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Persistent Renal Hypoxia and Histologic Changes at 4 Weeks after Cardiopulmonary Bypass in Sheep

Taku Furukawa, M.D., Clive N. May, Ph.D., Alemayehu H. Jufar, Ph.D., Roger G. Evans, Ph.D., Andrew D. Cochrane, M.B.B.S., Bruno Marino, B.A., Dip.Ed., Dip.Perf. (ANZCP),† Peter R. McCall, M.B.B.S., Ian E. Birchall, Ph.D., Sally G. Hood, B.Sc. (Hon.), Jaishankar Raman, M.B.B.S., Ph.D., Connie P. C. Ow, Ph.D., Anton Trask-Marino, B.Sc. (Hon.), Rinaldo Bellomo, M.D., Ph.D., Lachlan F. Miles, M.B.B.S., Ph.D., Yugeesh R. Lankadeva, Ph.D.

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ABSTRACT

Background: The sustained renal effects of exposure to cardiopulmonary bypass are unknown. This study aimed to test whether cardiopulmonary bypass (CPB) is associated with sustained renal tissue hypoxia and whether such hypoxia is associated with histologic injury.

Methods: The study included 12 adult female sheep undergoing CPB with a 2-h aortic cross-clamp. Systemic and renal hemodynamics and oxygen delivery, kidney function, and renal tissue oxygenation were measured before and during CPB, in the 48 h after CPB, and weekly for 4 weeks. The sheep were euthanized at 4 weeks and obtained renal tissue to perform histopathologic assessments for comparison with an independent cohort of five healthy animals that were euthanized without undergoing surgical or experimental interventions. These histologic assessments were performed by an independent, treatment-blinded pathologist.

Results: Compared with baseline, renal blood flow and renal medullary tissue oxygenation decreased significantly during CPB. In the first 48 h after CPB, there was a continuing significant decrease in medullary tissue oxygenation (from 39.2 ± 13.8 mmHg at baseline to 21.7 ± 16.2 mmHg at 48 h; P_{time} = 0.006) with stage 1 acute kidney injury in 42% of the animals. Moreover, in the following 4 weeks, medullary (16.1 ± 12.9 mmHg at 4 weeks; P_{time} = 0.005) and cortical (17.2 ± 6.5 mmHg at 4 weeks; P_{time} = 0.005) tissue oxygenation remained significantly lower than baseline. Finally, compared with healthy sheep, at 4 weeks after CPB, sheep kidneys had significantly more peritubular inflammation (8 of 8 *vs.* 1 of 5; P = 0.007), interstitial fibrosis (6 of 8 *vs.* 0 of 5; P = 0.021), and tubular casts (8 of 8 *vs.* 1 of 5; P = 0.007).

Conclusions: Exposure to CPB triggers sustained medullary and cortical tissue hypoxia and is associated with histopathologic renal injury. These findings suggest that the renal effect of exposure to CPB may be more profound and longer lasting than currently appreciated.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Acute kidney injury occurs commonly in patients who undergo cardiac surgery with cardiopulmonary bypass.
- Intraoperative renal tissue hypoxia occurs during cardiopulmonary bypass, particularly in the renal medulla. This supports the belief that renal hypoxia is part of the pathogenesis of postoperative acute kidney injury.

What This Article Tells Us That Is New

- This study assessed the duration and severity of renal tissue hypoxia after cardiopulmonary bypass and whether this hypoxia associates with histopathologic structural changes in the kidneys 4 weeks after cardiopulmonary bypass.
- This study was done using 12 adult female sheep that underwent cardiopulmonary bypass with a 2-h aortic cross-clamp time. The authors assessed systemic and renal hemodynamics and oxygen delivery, kidney function, and renal tissue oxygenation, before and during cardiopulmonary bypass, in the 48 h after cardiopulmonary bypass, and then weekly for 4 weeks.
- Four weeks after cardiopulmonary bypass, the authors observed sustained renal medullary and cortical tissue hypoxia and histopathologic renal injury.
- These findings support that exposure to cardiopulmonary bypass is associated with longer-term effects on kidney function, which may explain the transition of acute kidney injury to chronic kidney disease.

This article is featured in "This Month in ANESTHESIOLOGY," page A1. This article is accompanied by an editorial on p. 985. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). This article has a video abstract.

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Taku Furukawa, M.D.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

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Acute kidney injury (AKI) is a frequent and significant complication of cardiac surgery requiring cardiopulmonary bypass (CPB).[1] It occurs in approximately 30% of these patients, with up to 6% of AKI patients receiving renal replacement therapy subsequently.[1] AKI affects both short- and long-term postoperative outcomes significantly[2] and is associated with an increased risk of chronic kidney disease,[3,4] which subsequently diminishes quality of life and further increases healthcare costs.[5,6]

The pathophysiology of CPB-associated AKI is complex, but there is robust evidence that CPB induces intraoperative renal tissue hypoxia, particularly in the renal medulla, and that this may be implicated in the pathogenesis of this condition.[7–16] However, the duration and severity of renal tissue hypoxia during the postoperative period remains unknown. Furthermore, due to lack of histopathologic data, it is unclear whether CPB-induced tissue hypoxia is associated with structural changes that might explain the postulated causative link between AKI and chronic kidney disease.

To address these issues, we developed a sheep model of CPB with a 4-week postoperative recovery period. In this model, we monitored systemic and renal hemodynamics, renal function, and renal tissue oxygenation for 4 weeks after CPB. We aimed to investigate the effects of CPB on renal tissue oxygenation and histology for up to 4 weeks after CPB. We hypothesized that renal tissue hypoxia would persist into the postoperative period and that this prolonged hypoxia would be associated with histopathologic injury at 4 weeks after CPB.

