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Subnormothermic Oxygenated Machine Perfusion (24 h) in DCD Kidney Transplantation

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Background. Ex vivo kidney perfusion is an evolving platform that demonstrates promise in preserving and rehabilitating the kidney grafts. Despite this, there is little consensus on the optimal perfusion conditions. Hypothermic perfusion offers limited functional assessment, whereas normothermic perfusion requires a more complex mechanical system and perfusate. Subnormothermic machine perfusion (SNMP) has the potential to combine the advantages of both approaches but has undergone limited investigation. Therefore, the present study sought to determine the suitability of SNMP for extended kidney preservation. **Methods.** SNMP at 22–25 °C was performed on a portable device for 24 h with porcine kidneys. Graft assessment included measurement of mechanical parameters and biochemical analysis of the perfusate using point-of-care tests. To investigate the viability of kidneys preserved by SNMP, porcine kidney autotransplants were performed in a donation after **circulatory** death (DCD) model. SNMP was also compared with static cold storage (SCS). Finally, follow-up experiments were conducted in a subset of human kidneys to test the translational significance of findings in porcine kidneys. **Results.** In the perfusion-only cohort, porcine kidneys all displayed successful perfusion for 24 h by SNMP, evidenced by stable mechanical parameters and biological markers of graft function. Furthermore, in the transplant cohort, DCD grafts with 30 min of warm ischemic injury demonstrated superior posttransplant graft function when preserved by SNMP in comparison with SCS. Finally, human kidneys that underwent 24-h perfusion exhibited stable functional and biological parameters consistent with observations in porcine organs. **Conclusions.** These observations demonstrate the suitability and cross-species generalizability of subnormothermic machine perfusion to maintain stable kidney perfusion and provide foundational evidence for improved posttransplant graft function of DCD kidneys after SNMP compared with SCS.

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The imbalance between organ supply and clinical need is a fundamental challenge in kidney transplantation, resulting in only ~20% of patients on the waitlist receiving a transplant in a given year.[1] High discard rates of marginal kidney grafts exacerbated this problem. For instance, in 2021, 24.6% of deceased donor kidneys were recovered but not transplanted.[1] Marginal kidneys are discarded on the basis of high-risk clinical features, biopsy findings, and prolonged cold ischemic time. To use these kidney grafts more effectively, innovation in organ preservation, rehabilitation, and graft assessment is required. Ex vivo kidney perfusion with active oxygenation[2-4] has been recognized as a promising alternative to the current standard of

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care, which includes both static cold storage (SCS)[5] and hypothermic perfusion (HMP) without oxygenation.[6]

However, there is no consensus on the optimal perfusion conditions for ex vivo kidney perfusion, with the current focus on either hypothermic or normothermic conditions.[7-11] Although HMP (4–10 °C) allows the use of a simpler mechanical system and an "off the shelf" perfusate, the ability to assess the function of kidney grafts is limited by the lack of graft metabolic activity. In contrast, normothermic perfusion (34–37 °C) facilitates a comprehensive graft assessment under physiologic conditions but requires a more sophisticated perfusion system and a complex perfusate that includes oxygen carriers.[12] Alternatively, perfusion at an intermediate temperature range (22–30 °C), termed subnormothermic machine perfusion (SNMP), has some potential advantages that warrant further investigation, specifically (1) the oxygen requirement of the graft is low enough that it can be met with a simple acellular perfusate reducing the complexity of the circuit; (2) there is sufficient metabolic activity to enable rehabilitation of extended criteria organs, for instance, kidneys from donation after **circulatory** death (DCD); and furthermore (3) permits viability assessment of the graft before transplantation.

SNMP has shown promise in initial preclinical studies,[13-15] demonstrating improvements in urine production, creatinine clearance, and histological scoring relative to SCS, HMP, and normothermic machine perfusion (NMP). Although encouraging, important developments are necessary before SNMP can advance toward clinical utilization. For instance, many of the aforementioned studies used red blood cells (RBCs) in the perfusate as an oxygen carrier, creating competition for already-scarce blood products and limiting clinical application. Moreover, procured organs were perfused for a maximum duration of 8 h, potentially limiting metabolic recovery of the organ, particularly those from DCD donors, at subnormothermic temperatures. Most importantly, the viability of kidney grafts preserved by SNMP has not been validated in a clinically relevant transplant model.

The present study sought to address these concerns and demonstrate the suitability of SNMP for graft preservation and subsequent transplantation by using a porcine large animal model noted for its high anatomical and physiological similarity to humans.[16] Specifically, we hypothesized that our modified SNMP protocol would successfully maintain procured organs during 24 h without the use of blood products. Furthermore, we hypothesized that perfused kidneys would demonstrate, at minimum, equivalent physiological function after autotransplant relative to SCS. Mechanical parameters and biochemical analyses of the perfusate were used to assess grafts. Our second objective was to assess the viability of kidneys preserved by SNMP in a clinically relevant transplant model using similar criteria. Overall, our observations demonstrate stable SNMP for 24 h, which preliminary findings suggest is generalizable to human grafts. Furthermore, we report compelling evidence that SNMP exhibits superior performance relative to SCS in a clinically relevant DCD autotransplant model.

MATERIALS AND METHODS

Experimental Design

Animals

Yorkshire pigs weighing approximately 40 kg were acquired from the Looper farm (Granite Falls, NC). These animals were used for both perfusion-only and transplant studies. The Duke University Institutional Animal Care and Use Committee approved the experimental protocols. All animals received humane care according to criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (publication 86-23 revised 1985).

