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# Significant Dysfunction of Kidney Grafts Exposed to Prolonged Warm Ischemia Is Minimized Through Normothermic Ex Vivo Kidney Perfusion

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**Background.** Normothermic ex vivo kidney perfusion (NEVKP) is an emerging technique for renal graft preservation. We investigated whether NEVKP could improve early function of severely injured grafts and reduce the incidence of significant renal dysfunction (SRD) similar to delayed graft function in a model of donation after circulatory death. **Methods.** Kidneys from 30-kg Yorkshire pigs were removed following 120 minutes of warm ischemia (WI). These grafts were then preserved in static cold storage (SCS, n=6) or subjected to NEVKP (n=5) for 8 hours before heterotopic autotransplantation. SRD was defined as postoperative day (POD) 4 oliguria <500 mL/24h with serum K<sup>+</sup>>6.0 mmol/L. **Results.** All 4 surviving animals with 120 minutes WI grafts stored with SCS developed SRD, compared with 1/5 in the NEVKP group (P=0.02). The NEVKP group, when compared with SCS, also demonstrated significantly decreased serum creatinine peak values (1118.51±206.90 µmol/L versus 1675.56±98.15 µmol/L; P=0.002) and higher creatinine clearance (POD4: 9.05±6.97 mL/min versus 0.89±0.56 mL/min; P=0.05). By POD7, serum creatinine was not significantly different than baseline in the NEVKP (431.49±492.50 µmol/L versus 90.19±14.15 µmol/L, respectively; P=0.20) but remained elevated following SCS (1189.25±309.47 µmol/L versus 97.26±29.18 µmol/L, respectively; P<0.01). Histology demonstrated significantly decreased tubular injury scores compared with SCS grafts (P=0.03). **Conclusions.** Kidney grafts subjected to 120 minutes WI before retrieval showed significant improvement in function, prevention of SRD, and decreased injury following 8 hours of NEVKP.

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## INTRODUCTION

The success of kidney transplantation is limited by the scarcity of donor organs, with 94665 patients in the United States on the waiting list awaiting as of May 2020.<sup>1-4</sup> This

demand has led to interest in expanding the donor pool beyond standard-criteria kidney donors, including organs retrieved from donation after circulatory death (DCD) donors.

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The use of DCD kidney grafts has been increasing in clinical practice with 14 038 DCD kidney transplants performed in the United States in 2017.<sup>4,5</sup> Compared with standard-criteria grafts, the use of DCD kidneys is complicated by an increased rate of delayed graft function (DGF).<sup>6</sup> Definitions of DGF vary, but the most commonly used criterion is the need for dialysis within the first week following transplantation.<sup>6</sup> Patients who develop DGF have shown higher rates of acute rejection, increased serum creatinine in follow-up (indicating poorer graft function), higher incidence of overall graft failure, and increased mortality.<sup>7-9</sup> As a result of its impact on patient outcomes and its associated effects on the costs to the healthcare systems and availability of organs, methods to minimize or prevent DGF are of interest.

The causes of DGF in DCD grafts are incompletely understood, although prolonged warm and cold ischemic times are widely accepted as contributing factors to its development.<sup>8,9</sup> DCD grafts are particularly sensitive to ischemia-reperfusion injury (IRI) promoted by current standard storage strategies including static cold storage (SCS) and hypothermic machine perfusion (HMP).<sup>10,11</sup>

Normothermic ex vivo kidney perfusion (NEVKP) is an emerging technology for renal graft preservation that offers the opportunity to avoid cold storage. We perfuse grafts with an erythrocyte-based perfusate that delivers oxygen and nutrients at physiologic temperatures during the entire preservation time of 8 hours. In grafts that have been exposed to severely prolonged warm ischemic times and tolerate cold storage poorly, NEVKP can potentially prevent further damage from occurring by providing the conditions necessary for aerobic metabolism.

Our group has previously demonstrated the safety and efficacy of NEVKP in a heart-beating donor model of kidney transplantation with 8 hours of perfusion. We have also demonstrated superiority of NEVKP compared with SCS when storing kidneys subjected to 30 minutes of warm ischemia (WI) to mimic DCD, with improved postoperative serum creatinine and creatinine clearance.<sup>12</sup> Nevertheless, these grafts still demonstrated acceptable postoperative graft function with 8 hours of SCS and thus represent a degree of damage that would not preclude transplantation currently. In this study, we are interested in investigating whether grafts that would be otherwise be declined could be utilized for transplantation following NEVKP storage. Specifically, we address whether NEVKP can prevent or reduce significant renal dysfunction (SRD) similar to DGF in renal grafts that have been exposed to prolonged WI.

## MATERIALS AND METHODS

### Animals

Male Yorkshire pigs (30 kg) were obtained from Caughell farms (Fingal, ON). Animal protocols were approved by our institutional review board. All care provided to these animals followed the *Principles of Laboratory Animal Care* created by the National Society for Medical Research and the *Guide for the Care of Laboratory Animals* produced by the National Institutes of Health. Water and food ad libitum were provided while animals were kept in species-adapted housing.