Materials and Methods

All procedures were approved by the Animal Ethics Committee of the Florey Institute of Neuroscience and Mental Health (Melbourne, Australia; approval no. 18-119-FINMH). All data are reported according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE 2.0) guidelines.[17]

Seventeen healthy, adult Merino ewes (1.5 to 2.0 yr of age) were included in this study. We used only female sheep in our experiments because it is not possible to perform urethral bladder catheterization in male sheep, and handling larger, more aggressive rams carries significant occupational health and safety risks. Twelve animals, with a mean body weight of 36.2 ± 3.6 kg (mean ± SD) were subjected to a standardized CPB protocol outlined below. Five additional healthy animals served as a control group for histologic analysis. This analysis was performed by a pathologist blinded to treatment allocation.

The primary outcome was renal medullary tissue oxygen tension (tPo_2). We calculated that a sample size of eight animals would provide the study with 90% power ($\alpha = 0.05$) to detect a 50% reduction in medullary tPo_2 at 48-h postoperatively, relative to a pre-CPB nonanesthetized baseline.[8] We also accounted for an estimated dropout rate of 30% due to equipment failure or loss of test subjects due to sheep reaching ethical endpoint criteria for euthanasia. We therefore planned a total sample size of 12 animals. The secondary outcome was renal histopathologic changes in sheep that survived until 4 weeks after CPB.

Surgical Preparation

Before the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week. The sheep were housed in individual metabolic cages and provided with unrestricted access to 5 l of water and 800 g of oaten chaff per day. Before to the CPB experiment, all sheep underwent two aseptic surgical procedures under general anesthesia with isoflurane. In the first procedure, bilateral carotid arterial skin loops were prepared,[18] which

Clive N. May, Ph.D.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health and Department of Critical Care, Melbourne Medical School, University of Melbourne, Melbourne, Victoria, Australia.

Alemayehu H. Jufar, Ph.D.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

Roger G. Evans, Ph.D.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia; Cardiovascular Disease Program, Biomedicine Discovery Institute and Department of Physiology, Monash University, Melbourne, Victoria, Australia.

Andrew D. Cochrane, M.B.B.S.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

Bruno Marino, B.A., Dip.Ed., Dip.Perf. (ANZCP): Cellsaving and Perfusion Resources, Melbourne, Victoria, Australia.

Peter R. McCall, M.B.B.S.: Department of Anaesthesia, Austin Health, Heidelberg, Victoria, Australia.

Ian E. Birchall, Ph.D.: Neurohistology Laboratory, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

Sally G. Hood, B.Sc. (Hon.): Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

Jaishankar Raman, M.B.B.S., Ph.D.: Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne, Victoria, Australia.

Connie P. C. Ow, Ph.D.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

Anton Trask-Marino, B.Sc. (Hon.): Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia.

Rinaldo Bellomo, M.D., Ph.D.: Department of Critical Care, Melbourne Medical School, University of Melbourne, Australia; Department of Intensive Care, Austin Health, Heidelberg, Victoria, Australia; Australian and New Zealand Intensive Care Research Centre, Monash University, Melbourne, Victoria, Australia; Department of Intensive Care, Royal Melbourne Hospital, Melbourne, Victoria, Australia.

Lachlan F. Miles, M.B.B.S., Ph.D.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health and Department of Critical Care, Melbourne Medical School, University of Melbourne, Melbourne, Victoria, Australia; Department of Anaesthesia, Austin Health, Heidelberg, Victoria, Australia.

Yugeesh R. Lankadeva, Ph.D.: Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health and Department of Critical Care, Melbourne Medical School, University of Melbourne, Melbourne, Victoria, Australia; Department of Anaesthesia, Austin Health, Heidelberg, Victoria, Australia.

†Deceased.

provided easy access for arterial cannulation and thus measurement of arterial pressure and blood sampling. A time period of 3 to 4 weeks was required for the arteries to heal and become fully accessible for cannulation. At this time, a carotid loop and jugular vein were cannulated, and a second surgical procedure was performed to (1) place a transit-time flow probe (4 mm; Transonic Systems, USA) around the left renal artery; (2) cannulate the left renal vein; and (3) insert custom-made combination fiber-optic probes (Oxford Optronix, United Kingdom) into the renal medulla and cortex, as previously described.[18,19] This surgical instrumentation was performed 5 days before the CPB experiment to enable the continuous measurement of cardiovascular and renal hemodynamics, renal macro- and microcirculation, and kidney function in nonanesthetized sheep for a baseline period and then during and after CPB. The positions of the probe tips within the renal medulla and cortex were confirmed at post mortem.

Cardiopulmonary Bypass

The experimental timeline of the study is illustrated in figure 1. After 6 h of baseline recording in the nonanesthetized state, the animals were moved to the operating room. General anesthesia was induced with intravenous propofol (4 mg/kg, Feresofol; Fresenius Kabi Australia, Australia) and fentanyl (5 μg/kg, Fentanyl GH; Panpharma, Germany) and maintained with a combination of propofol (4 mg · kg^{-1} · h^{-1}), fentanyl (5 μg · kg^{-1} · h^{-1}), and inhaled sevoflurane (4 to 5%, Piramal Sevoflurane; Piramal Critical Care, USA). All animals received 2 ml · kg^{-1} · h^{-1} intravenous compound sodium lactate (Baxter Health Care, Australia) as maintenance fluid. A right thoracotomy was performed, and a transit-time flow probe (20 mm; Transonic Systems) was placed around the pulmonary artery to measure cardiac output. CPB was then established using methods previously described. A nonpulsatile flow of 2.4 l · min^{-1} · m^{-2} (approximately equivalent to 65 to 75 ml · kg^{-1} · min^{-1}), with body surface area calculated according to Bennett,[3] mean arterial pressure (MAP) of 65 to 75 mmHg, and core body temperature of 36°C (the basal body temperature in sheep is 39° to 40°C) were targeted, consistent with current best practice recommendations.[20] Intravenous boluses or a continuous infusion of metaraminol (Montrose Life Sciences, Australia) was used as required to maintain the target MAP. Phenylephrine (Neo-synephrine; Pfizer, Australia) was used as a secondary vasopressor if the target MAP could not be maintained with metaraminol alone. If MAP could not be maintained at 70 mmHg or higher with vasopressors or if mixed venous oxygen saturation was less than 70%, the pump flow was increased by 10%.

