm perfusion did not affect ROS production in circulating leukocytes, while cold perfusion decreased ROS production in circulating leukocytes compared with no perfusion. This was highly correlated with tubular lumen obstruction, $R^2 = 0.60$; $p = .03$. Leukocyte infiltration decreased more after warm perfusion than after cold perfusion as compared with no perfusion. However, warm perfusion did not affect ICAM-1 expression, while cold perfusion decreased ICAM-1 expression compared with no perfusion.

Sigmoid damage

I-FABP leakage in the sigmoid decreased to 1.3AU (95% CI 1.1—1.4; $p = .007$) after warm perfusion and to 1.2AU (95% CI 1.1—1.3; $p = .002$) after cold perfusion, compared with 1.5AU (95% CI 1.4—1.6) without perfusion. I-FABP leakage was positively correlated with tubular damage, $R^2 = 0.63$; $p < .001$, and inversely correlated with arginine/ADMA ratios and microcirculatory flow ratios, $R^2 = -.45$; $p = .015$ and $R^2 = -.59$; $p = .001$. Leakage was also correlated with ROS production in circulating leukocytes and mucosal leukocyte counts, $R^2 = 0.63$; $p < .001$ and $R^2 = 0.46$; $p = .014$.

DISCUSSION

This study investigated the effects of renal perfusion with cold and warm saline during renal ischaemia in a simulation of JAA repair on renal failure, systemic oxidative stress, and sigmoid damage. I/R injury during JAA repair in rats had a deleterious effect on renal function, systemic oxidative stress, and sigmoid damage. Brush border damage in renal tubules and obstruction of the tubular lumen was observed. Furthermore, JAA simulation decreased arginine levels resulting in a reduced microcirculatory flow in the sigmoid wall. Meanwhile, oxidative stress occurred as mitochondrial ROS production in circulating leukocytes increased. Infiltration of leukocytes in the sigmoid mucosa was increased as was expression of ICAM-1. Finally, leakage of I-FABP from mature enterocytes to the extracellular matrix increased. Interestingly, these parameters changed after renal cooling during SRC. This study verifies the early effect of renal preservation during JAA repair, and it not only reduces renal damage but also lowers systemic oxidative stress by: i) stabilizing arginine/ADMA ratios and microcirculation, ii) decreasing ROS production in circulating leukocytes, and iii) decreasing leukocyte counts in the sigmoid mucosa.

Ultimately, cold renal perfusion reduced sigmoid damage considering the decreased I-FABP leakage from enterocytes in the sigmoid mucosa, which was positively correlated with changes in plasma creatinine and tubular brush border damage.

JAA repair with SRC induces renal I/R on top of the lower limb I/R and might prompt AKI.[1—4,9] Loss of renal function has been described in patients with suprarenal clamp times under 40 min, and is often accompanied by MOF.[23,24] Increased suprarenal cross clamping time is correlated with the severity of renal deterioration, and is therefore kept to a minimum with average clamp times only a little above 30 min.[8,10—12] In previous animal studies, increased tubular damage and creatinine levels occurred with use of the same clamping conditions. These prolonged times were chosen to investigate renal I/R and the protective effect of renal cooling.

In patients with renal I/R injury after suprarenal aortic surgery other organs are often affected, significantly increasing mortality.[1—3,5—7,9,14,15,32,33] The correlation between remote organ damage and SRC is being unveiled by contemporary research. Loss of tubular cell function during AKI prompts an imbalance of synthesis and clearance of inflammatory mediators, for example the capacity to synthesise arginine and extract dimethylargnines.[20,21] This results in a reduced bioavailability of NO and disrupts haemodynamics via a systemic vasoconstrictive effect and is an independent risk factor of post-operative mortality.[19—21] As haemodynamics are disrupted, especially in the fragile circulatory system of the sigmoid, systemic oxidative stress damage occurs from increased release of oxygen free radicals. These oxygen free radicals contribute to systemic inflammatory tissue injury. AKI aggravates this effect by causing systemic excessive ROS production in circulating leukocytes.[25—27,34—36] Finally, AKI provokes infiltration of

