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Improved Normothermic Machine Perfusion After Short Oxygenated Hypothermic Machine Perfusion of Ischemically Injured Porcine Kidneys

Stina Lignell, MD,[1,2] Stine Lohmann, MD,[1,2] Kaithlyn M. Rozenberg, BSc,[3] Henri G. D. Leuvenink, PhD,[4] Merel B. F. Pool, MD,[4] Kate R. Lewis, MSc,[4] Cyril Moers, MD, PhD,[4] James P. Hunter, MD, Bsc(hons),[3] Rutger J. Ploeg, MD, PhD,[3] Marco Eijken, PhD,[2,5] Ulla Møldrup, MD,[6] Søren Krag, PhD,[7] Carla C. Baan, PhD,[8] Bjarne Kuno Møller, MD, PhD,[5] Anna Krarup Keller, MD, PhD,[6] and Bente Jespersen, MD, PhD[1,2]

Background. In an era where global kidney shortage has pushed the field of transplantation towards using more marginal donors, modified kidney preservation techniques are currently being reviewed. Some techniques require further optimization before implementation in full scale transplantation studies. Using a porcine donation after circulatory death kidney model, we investigated whether initial kidney hemodynamics improved during normothermic machine perfusion if this was preceded by a short period of oxygenated hypothermic machine perfusion (oxHMP) rather than static cold storage (SCS). **Methods.** Kidneys subjected to 75 minutes of warm ischemia were randomly assigned to either SCS (n = 4) or SCS + oxHMP (n = 4), with a total cold storage time of 240 minutes. Cold preservation was followed by 120 minutes of normothermic machine perfusion with continuous measurement of hemodynamic parameters and renal function. **Results.** oxHMP preserved kidneys maintained significantly lower renal resistance throughout the normothermic machine perfusion period compared to SCS kidneys ($P < 0.001$), reaching lowest levels at 60 minutes with means of 0.71 ± 0.35 mm Hg/mL/min/100 g (SCS) and 0.45 ± 0.15 mm Hg/mL/min/100 g (oxHMP). Accordingly, the oxHMP group had a higher mean renal blood flow versus SCS kidneys ($P < 0.001$). oxHMP kidneys had higher oxygen consumption during normothermic machine perfusion compared to SCS preserved kidneys ($P < 0.001$). Creatinine clearance remained similar between groups ($P = 0.665$). **Conclusions.** Preceding oxHMP significantly improved initial normothermic machine perfusion hemodynamics and increased total oxygen consumption. With the long period of warm ischemia, immediate kidney function was not observed, reflected by the findings of low creatinine clearance in both groups.

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Donor organ shortage remains a major obstacle to provide patients with end-stage renal failure, the superior treatment of kidney transplantation compared to dialysis. In the past decade, the kidney deficit has led to an increased use of organ donation after circulatory death (DCD) and to date, many researchers agree that the quality of DCD kidneys is often equivalent to donation after brain death kidneys.

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[1] *Department of Clinical Medicine, Aarhus University, Aarhus, Denmark.*

[2] *Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark.*

[3] *Nuffield Department of Surgical Sciences, Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom.*

[4] *Department of Surgery-Organ Donation and Transplantation, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.*

[5] *Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark.*

[6] *Department of Urology, Aarhus University Hospital, Aarhus, Denmark.*

[7] *Department of Histopathology, Aarhus University Hospital, Aarhus, Denmark.*

[8] *Department of Internal Medicine, Nephrology and Transplantation, Erasmus MC, University Medical Center, Rotterdam, The Netherlands.*

S. Lignell and S. Lohmann contributed equally and shared first authorship.

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Correspondence: Stina Lignell, MD, Department of Clinical Medicine, Aarhus University, Palle Juul-Jensens Blvd 99, 8200 Aarhus N, Denmark. (stina.lignell@outlook.com).

