Artificial Organs

WILEY

Artificial Organs

MAIN TEXT OPEN ACCESS

Simultaneous Ex Situ Normothermic Perfusion of Paired Kidneys in Pigs

Margaux Navez[1,2] | Nicholas Gilbo[2,3] | Morgan Vandermeulen[2,3] | Tiago Pinto Coelho[1] | Pauline Erpicum[1,4] | Marc Gilbert Lagny[2,5] | Clarisse Malisoux[5] | Claire Guarin[5] | Gabriel Thierry[6] | Pierre Delanaye[4] | Etienne Cavalier[7] | François Jouret[1,4] | Olivier Detry[2,3]

[1]Laboratory of Translational Research in Nephrology, Metabolism & Cardiovascular Biology, GIGA Institute, ULiege, Liege, Belgium | [2]Department of Surgery's Research Laboratory (CREDEC), Metabolism & Cardiovascular Biology, GIGA Institute, ULiege, Liege, Belgium | [3]Department of Abdominal Surgery and Transplantation, University Hospital of Liege (ULiege CHU), Liege, Belgium | [4]Division of Nephrology-Dialysis-Transplantation, University Hospital of Liège (ULiege CHU), Liege, Belgium | [5]Department of Cardiovascular Surgery, University Hospital of Liege (ULiege CHU), Liege, Belgium | [6]Department of Anesthesia–Intensive Care, University Hospital of Liege (ULiege CHU), Liege, Belgium | [7]Department of Clinical Chemistry, University Hospital of Liege (ULiège, CHU), Liege, Belgium

Correspondence: Margaux Navez (margaux.navez@uliege.be)

Received: 8 November 2024 | **Revised:** 25 March 2025 | **Accepted:** 22 April 2025

Funding: This work was supported by Research Training in Industry and Agriculture (FRIA); Fonds De La Recherche Scientifique-FNRS (J.0069.22 and T.0095.24); Fonds Léon Fredericq.

Keywords: kidney | normothermic machine perfusion | pig | transplantation

ABSTRACT

Background: Normothermic Machine Perfusion (NMP) is essential in renal transplantation to improve organ viability before transplantation. This study presents a proof of concept for simultaneous ex situ perfusion of paired porcine kidneys using exogenous creatinine and iohexol clearances to assess renal function, with the primary objective of examining intra-individual differences.

Methods: Five kidney pairs ($n = 10$) were harvested from pigs, preserved at 4°C for 3 h, and subjected to 6-h NMP. Each pair was perfused with a solution containing red blood cells, and perfusion parameters were monitored continuously. Biochemical parameters were assessed using hourly perfusate and urine samples. Kidney function was evaluated using creatinine, which was introduced during the priming procedure.

Results: No significant differences were observed between paired kidneys in terms of perfusion and biochemical parameters. Both kidneys maintained stable mean arterial pressures (67.40 ± 12.08 mmHg for right vs. 71.50 ± 4.36 mmHg for left) and flows (68.90 ± 38.61 mL/min vs. 54.00 ± 26.08 mL/min), with consistent electrolyte balance and pH levels. The high inter-individual variability in perfusion and biochemical parameters underscores the importance of paired comparisons.

Conclusions: This pig model of simultaneous NMP of paired kidneys demonstrates that the intra-individual variance is low, which makes it possible to test treatments prior to kidney transplantation using one kidney as a valid comparator of the other.

1 | Introduction

Kidney transplantation (KTx) is the best treatment for end-stage renal disease and is associated with lower morbidity and mortality rates than dialysis [1]. However, only approximately 20% of patients on the waiting list receive a kidney transplant each year, highlighting the fundamental challenges posed by the imbalance between organ availability and clinical demand [2, 3]. Static cold storage (SCS) remains the standard of care for kidney preservation owing to its simplicity, cost-effectiveness,

and efficiency. Nevertheless, prolonged cold ischemia can lead to acute tubular necrosis, resulting in delayed graft function and necessitating posttransplant dialysis care [4–6].

The use of kidneys from donations after circulatory death (DCD) and extended criteria donors (ECD) to expand the donor pool has become increasingly prevalent in transplantation. However, DCD grafts undergo a period of donor warm ischemia, which can severely damage the kidneys and exacerbate ischemia–reperfusion injury (IRI). In addition, DCD kidneys are more vulnerable to damage during cold preservation [7, 8]. High-risk clinical characteristics and extended cold ischemia periods contribute to the frequent discarding of marginal kidneys [9, 10].

Recent advances in organ preservation and graft evaluation are essential for optimizing the use of fragile kidney grafts. Studies have suggested that ex situ kidney perfusion may serve as an effective alternative to SCS [11–14]. While there is interest in both normothermic and hypothermic perfusions, there is no consensus on the ideal perfusion strategy for ex situ kidney perfusion [15, 16]. Hypothermic Machine Perfusion (4°C–10°C) allows the use of a simpler perfusion system and a commercially available perfusate, but the reduced metabolic activity of the graft limits the ability to evaluate kidney transplant viability.

Normothermic machine perfusion (NMP) is a promising method of organ preservation that uses a warmed, oxygenated, red cell-based, plasma-free solution with extracorporeal membrane oxygenation [17, 18]. By maintaining the organ in a state near physiological conditions, NMP may allow functional testing by restoring its function ex situ. Additionally, NMP may permit metabolic recovery by restoring adenosine triphosphate (ATP) levels depleted by warm and cold ischemia [19, 20]. NMP has the potential to optimize the use of DCD kidneys, as their suitability could be evaluated prior to transplantation, and treatments could also be administered within this context [21, 22]. Preserving these kidneys under optimal conditions before transplantation ensures immediate functionality and long-term effectiveness [23–25]. Pig kidney NMP models have previously been employed to assess drug efficacy, explore cell therapy, and study metabolism in various injury contexts, with the underlying assumption that both kidneys from the same animal would respond similarly during NMP. However, to the best of our knowledge, this assumption has not been validated experimentally.

This study aimed to develop a model for the simultaneous normothermic ex vivo perfusion of porcine kidney grafts obtained from the same donor, with one kidney serving as a paired control.

2 | Material and Methods

2.1 | Ethics and Animals

Landrace and Pietrain crossbreed laboratory pigs weighing 30–35 kg were acquired from the Walloon Agricultural Research Centre (CRA-W farm, Gembloux, Belgium). The animals were used for blood collection and kidney procurement. The Animal Ethics Committee of the University of Liege approved the experimental protocols (21-2447). All personnel involved in animal experiments were licensed by the Federation of European Laboratory Animal Science Associations licenses. All animals received humane care according to the 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 Institute of Health.

2.2 | Experimental Design

After surgical procurement, 5 kidney pairs underwent simultaneous ex situ NMP preservation 3 h after procurement, with creatinine and iohexol injected into the perfusion solution (Figure 1). Perfusate and urine samples were collected at 30-min intervals and hourly, respectively, throughout the 6-h NMP period. Continuous monitoring of flow and pressure was conducted using hourly data. Kidney biopsies were performed at the end of NMP.

2.3 | Animal Experiments

Animals were first sedated 30 min before entering the operating room with an intramuscular injection of tiletamine/zolazepam (4.4 mg kg^{-1} equivalent to 2.2 mg kg^{-1} of tiletamine and 2.2 mg kg^{-1} of zolazepam, Zoletil 100, Virbac, Leuven, Belgium: containing 250 mg of tiletamine and 250 mg of zolazepam); a peripheral catheter was placed in an auricular vein, and intravenous anesthesia was then induced before orotracheal intubation and maintained during pulmonary ventilation, dissection, and euthanasia. Mechanical ventilation was provided by a Datex-Ohmeda ADU S5 care station ventilator, and ventilatory parameters were adapted to allow sufficient oxygenation ($PaO_2 > 100$ mmHg) and avoid hypercapnic acidosis ($PaCO_2$ between 35 and 45 mmHg). Anesthesia was provided using a continuous infusion of propofol (Diprivan, 20 mg/mL, Aspen Pharma, Dublin, Ireland) at a rate between 2 and 4 mg $kg\,h^{-1}$ depending on hemodynamic parameters, and analgesia was performed using boluses of 0.2 mcg kg^{-1} of sufentanyl (Sufenta, 5 mcg/mL, Viatris, Canonsburg, Pennsylvanie, USA). Curarization was performed after intubation with boluses of 20 mg cisatracurium (Nimbex 2 mg mL^{-1}, Aspen Pharma, Dublin, Ireland) when signs of decurarization were evident. An arterial catheter was placed in the carotid artery to monitor the hemodynamic parameters and blood gas sampling. After a median laparotomy, the abdominal aorta and inferior vena cava were dissected and isolated. Following the intravenous administration of 25 000 IU of heparin (Leo Pharma B.V., Amsterdam, Netherlands), the abdominal aorta and inferior vena cava were cannulated. After thoracic aorta clamping, the kidneys were flushed through the aortic cannula with 2 L of cold (4°C) preservation solution (IGL-1; Institut Georges Lopez, Lissieu, France) and the abdominal cavity was cooled with 4°C saline solution. The pig's blood mass was then drained for salvage, and cardiac arrest was confirmed. Anesthesia and ventilation were stopped once death was confirmed.