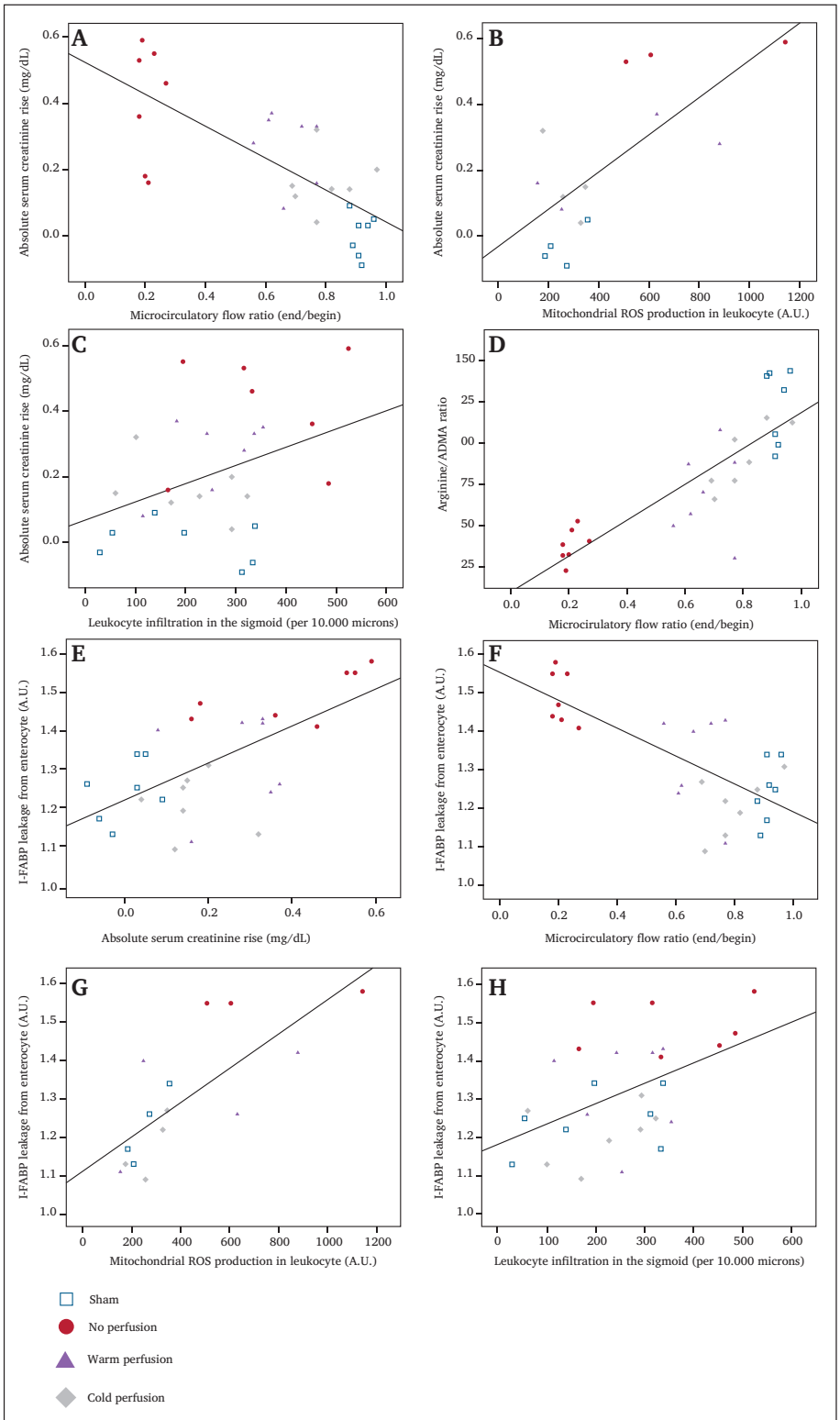

Figure 4. Correlation of systemic variables in all groups. Creatinine levels correlated with (A) microcirculatory flow in the sigmoid wall ($n = 7$ per group), (B) reactive oxygen species (ROS) production in circulating leukocytes ($n = 4$ in the sham, no perfusion and warm perfusion groups and $n = 3$ in the cold perfusion group, presented in arbitrary units (A.U.)) and (C) leukocyte infiltration in the sigmoid mucosa ($n = 7$ per group presented per 10.000 microns). (D) Correlation between arginine/asymmetrical dimethylarginine (ADMA) and microcirculatory flow in the sigmoid wall ($n = 7$ per group). Correlation between intestinal fatty acid binding protein (I-FABP) leakage (in A.U) and (E) absolute serum creatinine rise ($n = 7$ per group), (F) microcirculatory flow in the sigmoid wall ($n = 7$ per group), (G) ROS production in circulating leukocytes, ($n = 4$ in the sham, no perfusion and warm perfusion groups and $n = 3$ in the cold perfusion group in A.U.) and (H) leukocyte infiltration in the sigmoid mucosa (presented per 10.000 microns).

leukocytes in visceral organs, for example the sigmoid, by increased expression of ICAM-1.[28,29,37] The present study supports this theory, as decreased microcirculatory flow was found in the sigmoid, while systemic oxidative stress and leukocyte counts in the sigmoid mucosa increased after SRC. These three systemic effects after renal I/R might induce and exaggerate end organ damage. Strikingly, it was observed that protective renal cooling could reduce renal damage, which was strongly correlated with end organ damage in the sigmoid. The effect of cold renal perfusion was superior to that of warm renal perfusion with regard to preserving kidney function by diminishing renal deterioration and sigmoid damage, by attenuating renal function and microcirculation, and decreasing systemic ROS production in leukocytes.