### Kidney Retrieval and Transplantation

Porcine kidney retrieval and heterotopic autotransplantation were performed as previously described by our

laboratory.<sup>13</sup> In brief, animals were given anesthesia consisting of intramuscular injections of ketamine (20 mg/kg; Bimeda-MTC Animal Health Inc., Cambridge, Canada), midazolam (0.3 mg/kg; Pharmaceutical-Partners-of-Canada Inc., Richmond Hill, Canada), and atropine (0.04 mg/kg; Rafter-8 Products, Calgary, Canada). After intubation, isoflurane (1.5%; Pharmaceutical-Partners-of-Canada Inc., Richmond Hill, Canada) was administered. A midline incision was utilized for exposure, and the right kidney was dissected. Heparin (10 000 IU; Sandoz Canada Inc., Toronto, Canada) was administered intravenously 2 minutes before using vascular clamps on the right renal artery and vein, which was performed to induce 120 minutes of WI. The kidney was then resected, the renal artery was cannulated, and the kidney was immediately flushed with 300 mL of histidine-tryptophan-ketoglutarate (HTK) solution (Metapharm Inc., Brantford, Canada) at 4°C before placement on NEVKP circuit or SCS. The abdomen was closed, and the pig recovered from anesthesia during kidney storage.

Following 8 hours of kidney storage, pigs were reanesthetized with 5 mL Propofol IV (PharmaScience Inc., Montreal, Canada) and a continuous infusion of 150 mg/h Propofol IV was given with 1.5% isoflurane after intubation. The contralateral kidney was then resected and discarded. The stored kidney was flushed with 300 mL of HTK solution, and the following renal anastomoses were made: renal vein end-to-side to vena cava, renal artery end-to-side to aorta, and donor ureter side-to-side to recipient ureter. Perioperative care, drug administration, and follow-up were performed as previously described.<sup>13</sup>

### Normothermic Ex Vivo Kidney Perfusion

Our group's NEVKP strategy has been previously described.<sup>14</sup> In short, kidneys (n=5) were continuously perfused for 8 hours using a converted cardiopulmonary bypass machine (Sorin Group Inc., Markham, Canada). The perfusate was created to mimic physiologic conditions. This consisted of Ringer's lactate, Steen solution (XVIVO Perfusion AB, Goteborg, Sweden), washed erythrocytes, double reverse osmosis water, sodium bicarbonate (8.4%), calcium gluconate (10%, 100 mg/mL), and heparin. Continuous infusion of verapamil at 0.25 mg/h was provided to the arterial portion of the circuit, and a continuous infusion of amino acids, glucose, and insulin (Dextrose + Travasol + Humulin R, 1 mL/h) was added to the venous reservoir and titrated to achieve a target glucose concentration of 5–15 mmol/L. Oxygen and carbon dioxide gas (95%/5%; 2 L/min) were also administered continuously. Through the course of perfusion, additional Ringer's lactate was provided to replace urine and other insensible losses from the circuit at 10 mL/h.

### Static Cold Storage

Following retrieval and cold flush described above, the donor kidney was placed in a sterile organ bag (CardioMed Supplies Inc., Lindsay, Canada) containing cold HTK solution. The kidney was maintained at 4°C and stored for 8 hours (n=6).

### Whole Blood, Serum, and Urine Analysis

Venous blood gas analysis (RAPIDPoint 500 Systems; Siemens AG, Berlin, Germany) of perfusate samples was performed hourly during NEVKP. Recipient venous blood

from the previously placed central line was then collected for blood gas analysis and for serum measurements of creatinine (Piccolo Xpress, Union City, Canada) daily until postoperative day (POD) 7.

On the day preceding retrieval and transplantation (baseline), on POD3–4, and on POD6–7, animals were placed in a custom-designed cage where urine funnels through a filter and is collected for 24 hours. Creatinine clearance was measured following this collection with urine and serum analysis performed in the Core Laboratory on the Abbott Architect Chemistry Analyzer using the manufacturer's reagents (Abbott Laboratories, Abbott Park, IL). Creatinine clearance was calculated using a standard formula.<sup>15</sup>

$$\text{Creatinine Clearance} = (\text{Urine Creatinine} \times \text{Urine Volume}) / (\text{Serum Creatinine} \times \text{Time})$$

### Definition of Significant Renal Dysfunction

DGF is most commonly defined as the need for dialysis within the first week after kidney transplantation.<sup>6</sup> Dialysis is not feasible in pigs, and no equivalent porcine definition currently exists. However, low urine output and elevated serum potassium together reflect SRD. Our a priori definition of SRD is a serum potassium above 6.0 mmol/L on the fourth POD together with a urine output of <500 mL in the accompanying 24 hours period. Day 4 was chosen as this was the earliest time point we performed 24-hour urine collection with pigs in the custom-designed cage. Oliguria is not defined in the porcine setting. Normal urine output of 12–19 kg of Danish Landrace pigs at 9–13 weeks of age in a 24-hour period has been reported at 2845 ± 900 mL.<sup>16,17</sup> Although our pigs are larger, we choose 500 mL as low urine output as this represents a convenient threshold that is >2.5 standard deviations from this mean.