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Currently, DCD kidneys constitute 18% of deceased donor kidney transplantations in the United States,[1] 28% in the United Kingdom,[2] and 59% in the Netherlands.[3] Despite diminished concerns about DCD organs, primary nonfunction and delayed graft function are still commonly seen after DCD renal transplantation. This is thought to be due to inevitable ischemia-reperfusion injury and longer warm ischemia time compared to donation after brain death. For this reason, much research effort focuses on improving the quality of DCD and other high-risk kidneys by innovative preservation techniques to better condition and possibly even repair the kidney before transplantation.[4-7]

Hypothermic machine perfusion (HMP) has been shown to be superior to static cold storage (SCS) as it reduces delayed graft function and improves 1-year graft survival in deceased donor kidneys.[8,9] Supplemental oxygenation during HMP appears to have a favorable effect on function compared to both SCS and standard HMP on early graft function in some preclinical studies.[10-12] Recently, the beneficial effect of the addition of oxygen during HMP has been reported.[13]

Ex vivo normothermic machine perfusion (NMP) is currently studied for further improvements in glomerular, tubular, and endothelial integrity and function of the transplanted kidney.[14] NMP is more resource-intensive and difficult to master compared to cold storage. Additionally, organs with severe ischemic injury are more difficult to perfuse due to high resistance. Nevertheless, the benefits of NMP may outweigh its complexity as aerobic metabolism during NMP is thought to allow assessment of renal function and possibly enables reconditioning and introduction of therapies directly to the kidney before transplantation. The initial phase of kidney NMP is highly crucial and necessitates constant supply of oxygen and nutrients to ensure optimal aerobic cell respiration and thus organ function. These conditions can only be achieved by a stable and adequate perfusion, which in turn depends on hemodynamics within the kidney.

Still, knowledge of cold storage management's impact on subsequent NMP is poor and the ideal cold kidney management before NMP should be elaborated in the continued investigation of refined NMP protocols. Whether dynamic, oxygenated cold preservation could optimize kidney hemodynamics remains unknown.

The primary aim of this study was to investigate in a porcine model whether a preceding short period of oxygenated HMP (oxHMP) compared to SCS alone had the ability to improve the initial perfusion hemodynamics and oxygen consumption during subsequent ex vivo NMP of severely injured DCD kidneys.

MATERIALS AND METHODS

Study Design

A preclinical pig model with 75 minutes of renal warm ischemia[15] was used simulating significant functional warm ischemia as in DCD. Eight left kidneys from donor pigs were randomized to SCS (n = 4) or SCS plus 90 minutes of oxHMP (n = 4) followed by 2 hours of NMP, see Figure 1. All kidneys were exposed to a total cold ischemia time of 240 minutes.

Animals and Ethics

Animal care and procedures followed guidelines by the European Union (directive 2010/63/EU) and local regulations. The study was approved by the Animal Experiments Expectorate (reference-number 2016-15-0201-01145). All involved personnel had Federation for Laboratory Animal Science Associations licenses.

Ten 60 kg female Danish Landrace/Yorkshire crossbred pigs were used for this study. Two pigs with pretyped blood groups of A and O were used only for blood donation to establish a porcine blood bank for NMP usage. From the remaining 8 pigs, the left kidneys and 4 units of blood were retrieved. For blood donation protocol, see **Table S1, SDC**, http://links.lww.com/TXD/A306.

Surgical Procedure

Anesthetics

After premedication with ketamine (5.0 mg/kg) and midazolam (0.5 mg/kg), animals were intubated. Anesthesia was maintained by continuously administrated sevoflurane (gas, 2%–3%) and fentanyl (12.5 μg/kg/h) throughout the surgical procedure. Heart rate, respiratory rate, and oxygen saturation were monitored and $PaCO_2$ were kept between 4.5 and 5.5 kPa. One liter of Ringer's acetate was administrated intravenously before phlebotomy. After kidney and subsequent blood retrieval, pigs were terminated by an overdose of pentobarbital (100 mg/kg) while in general anesthesia.

Kidney Retrieval

The left kidney was approached retroperitoneally through a midline incision. Dissection of the renal artery, vein, and ureter was followed by nephrectomy. Subsequently, the kidney was placed in an organ bag and stored in a heating cabinet at 38°C (normal porcine body temperature) for 75 minutes to simulate warm ischemia in DCD. Afterward, the kidney was put on ice and immediately cold flushed with 20 mL of saline containing 5000 international units of heparin followed by 300 mL of Belzer UW Cold Storage Solution (Bridge to Life Ltd, Columbia, SC). The renal artery and ureter were cannulated and the kidney was placed in a new organ bag, submerged in the above-mentioned fluid, and stored at 4–6°C until the end of SCS.