Considering the lower normal/baseline hemoglobin levels (typically between 8 and 10 g/dl) in sheep compared with humans, if the blood hemoglobin concentration decreased to less than 6 g/dl, 500 ml of donor blood was administered. This decrease is similar to the percentage decrease observed in human CPB.[21]

The aortic cross-clamp was applied for 2 h, and antegrade blood cardioplegia (4:1) was administered for myocardial protection; an initial dose of 1,000 ml (AHK5534; Baxter) was followed by further doses of 300 ml (AHK5535; Baxter) at 15-min intervals. During the final 15 min of the 2-h period of aortic cross-cramp, the animals were rewarmed to 39°C (basal sheep body temperature). The aortic cross-clamp was then removed, and the animals were weaned off CPB once the hemodynamic stability was achieved. Protamine was administered (3 mg/kg, Fisons Protamine Sulfate; Sanofi, Australia), followed by decannulation. Residual blood salvaged from the CPB reservoir was transfused until the volume status was deemed optimal based on the clinical judgment of the treating cardiac

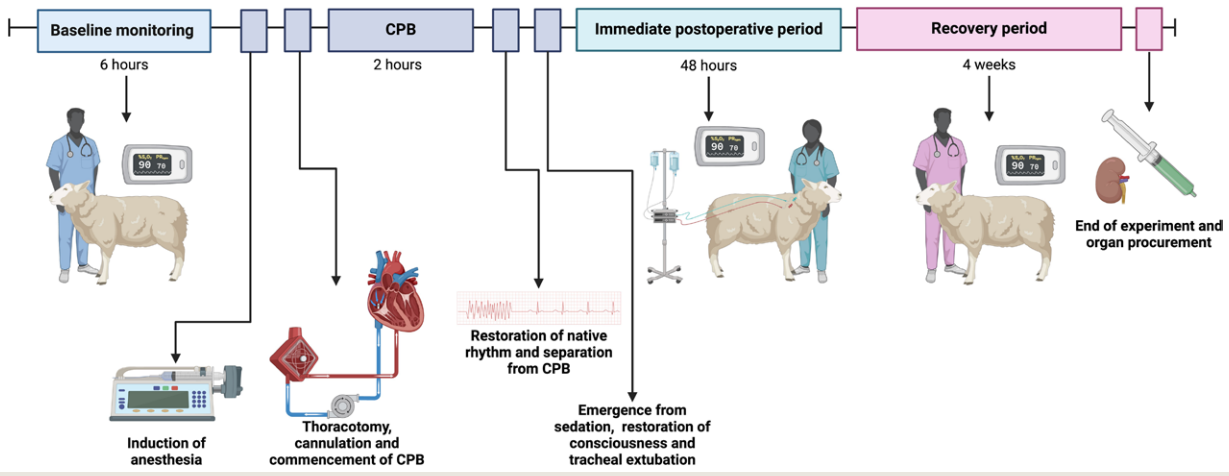

Fig. 1. Schematic description of experimental timeline. Created with BioRender.com. CPB, cardiopulmonary bypass.

surgeons and anesthetists, using a combination of visual inspection of the right heart by the surgeons and hemodynamic monitoring of arterial blood pressure, cardiac output, and central venous pressure by the anesthetist. After chest closure, anesthesia was discontinued, and the trachea was extubated when clinically appropriate. The sheep were then transferred back to individual metabolic cages for postoperative observation.

Post-CPB Period

After being returned to their cages, the animals were monitored, and data were recorded continuously for 48 h with all variables averaged hourly. Urine output was also recorded hourly. Blood samples were obtained at 12-, 24-, and 48-h timepoints, and urine samples were collected over 1 h at each timepoint. The animals received a continuous intravenous infusion of compound sodium lactate at 2 to 4 ml · kg^{-1} · h^{-1}, tapered over 24 h, with 200- to 250-ml boluses administered as needed based on hemodynamic status. If hemoglobin dropped from the normal level in sheep of 8.5 g/dl to less than 6.5 g/dl, 500 ml of donor blood was given. Norepinephrine was infused as needed to maintain MAP above 65 mmHg. An initial flow of 4.0 l/min of supplemental oxygen was administered *via* a nasal cannula; this was weaned over 4 h, guided by arterial Po_2. Once the animals regained consciousness, they had unrestricted access to food and water.

From 1 to 4 weeks after CPB, variables were recorded for 6 h once weekly. Urine samples were collected over 30-min periods, with blood sampled at the midpoint of each period.

At the end of the experiment, the animals were euthanized humanely with intravenous sodium pentobarbital (100 mg/kg, Lethobarb; Virbac, Australia), and the kidneys were collected immediately for histopathologic analysis.