Whole blood was collected during renal flush-cooling through the vena cava cannula and processed using a cell-saving system (Xtra autotransfusion system; LivaNova, Zaventem, Belgium) to yield concentrated red blood cells without the presence of

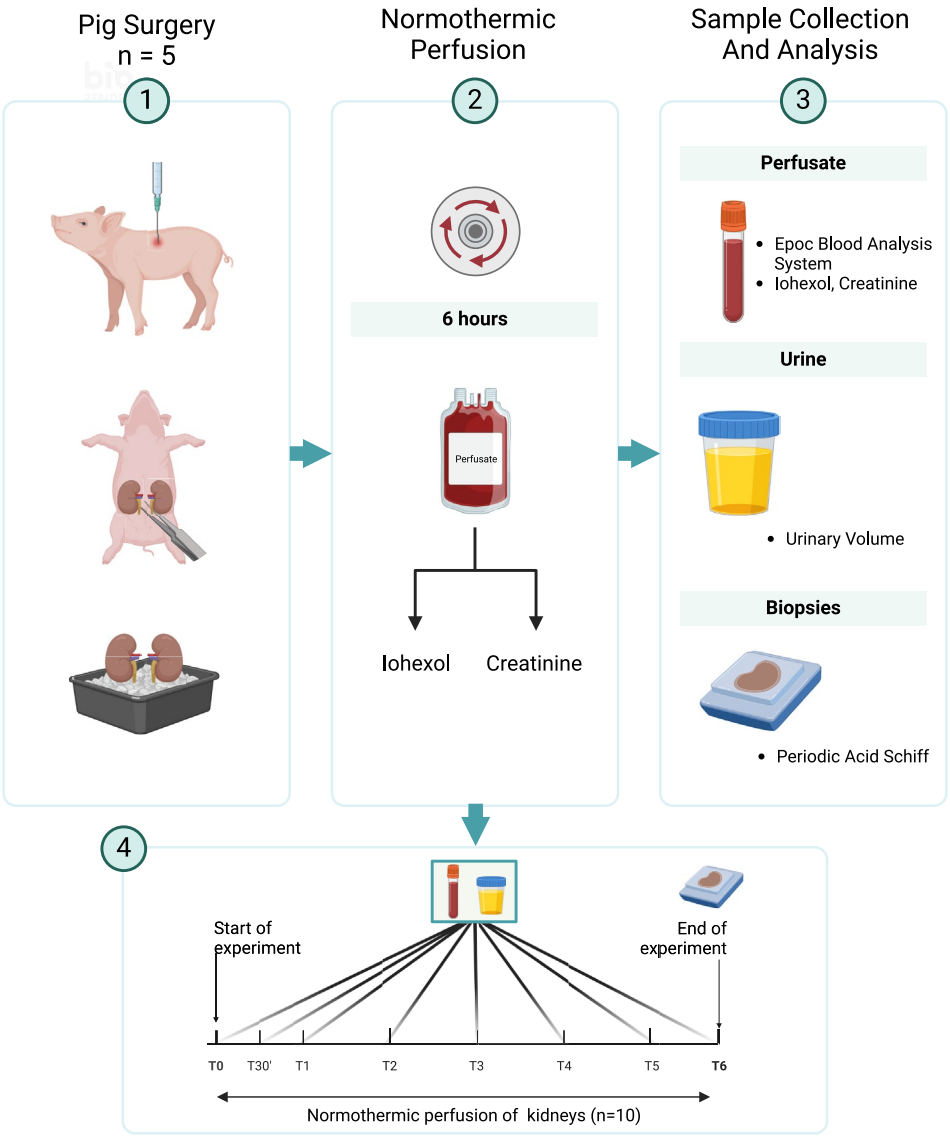

FIGURE 1 | Overview of the experimental design. Ten porcine kidneys underwent concurrent machine perfusion preservation approximately 3 h post-procurement, with the injection of creatinine and iohexol in the perfusion solution ($n = 10$). [Color figure can be viewed at wileyonlinelibrary.com]

leukocytes. After bilateral nephrectomy, renal arteries and veins were dissected and cannulated. Kidneys underwent between 3 and 3 h 30 SCS. Before connecting them to the perfusion circuit, both kidneys were flushed on the back table with 500 mL of IGL-1 at 4°C.

2.4 | Normothermic Machine Perfusion Set-Up

The two kidneys from the same pig underwent simultaneous 6-h NMP using identical settings on separate NMP circuits (Figure 2). Each consisted of one kidney reservoir (Organ Recovery Systems Inc., Chicago, IL, USA), roller pump, membrane oxygenator (Dideco Perfusion Tubing Systems, LivaNova, Zaventem, Belgium), and heat exchanger (Stockert GmbH, Freiburg, Germany) connected to coated tubing (Soring Group LivaNova, Belgium). The arterial pressure (WPI, Friedberg, Germany) and flow rates (Sonotec, Halle, Germany) were continuously measured. The renal artery was cannulated and connected to the device, while the renal vein was left open for drainage. The ureter was then cannulated for urine collection.

The perfusion circuits were primed with a perfusion solution composed of homologous red blood cells and an isotonic electrolyte solution (Plasmalyte, Baxter, Lessines, Belgium) to achieve a hematocrit of 20%–30% (Table 1). Flolan (0.5 mg, GSK, Wavre, Belgium) was continuously infused, and urine was replaced by Plasmalyte in a 1:1 ratio. A parenteral nutrient solution (Aminomix 2 Novum; Fresenius Kabi, Willebroek, Belgium) was administered to maintain a perfusate glucose level above 100 mg/dL, and 10 mL of sodium bicarbonate (8.4%, B Braun, Melsungen, Deutschland) was added to the perfusate to adjust basal pH. The perfusion temperature was maintained at 38°C, with an airflow rate of 100 mL/min of oxygen and an FiO2 of 21% administered through the oxygenator. The perfusion solution was prepared and divided into two separate circuits. After reperfusion, the mean arterial perfusion pressure was gradually increased to 60–80 mmHg in a pressure-controlled system.