Blood Collection and Processing

Concurrent to the kidney retrieval, a 12F sheath (Radifocus Introducer II; Terumo Europe, Leuven, Belgium) was placed in the external jugular vein. Blood retrieval started directly after nephrectomy, and 1.6–1.8 L of whole blood was drained and divided into 4 quadruple-blood bags (Macopharma, Mouvaux, France), containing a citrate-phosphate-dextrose solution as anticoagulant. The blood was ABO-typed (Ortho BioVue card; Ortho Clinical Diagnostics, Raritan,

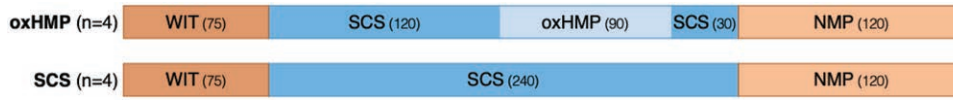

FIGURE 1. Kidney graft management in the 2 intervention groups after nephrectomy. Warm ischemia time (WIT), static cold storage (SCS), oxygenated hypothermic machine perfusion (oxHMP), normothermic machine perfusion (NMP), and time presented in parentheses in minutes.

NJ), fractionated (Baxter Optipress II Blood Component Separator; Baxter, Chicago, IL), and leukocyte depleted. Lastly, the red blood cells (RBCs) were transferred to the inline storage bag containing salt-adenine-glucose-mannitol and kept at 4°C until usage. At the day of kidney NMP, pre-typed RBC were selected from stock. To ensure RBC and kidney compatibility cross-matching was performed with a serum sample from the recipient (NMP-kidney). One hour before NMP, the chosen RBC were washed twice in PBS to eliminate the salt-adenine-glucose-mannitol and reduce the amount of extracellular electrolytes, as described by the European guidelines for blood transfusion.[16]

Machine Perfusion

Kidneys were perfused during both oxHMP and NMP with the pressure controlled Groningen machine perfusion system (Software; SophistiKate, UMCG, Groningen, The Netherlands) consisting of a disposable organ chamber, a centrifugal pump maintaining pulsatile flow (Medos Deltastream DP2; Xenios AG, Heilbronn, Germany), and an oxygenator/heat exchanger (Maquet QUADROX-I Neonatal; Getinge Group, Sweden). All connected by custom-made coated tubings (Medtronic, Minneapolis, MN) with sampling ports before and after the oxygenator. The machine perfusion system is shown in Figure 2. To minimize cost, the same system was applied for both oxHMP and NMP. After oxHMP, the cold preservation fluid was easily drained from the system before primed with the perfusate, necessitating a short period of SCS.

The fluid of oxHMP and the perfusate of NMP were oxygenated with 0.5 L/min carbogen gas (95% O_2, 5% CO_2).

For oxHMP, the system was primed with Belzer's Machine Perfusion Solution (Bridge to Life Ltd, Columbia, SC) and temperature was kept between 4.0 and 8.0°C, while mean arterial pressure was set at 30 mm Hg.

The NMP setup was primed with a newly developed and thoroughly optimized perfusate, see Table 1. When the system reached a temperature of 20°C, 240 mL of washed, cross-matched allogeneic erythrocytes was added aiming for a porcine hematocrit of 30%. Perfusate temperature was kept 2 and a half degree below porcine body temperature to slightly lower the metabolic rate. The mean arterial pressure was kept constantly at 70 mm Hg. Before connection of the kidney to the NMP system, blood gas analyses were carried out (ABL-90 FLEX Blood Gas Analyzer; Radiometer, Brønshøj, Denmark) and if necessary, pH was adjusted with 8.4% sodium bicarbonate to obtain a physiological pH of 7.35. A nutrition solution was administrated during NMP at a rate of 1.5 mL/h, see Table 1. Perfusion characteristics were continuously recorded and blood samples (NMP only) were collected every 30 minutes from the arterial line and from the renal vein.

Outcome Measurements

The primary outcome was hemodynamic parameters during NMP assessed as renal resistance (RR) and renal blood flow (RBF) per 100 g of tissue. Secondary outcome measures were creatinine clearance, diuresis, oxygen consumption, lactate production, and glucose consumption.