Experimental Measurements

Variables, including arterial pressure, renal blood flow, renal cortical and medullary tPo_2, and temperature, were recorded digitally as described previously.[19] Renal vascular conductance was calculated by dividing renal blood flow by the product of MAP and body weight. Renal oxygen delivery was determined as the product of renal blood flow and arterial oxygen content. Blood and urine samples were collected for creatinine and sodium measurements. Additional blood samples were collected from arterial, jugular, and renal venous catheters for oximetry and biochemistry (ABL system 625; Radiometer Medical, Denmark). Additional details are provided in Supplemental Digital Content 1 (https://links.lww.com/ALN/D924).

Histologic Analysis

After euthanasia, the kidneys were cut transversely and fixed for 14 days in 10% neutral buffered formalin. Three representative segments containing cortex, medulla, and a papilla from each sample were processed for paraffin embedding and sectioning. Kidney sections stained with hematoxylin and eosin, periodic acid Schiff's, and Masson's trichrome were assessed by a pathologist blinded to treatment allocation (I.E.B.), for changes consistent with acute tubular necrosis, *i.e.* tubular dilatation, epithelial thinning, regenerative changes, and vacuolation. The interstitial tissue was assessed by a pathologist blinded to treatment allocation. Assessment focused on peritubular inflammatory infiltrates and fibrosis. The changes were graded semiquantitatively as: 0 = no change; + = mild focal change (0 to 10%); ++ = moderate focal change (10 to 50%); and +++ = severe diffuse change (greater than or equal to 50%), as described previously.[13]

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism for Windows, version 10 (GraphPad Software, USA). The data are presented as mean ± SD or median [interquartile range] as appropriate. Normality of data was assessed using the Shapiro–Wilk test. Comparisons between baseline and CPB period were performed using a *t* test or a Wilcoxon matched-pairs signed-rank test. For post-CPB measurements, mixed-effects modeling was applied with a Greenhouse–Geisser correction for the main effect of "time" (*P* values are reported as P_{time}). Histopathologic data were analyzed using Fisher's exact test for categorical data and the Mann–Whitney U test for ordinal data, as described in a previous publication.[13] Two-sided *P* values less than or equal to 0.05 were considered significant.

Results

Study Completion

Twelve sheep completed the early (48 h) experimental period. Four animals were euthanized between 48 h and 4 weeks for the following reasons: immobility at 48 h postoperatively due to mild preexisting leg soreness unrelated to the experiment, which impaired recovery (n = 1); development of a stroke on postoperative day 3 (n = 1); and malfunction of a carotid arterial loop (n = 2). These issues were judged unrelated to the study measurements, and therefore, the data from these animals were included in the analysis of variables during CPB and the first 48 h after CPB but were excluded from the analysis of the extended post-CPB period and histologic examination. To maintain MAP after CPB, phenylephrine was required in one animal (total dose, 10 mg), and three animals required a norepinephrine infusion (dose range). Six animals received a donor blood transfusion.

First 48 h after CPB

As previously reported,[7–13] compared with baseline, over the 2-h CPB period, there were significantly decreases

in renal blood flow, renal oxygen delivery, renal vascular conductance, and medullary tPo_2, while cortical tPo_2 remained unchanged (see supplemental table S1, https://links.lww.com/ALN/D925). In the first 48 h after CPB, there was a significant decrease in medullary tPo_2 (39.2 ± 13.8 mmHg at baseline to 21.7 ± 16.2 mmHg at 48 h; P_{time} = 0.006; fig. 2a). Similarly, cortical tPo_2 decreased from 36.1 ± 13.1 mmHg at baseline to 27.5 ± 11.1 mmHg at 48 h (P_{time} = 0.06; fig. 2b). Renal blood flow, renal vascular conductance, and renal oxygen delivery also decreased over time (P_{time} = 0.029, 0.006, and 0.001, respectively; fig. 2, c and d). Moreover, renal vein oxygen saturation decreased significantly during this period (from 86.0 ± 2.4 at baseline to 78.1 ± 3.2 at 48 h; P_{time} = 0.0006; fig. 2f).

Mean creatinine clearance and median urine output did not change significantly (table 1). Five animals, however, met the Kidney Disease: Improving Global Outcomes (KDIGO) criteria[22] for stage 1 AKI based on urine output, while none met the criteria based on an increase in serum creatinine. Other variables are shown in table 1 and supplemental table S1 (https://links.lww.com/ALN/D925).

Extended Post-CPB Period (1 to 4 Weeks)

During the extended post-CPB period, both medullary (16.1 ± 12.9 mmHg at 4 weeks; P_{time} = 0.0047; fig. 3a) and cortical tPo_2 (17.2 ± 6.5 mmHg at 4 weeks; P_{time} = 0.0049; fig. 3b) showed significant and sustained decreases, along with a decrease in renal oxygen delivery (P_{time} < 0.0001; fig. 3c). Plasma creatinine also decreased during this period (P_{time} = 0.006; table 2). There were no significant changes in renal blood flow, renal vascular conductance, urine output, or creatinine clearance (P_{time} > 0.05; table 2). Other