Porcine Kidney Procurement for Perfusion-only Experiments

Animals were induced using midazolam and ketamine and placed on active ventilation, with anesthesia maintained using isoflurane. A midline laparotomy was made, and the bilateral kidneys were exposed sequentially. Renal vasculature and the ureter were carefully dissected free from surrounding structures. Each pig was assigned to the living donor (LD; n = 4) group or the DCD (n = 12) group. For the LD group, the kidney was procured after systematic heparinization and immediately flushed with 250 mL cold Belzer University of Wisconsin solution via the renal artery. For the DCD group, the renal artery and vein were tied at their origin, 5 min after systematic heparinization (500 U/kg), to simulate warm ischemic time. Kidneys were subjected to 30 min of warm ischemic time before the cold flush.

Discarded Human Kidney Procurement

Discarded human kidneys (n = 3) were acquired for research purposes after they were declined by all transplant centers because of clinical risk factors and prolonged cold ischemia time. These organs were subjected to the same ex vivo perfusion protocol to demonstrate the translatability of the SNMP approach.

Subnormothermic Machine Perfusion

Porcine kidneys and discarded human kidneys were both perfused on an automated, portable perfusion platform designed and built by BMI OrganBank (Winston-Salem, NC). A simplified schematic of the perfusion circuit is outlined in Figure 1. The organ was perfused using a proprietary acellular perfusate principally composed of human albumin, bicarbonate-based dialysate (B. Braun Medical Inc, Melsungen, Germany), calcium gluconate, heparin, and multivitamins. The perfusate was additionally supplemented with dexamethasone, continuous infusion of the vasodilator verapamil, and piperacillin/tazobactam. Once the organ was placed on the perfusion device, the perfusate received periodic additions of a nutritional mix of Clinimix (Baxter International Inc, Deerfield, IL) and regular insulin. The perfusate was maintained at room temperature (22–25 °C), oxygenated with carbogen gas (95% O_2 and 5% CO_2), and circulated at an approximate flow rate of 2–3 L/min. Following procurement, the renal artery was cannulated and connected to the device, whereas the renal vein was left open for drainage. The ureter was similarly cannulated for external urine collection. Urine was recirculated back into the perfusate every 1–4 h, depending on the rate of urine production. Following an initial warming period of 30 min, the mean arterial pressure was gradually increased to 60–70 mm Hg in a pressure-controlled system. Kidney biopsies were taken pre-preservation and postpreservation. Samples of perfusate and urine were collected every 3 h for biochemical analysis. Periodic sampling of the perfusate

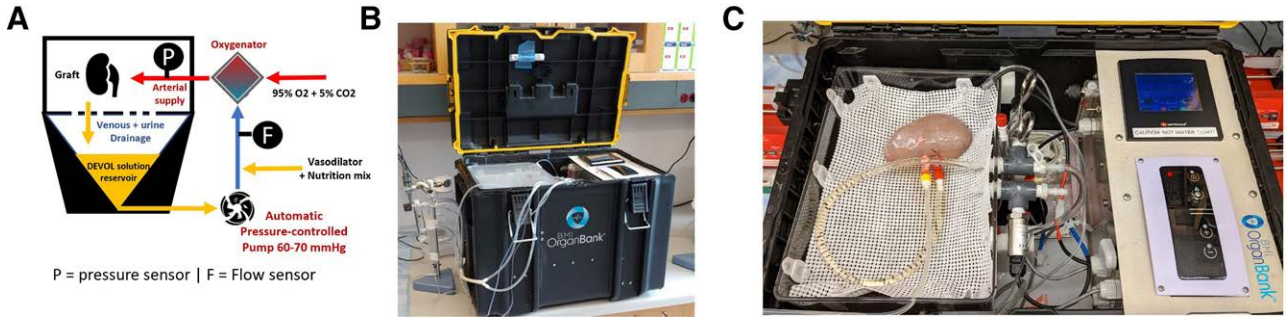

FIGURE 1. Subnormothermic ex vivo kidney perfusion using an automated, portable perfusion platform (OrganBank Transport device supplied by BMI OrganBank). A, Cartoon schematic depicts the perfusion circuit used in this study. The perfusate is oxygenated with a gas mix (95% O_2, 5% CO_2). The flow is continuous and adjusted automatically based on pressure readings to achieve a constant pressure of 60–70 mm Hg. Urine is collected externally and then recirculated into the perfusate. B, Perfusion device is fully portable and easily accommodated on a standard benchtop. C, Porcine kidney is shown undergoing oxygenated subnormothermic machine perfusion (SNMP) using the device.

was conducted, and measurements of blood chemistry and composition were conducted using an iSTAT handheld point-of-care analyzer with CG8+ and Chem8+ cartridges (Abbot Point of Care, Princeton, NJ).

Porcine Kidney Autotransplantation

Kidney viability after storage was assessed using a porcine autotransplant model (Figure 2) in a total of 8 animals. Groups were assigned as follows: DCD SCS (n = 4) versus DCD SNMP (n = 4). Animals underwent donor right nephrectomy on day 1. For the DCD model, 30 min of warm ischemic injury was applied in situ before kidney recovery, as described previously. Following a 24-h preservation period, the right kidney was transplanted back into the same animal with a uretero-ureterostomy technique and ureteral stent similar to previously described methods.[17] Left nephrectomy was performed before abdominal closure. The 24-h preservation time was chosen as a clinically relevant duration of preservation.[18] Postoperative care included administration of perioperative antimicrobials, aspirin, and intravenous fluids as needed. Blood draws were performed twice per day via a central line to monitor blood chemistry. Animals were euthanized on posttransplant day 3 or 7 for tissue collection.