Oxygen consumption (VO_2) was defined as mL O_2/min/100 g, calculated every 30 minutes during 120 minutes of NMP. The following equations were used for oxygen consumption.[17]

$$\text{Oxygen consuption}: VO_2 = RBF\left(CaO_2 - CvO_2\right)$$

$$\text{Arterial oxygen content}: CaO_2 = \begin{pmatrix}(aHb \times 1.36 \times \%SatHb) + \\ (0.0031 \times PaO_2)\end{pmatrix}$$

$$\text{Venous oxygen content}: CvO_2 = \begin{pmatrix}(vHb \times 1.36 \times \%SatHb) + \\ (0.0031 \times PvO_2)\end{pmatrix}$$

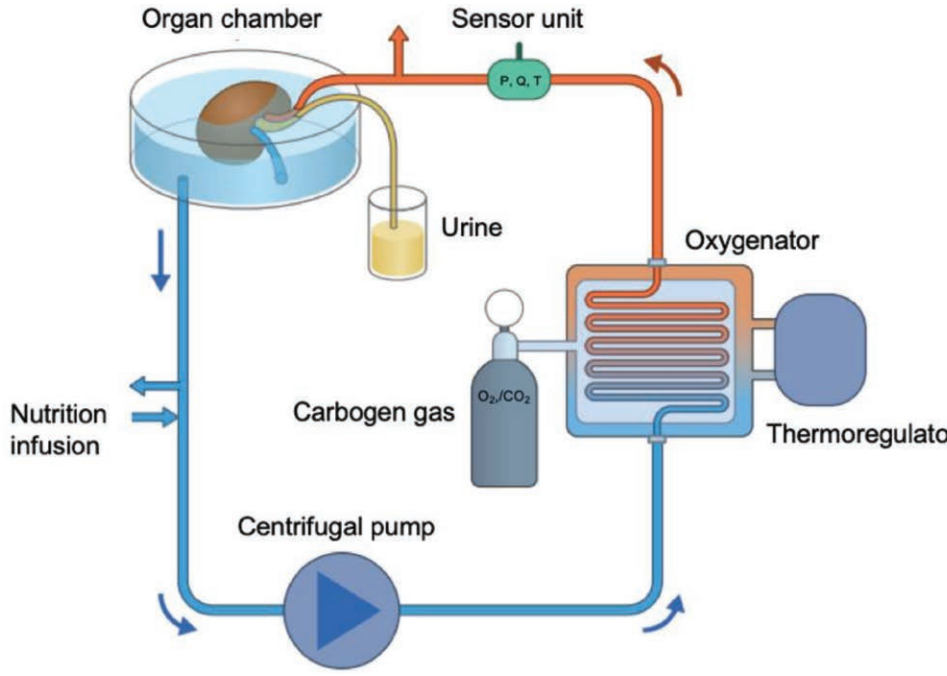

FIGURE 2. Schematic illustration of the machine perfusion circuit. Tubings with sample ports on the arterial and venous side are connected the centrifugal pump, oxygenator, and organ chamber. Carbogen gas (95% O_2, 5% CO_2) is administrated to the oxygenator and an external thermoregulator is used for temperature control. A nutrition solution is administrated into the venous line before the centrifugal pump. Pressure (P), flow (Q), and temperature (T) were recorded continuously.

TABLE 1.

The components of the perfusion solution and nutrition added during NMP

Perfusion solution	Volume (mL)
Ringers solution[a]	120
Calcium gluconate 10%	3
Glucose 5%	6
$NaHCO_3$ 8.4%	10
Amoxicillin clavulanate (60 mg/mL)	1
Sodium nitroprusside (0.2 mg/mL)	0.1
Bovine albumin dissolved in Ringers solution (0.05 g/mL)	240
Mannitol 10 mg[b]	0
Creatinine 69 mg[b]	0
Leukocyte-depleted washed RBC	240
Nutrition solution 1.5 mL/h	**Volume (mL)**
Nutriflex special	20
$NaHCO_3$ 8.4%	5
Insulin 100 U/mL	1

[a]Additionally administrated as urine replacement during NMP.
[b]Added in powder form.
$NaHCO_3$, sodium bicarbonate; NMP, normothermic machine perfusion; RBCs, red blood cells.