Sigmoid ischaemia after JAA repair is associated with high mortality, and therefore, early detection is required to prevent transmural ischaemia, ulceration, and perforation. Initially, the sigmoid mucosa is affected, which is accompanied by leakage of digestive enzymes, e.g. I-FABP, into subepithelial spaces. Earlier studies proved that I-FABP is mainly expressed by mature enterocytes in the intestinal villi and can be used as a marker for enterocyte necrosis.[38] It is understood that during intestinal necrosis I-FABP leaks from the necrotic enterocytes in the villi via the subepithelial spaces towards the crypts.[38–40] This leakage of digestive enzymes results in an increase of serum I-FABP levels.[39,41] In accordance with these studies, higher I-FABP concentrations were found in the sigmoid crypts after SRC and I-FABP was observed in subepithelial spaces, intestinal lumen, and the lamina propria, as a result of I-FABP leakage from mature enterocytes. This indicates recent inception of sigmoid injury. The severity of intestinal necrosis correlates with I-FABP leakage from enterocytes.[41,42]

This study provides a uniform model, much like a clinical setting, to investigate the systemic effects of renal preservation during JAA repair. The simulation of JAA repair in rats with its clamping sequence (suprarenal aortic cross clamping followed by infrarenal aortic cross clamping) and ischaemia/reperfusion times was chosen based on earlier published studies, in which a clear effect of renal preservation with warm and cold saline was seen at the end of the experiments. As a result of these prolonged clamp times this sequence is valuable to investigate the effects of renal cooling on renal failure and remote organ damage.[22,23]

The weaknesses of this study are that differences between rats and humans cannot be ruled out. Animal ethical dilemmas meant that no post-operative course or longer reperfusion times could be evaluated. Furthermore, the study did not include a group that solely underwent infrarenal clamping, which might have distinguished between the effect of renal I/R and changes in the circulatory system caused by lower limb I/R injury or clamping proximal of the IMA. However, in these rat models it is not possible to perfuse the renal arteries with cold saline without suprarenal aortic cross clamping, so the effect of renal preservation cannot be investigated in an experimental setup with infrarenal aortic cross clamping alone. The effect of this clamping sequence in another animal study has been published previously.[22] Above all, (temporary) exclusion of the IMA might not contribute to sigmoid ischaemia, with patent coeliac and superior mesenteric arteries, and is therefore not revascularised during both endovascular and open repair for infrarenal aneurysms, with negligible rates of sigmoid ischaemia in contrast to juxtarenal aneurysm repair.[15] Hence, the observed sigmoid damage is most likely related to the effects of renal I/R injury combined with lower limb injury, and reduction of the renal deterioration from renal cooling resulted in preserved intestinal integrity, especially because use of healthy rats are expected to have a patent visceral vascular system. In this study increased ROS production was identified in circulating leukocytes, along with infiltration of leukocytes in the sigmoid mucosa. However, these effects could be explained by lower limb I/R and re-establishment of the circulation to the lower extremities after declamping the aorta, which results in release of ROS and leukocytes during reperfusion.[43,44] A significant reduction in ROS production and sigmoid infiltration of leukocytes was found after renal cooling, but as renal cooling had no effect in the limbs during renal ischaemia and renal reperfusion after the clamp was moved infrarenally, the contribution of renal I/R on sigmoid damage is distinct. The core temperature was also maintained at 36 °C, which makes less likely an indirect effect of renal cooling by lowering the temperature in the sigmoid. Furthermore, indirect cooling of the intestines would have had a vasoconstrictive effect in the sigmoid wall; in contrast, this study found increased microcirculatory flow in the sigmoid wall. Another limitation of this study is that it does not prove a clear relationship, and the exact pathways involved in renal protection affecting systemic parameters remain unknown. Future studies could include a pharmacological approach with antioxidants or detection of intracellular pathways to elucidate the latter.

Nevertheless, the present study clearly shows the protective effect of selective renal perfusion. Cold perfusion was superior to warm perfusion in reduction of renal damage, parameters affecting systemic inflammation, sigmoid damage, and increase of microcirculation.

In conclusion, renal I/R and the effect of lower limb I/R during a simulation of JAA repair in rats not only leads to renal injury, but also influences parameters for systemic inflammation and microcirculation. The latter might contribute to the sigmoid damage, observed in the present study by increased I-FABP leakage from enterocytes after SRC, a marker of intestinal integrity. Selective cold perfusion of the kidneys during suprarenal aortic cross clamping during JAA repair preserved renal function and might have reduced systemic deleterious effects of ischaemia reperfusion, as shown by prevention of early sigmoid damage in this experimental study.

CONFLICT OF INTEREST

None.

FUNDING

The experiments were sponsored by an unrestricted grant of the Amsterdam University Medical Center, location VUmc. No external funding was required.

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