Histology

Kidney tissues were fixed in 10% formalin and subsequently embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin for tubular injury scoring using a method modified from that previously described.[19] Renal tubular damage was graded over 4 levels based on the tubular vacuolization, hyaline droplets, and proteinaceous cast formation. All measures were scored from 0 to 3 (0: <3% tubular involvement; 1: 3%–25% involvement; 2: 25%–50% involvement; 3: >50% involvement).

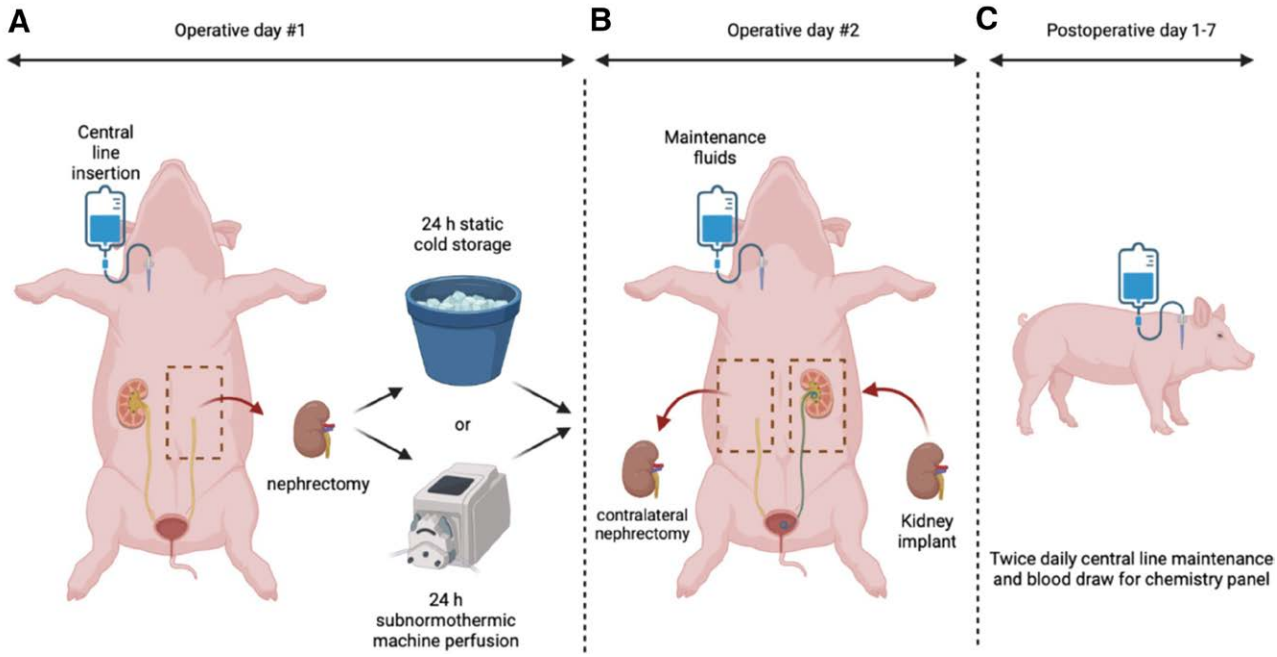

FIGURE 2. Cartoon schematic of the porcine autotransplant procedure. A, On operative day 1, pigs underwent donor nephrectomy. DCD kidneys were subjected to 30 min of warm ischemia before preservation by SCS or SNMP. B, Following 24 h of SCS or SNMP, the pig was again anesthetized, the kidney autotransplant was performed, and the contralateral kidney was removed. C, Pigs were subsequently monitored for 3 or 7 d postsurgery and the analysis of blood chemistry was conducted periodically. DCD, donation after **circulatory** death; SCS, static cold storage; SNMP, subnormothermic machine perfusion.

Statistical Analysis

GraphPad Prism Software (version 10.1.0; GraphPad Software Inc, La Jolla, CA) was used for statistical analysis. All values are presented as mean ± SEM. Data presented in Figure 3E were analyzed by 2-factor ANOVA. In all other multifactor analyses, because of random missing values (eg, unrecorded vascular resistance values for 1 DCD kidney at 1 and 3 h), restricted maximum likelihood mixed-effects modeling was used. In all mixed-effects analyses, fixed effects included Time, Group, and Time × Group interaction, and all post hoc analyses were conducted using the Sidak multiple comparisons test. In all analyses, a P value of <0.05 defined the threshold for statistical significance.

RESULTS

Porcine Kidneys Demonstrate Stable Mechanical Perfusion Parameters During 24-h SNMP

In 16 porcine kidneys (4 LDs, 12 DCDs) and an additional 8 kidneys that were subsequently autotransplanted successfully, 24-h stable perfusion was observed (Figure 3). Furthermore, 3 human kidneys were also stably perfused, demonstrating the cross-species generalizability of the technique (**Figure S1, SDC,** http://links.lww.com/TXD/A647). Notably, a single porcine kidney demonstrated a poor initial flush, resulting in increased vascular resistance and reduction of arterial flow to zero. Perfusion for this kidney was terminated at hour 12 and excluded from the analysis (data not shown). As shown in Figure 1, SNMP enjoys a comparatively simple circuit design relative to normothermic units and is sufficiently compact to fit into a table-top, portable unit.