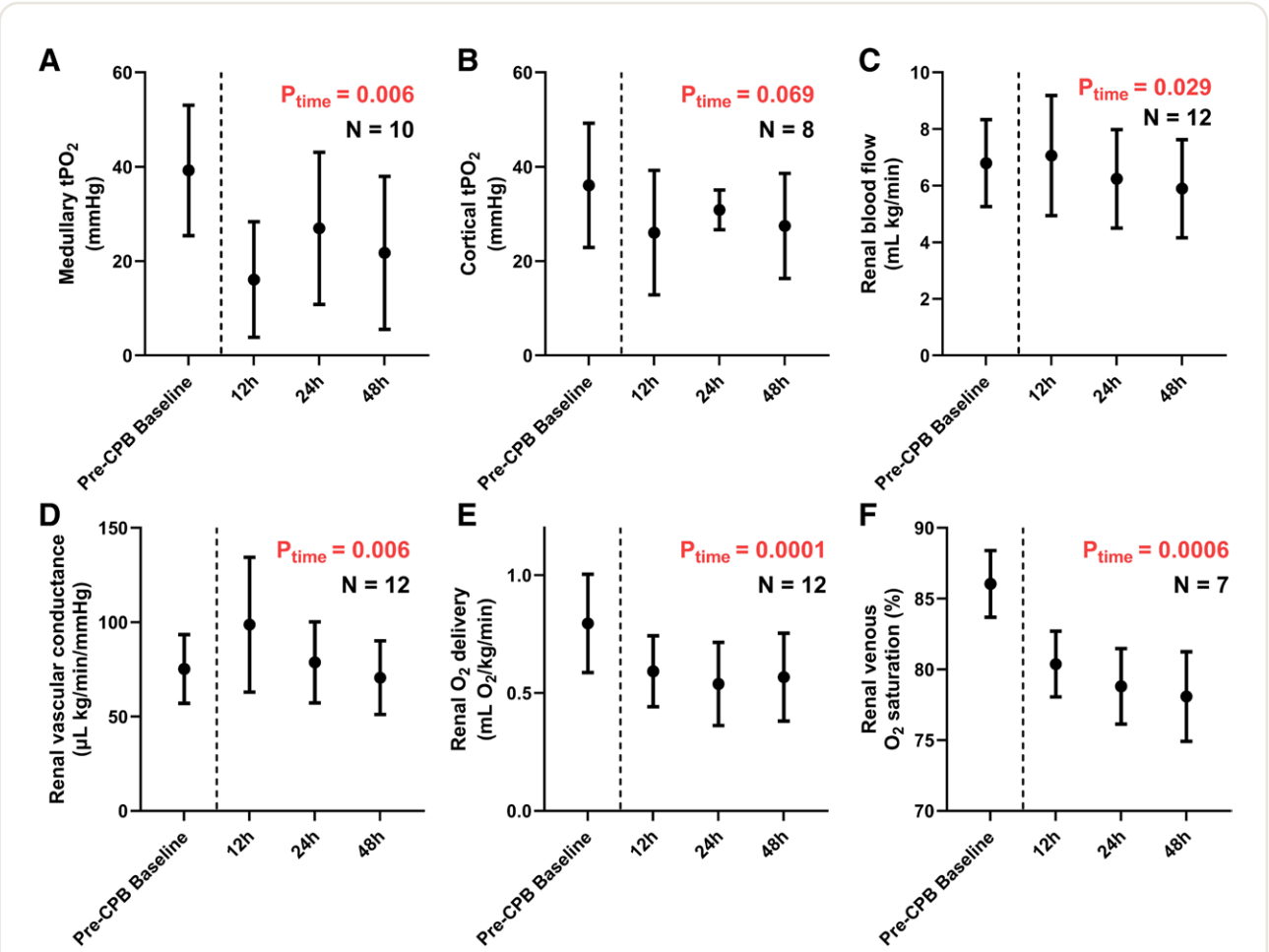

Fig. 2. Changes in renal tissue oxygenation and hemodynamics in the initial 48 h post-CPB. (*A*) Renal medullary tissue oxygen tension (tPo_2). (*B*) Cortical tPo_2. (*C*) Renal blood flow. (*D*) Renal vascular conductance. (*E*) Renal oxygen delivery. (*F*) Renal venous oxygen saturation. Sample size is indicated on each panel, because some of the data are missing due to equipment failure. The data were subjected to mixed-effects model with a Greenhouse–Geisser correction applied to the main effect of "time." The data are presented as means and SD. CPB, cardiopulmonary bypass.

Table 1. Renal and Systemic Variables in the Immediate Post-CPB Period (48 h)

Variable	N	Pre-CPB Baseline	12 h	24 h	48 h	*P* Value
Renal oxygen consumption, ml O_2 · kg^{-1} · min^{-1}	7	0.094 ± 0.027	0.11 ± 0.030	0.097 ± 0.024	0.11 ± 0.027	0.35
Renal oxygen extraction, %	7	13.4 ± 3.22	20.3 ± 3.22	21.6 ± 3.21	21.9 ± 5.84	0.006
Urine output, ml · kg^{-1} · h^{-1}	12	1.1 [0.59, 1.6]	0.69 [0.42, 1.6]	1.4 [0.42, 3.1]	0.64 [0.43, 1.4]	0.16
Plasma creatinine, µmol/L	12	60.3 ± 13.6	62.8 ± 17.4	58.1 ± 14.4	50.6 ± 12.5	0.009
Creatinine clearance, ml/min	12	74.8 ± 25.9	71.7 ± 25.3	79.8 ± 24.1	76.1 ± 23.4	0.73
Mean arterial pressure, mmHg	12	91.3 ± 13.9	73.4 ± 10.0	79.7 ± 6.0	84.0 ± 9.6	0.001
Cardiac output, ml · kg^{-1} · min^{-1}	8	NA	129 ± 27	136 ± 25	113 ± 18	0.020

The data are expressed as mean ± SD or median [quartile 1, quartile 3], depending on the distribution (assessed by the Shapiro–Wilk test). Renal oxygen consumption was calculated as renal blood flow × ([arterial O_2 content] – [venous O_2 content]). Renal oxygen extraction was calculated as 100 × ([arterial O_2 content] – [venous O_2 content])/[arterial O_2 content]. Blood oxygen content was calculated as (0.0139 × [Hb] × SO_2) + (0.003 × Po_2). Creatinine clearance was calculated as ([urine creatinine] × [urine output]/[plasma creatinine]). The *P* values were derived from mixed-effects modeling with a Greenhouse–Geisser correction for the main effect of "time." Sample size is indicated on each variable, because some of the data are missing due to equipment failure.