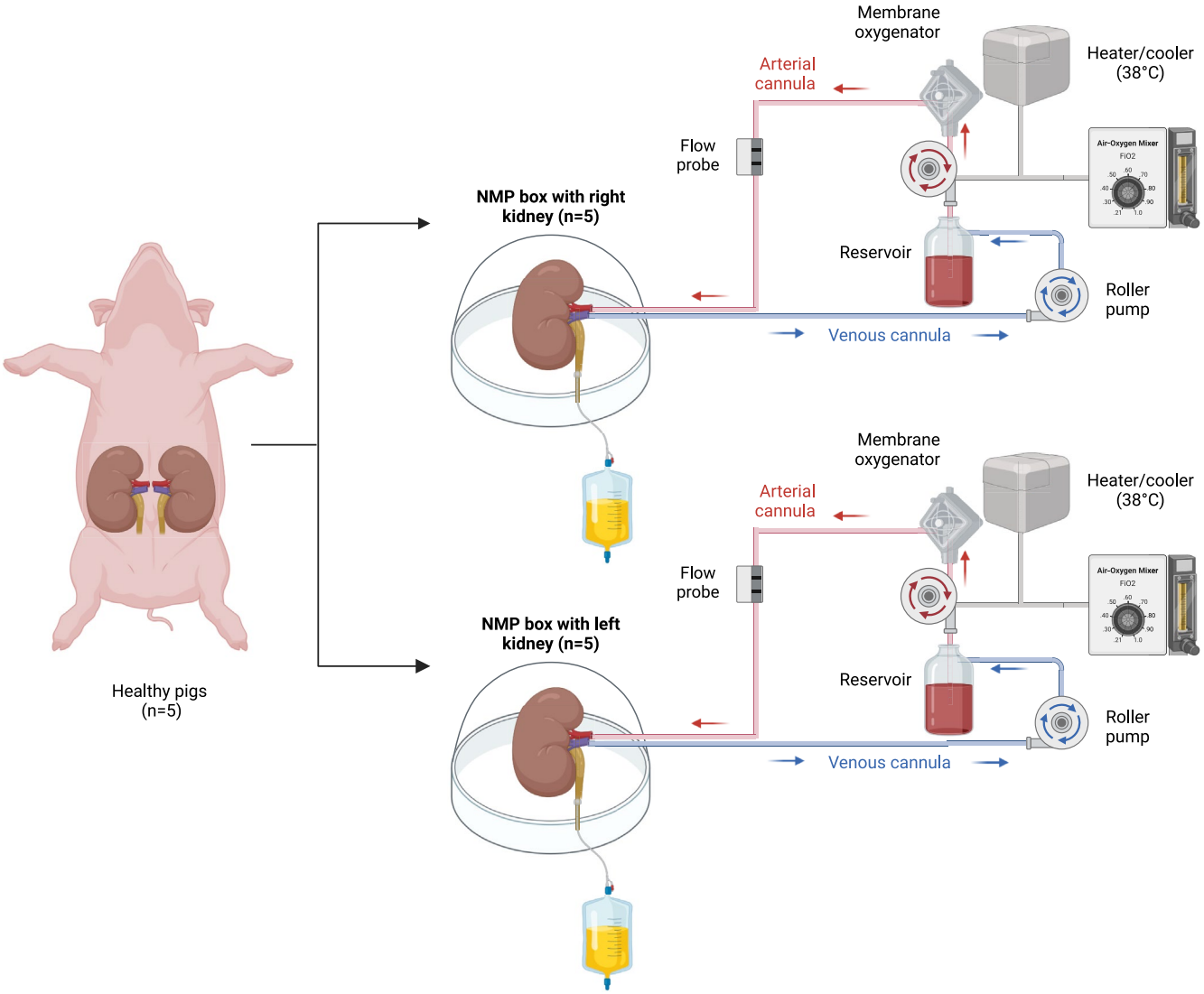

FIGURE 2 | Overview of the simultaneous perfusion of two kidney grafts from the same pig. [Color figure can be viewed at wileyonlinelibrary.com]

Intrarenal Resistance (IRR) was calculated by dividing the pressure by the flow and was expressed in mmHg/mL/min/100 g of tissue.

2.5 | Sample Collection and Analyses

The sampling line was attached to the arterial inflow cannula. Perfusate samples were collected using SST BD Microtainer (New Jersey, USA) blood collection tubes and subsequently centrifuged at 2000 g for 5 min at 4°C. The resulting supernatant was aliquoted and preserved at −80°C until further analysis. All samples were analyzed, and all data points were included in the analysis. One pig was excluded because its pH level fell below the threshold of 7. All other pigs used in this study met the inclusion criteria.

Biochemical parameters were assessed in the perfusate obtained from the arterial line using an Epoc Blood Analysis System (Siemens Healthcare NV, Dilbeek, Belgium). Kidney function was evaluated using a prespecified dose of creatinine (GE Healthcare, Diegem, Belgium) and iohexol (Omnipaque 240 mg I/mln, GE Healthcare, Diegem, Belgium), which were introduced into the perfusion solution during the priming phase. These compounds were then cleared from the perfusate by the kidneys over time.

Kidney biopsies were taken at the end of perfusion, and histological samples were harvested, fixed in 4% formalin, embedded in paraffin, and stained with hematoxylin–eosin and Periodic Acid-Schiff (PAS). Histopathological analysis was performed by an expert nephropathologist in a blinded manner.

2.6 | Statistical Analyses

Given the exploratory nature of this study, which aimed to establish a simultaneous perfusion model, formal sample size calculation was not feasible. A sample size of $n = 5$ animals was chosen to account for the biological variation typically observed between experimental units in large-animal models. Results are presented

per kidney or expressed as the mean ± standard deviation. Data were visualized using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA, version 10). A generalized linear mixed model was employed to evaluate the effects of time, the kidney, and the interaction between time and the kidney. A compound symmetry covariance matrix was used to account for the repeated measures. Residual normality and homoscedasticity assumptions were checked, and the data were log-transformed when necessary. For the two parameters, beta regression was applied owing to the specific data characteristics. Model quality was assessed using Akaike's Information Criterion and Bayesian Information Criterion, with smaller values indicating a better model fit. Statistical significance was defined at the 5% level ($p < 0.05$) using SAS (version 9.4) and R (version 4.2.2), with the lnormimp package for censored data imputation.

TABLE 1 | Composition of the perfusate used for the simultaneous NMP of kidneys.

	NMP perfusate solution
Priming solution	
Autologous concentrated red cells	500 mL
Plasmalyte	500 mL
Additives in bolus	
Albumin 20%	100 mL
Cefuroxime	1500 mg
Sodium bicarbonate (8.4%)	10 mL
Mannitol (15%)	13.4 mL
Creatinine	75 mg
Iohexol	518 mg
Infusions during perfusion	
Epoprostenol	8.3 μg/h
Plasmalyte	10 mL/h

3 | Results

3.1 | Animal Characteristics

The characteristics and preoperative blood parameters of the five pigs are presented in Table 2. No significant difference was observed between the right (119.0 ± 19.1 g) and left kidney weights (118.4 ± 23.2 g), $p = 0.62$. The mean hemoglobin level was 9.38 ± 0.81 g/dL, while the creatinine level averaged 0.84 ± 0.21 mg/dL. The pH levels had a mean of 7.42 ± 0.03, and the mean pO_2 level was 194.8 ± 54.9 mmHg. The mean glucose level was 129.8 ± 15.6 mg/dL. The minor inter-individual variations in the cold ischemic period showed no correlation with the perfusion parameters.

3.2 | Hemodynamic and Metabolic Function Parameters

Five kidney pairs were perfused; the perfusion parameters are listed in Table 3. Hemodynamic parameters, including arterial flow and intrarenal resistance, are illustrated in Figure 3A for arterial flow and Figure 3B for IRR. Their comparison during the 6-h perfusion in five right kidneys versus five left kidneys is presented in Table 4.

3.2.1 | Arterial Pressure, Flow, and Intrarenal Resistance

Arterial pressure increased significantly over time ($p < 0.0001$). The arterial flows showed no significant difference, with right kidneys exhibiting a mean flow of 51.59 ± 30.67 mL/min/100 g and left kidneys 45.90 ± 19.29 mL/min/100 g ($p = 0.56$). The flow rate decreased significantly over time ($p < 0.0001$) regardless of the kidney side (left or right). There was no difference in IRR of right (1.60 ± 1.06 mmHg/mL/min/100 g) vs. left kidneys (1.58 ± 0.64 mmHg/mL/min/100 g, $p = 0.92$). Resistance increased significantly over time ($p < 0.0001$), regardless of the kidney side (left or right). The paired t-test results for arterial pressure, arterial flow, and IRR indicated that there was

TABLE 2 | Overview of animals and perfusion characteristics of basal pig.

	Pig 1	Pig 2	Pig 3	Pig 4	Pig 5	Mean ± SD
Animal weight (kg)	33	32	33	32	35	33.0 ± 1.2
Right kidney weight (g)	110	153	113	110	109	119.0 ± 19.1
Left kidney weight (g)	111	157	95	118	111	118.4 ± 23.2
Hemoglobin (g/dL)	8.4	9.6	9.1	10.6	9.2	9.38 ± 0.81
Creatinine (mg/dL)	0.67	0.90	1.15	0.63	0.85	0.84 ± 0.21
pH	7.459	7.389	7.411	7.416	7.432	7.42 ± 0.03
pO_2 (mmHg)	187.5	281.9	160.4	205.3	138.9	194.8 ± 54.9
Glucose (mg/dL)	120	135	108	147	139	129.8 ± 15.6
Cold ischemic time (min)	178	210	207	190	183	193.6 ± 14.3

TABLE 3 | Hemodynamic and metabolic function parameters per pair of kidneys[a].