Mechanical parameters for perfusion demonstrate stable performance of the SNMP platform for the full 24-h duration in both LD and DCD groups (Figure 2). Arterial input pressure was held near-constant between 60 and 70 mm Hg (Figure 3A), yielding consistent total renal arterial output flow between 300 and 350 mL/min for the duration of perfusion (Figure 3C). A significant interaction of time and group was observed for both vascular resistance (Figure 3B) and renal artery flow (Figure 3C), although post hoc testing did not resolve any specific group difference among time points (resistance: Time × Group; $F_{(5,68)} = 2.463$; $P = 0.041$: flow: Time × Group; $F_{(5,68)} = 3.039$; $P = 0.015$). Grafts appeared healthy at the end of the 24 h of perfusion and, when reperfused with RBCs, exhibited homogeneous reperfusion (Figure 3D). As expected, moderate increases in graft weight were observed in both groups postperfusion, indicative of tissue edema (Figure 3E). In a pilot experiment, 3 human

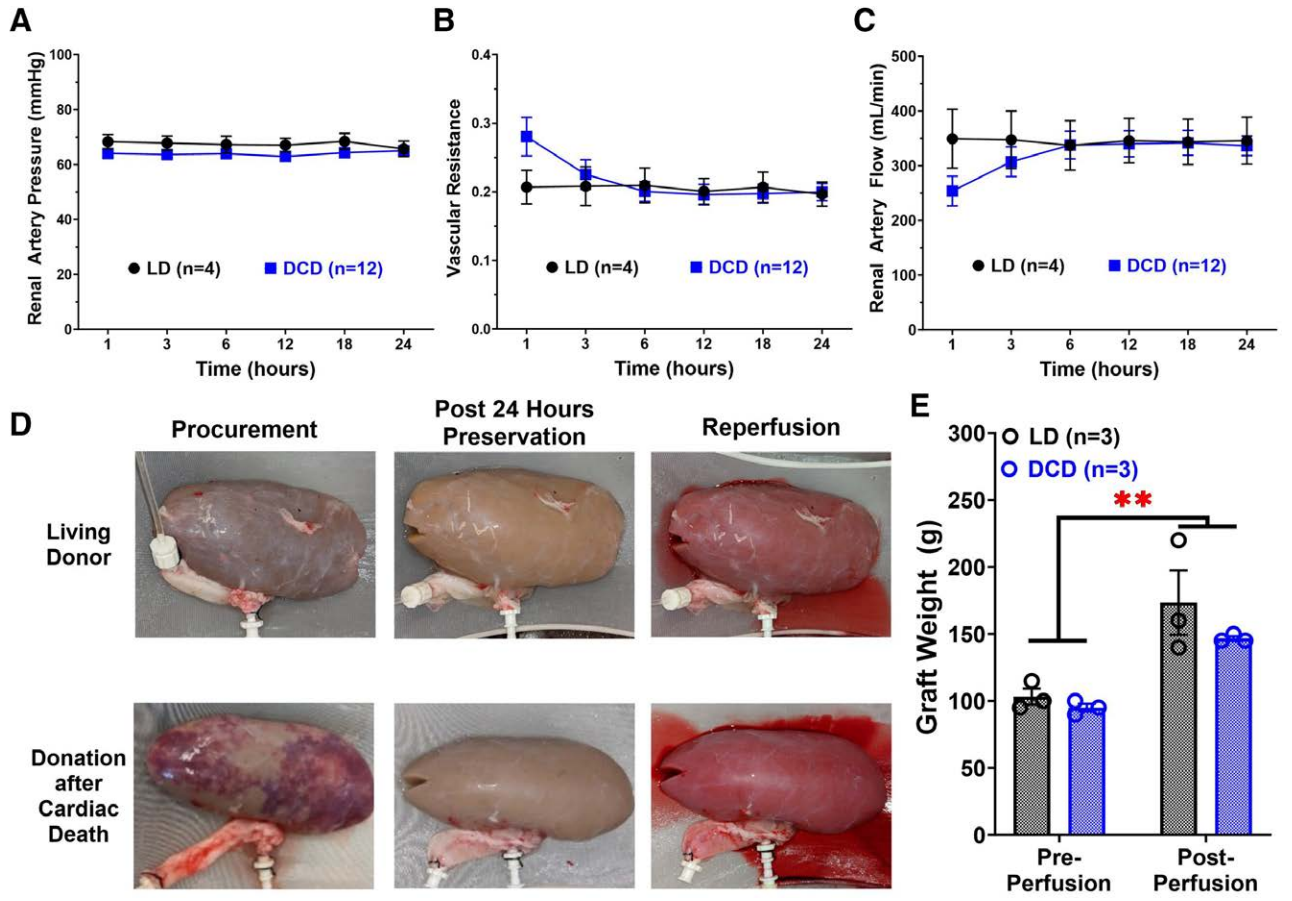

FIGURE 3. Mechanical perfusion parameters in LD and DCD porcine kidneys. A, Input pressure was maintained at 65–70 mm Hg for the duration of perfusion. B, Vascular resistance remained stable for LD kidneys, which, was similarly observed in DCD kidneys despite an initial period of elevated resistance. C, Target flow of 300–350 mL/min was achieved in both groups within 3 h of perfusion and remained stable for the duration of perfusion. D, Both LD and DCD kidneys exhibited healthy gross morphology after 24 h of perfusion and after reperfusion with RBCs. E, Pre- and postperfusion graft weights were comparable in a subset of LD and DCD kidneys. Postperfusion weight increased by approximately 50% in both groups, indicative of modest edema. DCD, donation after **circulatory** death; LD, living donor; RBC, red blood cell. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

kidneys were obtained from male donors aged 40–50 y with no history of hypertension that was discarded because of atherosclerosis or prolonged cold ischemic time. Following 24 h of perfusion, we observed stable parameters similar to those seen in LD porcine kidney, indicative of cross-species generalizability of the technique (**Figure S1, SDC,** http://links.lww.com/TXD/A647). Specifically, grafts exhibited comparable mechanical (**Figure S1A–C, SDC,** http://links.lww.com/TXD/A647) and functional (**Figure S1D–F, SDC,** http://links.lww.com/TXD/A647) measures relative to LD porcine kidneys for the duration of perfusion. Although preliminary, these results nevertheless highlight both the adequacy of pigs for modeling human physiology and the cross-species efficacy of SNMP.