### Histology

Donor kidney tissue was obtained by needle biopsy immediately after renal vascular anastomosis and reperfusion in the donor animal. Hemostasis was achieved with electrocautery and direct pressure. POD7 renal biopsies were collected by wedge resection with the animal under anesthesia. Tissue was placed in 10% neutral buffered formalin and transferred to 70% ethanol after 48 hours. After paraffin-embedding, sectioning, and staining, 3 μm periodic acid-Schiff-stained sections were used to score tubular injury and interstitial inflammation on a scale of 0-to-3, respectively, by a single pathologist blinded to the experimental group.<sup>18,19</sup> Tubular injury, including brush border loss, tubular dilation, epithelial vacuolation, thinning and sloughing, and luminal debris, were scored in 10 high-power fields and averaged to assess overall tubular injury. Interstitial inflammation was scored in 10 low-power fields and averaged.<sup>18,19</sup>

### Statistical Analysis

Significance was defined as  $P < 0.05$  between NEVKP and SCS groups. A log-rank test was used for calculation of differences in mortality. The Student *t* test assuming unequal variance was used to compare differences of parametric variables, and a paired *t* test was used to test significance of differences in normally distributed continuous parameters over time within the same group. Binomial categorical data were assessed through the Fisher's Exact Test. Significance of

semi-quantitative histological scores was determined with the Mann-Whitney *U* test.

## RESULTS

### Animal Baseline Characteristics and Surgical Procedure

Pigs utilized for autotransplantation did not vary in weight between SCS ( $n=6$ ) and NEVKP ( $n=5$ ) ( $31.4 \pm 1.5$  versus  $31.7 \pm 1.1$  kg, respectively;  $P=0.66$ ). Similarly, total vascular anastomosis time did not significantly differ ( $43.8 \pm 16.2$  min versus  $37.2 \pm 13.6$  min, respectively;  $P=0.49$ ). Additional operative parameters did not vary between groups (Table S1, SDC, <http://links.lww.com/TXD/A262>). Vital signs were continuously monitored and were maintained in physiological range.

### Characteristics of Normothermic Ex Vivo Perfusion

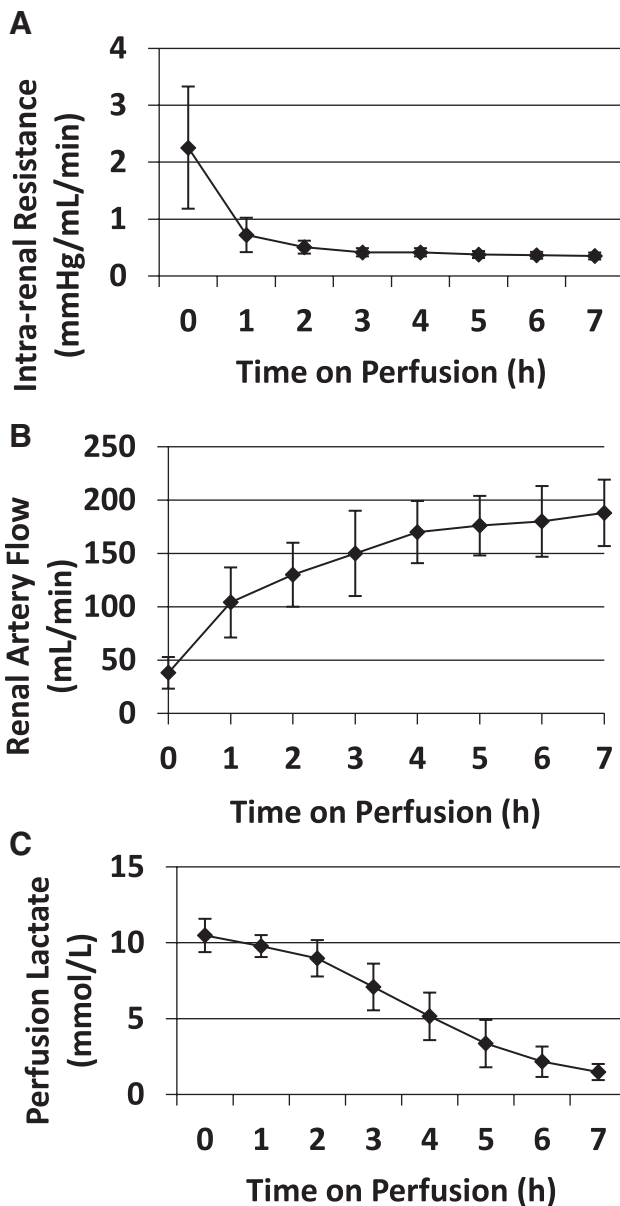
Through NEVKP storage, intrarenal resistance decreased from  $2.26 \pm 0.9$  mmHg/mL/min at 0 hour to  $0.37 \pm 0.6$  mmHg/mL/min at 7 hours ( $P < 0.01$ ), and renal blood flow increased from  $38.0 \pm 14.8$  mL/min at 0 hour to  $188.0 \pm 31.1$  mL/min at 7 hours ( $P < 0.01$ ) (Figure 1A and B, respectively). Perfusate lactate concentrations also decreased from  $10.48 \pm 0.93$  mmol/L at 0 hour to  $1.48 \pm 0.85$  mmol/L at 7 hours ( $P < 0.01$ ) (Figure 1B). Physiologic acid-base parameters (including pH, bicarbonate, and base excess) and electrolyte concentrations (serum sodium, potassium, calcium, and chloride) were maintained throughout the duration of perfusion without any additional supplementation (Figure S1, SDC, <http://links.lww.com/TXD/A262>). Hematocrit was maintained between 25% and 30%. Total urine output during perfusion was minimal ( $23.0 \pm 21.4$  mL/8 h) and started at higher rates but decreased over time on perfusion.