	Pair 1		Pair 2		Pair 3		Pair 4		Pair 5		
Kidney pair	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	***p***
Arterial pressure (mmHg)	76.8 ± 9.1	76.5 ± 10.1	79.6 ± 12.9	77.6 ± 14.2	71.4 ± 8.7	67.8 ± 8.6	64.8 ± 11.9	63.7 ± 10.0	49.9 ± 25.0	67.2 ± 18.7	0.51
Arterial flow (mL/min/100 g)	53.8 ± 18.0	66.8 ± 25.9	41.6 ± 22.9	30.1 ± 14.3	42.0 ± 16.1	44.7 ± 14.3	18.9 ± 7.2	24.0 ± 8.7	101.6 ± 28.8	63.8 ± 12.4	0.56
Resistance index (mmHg/mL/min/100 g)	1.5 ± 1.0	1.4 ± 1.3	1.2 ± 0.8	1.2 ± 0.5	1.6 ± 0.9	1.9 ± 0.8	3.3 ± 1.6	2.5 ± 1.9	0.4 ± 0.1	0.8 ± 0.2	0.92
pH	7.67 ± 0.07	7.74 ± 0.10	7.36 ± 0.13	7.39 ± 0.18	7.42 ± 0.16	7.37 ± 0.04	7.26 ± 0.08	7.27 ± 0.07	7.44 ± 0.12	7.49 ± 0.16	0.15
Sodium (mmol/L)	155.8 ± 10.02	150.7 ± 9.3	141.0 ± 10.9	142.5 ± 13.3	150.1 ± 5.1	147.4 ± 5.5	134.2 ± 3.8	135.3 ± 3.8	147.0 ± 4.9	147.5 ± 6.4	0.90
Chloride (mmol/L)	97.4 ± 7.9	94.9 ± 9.0	101.6 ± 4.6	104.3 ± 5.7	103.2 ± 2.5	101.0 ± 3.5	97.1 ± 5.2	97.4 ± 5.2	100.2 ± 4.6	101.5 ± 4.2	0.69
Calcium (mmol/L)	0.63 ± 0.35	0.76 ± 0.30	0.75 ± 0.17	0.80 ± 0.15	1.12 ± 0.14	1.08 ± 0.13	1.00 ± 0.12	1.01 ± 0.12	0.50 ± 0.08	0.54 ± 0.06	0.20
Potassium (mmol/L)	2.8 ± 0.8	2.8 ± 1.1	2.4 ± 0.9	2.3 ± 1.3	2.5 ± 1.0	2.4 ± 0.8	5.8 ± 0.6	5.3 ± 0.7	2.9 ± 0.7	2.8 ± 1.0	0.04
Hemoglobin (g/dL)	10.6 ± 0.8	11.1 ± 1.2	11.1 ± 1.0	12.0 ± 1.1	6.9 ± 0.4	7.1 ± 0.4	6.7 ± 0.7	6.8 ± 0.4	6.4 ± 0.4	6.1 ± 0.7	0.35
Hematocrit (%)	31 ± 2.2	32.7 ± 3.4	32.6 ± 3.1	35.2 ± 3.2	20.3 ± 1.4	20.9 ± 1.4	19.5 ± 1.9	19.9 ± 1.1	18.9 ± 1.1	17.9 ± 2.0	0.41
Glucose (mg/dL)	84.4 ± 22.8	91.7 ± 28.1	173.3 ± 34.4	178.1 ± 34.9	120.0 ± 25.9	134.4 ± 28.6	113.5 ± 26.9	110.6 ± 23.9	126.4 ± 14.7	134.2 ± 17.1	0.12
Arterial PO_2	89.1 ± 20.1	100.0 ± 7.0	120.0 ± 21.6	110.3 ± 6.1	107.7 ± 38.8	107.8 ± 40.9	115.5 ± 13.7	110.3 ± 26.0	91.9 ± 26.3	103.0 ± 29.1	0.27
Arterial PCO_2	39.9 ± 9.1	33.7 ± 11.0	37.7 ± 16.3	32.5 ± 5.2	40.6 ± 7.2	38.2 ± 0.8	34.2 ± 11.1	35.9 ± 11.4	39.6 ± 6.4	44.1 ± 15.0	0.96
Temperature (°C)	35.9 ± 1.8	37.4 ± 2.0	33.1 ± 5.0	35.3 ± 1.9	35.5 ± 2.1	35.1 ± 1.5	33.4 ± 1.2	34.1 ± 1.6	36.5 ± 2.3	34.9 ± 1.8	0.60

[a]Values represented mean ± SD. Time-averaged longitudinal mean value compiled from 30′—measurements over the course of the perfusion.

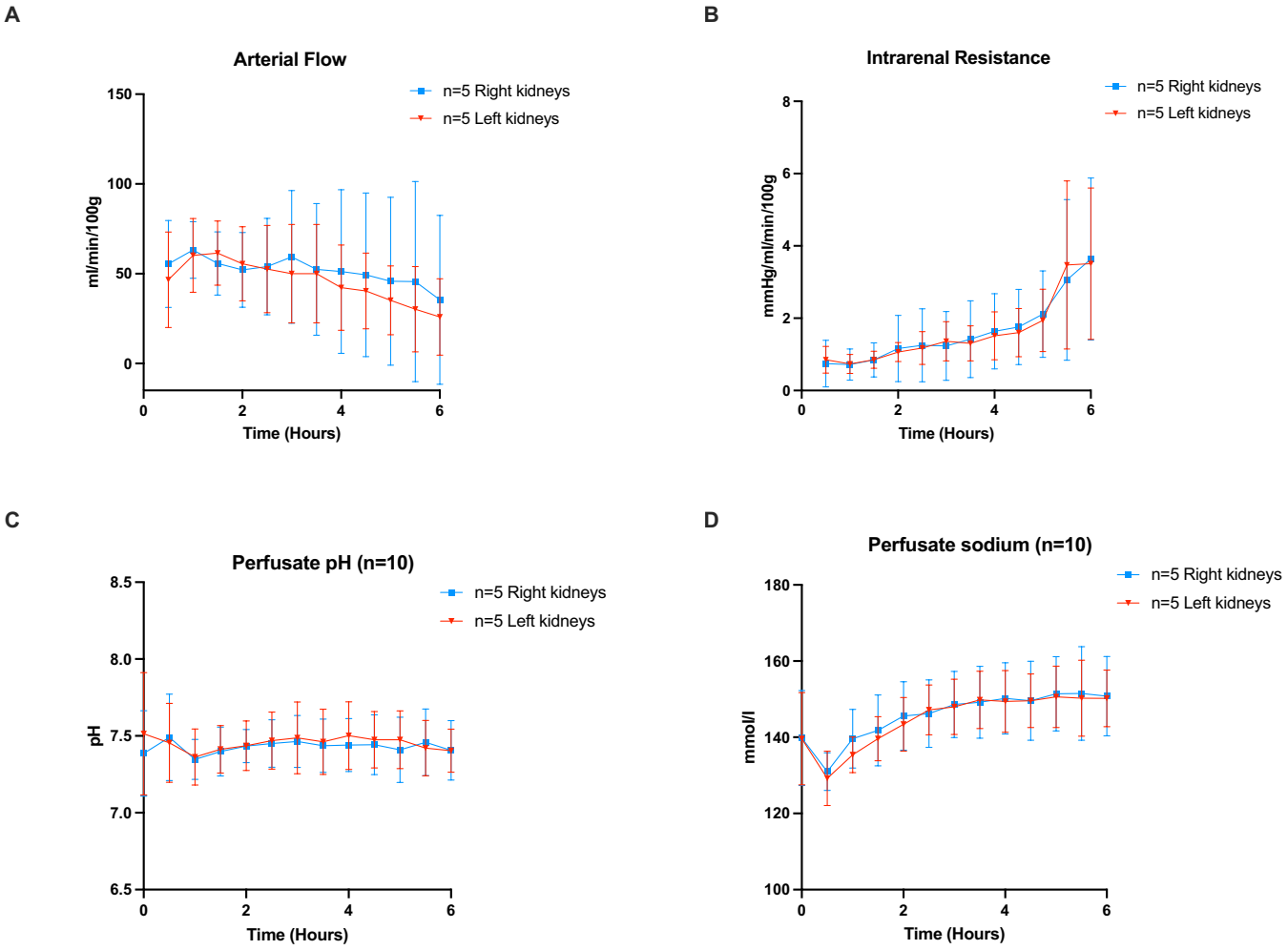

FIGURE 3 | (A) Arterial flow ($n = 10$) in mL/min/100 g in right and left kidney perfusions. (B) Intrarenal resistance ($n = 10$) in mmHg/mL/min/100 g in right and left kidney perfusions. (C) Perfusate pH ($n = 10$) in right and left kidney perfusions. (D) Perfusate sodium ($n = 10$) in mmol/L in right and left kidney perfusions. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 4 | Comparison of perfusion characteristics between right and left kidneys from the same pig.