Biochemical Analyses of Perfusate Reveal Comparable Values Between LD and DCD Over Time

A significant potential advantage of SNMP over HMP is the ability to evaluate graft function, and while the optimal parameters for doing so remain to be established, we report promising biochemical observations in perfusion that indicate viable organ function. As shown in Figure 4A, after 1 h of perfusion, both LD and DCD groups displayed ~144 mmol/L sodium, which approximates to that observed in the blood of ~25 kg Yorkshire pigs (134–138 mmol/L).[20,21] A modest, although significant, cumulative 8% increase in concentration was observed over time, peaking at ~156 mmol/L at 24 h but did not differ between LD and DCD groups (Time; $F_{(1.352,18.38)} = 53.0$; $P < 0.0001$). For potassium levels, a small, although statistically significant, main effect of time was again observed in perfusate as well as a significant interaction of time and group (Figure 4B: Time; $F_{(0.37,5.034)} = 8.318$; $P = 0.046$; Time × Group: $F_{(5,68)} = 2.759$; $P = 0.025$). Subsequent post hoc testing did not reveal any significant difference between groups for any time point. The biological significance of this is unclear, although we note that levels in both groups fall within the normal physiological range observed in the blood of swine (3.8–4.4 mmol/L).[20,21] Ionized calcium, on the other hand, displayed a minor, although statistically significant, reduction in concentration over time (Time: $F_{(1.265,17.2)} = 30.24$; $P < 0.0001$), although again, these values approximate levels reported in healthy pigs (eg, ~1.3 mmol/L; Figure 4C).[20]

Regulation of electrolyte levels by the kidneys contributes to the circulating pH of the blood, which then dictates oxygenation of hemoglobin, among other physiological effects. Additional important contributors to pH include lactate and bicarbonate, both of which are quantified in Figure 4D and E. Lactate demonstrated a modest increase in perfusate concentration at the 18- and 24-h sampling times, and bicarbonate similarly exhibited accumulation over time, with peak values in both occurring within the acceptable physiological range (lactate time: $F_{(1.98,26.93)} = 11.52$; $P < 0.001$: bicarbonate time: $F_{(1.831,24.17)} = 43.46$; $P < 0.0001$). The net effect of these changes in blood chemistry is similarly modest, yielding perfusate pH values at a physiologic temperature of approximately 7.25 at 1 h to 7.3 and 7.4 for LD and DCD at 24 h, respectively (Figure 4F: Time; $F_{(2.239,30.0)} = 7.42$; $P = 0.002$). Importantly, post hoc testing did not reveal any significant differences between LD and DCD at any of the time points for lactate, bicarbonate, or pH.

In a subset of perfused kidneys, periodic assessments of graft function were conducted at intervals specified in Figure 5. Figure 5A demonstrates that levels of creatinine were consistent in both groups for the duration of perfusion (Group: $F_{(1,6)} = 0.408$; $P = 0.547$; n = 3–5 per group). Blood urea nitrogen (BUN), on the other hand (Figure 5B), displayed a significant accumulation in both groups starting approximately 12 h into organ perfusion (Time: $F_{(1.17,6.32)} = 115.0$; $P < 0.0001$: n = 3–5 per group). Ultimately, levels peaked at approximately 15 mg/dL in both groups by the end of perfusion (Group: $F_{(1,6)} = 0.608$; n = 3–5 per group). Similarly, levels of aspartate aminotransferase (AST) increased significantly during the 24-h perfusion period (Figure 5C). The extent to which this accumulation reflects graft injury remains unclear, although we note that LD and DCD groups did not differ in either the rate of accumulation or peak AST levels achieved (Group: $F_{(1,14)} = 1.09$; $P = 0.314$; n = 4–12 per group). Other perfusate values at 12 h and 24 h are shown in Table 1. Biopsies of LD and DCD kidneys were also conducted, and they were evaluated and scored for measures of graft health, including vacuolization, hyaline droplets, and proteinaceous casts. Figure S2 (**SDC,** http://links.lww.com/TXD/A648) demonstrated that no apparent differences in any of these measures were observed pre- or postperfusion.

SNMP Improves DCD Kidney Graft Function Relative to SCS in a Porcine Autotransplant Model

To evaluate the posttransplant viability of perfused grafts, a series of autotransplants were conducted after 24 h of graft preservation. Figure 2 describes the general procedures for surgery and postoperative monitoring. Posttransplant, DCD animals receiving an SCS graft exhibited a slow rise in serum creatinine levels starting approximately 24 h posttransplant that peaked between 72 and 86 h postoperatively and ultimately resolved at the end of the 7-d observation period (Figure 6A: Time: $F_{(1.39,5.95)} = 12.05$; $P = 0.011$). Animals that received an SNMP-preserved DCD kidney exhibited significantly reduced overall serum creatinine levels relative to SCS across the 7-d period (Group: $F_{(1,6)} = 10.31$; $P = 0.018$). BUN likewise displayed significant main effects of time and preservation group, with both SCS and SNMP groups realizing peak circulating levels approximately 72 h posttransplant. Importantly, animals in the SNMP group demonstrated significantly lower levels overall (Figure 6B: Time: $F_{(1.17,5)} = 11.76$; $P = 0.017$; Group: $F_{(1,6)} = 9.16$; $P = 0.023$). Circulating levels of potassium did not change appreciably over time, nor did they differ between groups (Figure 6C: Time: $F_{(2.23,9.56)} = 3.02$; $P = 0.092$; Group: $F_{(1,6)} = 0.797$; $P = 0.406$).