### Recipient Survival

All 5 animals that received kidney grafts subjected to 120 minutes of WI before NEVKP storage survived the 7-day follow-up period. In comparison, 2/6 animals that received grafts following SCS were sacrificed before completion of the follow-up period (Figure 2). The sample size of the SCS group was increased to 6 to assess if the first animal to undergo early euthanasia was an outlier. Both animals demonstrated clinical deterioration. One was sacrificed because of severe lactic acidosis on POD1, and the other was sacrificed on POD2 because of respiratory distress secondary to hydrothorax identified on necropsy. The difference in survival between groups did not reach statistical significance ( $P=0.13$ ).

### Grafts Subjected to 120 Minutes of WI Before Procurement Demonstrated Significant Renal Dysfunction After Storage With SCS

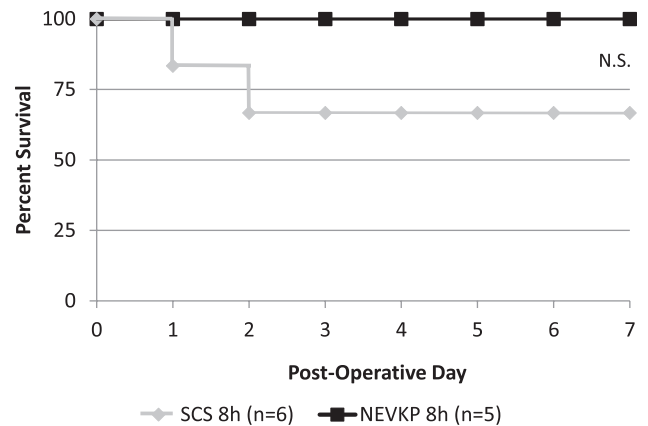
All 4 surviving animals that received grafts exposed to 120 minutes WI before procurement and stored for 8 hours in SCS before autotransplantation met the criteria for SRD in that the 24 hours urine production measured between POD3–4 was <500 mL, and serum potassium was >6.0 mmol/L on POD4. In comparison, only 20% of animals in the NEVKP group fulfilled the criteria for SRD ( $P=0.02$ ). We could not identify any pretransplantation perfusion characteristics that would predict the poor outcome in this 1 specimen, including intrarenal resistance or lactate clearance.



**FIGURE 1.** Improving perfusion characteristics of 120min warm ischemia grafts while on normothermic ex vivo kidney perfusion ( $n=5$ ). A, intra-renal resistance; B, renal artery flow; and C, perfusion lactate concentration. Expressed as mean  $\pm$  SD.

### Recipients of NEVKP-Stored Marginal Grafts Demonstrate Improved Postoperative Renal Function

Prolonged WI grafts stored with NEVKP demonstrated improved kidney function following heterotopic transplantation, whereas those stored in cold static conditions exhibited SRD. Rising serum creatinine curves from both groups were similar on POD1–2 (Figure 3A). In contrast, pigs receiving NEVKP-stored grafts had lower serum creatinine values from POD3–7 when compared with the SCS group and reaching significance on POD4–7 (Figure 3A;  $P<0.05$ ). By POD7, serum creatinine values in the NEVKP-stored group were not statistically significant from baseline values ( $4.88 \pm 5.57$  mg/dL [ $431.49 \pm 492.50$   $\mu\text{mol/L}$ ] versus  $1.02 \pm 0.16$  mg/dL [ $90.19 \pm 14.15$   $\mu\text{mol/L}$ ], respectively;  $P=0.20$ ), whereas serum creatinine remained elevated in the SCS group