	Right kidneys ($n = 5$)	Left kidneys ($n = 5$)	Paired *t*-test *p*
Arterial pressure in mmHg	67.40 ± 12.08	71.50 ± 4.36	**0.51 (ns)**
Arterial flow in mL/min/100 g	51.59 ± 30.67	45.90 ± 19.29	**0.56 (ns)**
IRR in mmHg/mL/min/100 g	1.60 ± 1.06	1.58 ± 0.64	**0.92 (ns)**
pH	7.43 ± 0.17	7.46 ± 0.18	**0.15 (ns)**
Sodium in mmol/L	147.70 ± 8.53	147.50 ± 6.63	**0.90 (ns)**
Potassium in mmol/L	3.04 ± 1.46	2.80 ± 1.38	0.04 (*)
Calcium in mmol/L	0.76 ± 0.28	0.82 ± 0.21	**0.20 (ns)**
Hematocrit in %	24.60 ± 6.35	25.40 ± 8.05	**0.41 (ns)**
Hemoglobin in g/dL	8.36 ± 2.11	8.66 ± 2.70	**0.35 (ns)**
Glucose in mg/dL	123.30 ± 33.46	130.10 ± 30.34	**0.12 (ns)**
Cumulative urine (mL)	75.60 ± 44.17	90.40 ± 47.55	**0.18 (ns)**

Note: NS = not significant. Bold values are marked with (ns).

no difference between the right and left kidneys during the 6-h NMP ($p > 0.05$). Of note, for further experiments testing conditioning strategies, 47% of data variability was explained by inter-individual differences, which underlines the importance of intra-individual simultaneous paired comparisons. The GLMM model is represented in the Table S1.

3.2.2 | pH Levels

The pH course of each perfused kidney is shown in Figure 3C. The average pH values for the right and left kidneys were 7.43 ± 0.17 and 7.46 ± 0.18 ($p = 0.15$), respectively, and no significant changes were detected during the 6-h NMP period. The variability in the pH levels was largely attributed to inter-animal differences, accounting for 59% of the total variability. No statistically significant differences were observed between the left and right kidneys ($p > 0.05$). Figure 4A presents paired data that enable visual assessment of inter-organ differences between the right and left kidneys of each animal. The individual variations were minor, as the mean difference was close to zero, suggesting no significant difference between the right and left kidneys.

3.3 | Electrolytes Measurements: Sodium and Potassium

As illustrated in Figure 3D, the sodium levels in both kidneys started at approximately 140 mmol/L and showed minor fluctuations during perfusion. Sodium concentrations measured 147.70 ± 8.53 mmol/L for right kidneys and 147.50 ± 6.63 mmol/L for left kidneys, showing no significant difference ($p = 0.90$). However, the sodium levels increased over time ($p < 0.0001$), with 38% of the variability explained by individual pig differences. As shown in Figure 4B, the mean difference was slightly positive, indicating a slight tendency toward higher sodium levels on the left side. While some animals exhibited larger discrepancies, the average suggests that these differences are not systematic. Potassium levels (Table 3) remained stable but were significantly higher in both kidneys of pair 4 than in the other kidneys ($p < 0.0001$). No significant differences were observed between the left and right kidneys ($p > 0.05$). In Figure 4C, potassium levels indicate that variations exist between individuals, but are mostly balanced, indicating a lack of significant preference.

3.4 | Glucose Measurements

Glucose concentrations declined significantly and progressively over the perfusion period, reflecting consumption despite the administration of a parenteral solution to regulate glucose levels. This decline was similar in both kidneys. Glucose concentrations were 123.30 ± 33.46 mg/dL in the right kidney and 130.10 ± 30.34 mg/dL in the left kidney ($p = 0.12$). In Figure 4D, the glucose measurements show considerable individual variability, but the mean difference suggests a balanced distribution.

3.5 | Hematological Parameters: Hemoglobin and Hematocrit Levels

Both parameters exhibited no significant differences between the kidneys, indicating stable perfusion. No significant time

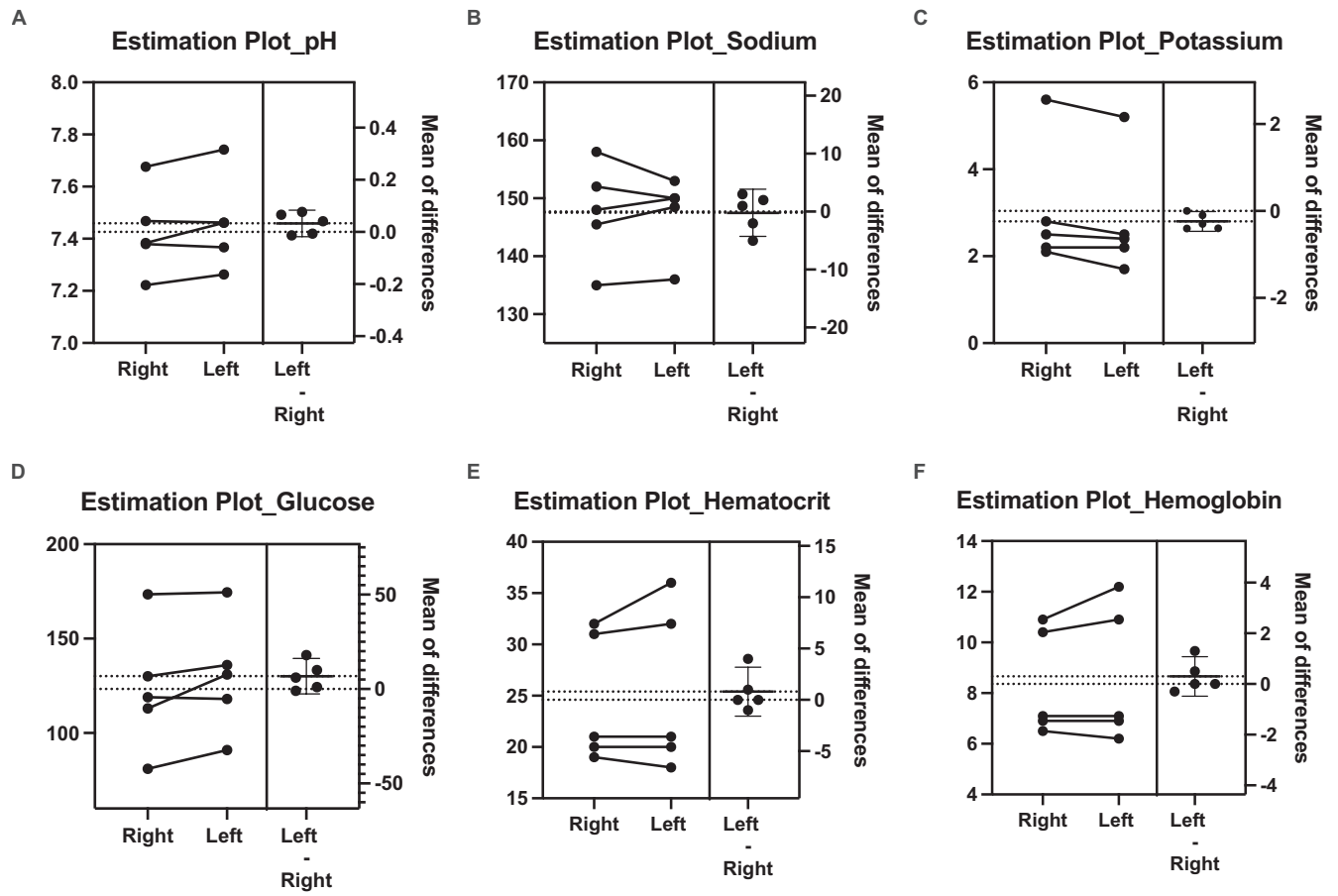

FIGURE 4 | (A–F) Estimation plots comparing physiological measurements taken from the right and left kidneys of the same pig during a 6-h normothermic perfusion. The six panels illustrate differences between sides for pH, sodium, potassium, hemoglobin, hematocrit, and glucose levels.

or kidney effect was observed for hematocrit and hemoglobin levels, with 21% and 19% variability attributed to pig differences, respectively. Hematocrit values ranged from 20% to 35% with a slightly positive mean difference, suggesting a small tendency for higher levels on the left side, although these individual differences were not consistent across all animals in Figure 4E. Figure 4F shows hemoglobin levels ranging from approximately 6 to 13 g/dL, with a mean difference near zero, indicating no significant side differences, despite some individual variations.

3.6 | Urine Production

The functional assessment was further supported by hourly urine output measurements. Cumulative urine output did not differ significantly between the right and left kidneys, with both maintaining steady production over the 6-h period. Cumulative urine volume increased significantly over time ($p < 0.0001$), with a significant kidney effect and no interaction effect, and 15% of the variability was explained by pig differences.