DISCUSSION

The present study sought to evaluate the suitability of SNMP for the extended duration of kidney preservation, and we report consistent and stable 24-h perfusion in porcine kidneys. Importantly, we observe similar preservation in LD and DCD kidneys that were subjected to 30 min of warm ischemic, suggesting that SNMP is adequate to recover graft function after ischemic injury. Indeed, the DCD model used in this study represents a high-risk clinical scenario frequently encountered in the current US allocation system, and our observations support the conclusion that ex vivo kidney perfusion, including SNMP, could be a viable method for rehabilitating extended criteria grafts.

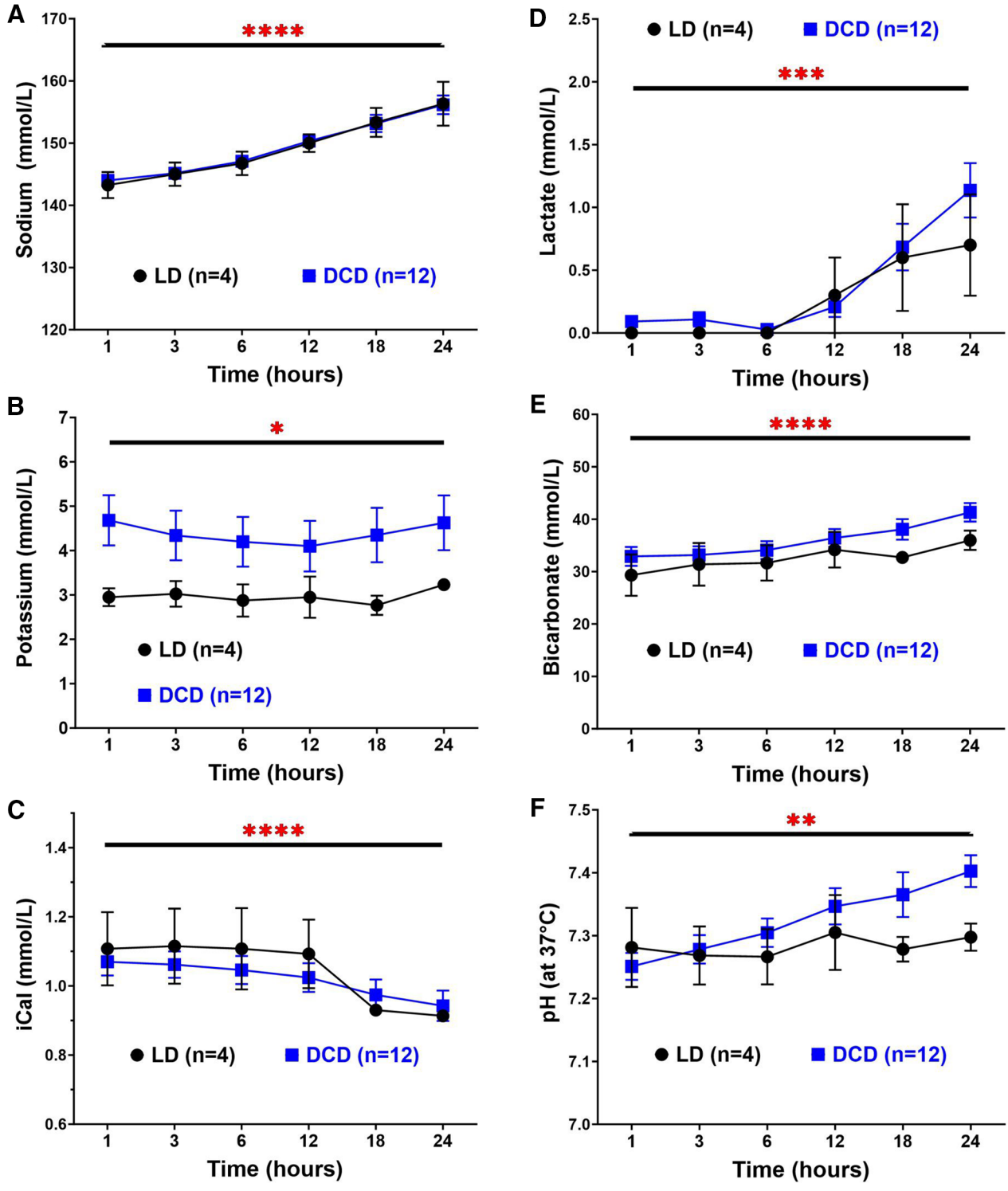

FIGURE 4. Biochemical analysis of circulating perfusate during 24 h. A, LD and DCD groups displayed similar increases in sodium concentration in the perfusate over time, reaching a peak of ~155 mM at 24 h ($P < 0.001$). B, Similarly, despite a main effect of time, no significant difference in potassium levels between LD and DCD groups was observed ($P = 0.311$). C, iCal concentration displayed a modest decline in both groups but otherwise remained within tolerable limits ($P < 0.0001$). D, Lactate remained stable for approximately 12 h, after which modest accumulation was observed in both LD and DCD groups ($P < 0.001$). E, Bicarbonate concentration similarly accumulated during the course of perfusion equally between groups ($P < 0.0001$). F, Although perfusate lactate and bicarbonate moderately increased over time, pH remained largely stable, ending in the range of ~7.3–7.4, well within tolerable physiological limits ($P < 0.01$). DCD, donation after **circulatory** death; iCal, ionized calcium; LD, living donor. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

Although the potential benefits of SNMP on kidney graft preservation have been previously demonstrated in perfusion experiments of limited duration,[13,15,22-26] this is the first study to validate this approach over such an extended interval and signals the possibility of furthermore establishing perfusion protocols for prolonged, multiday preservation.