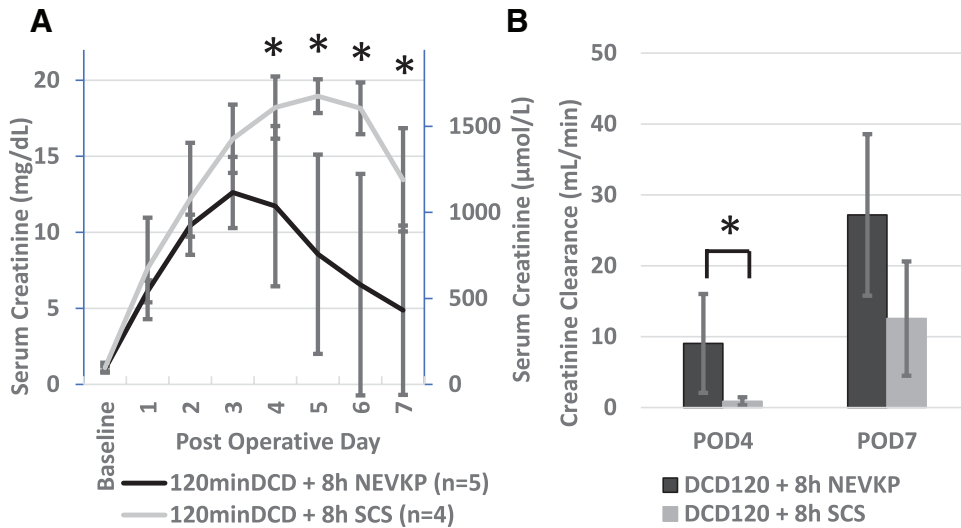


**FIGURE 2.** Recipient survival following transplantation of grafts subjected to 120min warm ischemia and either static cold storage (SCS) or normothermic ex vivo kidney perfusion (NEVKP). Survival is depicted in a Kaplan-Meier plot. NS, not significant.

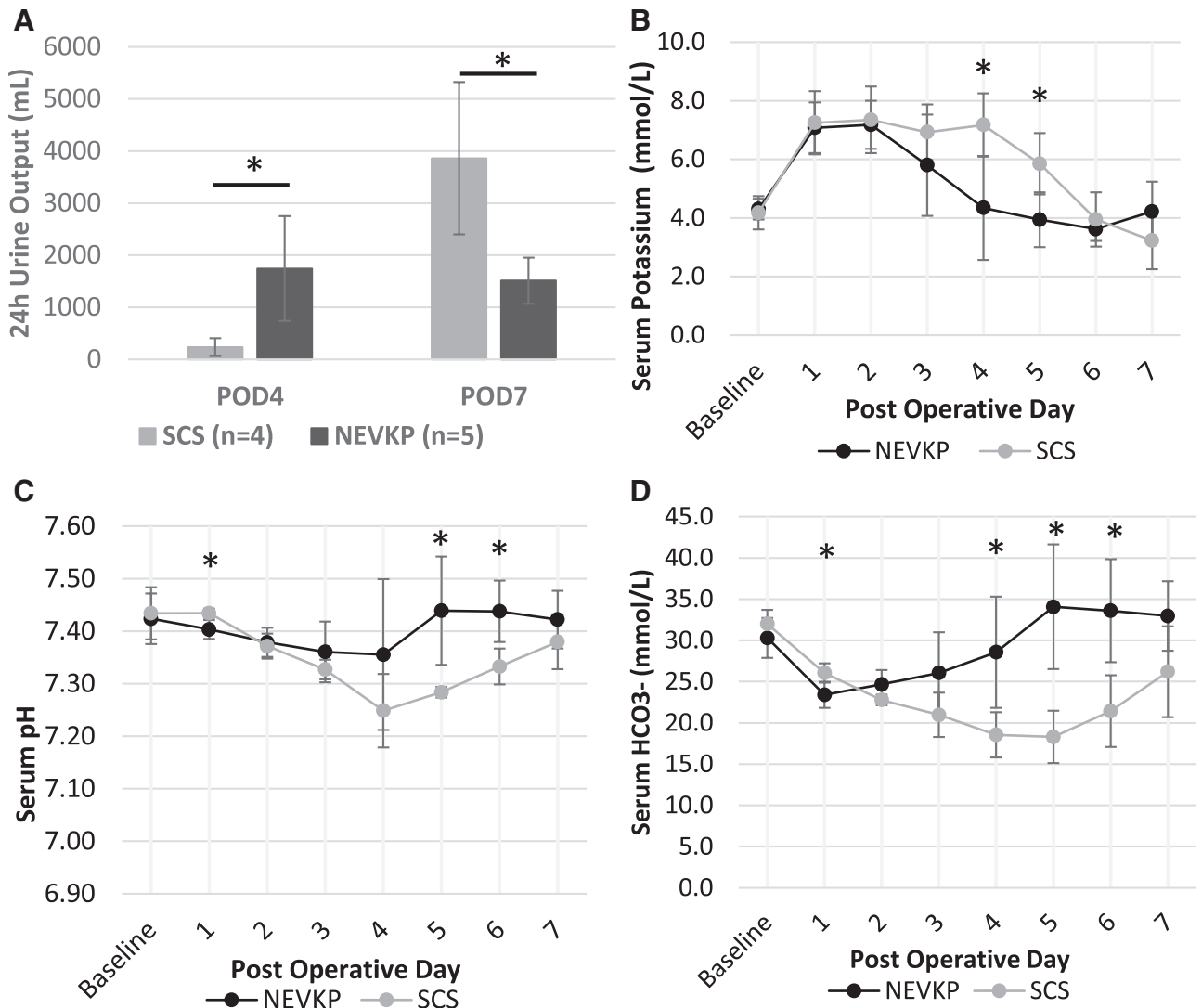
compared with baseline (Figure 3A;  $13.45 \pm 3.50$  mg/dL [ $1189.25 \pm 309.47$   $\mu\text{mol/L}$ ] versus  $1.1 \pm 0.33$  mg/dL [ $97.26 \pm 29.18$   $\mu\text{mol/L}$ ], respectively;  $P<0.01$ ). Peak serum creatinine values were also significantly decreased in recipients that received NEVKP-stored grafts. The peak creatinine in this group was  $12.62 \pm 2.34$  mg/dL [ $1115.86 \pm 206.90$   $\mu\text{mol/L}$ ] on POD3 compared with the peak serum creatinine value of  $18.95 \pm 1.11$  mg/dL [ $1675.56 \pm 98.15$   $\mu\text{mol/L}$ ] on POD5 in the SCS group ( $P=0.002$ ) (Figure 3A). Creatinine clearance was improved in NEVKP-stored grafts compared with SCS on POD4 ( $9.05 \pm 6.97$  mL/min versus  $0.89 \pm 0.56$  mL/min;  $P=0.05$ ) and trends higher on POD7 ( $27.14 \pm 11.39$  mL/min versus  $12.55 \pm 8.06$  mL/min;  $P=0.06$ ) (Figure 3B). Individual animal postoperative renal performance is depicted in Figure S2 (SDC, <http://links.lww.com/TXD/A262>).