3.7 | Assessment of Renal Function: Creatinine and Iohexol Clearances

Creatinine clearance remained consistent across all kidneys, reflecting stable glomerular filtration rates throughout perfusion. Creatinine concentration significantly decreased over time ($p < 0.0001$), with 21% variability attributed to inter-pig differences (Figure 5A). Paired *t*-test analyses comparing the right and left kidneys revealed no statistically significant differences in the elimination of creatinine ($p = 0.43$) from the perfusate. Similarly, iohexol levels decreased significantly over time during NMP ($p < 0.0001$), with no intra-individual right versus left difference. The inter-individual difference between pigs was responsible for 6.4% of the variability. A similar pattern of creatinine and iohexol elimination during NMP was observed across all samples ($n = 10$). The elimination of both tracers mainly occurred during the first 2 h *post*-infusion (Figure 5B). The GLMM indicated a significant association between iohexol and creatinine clearances ($p < 0.0001$), which was independent of time ($p = 0.14$). Note that a time-dependent reduction in clearance was observed at later time points, particularly at 180 min compared to baseline ($p = 0.0134$). Iohexol clearance was characterized by a coefficient of 0.0118 ± 0.0017 ($p < 0.0001$). Of note, the AIC (−7.0) and BIC (−7.8) criteria confirmed the robustness of the model fit (Table S2).

3.8 | Histological Results

Histological analysis of the kidney biopsies was performed exclusively at the end of perfusion. PAS staining revealed features suggesting acute tubular necrosis with marked loss of the brush border and thinning of the tubular epithelium lining the proximal tubule, with no evidence of cellular desquamation into the lumen. Peritubular capillaritis was observed in all the samples to the same extent (Figure 6).

4 | Discussion

The primary aim of this study was to develop a pig model for the simultaneous ex vivo normothermic perfusion of two kidneys from the same animal in order to demonstrate the potential use of one kidney as a control for the other. This model opens the possibility of studying conditioning strategies to prevent or limit IRI. Furthermore, our findings demonstrate several critical aspects of renal physiology and function during NMP, offering valuable contributions to the knowledge on KTx and preservation.

Throughout the 6-h normothermic perfusion period, we observed stable hemodynamic and metabolic parameters, indicating effective perfusion and organ functionality. Arterial pressure, flow, and intrarenal resistance were maintained, and no significant differences were observed between the right and left kidneys of each animal. The stability of these parameters suggests that our perfusion model successfully preserved renal hemodynamics, which is crucial for maintaining organ viability during preservation [25–27].

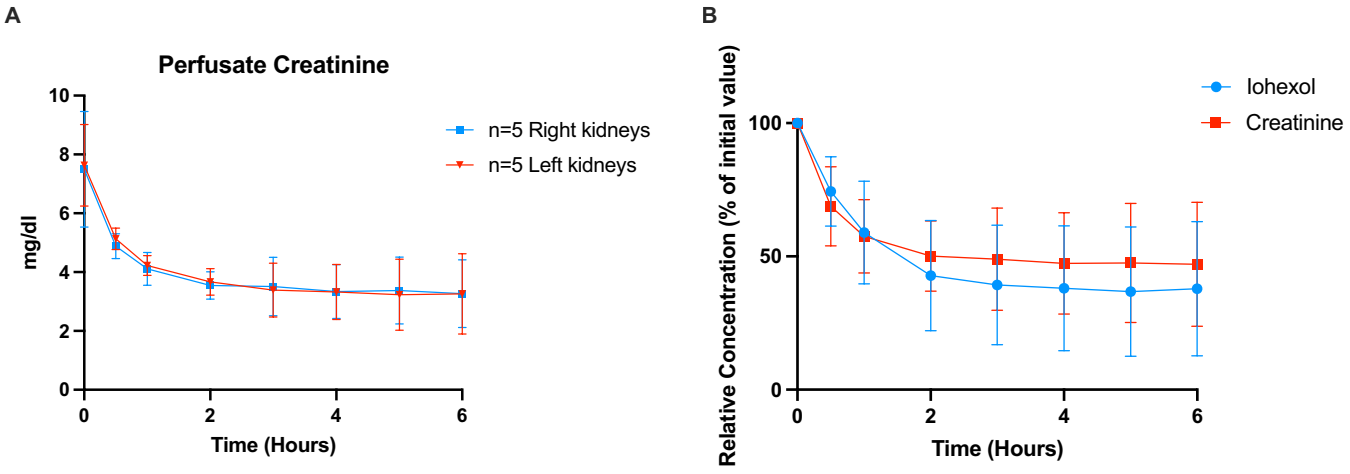

FIGURE 5 | (A) Perfusate creatinine in mg/dl: Mean and SD of 5 right and 5 left kidney perfusions ($n = 10$). (B) Relative concentration of creatinine and iohexol during perfusion ($n = 10$). [Color figure can be viewed at wileyonlinelibrary.com]

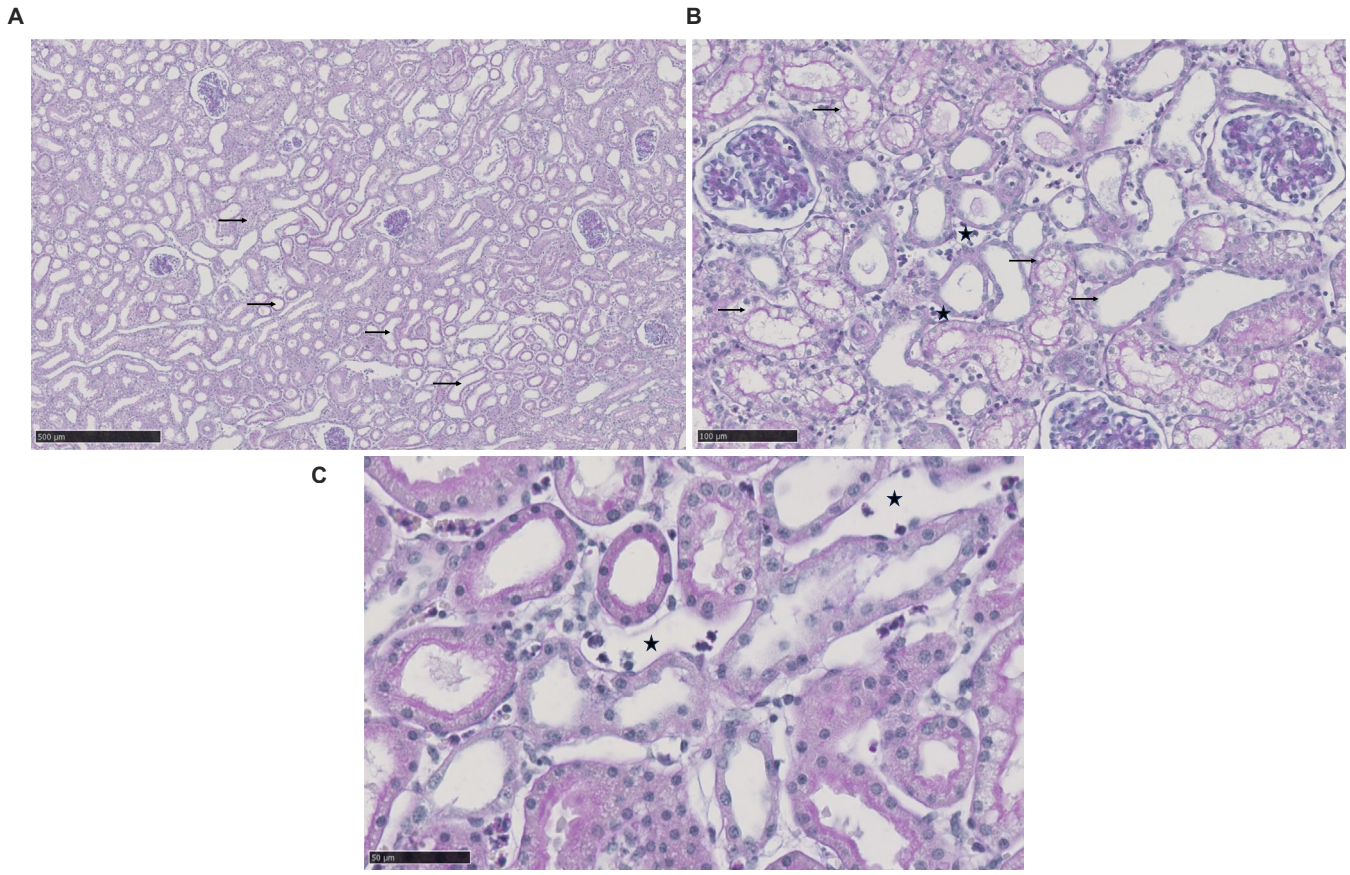

FIGURE 6 | (A and B) Acute tubular necrosis with thinning of the tubular epithelium and cytoplasmic vacuolization of proximal tubular epithelial cells. Original magnification ×5 (A); ×20 (B), PAS staining. (C) peritubular capillaritis. Original magnification ×40, PAS staining. Scale bars = 500 µm (A), 100 µm (B), 50 µm (C). [Color figure can be viewed at wileyonlinelibrary.com]

Electrolyte levels, including sodium and potassium levels, remained stable throughout the perfusion period, indicating the effective regulation of ionic balance. The absence of significant differences between the right and left kidneys further supports the reliability of our perfusion model for maintaining electrolyte homeostasis. These results are consistent with previous reports highlighting the ability of NMP to preserve electrolyte balance and support renal metabolic functions [28–30]. Glucose concentrations decreased progressively over the perfusion period, reflecting metabolic consumption despite the administration of a parenteral nutrient solution. This decline in glucose levels suggests active metabolic processes during NMP, as confirmed by previous studies demonstrating the restoration of ATP levels and metabolic activity in kidneys subjected to NMP [31, 32]. The comparable metabolic activity observed in both kidneys indicated that this model effectively supported glucose metabolism, an essential aspect of organ preservation. Consistent urine production observed during the 6-h perfusion period serves as a functional indicator of renal activity and viability. The cumulative urine output did not differ significantly between the right and left kidneys. This study highlights the potential of NMP as a transformative approach for KTx. By maintaining physiological conditions and enabling real-time functional assessment, NMP can optimize organ utilization, particularly for kidneys, from DCD and ECD sources.