CPB, cardiopulmonary bypass; NA, not available.

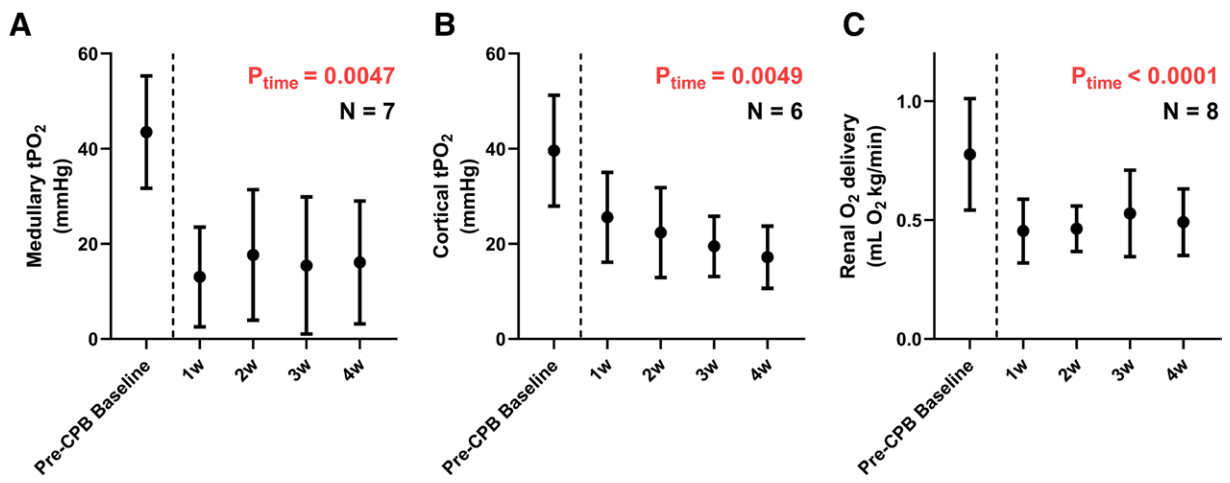

Fig. 3. Changes in renal macro- and microcirculation from 1 to 4 weeks post-CPB. (*A*) Renal medullary tissue oxygen tension (tPo_2). (*B*) Cortical tPo_2. (*C*) Renal oxygen delivery. Sample size is indicated on each panel, because some of the data are missing due to equipment failure or loss of subjects. The data were subjected to a mixed-effects model with a Greenhouse–Geisser correction applied to the main effect of "time." The data are presented as means and SD. CPB, cardiopulmonary bypass.

variables are shown in table 2 and supplemental table S3 (https://links.lww.com/ALN/D925).

Renal Histopathology

As reported in table 3, compared with healthy sheep (fig. 4, A and B), those exposed to CPB exhibited a higher prevalence and severity of peritubular inflammation, characterized by focal accumulations of mononuclear inflammatory cells (8 of 8 *vs.* 1 of 5, $P = 0.007$; severity score: 1 [1 to 1] *vs.* 0 [0 to 0.5], $P = 0.0062$; table 3; fig. 4, C and D). Interstitial fibrosis was also significantly more prevalent and severe in the CPB group (6 of 8 *vs.* 0 of 5, $P = 0.021$; severity score: 1 [0.3 to 2] *vs.* 0 [0 to 0], $P = 0.021$; table 3; fig. 4, E and F). Finally, hyaline and cellular tubular casts were more frequently observed in the CPB group (8 of 8 *vs.* 1 of 5, $P = 0.0070$; severity score: 1 [1 to 2] *vs.* 0 [0 to 0.5], $P = 0.0054$; table 3; fig. 4, G and H).

Discussion

Key Findings

In a large mammalian model of CPB, in the first 48 h postoperatively, CPB induced significant renal medullary hypoxia and a similar degree of cortical hypoxia, along with decreased renal oxygen delivery and renal venous blood desaturation. Over the following 4 weeks, the renal medullary and cortical hypoxia and the decrease in renal oxygen delivery were sustained. Finally, at 4 weeks after CPB, this sustained hypoxia was associated with substantial renal histopathologic injury,

Table 2. Systemic and Renal Variables from 1 to 4 Weeks Post-CPB

Variable	N	Pre-CPB Baseline	1 Week	2 Weeks	3 Weeks	4 Weeks	*P* Value
Renal blood flow, ml · kg^{-1} · min^{-1}	8	6.41 ± 1.51	5.38 ± 1.18	5.18 ± 0.73	5.63 ± 1.77	5.33 ± 1.47	0.11
Renal vascular conductance, ml · kg^{-1} · min^{-1} · mmHg^{-1}	8	71.4 ± 18.1	63.8 ± 11.6	60.8 ± 9.76	60.5 ± 11.8	66.3 ± 14.9	0.25
Urine output, ml · kg^{-1} · h^{-1}	8	0.86 [0.51, 1.4]	0.71 [0.53, 1.3]	1.0 [0.82, 1.4]	1.0 [0.78, 1.5]	1.2 [0.70, 2.2]	0.38
Plasma creatinine, μmol/l	8	62.6 ± 13.8	48.9 ± 16.0	47.0 ± 7.3	53.1 ± 8.5	57.6 ± 9.5	0.006
Creatinine clearance, ml/min	7	80.3 ± 31.3	78.4 ± 33.3	92.1 ± 16.4	91.8 ± 16.6	76.2 ± 19.8	0.50
Mean arterial pressure, mmHg	8	90.4 ± 13.0	84.2 ± 7.2	88.4 ± 8.0	87.4 ± 5.0	85.0 ± 8.7	0.29
Cardiac output, ml · kg^{-1} · min^{-1}	5	NA	129 [116, 146]	119 [111,122]	127 [113, 132]	109 [95.6, 119]	0.021

The data are expressed as mean ± SD or median [quartile 1, quartile 3]. The *P* values were from mixed-effects modeling with a Greenhouse–Geisser correction for the main effect of "time." Sample size is indicated on each variable, because some of the data are missing due to equipment failure or loss of subjects.