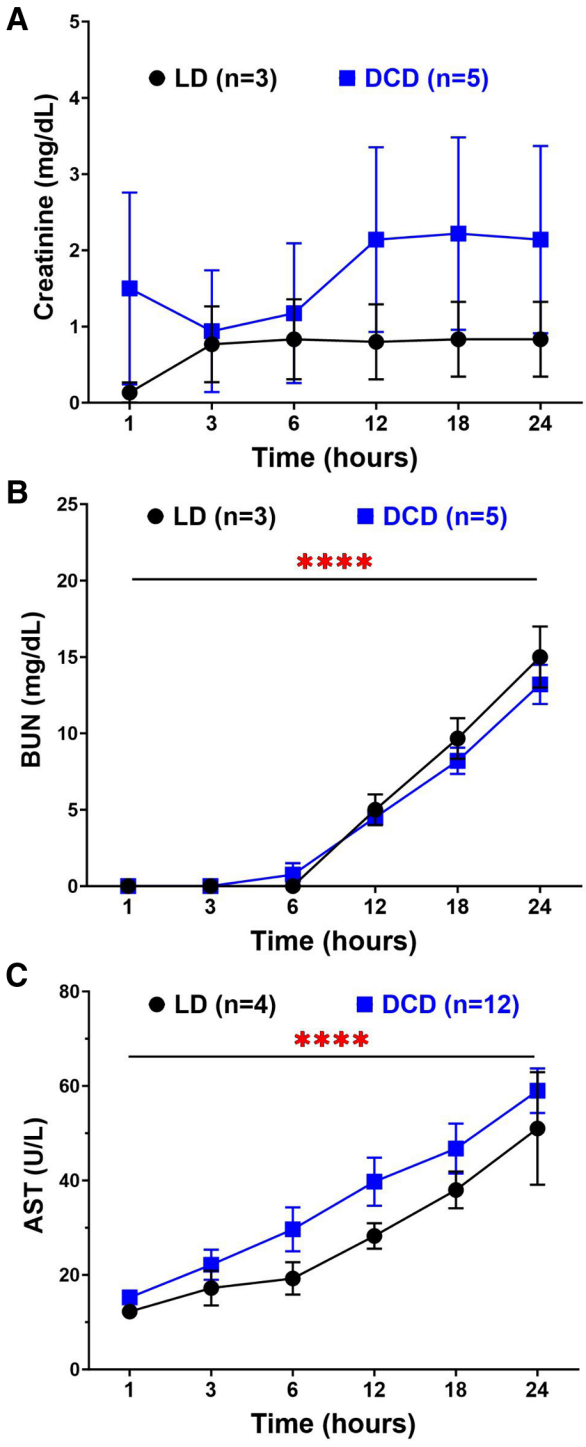

FIGURE 5. Functional tests of graft viability. A and B, In a subset of perfused kidneys, perfusate levels of creatinine and BUN are quantified during the 24-h period. As shown in panel A, creatinine remained stable during perfusion and well within tolerable limits, indicative of adequate glomerular filtration. BUN, however, displayed significant accumulation after 6 h of perfusion, reaching a peak concentration of ~15 mg/dL but within the typical range of arterial and venous levels (eg, 8–26; $P < 0.0001$). C, Concentration of AST increased significantly during the duration of perfusion, indicative of some degree of cell death ($P < 0.0001$). AST, aspartate aminotransferase; BUN, blood urea nitrogen; DCD, donation after **circulatory** death; LD, living donor. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

TABLE 1.

Additional biochemical measures of perfusate

Analyte	LD (12 h)	DCD (12 h)	LD (24 h)	DCD (24 h)
pO_2, mm Hg	603.8 ± 28.5	600.4 ± 16.6	596.1 ± 8.4	552.3 ± 19.6
pCO_2, mm Hg	36.8 ± 2.2	36.5 ± 0.8	39.1 ± 0.8	36.1 ± 0.7
Cl^-, mmol/L	105.3 ± 2.3	106.3 ± 1.2	112.7 ± 3.2	112.2 ± 1.4
Alk Phos, U/L	10.5 ± 3.2	18.3 ± 3.0	6.0 ± 0.6	18.3 ± 2.6
ALT, U/L	11.0 ± 0.4	12.1 ± 0.7	14.0 ± 1.5	12.5 ± 1.1

Alk Phos, alkaline phosphatase; ALT, alanine transaminase; DCD, donation after **circulatory** death; LD, living donor.

That we report preliminary demonstration of successful cross-species perfusion of human kidneys further underscores the intrinsic simplicity, adaptability, and translational value of the SNMP platform.

Ex vivo kidney perfusion is largely conducted under either hypothermic[2,3] or normothermic conditions.[27-34] Although both approaches have demonstrable benefits over SCS,[35] both have specific limitations that potentially restrict wider clinical utilization. For instance, under hypothermic conditions, graft assessment is limited because of low metabolic activity and the lack of urine production, precluding viability testing of marginal kidneys. Normothermic perfusion, in contrast, permits viability testing but requires a sophisticated mechanical system with a complex perfusate that necessitates an oxygen carrier, typically in the form of RBCs. The use of RBCs is particularly problematic because this creates additional competition for an already-scarce resource, particularly in the hospital setting. Successful transplantation has only been achieved after short-term normothermic perfusion,[4,27,28] and it remains to be seen whether these systems can be adapted for longer durations without additional supplementation of perfusate and further exacerbating the problem of RBC utilization. Indeed, among the more attractive features of SNMP is the option to use an acellular perfusate that eliminates the need for blood products. In addition to lower complexity, which improves portability and reduces cost, SNMP, in principle, also enables the assessment and monitoring of graft function throughout perfusion. Although the present work did not specifically test the predictive value of functional measures such as circulating creatinine during perfusion on subsequent graft viability in situ, we, nevertheless, report compelling evidence that merits additional investigation.