Creatinine clearance (Cl_{cr}) was calculated every hour using the following equation and expressed in mL/min/100 g kidney tissue.

$$\text{Creatinine clearance}: Cl_{Cr} = \frac{(Cr_{Urine} \times Vol_{urine})}{Cr_{plasma}}$$

Neutrophil Gelatinase-associated Lipocalin

Urine neutrophil gelatinase-associated lipocalin (NGAL) was determined by the pig NGAL sandwich ELISA (Kit 044; Bioporto, Hellerup, Denmark). The assay was set up according to manufacturer's protocol and samples were diluted by a factor of 10^{-4}. Absorbance was read at 450 nm (630 nm reference) on an 800 TS-microplate absorbance reader with Gen5 software (BioTek, Winooski, VT). Analyses were carried out by laboratory technician at the Department of Renal Medicine, Aarhus University Hospital.

Histomorphological Assessment

Tissue samples were fixed in 4% formalin immediately after collection and stored for 24 hours, followed by storage in PBS until dehydrated and embedded in parafine, known as formalin-fixed parafin-embedding (FFPE). Sections of formalin-fixed parafin-embedding tissue were stained with hematoxylin and eosin, Masson trichrome, and periodic acid-Schiff. Sections were analyzed by an expert renal pathologist blinded to intervention and cases. Several parameters were assessed: inflammation, tubular atrophy, fibrosis, and tubular and glomerular damage. Each parameter was scored semi-quantitatively on a scale from 0 to 4 based on the degree of injury: 0 if <2% was affected, 1 (2%–5%), 2 (6%–25%), 3 (26%–50%), and 4 (51%–100%).

Statistical Analysis

STATA software version 15.1 (StataCorp, College Station, TX) was used for statistical analyses. Data were normally distributed and presented as mean values with SD. Student *t* test was used to test for differences between groups.

To test for differences of parametric continuous variables over time, a linear mixed-effects model with group and time as fixed effects and pig as random effect was used to analyze repeated measurements. Statistical significance was defined as $P < 0.05$.

RESULTS

Kidney and Machine Perfusion Characteristics

All 8 kidneys were subjected to 75 minutes of warm ischemia and completed 120 minutes of NMP without any adverse events. Baseline kidney weight in the SCS and oxHMP group were comparable (151 ± 15 versus 136 ± 14 g, $P = 0.190$) likewise gained weight after NMP ($P = 0.881$). There was no significant difference between groups with regard to total cold preservation time (SCS 240.0 versus oxHMP 240.5 min, $P = 0.620$). During oxHMP, the temperature was 6.9 ± 0.4°C. Perfusion settings, blood gas levels, and pH during NMP are displayed in Table 2, with no differences between groups.

Hemodynamics

During NMP, the RR decreased substantially in both groups during the first hour, reaching the lowest level at 60 minutes (SCS 0.71 ± 0.35 versus oxHMP 0.45 ± 0.15 mm Hg/mL/min/100 g). The mixed model analysis showed that mean RR as a function of time differed in the 2 groups, where the RR of oxHMP preserved kidneys decreased more compared to the SCS preserved kidneys of which RR increased during NMP ($P < 0.001$). Accordingly, different RBF changes were seen over time ($P < 0.001$). Peak levels of RBF were 115 ± 45 mL/min/100 g (SCS) and 165 ± 42 mL/min/100 g (oxHMP). After reached peak levels, RBF dropped in the SCS group, whereas it appeared to remain more stable in the oxHMP group. Hemodynamic parameters are illustrated in Figure 3.

Renal Function, Injury, and Metabolism

During the first 60 minutes of NMP, SCS, and oxHMP kidneys had similar progression of oxygen consumption. However, the oxHMP group's oxygen consumption kept increasing during perfusion and peaked at a mean of 1.5 ± 0.2 mL O_2/min/100 g, whereas SCS kidneys' mean oxygen consumption reached highest level after 30 minutes of NMP with a mean

TABLE 2.

Machine perfusion characteristics during 0–120 minutes of NMP after SCS or oxHMP

Perfusion characteristics	SCS (n = 4)		oxHMP (n = 4)		
	Mean	SD	Mean	SD	*P*
MAP (mm Hg)[a]	70	NA	70	NA	NA
HR (bpm)[a]	60	NA	60	NA	NA
Temperature(°C)	35.3	0.3	34.9	0.7	0.412
Pao_2 (kPa)	65.3	5.2	65.6	1.3	0.916
$Paco_2$ (kPa)	5.1	0.4	5.1	0.4	0.895
Sao_2 (%)	99.5	0.4	99.5	0.2	1.000
pH	7.35	0.04	7.35	0.02	0.771