CPB, cardiopulmonary bypass; NA, not available.

Table 3. Renal Pathologic Changes in the Study (n = 8) and Healthy Control Groups (n = 5)

	Cardiopulmonary Bypass with Recovery								Control				
Histopathology	R1	R2	R3	R4	R5	R6	R7	R8	C1	C2	C3	C4	C5
Acute tubular necrosis	0	0	0	0	0	0	0	0	0	0	0	0	0
Peritubular inflammation	+	+	+	+	++	+	+	+	0	0	+	0	0
Tubular casts	+	+	+	+	++	++	+	+	0	+	0	0	0
Interstitial fibrosis	+	+	0	+	+	++	++	0	0	0	0	0	0

Zero (0), no histologic renal tubular injury, inflammation, tubular casts or fibrosis; +, mild histologic renal tubular injury, inflammation, tubular casts, or fibrosis; ++, moderate histologic renal tubular injury, inflammation, tubular casts, or fibrosis; +++, severe histologic renal tubular injury, inflammation, tubular casts, or fibrosis.

including peritubular inflammation, interstitial fibrosis, and tubular casts.

Relationships with Previous Studies

To the best of our knowledge, no other study has reported data on renal tissue oxygenation beyond the duration of CPB and the immediate post-CPB period. However, such short-term studies of renal perfusion and oxygenation during CPB have shown findings consistent with those reported in this article.[7–16] For example, in an ovine model, CPB triggered rapid reductions in renal blood flow, medullary oxygenation, renal vascular conductance, and renal oxygen delivery.[13] Our acute findings in this study are consistent with such previous investigations. Importantly, in this study, we extended our observations to show that the CPB-induced renal tissue hypoxia persists for a month after CPB.

Clinical studies have also supported the concept that medullary tissue hypoxia occurs during and after CPB in humans. By using continuous measurements of bladder urinary Po_2, a validated surrogate of medullary tissue oxygenation,[23,24] a correlation has been demonstrated between a low urinary Po_2, its severity and duration, and the development of AKI.[25–27] Such clinical observations support the clinical relevance of our sheep model of CPB.

A previous study reported that CPB induces peritubular inflammation after 2-h exposure to CPB.[13] Another preclinical study demonstrated that histopathologic injuries occurred at 72 h after CPB, including cast formation, epithelial necrosis, tubular sloughing, and glomerular edema.[28] Notably, our study shows that such changes persist for at least 4 weeks after CPB. The presence of sustained inflammation and interstitial fibrosis, which are important potential precursors of chronic kidney disease,[29,30] suggests that the changes observed in the immediate postoperative period do not resolve by 4 weeks after CPB.

The pathologic changes observed in our study occurred despite mild and transient AKI in less than half of the experimental animals. The presence of these histologic changes may explain how even mild AKI after cardiac surgery with CPB increases the risk of developing chronic kidney disease up to fivefold.[3,4] These findings also suggest that the normalization of conventional renal functional indices (creatinine and urine output) may be misleading. Moreover, these observations may explain the reported decrease in renal functional reserve[31] seen after CPB in humans. In this regard, previous clinical studies have suggested that in patients undergoing cardiac surgery with CPB, a reduced baseline renal functional reserve is predictive of postoperative AKI[32] and that CPB-associated AKI leads to a reduction in renal

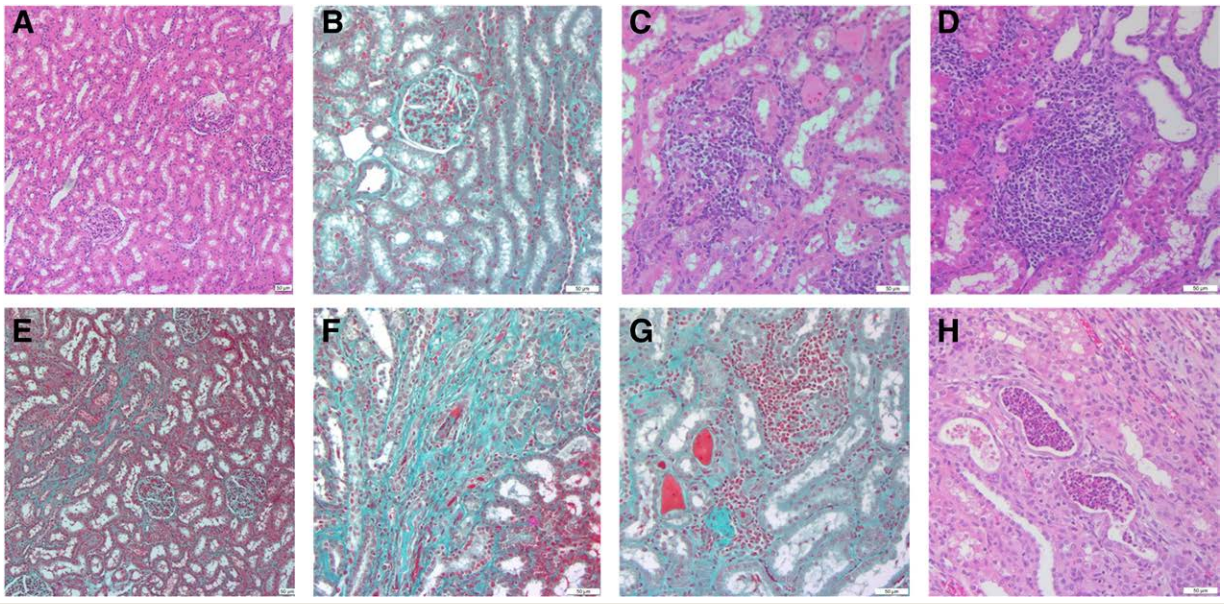

Fig. 4. Representative images of histologic analysis. (*A*, *B*) Normal kidney tissue. (*C*, *D*) Mild and moderate peritubular inflammation. (*E*, *F*) Mild and moderate interstitial fibrosis. (*G*) Hyaline casts. (*H*) Cellular casts. *White bars* represent 50 μm.