NEVKP prevented SRD, improved urine output, and improved regulation of serum electrolytes/acid-base homeostasis postoperatively. Postoperative urine output began earlier in recipients of NEVKP-stored grafts and resulted in higher urine output on POD4 ( $1745 \pm 1006$  mL). In contrast, urine output remained low in recipients of SCS-stored grafts, with all animals producing  $<500$  mL of urine in the 24 hours collection period ( $235 \pm 172$  mL;  $P=0.03$ ). However, urine output began in the SCS group by the end of the observation period with all animals producing more than 500 mL at POD7 ( $3863 \pm 1463$  mL; Figure 4A). Individual urine output values are presented in Figure S3a (SDC, <http://links.lww.com/TXD/A262>). Serum electrolytes and acid-base status were better regulated with more rapid return to baseline values following NEVKP compared with SCS in grafts subjected to prolonged ischemia. Elevated serum potassium levels were seen in both NEVKP and SCS-stored groups on POD1–3; however, levels begin to return toward baseline on POD3 and are similar to baseline on POD4–7 in the NEVKP group, whereas potassium remained elevated in the SCS group on POD4–5 (POD4:  $4.3 \pm 1.8$  mmol/L versus  $7.2 \pm 1.1$  mmol/L;  $P=0.02$ ; POD5:  $3.9 \pm 0.9$  mmol/L versus  $5.9 \pm 1.1$  mmol/L, respectively;  $P=0.03$ ; Figure 4B) Individual serum potassium values are presented in Figure S3b (SDC, <http://links.lww.com/TXD/A262>). The pH was acidotic in the SCS-stored group and remained physiological in the NEVKP group at POD6 (POD5:  $7.28 \pm 0.01$  versus  $7.44 \pm 0.10$ ;  $P<0.05$ ;





**FIGURE 3.** Postoperative renal graft function measured through (A) serum creatinine expressed as mean±SD and (B) creatinine clearance obtained following 24h urine collection also expressed as mean±SD. \**P*<0.05. DCD, donation after circulatory death; NEVKP, normothermic ex vivo kidney perfusion; POD, postoperative day; SCS, static cold storage.



**FIGURE 4.** Parameters of significant renal dysfunction A. POD4—24h urine output and postoperative serum electrolyte and acid-base regulation including B. Serum potassium. C. Serum pH and D. Serum bicarbonate levels. Values are expressed as mean±SD. \**P*<0.05. HCO<sub>3</sub><sup>-</sup>, bicarbonate; NEVKP, normothermic ex vivo kidney perfusion; POD, postoperative day; SCS, static cold storage.

POD6:  $7.33 \pm 0.03$  versus  $7.44 \pm 0.06$ ;  $P < 0.05$ ; Figure 4C). This acidosis had a metabolic component with decreased serum bicarbonate levels in the SCS group compared with baseline values in the NEVKP group from POD4–6 (POD4:  $18.55 \pm 2.74$  mmol/L versus  $28.56 \pm 6.75$  mmol/L;  $P < 0.05$ ; POD5:  $18.30 \pm 3.17$  mmol/L versus  $34.08 \pm 7.55$  mmol/L;  $P < 0.01$ ; POD6:  $21.43 \pm 4.35$  mmol/L versus  $33.60 \pm 6.24$  mmol/L;  $P < 0.05$ ; Figure 4D).

### Decreased Histopathologic Renal Injury Is Observed in NEVKP-stored Compared With SCS-stored Marginal Grafts

Renal biopsies taken at the time of sacrifice on POD7 demonstrated decreased tubular injury with less brush border loss, tubular dilation, epithelial vacuolation, and cast formation as luminal debris in the NEVKP grafts compared with the SCS group (median score 2.0 [range, 1.0–3.0] versus median score 3.0 [range, 3.0–3.0];  $P < 0.05$ ; Figure 5). Interstitial inflammation remained similar between the NEVKP and SCS groups on POD7 (median score 2.0 [range, 1.0–2.0] versus median score 2.0 [range, 1.0–3.0];  $P = 0.42$ ). There was no histological difference between grafts from the NEVKP and SCS groups immediately after transplantation regarding tubular injury (median score 2.0 [range, 1.0–3.0] versus median score 2.0 [range, 1.0–3.0];  $P = 1.00$ ) and interstitial inflammation (median score 1.0 [range, 1.0–3.0] versus median score 1.0 [range, 1.0–1.0];  $P = 0.24$ ).