Despite these promising findings, the important limitations of this study warrant discussion. First, this study was designed primarily as a demonstration of principle and feasibility and thus did not explicitly seek to interrogate the full physiological significance of reported functional measures. For instance, additional experiments could explicitly investigate what, if any, impact observed cumulative increases in AST shown in Figure 5C contribute to the presentation of delayed graft function, or interrogate the discrete biomolecular cascades governing such increases. Furthermore, future investigations could specifically and rigorously delineate how measures such as creatinine, BUN, and AST collectively predict the manifestation of delayed graft function post-transplantation. Second, we restricted our comparison with SNMP and SCS, which remains the global standard of care in kidney transplantation. Follow-up studies could directly compare the performance of SNMP with either hypothermic or normothermic perfusion using the autotransplant model

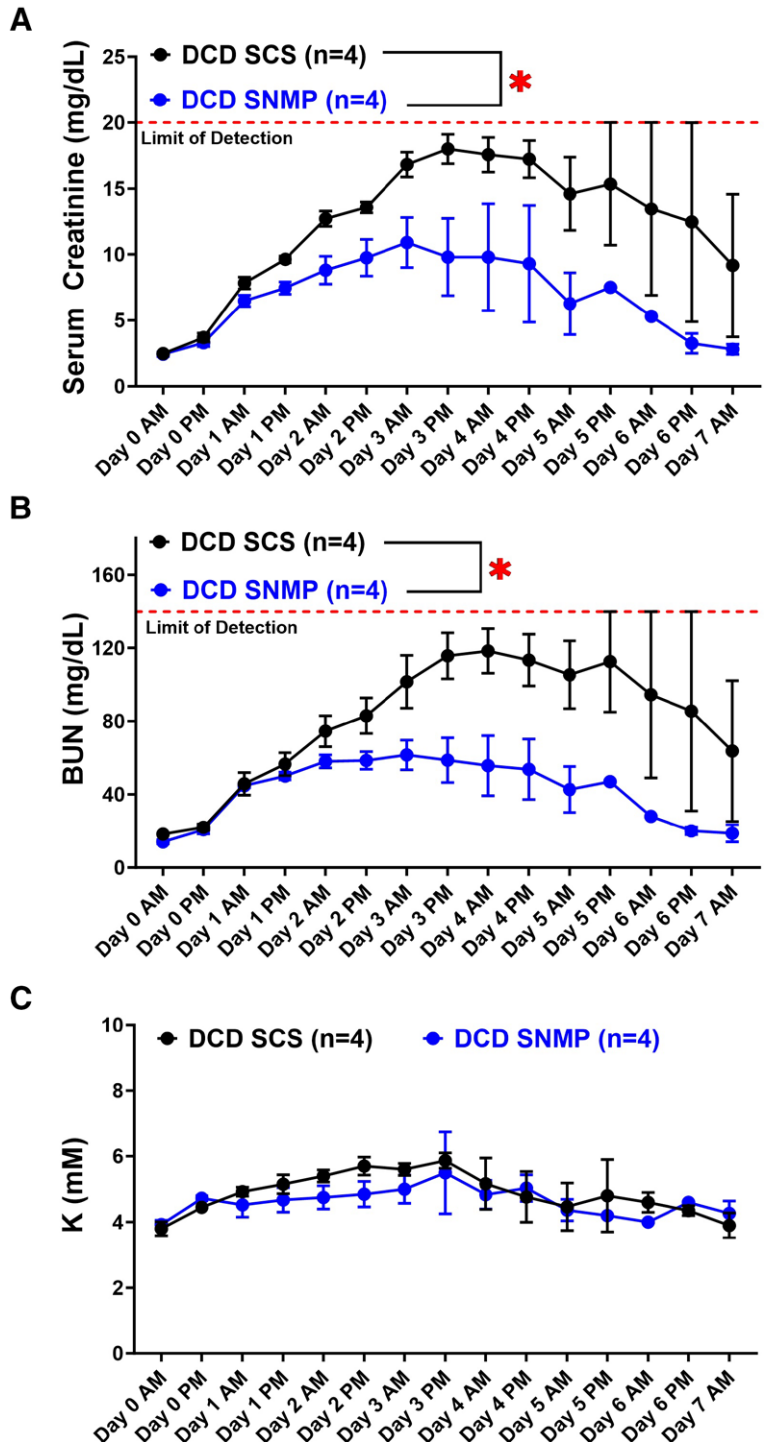

FIGURE 6. Assessment of graft function over time after porcine kidney autotransplantation. A, Analysis of serum creatinine revealed a significant main effect of the preservation method, with SNMP kidneys exhibiting reduced serum creatinine overall posttransplant ($P = 0.018$). B, In addition to creatinine, animals in the DCD SNMP group displayed significantly lower BUN relative to SCS ($P = 0.023$). C, Circulating potassium also remained stable in both groups throughout the 7-d postoperative period. BUN, blood urea nitrogen; DCD, donation after **circulatory** death; SCS, static cold storage; SNMP, subnormothermic machine perfusion. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

outlined herein. Future studies exploring these comparisons would be informative for further clinical development of the optimal approach in ex vivo kidney perfusion.

In conclusion, the present work demonstrates the viability of SNMP for prolonged ex vivo kidney perfusion, and it provides compelling evidence for the superiority of this approach compared with SCS in a clinically relevant porcine DCD autotransplant model.

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