There is no clear consensus on perfusion protocols, including the use of red blood cells or hemoglobin-based oxygen carrier-based perfusion fluid [31, 33]. Strategies for circuit structure, perfusate composition, and assessment of perfused organ viability vary across published studies, necessitating ongoing research to determine the optimal method for organ perfusion [34]. Studies have shown that using whole blood in liver perfusion can provoke a heightened inflammatory response due to the presence of immune cells, resulting in elevated cytokine levels and greater tissue damage [35]. However, researchers indicated that during kidney perfusion, there was no significant difference in cytokine levels or renal function between whole blood and RBC-based perfusates, highlighting the benefits of leukocyte-depleted blood in reducing excessive inflammation [36]. Considering this evidence, we selected an RBC-based perfusate to maintain sufficient oxygenation while minimizing immune activation. The red blood cells used for perfusion in this study were sourced from the same pig from which the kidneys were harvested, further enhancing the relevance and interest of this study.

Our pilot study pioneers the application of iohexol clearance as a reproducible method for assessing glomerular filtration in porcine kidneys during NMP. Thus far, most studies about NMP have evaluated renal function using creatinine as the conventional biomarker [14, 23]. However, creatinine levels are subject to significant, well-known non-GFR-related variability,

which may similarly impact measurements in pig kidneys. Furthermore, the endogenous circulating porcine creatinine may bias the clearance of human creatinine used as GFR tracer during the NMP. In order to circumvent these creatinine-related limitations in GFR assessment, iohexol clearance has been developed and validated in research and clinical routine [37]. Note that iohexol clearance has not been tested in human kidneys under dynamic NMP. Still, we demonstrate here an intra-individual right versus left concordance of iohexol clearance across kidney pairs. The inter-pig variability in clearance rates, likely due to differences in vascular resistance or NMP settings, did not affect the intra-pair reproducibility. Future research will explore iohexol clearance in larger cohorts and assess the effects of extended perfusion times and ischemia on GFR to validate our pilot study across diverse preclinical and clinical settings.

Our study has certain limitations. The use of a well-controlled pig kidney model with short cold ischemia and a limited sample size makes it difficult to establish the precise reference ranges for normal kidney function during NMP. Rather than defining strict thresholds, this study aimed to assess the feasibility of the model. To further investigate the sensitivity of NMP in detecting kidney dysfunction, it would be valuable to develop a model incorporating a defined period of warm ischemia to induce ischemic injury. Additionally, while the dual kidney perfusion approach ensures standardized conditions and minimizes variability in experimental comparisons, it does not replace clinical single-organ perfusion. Instead, it provides a controlled framework for evaluating therapeutic interventions to mitigate ischemia–reperfusion injury.

In conclusion, this study establishes the use of a paired kidney NMP model, in which one kidney serves as a control for the other. This experimental design provides a robust framework for evaluating therapeutic interventions in the context of NMP [38–41]. This allows for the assessment of the true impact of these treatments on the kidneys under identical conditions and from the same donor. These findings contribute to the growing evidence supporting NMP as a valuable tool in KTx, with the potential to improve organ utilization and patient outcomes. Further research and clinical trials are necessary to validate these findings and establish NMP as a standard practice for KTx.

Author Contributions

M.N., N.G., P.E., F.J., and O.D. designed the study. M.N., N.G., M.V., T.P.C., M.G.L., C.M., C.G., G.T., E.C., and O.D. performed the surgeries and experiments. M.N., E.C., F.J., and O.D. contributed to the analysis and interpretation of results. M.N. conducted statistical analyses. M.N., N.G., F.J., and O.D. were involved in the data discussion and revised the manuscript for intellectual content. M.N. participated in the writing of the paper. M.N. prepared figures and tables. All authors have reviewed the results and approved the final version of the manuscript.

Acknowledgments

We thank the University Hospital Center for Biostatistics and Research Methods (B-STAT), and particularly Nadia Dardenne, for their assistance with the statistical data, and Jean-Paul Cheramy-Bien for technical assistance. This study was funded by grants, with the funding sources not being involved in the design, execution, or interpretation of the research. M.N. has been awarded a grant for Research Training in Industry and Agriculture (FRIA). N.G., T.P.C., and F.J. are fellows of the Fonds de la Recherche Scientifique—FNRS, and this work was supported by the FNRS under Grants n° J.0069.22 and T.0095.24. This study was also supported by the University of Liege (Crédit Forfaitaire de Fonctionnement, Fonds Léon Fredericq).

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. R. A. Wolfe, V. B. Ashby, E. L. Milford, et al., "Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant," *New England Journal of Medicine* 341, no. 23 (1999): 1725–1730.

2. K. L. Lentine, J. M. Smith, J. M. Miller, et al., "OPTN/SRTR 2021 Annual Data Report: Kidney," *American Journal of Transplantation* 23, no. 2 (2023): S21–S120.

3. A. Lewis, A. Koukoura, G. I. Tsianos, A. A. Gargavanis, A. A. Nielsen, and E. Vassiliadis, "Organ Donation in the US and Europe: The Supply vs Demand Imbalance," *Transplantation Reviews (Orlando, Fla.)* 35, no. 2 (2021): 100585.

4. J. M. Kaths, J. Echeverri, Y. M. Chun, et al., "Continuous Normothermic Ex Vivo Kidney Perfusion Improves Graft Function in Donation After Circulatory Death Pig Kidney Transplantation," *Transplantation* 101, no. 4 (2017): 754–763.

5. M. N. Levy, "Oxygen Consumption and Blood Flow in the Hypothermic, Perfused Kidney," *American Journal of Physiology-Legacy Content* 197, no. 5 (1959): 1111–1114.

6. A. Cirillo, M. Vandermeulen, P. Erpicum, et al., "Untargeted NMR-Based Metabolomics Analysis of Kidney Allograft Perfusates Identifies a Signature of Delayed Graft Function," *Metabolomics* 20, no. 2 (2024): 39.

7. D. M. Summers, R. J. Johnson, A. Hudson, D. Collett, C. J. Watson, and J. A. Bradley, "Effect of Donor Age and Cold Storage Time on Outcome in Recipients of Kidneys Donated After Circulatory Death in the UK: A Cohort Study," *Lancet* 381, no. 9868 (2013): 727–734.

8. D. M. Summers, C. J. E. Watson, G. J. Pettigrew, et al., "Kidney Donation After Circulatory Death (DCD): State of the Art," *Kidney International* 88, no. 2 (2015): 241–249.

9. L. K. Kayler, J. Magliocca, I. Zendejas, T. R. Srinivas, and J. D. Schold, "Impact of Cold Ischemia Time on Graft Survival Among ECD Transplant Recipients: A Paired Kidney Analysis," *American Journal of Transplantation* 11, no. 12 (2011): 2647–2656.

10. M. O. Hamed, Y. Chen, L. Pasea, et al., "Early Graft Loss After Kidney Transplantation: Risk Factors and Consequences," *American Journal of Transplantation* 15, no. 6 (2015): 1632–1643.

11. I. Jochmans, A. Brat, L. Davies, et al., "Oxygenated Versus Standard Cold Perfusion Preservation in Kidney Transplantation (COMPARE): A Randomised, Double-Blind, Paired, Phase 3 Trial," *Lancet* 396, no. 10263 (2020): 1653–1662.

12. C. Moers, J. M. Smits, M.-H. J. Maathuis, et al., "Machine Perfusion or Cold Storage in Deceased-Donor Kidney Transplantation," *New England Journal of Medicine* 360, no. 1 (2009): 7–19.