## DISCUSSION

NEVKP is a novel technique for the preservation of kidneys before transplantation. To our knowledge, this is the first report to demonstrate the development of SRD similar to human DGF in a large animal survival model following SCS and the first study directly comparing the effect of replacing cold storage with NEVKP preservation for these extremely injured kidneys.

Specifically, we demonstrated that all kidneys subjected to 120 minutes of WI and stored in SCS fulfilled our criteria for SRD on POD4 with low urine output and increased serum potassium. These grafts began to show signs of functional recovery with decreasing serum creatinine and increasing urine output at POD7 indicating that PNF was not present. SRD was prevented in 80% of grafts stored with NEVKP. Moreover, survival was better in the NEVKP group with no animals requiring early sacrifice, although we recognize our study was not statistically powered to detect this endpoint. This survival difference could not be attributed to a technical error. Although the vascular anastomosis time was slightly elevated in the SCS group, it was not statistically different given the variation in both groups. It is unlikely this accounted for the observed differences.

In the 2 animals in the SCS group requiring early euthanasia, the causes of lactic acidosis, hemothorax, and respiratory distress were not identified on necropsy. However, it is possible that a systemic inflammatory response from the ischemic graft caused these events.<sup>20</sup> An increase in oxidative stress following a shift to anaerobic metabolism leads to cell damage and death, and a release of proinflammatory mediators. Upon reperfusion, these mediators are released systemically and can promote vascular permeability, venous congestion, and

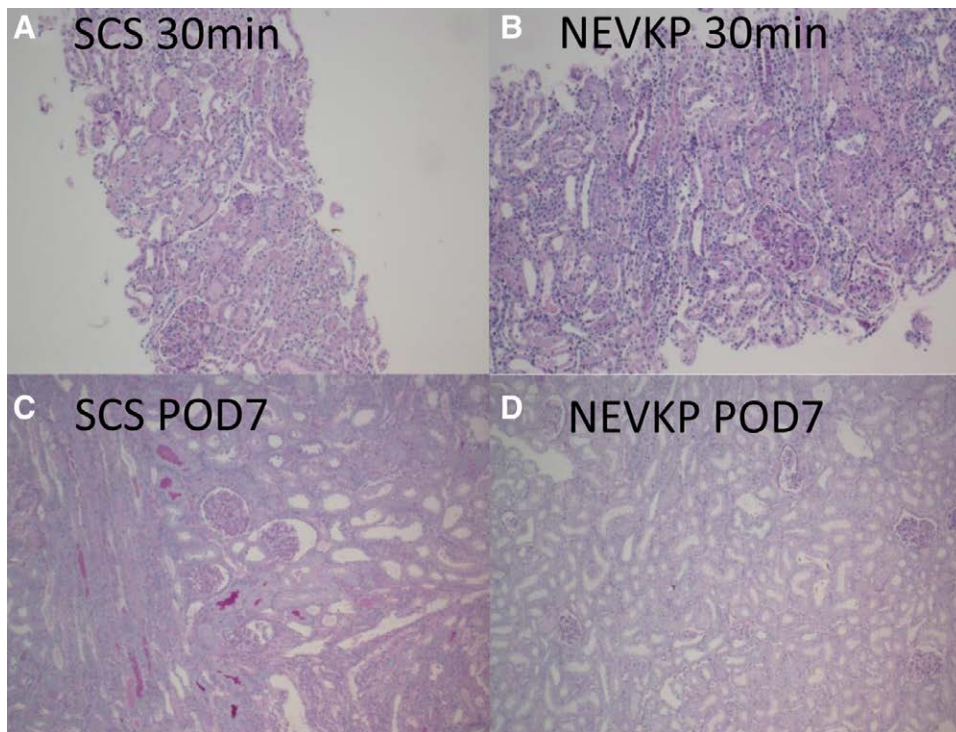
apoptosis/necrosis in distant targets.<sup>21</sup> NEVKP may protect the recipient from these responses.

The precise mechanisms accounting for NEVKP's superiority in this model remain speculative. We hypothesize that aerobic metabolism is maintained, and further IRI is minimized with NEVKP. In contrast, ongoing ischemia during SCS may exacerbate IRI following transplantation.<sup>21,22</sup> Moreover, proinflammatory by-products caused by prolonged WI may be removed and diluted from the graft during NEVKP. The histological findings also suggest a possible role for immunomodulation. Although tubular injury was decreased following NEVKP storage, similar numbers of infiltrating mononuclear cells were observed. It is possible these cells are not activated due to a reduction in proinflammatory mediators resulting from limiting IRI. It is also possible these cells represent a different subset of leukocytes that promote immunomodulation or regeneration, as seen in other settings.<sup>23,24</sup>