functional reserve at 3 months after surgery.[33] Additionally, the findings of a recent large clinical trial of perioperative amino acid infusion to recruit renal functional reserve in patients undergoing cardiac surgery with CPB appear consistent with the above observations.[34]

Study Implications

Our findings demonstrate that renal tissue hypoxia induced by CPB can last for up to 4 weeks. Because no cardiac surgical procedure was performed, these changes can logically only be attributed directly to the CPB itself. Moreover, our study implies that this sustained renal tissue hypoxia is associated with structural injuries to the kidneys, including the development of fibrosis, and that such changes were not associated with abnormalities in creatinine or urine output. This highlights the need for more sensitive markers of renal tissue hypoxia and AKI and the importance of testing interventions aimed at attenuating such tissue hypoxia in the perioperative period. Finally, our observations of sustained renal tissue injury at 4 weeks, combined with previous clinical data showing that renal functional reserve is decreased 90 days after CPB in humans, suggest the need for even longer recovery periods in preclinical studies to investigate the association of structural injury, loss of renal functional reserve, and the potential transition to chronic kidney disease.

Strengths and Limitations

Our study has several strengths. We conducted a comprehensive assessment of the temporal changes in systemic hemodynamics, renal blood flow and oxygen delivery, and kidney function over a 4-week period after CPB. To our knowledge, this is the first study to investigate renal post-CPB changes for such an extended period. Our large-animal model closely replicates all major aspects of CPB in humans, including pump flow, target perfusion pressure, extracorporeal circuit, temperature management, anticoagulation strategies, and the choice of anesthetic drugs, making it relevant to human disease.[20,35] The intra- and postoperative studies were codesigned and implemented with practicing cardiac anesthesiologists, cardiac surgeons, clinical perfusionists, and intensivists to minimize deviations from clinical practice. Finally, the percentage change in hemoglobin from baseline to after CPB and after 28 days was identical to that seen in patients undergoing cardiac surgery.[21]

We acknowledge some limitations. First, the experiment was conducted in young healthy sheep; this may explain the lack of a postoperative increase in plasma creatinine. However, given the frequent presence of diabetes, hypertension, and vascular disease in patients undergoing CPB, it seems logical to hypothesize that the renal histopathologic changes seen in our healthy animals would be even more severe in human patients. Second, given the small sample size and little variability in the low level of tissue oxygenation, it is not possible to conduct additional meaningful analysis to relate such tissue hypoxia to changes in creatinine clearance and/or tissue fibrosis. Third, in the absence of strong clinical evidence of AKI (such as changes in plasma creatinine and/or creatinine clearance) in this healthy cohort of sheep, the causal relationship between renal hypoxia, AKI, and histologic changes cannot be assessed in this study. Additionally, due to the observational nature of our

study, causality cannot be established. However, our findings provide a strong rationale for future studies to investigate whether targeted interventions preventing renal hypoxia in CPB can mitigate postoperative AKI and subsequent histologic injury. Fourth, we used only female animals because chronic catheterization of the bladder in male sheep is technically impossible without a suprapubic approach. Fifth, in our model, the heart was accessed *via* a lateral thoracotomy because systemic and renal hemodynamics are compromised when the sheep is placed in the dorsal recumbency position for median sternotomy.[36] Finally, we utilized flunixin, an anti-inflammatory drug, for postoperative analgesia, which may have affected our findings. However, flunixin has a short half-life and in previous studies using a sheep model live Gram-negative septic shock for 32 to 48 h with more severe AKI that also employed flunixin treatment, we did not detect any signs of renal histopathologic injury.[37,38]

Conclusions

In a clinically relevant large-mammal model, CPB triggered significant pathophysiologic renal events, characterized by sustained renal cortical and medullary tissue hypoxia for 4 weeks after CPB, which was associated with histopathologic injury. These findings provide a potential explanatory pathway for the association between cardiac surgery–associated AKI and chronic kidney disease. They also highlight the need to test interventions aimed at maintaining renal tissue oxygenation to mitigate the risk, severity, and progression of AKI. Finally, they suggest that further preclinical studies of recovery from CPB should consider extending the postoperative monitoring period beyond 4 weeks.

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Competing Interests

Dr. Miles has served on a medical advisory committee for BD Advanced Patient Monitoring (formerly Edwards Lifesciences) in an unpaid capacity within the last 5 yr; the work performed as part of this committee is unrelated to this article. The other authors declare no competing interests.

Correspondence

Address correspondence to Dr. Lankadeva: Florey Institute of Neuroscience and Mental Health, 30 Royal Parade, Parkville, Victoria 3052, Australia. yugeesh.lankadeva@florey.edu.au

Supplemental Digital Content

Supplemental Digital Content 1. Supplemental Methods, https://links.lww.com/ALN/D924
Supplemental Digital Content 2. Supplemental Tables, https://links.lww.com/ALN/D925

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