13. L. I. Mazilescu, P. Urbanellis, M. J. Kaths, et al., "Prolonged Normothermic Ex Vivo Kidney Perfusion Is Superior to Cold Nonoxygenated and Oxygenated Machine Perfusion for the Preservation of DCD Porcine Kidney Grafts," *Transplantation Direct* 7, no. 10 (2021): e751.

14. L. I. Mazilescu, P. Urbanellis, S. J. Kim, et al., "Normothermic Ex Vivo Kidney Perfusion for Human Kidney Transplantation: First North American Results," *Transplantation* 106, no. 9 (2022): 1852–1859, https://doi.org/10.1097/tp.0000000000004098.

15. S. A. Hosgood, R. J. Brown, and M. L. Nicholson, "Advances in Kidney Preservation Techniques and Their Application in Clinical Practice," *Transplantation* 105, no. 11 (2021): e202–e214.

16. S. Juriasingani, M. Akbari, P. Luke, and A. Sener, "Novel Therapeutic Strategies for Renal Graft Preservation and Their Potential Impact on the Future of Clinical Transplantation," *Current Opinion in Organ Transplantation* 24, no. 4 (2019): 385–390.

17. S. A. Hosgood and M. L. Nicholson, "First in Man Renal Transplantation After Ex Vivo Normothermic Perfusion," *Transplantation* 92, no. 7 (2011): 735–738.

18. E. Rijkse, S. Bouari, H. J. A. N. Kimenai, et al., "Additional Normothermic Machine Perfusion Versus Hypothermic Machine Perfusion in Suboptimal Donor Kidney Transplantation: Protocol of a Randomized, Controlled, Open-Label Trial," *International Journal of Surgery Protocols* 25, no. 1 (2021): 227–237, https://doi.org/10.29337/ijsp.165.

19. A. Bagul, S. A. Hosgood, M. Kaushik, M. D. Kay, H. L. Waller, and M. L. Nicholson, "Experimental Renal Preservation by Normothermic Resuscitation Perfusion With Autologous Blood," *British Journal of Surgery* 95, no. 1 (2008): 111–118, https://doi.org/10.1002/bjs.5909.

20. S. A. Hosgood, M. Patel, and M. L. Nicholson, "The Conditioning Effect of Ex Vivo Normothermic Perfusion in an Experimental Kidney Model," *Journal of Surgical Research* 182, no. 1 (2013): 153–160.

21. S. Lohmann, M. B. F. Pool, K. M. Rozenberg, et al., "Mesenchymal Stromal Cell Treatment of Donor Kidneys During Ex Vivo Normothermic Machine Perfusion: A Porcine Renal Autotransplantation Study," *American Journal of Transplantation* 21, no. 7 (2021): 2348–2359.

22. E. R. Thompson, L. Bates, I. K. Ibrahim, et al., "Novel Delivery of Cellular Therapy to Reduce Ischemia Reperfusion Injury in Kidney Transplantation," *American Journal of Transplantation* 21, no. 4 (2021): 1402–1414.

23. S. A. Hosgood, C. J. Callaghan, C. H. Wilson, et al., "Normothermic Machine Perfusion Versus Static Cold Storage in Donation After Circulatory Death Kidney Transplantation: A Randomized Controlled Trial," *Nature Medicine* 29, no. 6 (2023): 1511–1519.

24. D. Choudhary, A. Sharma, S. Singh, et al., "Application of Ex Vivo Normothermic Machine Perfusion in Deceased Donors With Acute Kidney Injury With Successful Renal Transplantation: A Preliminary Experience," *Transplantation direct* 8, no. 11 (2022): e1391.

25. M. Foguenne, S. MacMillan, P. Kron, et al., "Current Evidence and Future Perspectives to Implement Continuous and End-Ischemic Use of Normothermic and Oxygenated Hypothermic Machine Perfusion in Clinical Practice," *Journal of Clinical Medicine* 12, no. 9 (2023): 3207.

26. B. J. Hasjim, J. M. Sanders, M. Alexander, R. R. Redfield, and H. Ichii, "Perfusion Techniques in Kidney Allograft Preservation to Reduce Ischemic Reperfusion Injury: A Systematic Review and Meta-Analysis," *Antioxidants (Basel)* 13, no. 6 (2024): 642.

27. S. Stimmeder, B. Leber, R. Sucher, and P. Stiegler, "Genetic Modulation: Future Trends Toward Graft Optimization During Machine Perfusion," *Transplantation* 108, no. 3 (2024): 614–624.

28. G. Zaza, F. Neri, M. Bruschi, et al., "Proteomics Reveals Specific Biological Changes Induced by the Normothermic Machine Perfusion of Donor Kidneys With a Significant Up-Regulation of Latexin," *Scientific Reports* 13, no. 1 (2023): 5920.

29. A. Weissenbacher, J. P. Stone, M. L. Lo Faro, et al., "Hemodynamics and Metabolic Parameters in Normothermic Kidney Preservation Are Linked With Donor Factors, Perfusate Cells, and Cytokines," *Frontiers in Medicine* 8 (2022): 801098.

30. A. Weissenbacher, H. Huang, T. Surik, et al., "Urine Recirculation Prolongs Normothermic Kidney Perfusion via More Optimal Metabolic Homeostasis—A Proteomics Study," *American Journal of Transplantation* 21, no. 5 (2021): 1740–1753.

31. T. R. Elliott, M. L. Nicholson, and S. A. Hosgood, "Normothermic Kidney Perfusion: An Overview of Protocols and Strategies," *American Journal of Transplantation* 21, no. 4 (2021): 1382–1390.

32. S. Lignell, S. Lohmann, K. M. Rozenberg, et al., "Improved Normothermic Machine Perfusion After Short Oxygenated Hypothermic Machine Perfusion of Ischemically Injured Porcine Kidneys," *Transplantation direct* 7, no. 2 (2021): e653.

33. Y. de Vries, A. P. M. Matton, M. W. N. Nijsten, et al., "Pretransplant Sequential Hypo- and Normothermic Machine Perfusion of Suboptimal Livers Donated After Circulatory Death Using a Hemoglobin-Based Oxygen Carrier Perfusion Solution," *American Journal of Transplantation* 19, no. 4 (2019): 1202–1211.

34. V. A. Lantinga, A. S. Arykbaeva, N. A. Spraakman, et al., "Impact of Device Variability and Protocol Differences on Kidney Function During Normothermic Machine Perfusion: A Comparative Study Using Porcine and Human Kidneys," *Artificial Organs* 49, no. 1 (2024): 93–107, https://doi.org/10.1111/aor.14851.

35. N. Gilbo, J. Blondeel, T. Wylin, et al., "The Dynamics of Cytokine Release During 24 Hours Continuous Normothermic Machine Perfusion Liver Preservation: An Explorative Porcine Study," *Artificial Organs* 48, no. 6 (2024): 606–618.

36. J. De Beule, D. Keppens, H. Korf, and I. Jochmans, "Differential Cytokine Levels During Normothermic Kidney Perfusion With Whole Blood- or Red Blood Cell-Based Perfusates—Results of a Scoping Review and Experimental Study," *Journal of Clinical Medicine* 11, no. 22 (2022): 6618.

37. N. Ebert, E. Schaeffner, J. C. Seegmiller, et al., "Iohexol Plasma Clearance Measurement Protocol Standardization for Adults: A Consensus Paper of the European Kidney Function Consortium," *Kidney International* 106, no. 4 (2024): 583–596.

38. A. M. Tolomeo, R. Malvicini, D. Ventrella, et al., "Protective Effects of Mesenchymal Stem Cells-Derived Extracellular Vesicles Against Ischemia-Reperfusion Injury of Hearts Donated After Circulatory Death: Preliminary Study in a Pig Model," *Biomedicine & Pharmacotherapy* 178 (2024): 117256.

39. M. Vandermeulen, P. Erpicum, L. Weekers, et al., "Mesenchymal Stromal Cells in Solid Organ Transplantation," *Transplantation* 104, no. 5 (2020): 923–936.

40. P. Erpicum, L. Weekers, O. Detry, et al., "Infusion of Third-Party Mesenchymal Stromal Cells After Kidney Transplantation: A Phase I-II, Open-Label, Clinical Study," *Kidney International* 95, no. 3 (2019): 693–707.

41. P. Erpicum, P. Rowart, L. Poma, J. M. Krzesinski, O. Detry, and F. Jouret, "Administration of Mesenchymal Stromal Cells Before Renal Ischemia/Reperfusion Attenuates Kidney Injury and May Modulate Renal Lipid Metabolism in Rats," *Scientific Reports* 7, no. 1 (2017): 1–13.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.