Few previous studies have indicated potential mechanism for NEVKP's superiority. Hosgood et al suggested that NEVKP can condition the kidney against further damage by promoting the expression of heat-shock protein 70. This encourages cell survival and maintains cell homeostasis by preventing inappropriate binding and folding of proteins in response to stress.<sup>25</sup> They also demonstrate that NEVKP upregulates IL-6 that promotes antiinflammatory effects and repair despite potentially promoting inflammation in the kidney at first.<sup>25</sup> Brasile et al utilized a canine kidney transplantation model using 2 hours of WI and 24 hours of perfusion at 32°C with an acellular solution containing fibroblast growth factor. They speculated that aerobic metabolism was sufficiently restored to increase expression of proliferating nuclear cell antigen involved in protein synthesis. They also demonstrated restoration of cytoskeletal architecture. However, both findings were depended on the presence of fibroblast growth factor in the perfusate.<sup>26</sup> Our laboratory previously demonstrated that NEVKP itself directly repairs warm ischemic damage with improved renal function compared with grafts that were not stored and subjected to immediate retransplantation.<sup>27</sup> A combination of factors discussed above may contribute to the improved graft function following NEVKP storage.

Very few large animal studies have been conducted in which the effects of prolonged WI on kidney grafts were explored. An early study by Brasile et al demonstrated warm perfusion technology prevented PNF in a canine kidney autotransplantation model following 2 hours of WI and 18 hours of warm perfusion at a 32°C using an acellular solution. Control group kidneys were either immediately transplanted or first subjected to HMP. The grafts were described as having PNF since the animals required early sacrifice due to increasing serum creatinine and poor overall health. Warm perfused kidneys ( $n = 5$ ) resulted in improved serum creatinine and survival of the animals for the 10-day period of observation.<sup>28</sup> The presence of PNF in the control animals may represent a reduced ability of canine kidneys to tolerate WI. Alternatively, earlier euthanasia in control animals may have prevented the demonstration of recoverable renal function. These control groups were also limited by the sample size ( $n = 2$ ).

The Hosgood-Nicholson group also addressed the impact of prolonged WI in porcine renal grafts through experiments that incrementally increased the WI time of porcine renal grafts for 7–120 minutes before procurement followed by flushing and storage with cold hyperosmolar citrate solution.



**FIGURE 5.** Representative PAS staining of transplanted kidneys: A, 30min following reperfusion after 8h SCS (10× magnification), and (B) 30 min following reperfusion after 8h NEVKP storage (10× magnification demonstrating equal degrees of tubular brush border loss and luminal debris between these groups). C, SCS-stored kidneys at POD7 (5× magnification) showed tubular casts and also greater brush border loss and dilatation compared with (D). NEVKP-stored kidneys at POD7 (5× magnification). NEVKP, normothermic ex-vivo kidney perfusion; POD, postoperative day; SCS, static cold storage.

Subsequently, to simulate posttransplantation reperfusion, graft function was assessed through 3 or 6 hours of ex vivo warm perfusion.<sup>29,30</sup> These studies demonstrated incremental deterioration of renal hemodynamics, creatinine clearance, urine output, and fractional excretion of sodium with increasing warm ischemic times. However, important limitations in their experimental design remain. The 3–6 hours of ex vivo reperfusion to simulate transplantation and to assess graft injury is too short and lacks the in vivo mediators that can exacerbate graft injury. Also, there was no direct comparison of different storage strategies to mitigate the effect of warm ischemic injury. Our study has addressed these limitations.

Our findings in the porcine kidney transplant model have important clinical implications. First, 120 minutes of complete WI induces more extensive injury than seen in controlled DCD settings with an agonal phase over 120 minutes. This suggests that even more damaged grafts could be considered for clinical use following NEVKP. Second, DGF in SCS DCD grafts has a negative impact on long-term graft outcome and can be associated with increasing costs and utilization of healthcare resources such as the need for retransplantation.<sup>31</sup> Reducing DGF in the DCD setting, like the reduction of SRD in our model, could mitigate these consequences.

Our study has several shortcomings that require consideration. The long-term impact on graft function of NEVKP was not assessed due to the logistical barriers in serially assessing large animals. An autotransplantation model was also necessary as an immunosuppression, and crossmatching protocol are not established in the porcine setting. The effects of alloimmunity and interaction with IRI were not evaluated. The small sample size imposed by the large animal study cost, along with the inability to assess graft function of animals

that were sacrificed in the SCS group could also introduce selection bias that may result in underestimating the improvement and effect of NEVKP storage. We also did not assess the effects of HMP storage. HMP may confer some protective effect on the grafts compared with SCS but not to the degree observed with NEVKP due to ongoing hypoxia as suggested by previous work in models with less WI injury.<sup>32</sup>

This study was the first to demonstrate that NEVKP, compared with SCS, improves the immediate posttransplantation function by preventing the development of SRD in DCD kidneys subjected to prolonged ischemia. Importantly, the animal model we used has many clinical similarities to DGF observed after human kidney transplantation. Grafts that would otherwise be declined for transplantation currently because of profound WI could be considered for use with NEVKP while decreasing the incidence of SRD and potentially preventing long-term complications. This would have a dramatic impact on the organ donation pool and allow improved access for life-saving transplantation for many patients.

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