[a]Fixed machine perfusion settings.
bpm, beats per minute; HR, heart rate; MAP, mean arterial pressure; NA, not applicable; NMP, normothermic machine perfusion; oxHMP, oxygenated hypothermic machine perfusion; Sao_2, arterial oxygen saturation; SCS, static cold storage.

of 1.3 ± 0.2 mL O_2/min/100 g. Thus, the interaction of oxygen consumption and time differed in the 2 groups of which oxHMP kidneys had significantly higher levels compared to SCS preserved kidneys ($P < 0.0001$), illustrated in Figure 4A. There was no difference in glucose consumption during the observational period ($P = 0.350$, see Figure 4B).

Creatinine clearance was generally low during NMP with mean values after 60 minutes of 1.1 ± 0.8 mL/min/100 g (SCS) and 1.2 ± 0.9 mL/min/100 g (oxHMP) ($P = 0.905$). Creatinine clearance tended to increase over time in the oxHMP group and remained unchanged in the SCS group at the end of perfusion (SCS 1.1 mL/min/100 g versus oxHMP 1.4 mL/min/100 g, $P = 0.665$) see Figure 5A. Urine production showed similar rates after 60 minutes (SCS 58 ± 36 versus oxHMP 67 ± 48 mL/h/100 g, $P = 0.79$) and 120 minutes (SCS 44 ± 35 versus oxHMP 59 ± 42 mL/h/100 g, $P = 0.606$) in both groups, see Figure 5B. Urinary NGAL was measured at the end on NMP and showed similar levels between groups.

Histomorphological Assessment

Kidney tissue samples were scored on a scale from 0 to 4 and individual scores are reported in Figure 6. The histological assessment only revealed tubular damage with presence of tubular dilatation and, in some cases, tubular necrosis. The histological changes were similar between groups.

DISCUSSION

This NMP study on severely ischemically damaged porcine DCD kidneys examined the impact of a short period of oxHMP before NMP. The study demonstrated that compared to going directly from SCS to NMP, kidney hemodynamics as well as oxygen consumption were improved if oxHMP preceded NMP.

Extensive preclinical research has been made during the last decade on how to improve kidney NMP by altering NMP settings. Despite this, there is still no consensus on a standardized NMP protocol. Our findings add important knowledge to the field. First, by adding 90 minutes of oxHMP at the end of the cold preservation period, we were able to significantly decrease initial RR during NMP in comparison with kidneys that solely underwent SCS. Second, kidneys subjected to oxHMP showed a significant increase in oxygen consumption during the NMP.

Preclinical NMP studies have demonstrated improved renal function after transplantation compared to SCS.[7,18] However, in these reports, NMP does not obviate a period of cold preservation during NMP preparation. To improve initial hemodynamics during NMP, our strategy was to include a brief period of oxHMP, aiming for a reduction in initial RR during the critical period of NMP startup. In the clinic, HMP has been shown to be more advantageous compared with SCS in kidney DCD donation,[19,20] and HMP is now at some centers standard treatment. Further HMP optimization is attempted by oxygen supply,[6,10,21-27] with the objective to reestablish ATP levels in kidneys suffered from prolonged warm ischemia. Still, the impact of these different cold preservation methods in relation to NMP have been scarcely studied, and our study shows that oxHMP has a positive impact on initial NMP resistance, which is in line with a few conducted studies investigating oxHMP in preclinical autotransplantation and ex vivo reperfusion models.[6,23] The main findings suggest that oxHMP is advantageous as it improves kidney microcirculation due to reduced vascular resistance, although Gallinat et al[23] point out that this only applies kidneys suffering from prolonged warm ischemia and not kidneys from heart-beating donors. This study supports the positive findings after oxHMP of injured kidneys, and our study additionally provides information about the beneficial effect, which might be present even after a brief period of oxHMP. A short duration of oxHMP would contribute to keeping total preservation durations low, an important factor in transplantation.

The protocol chosen included 75 minutes of warm ischemia resulting in severely damaged kidneys, which could however regain function as shown in our previous studies.[15,28] In the present study, oxHMP significantly increased oxygen consumption throughout NMP, suggesting increased levels of metabolism in oxHMP kidneys. This assumption is supported by Venema et al,[27] who also described improved ATP levels during oxHMP. The restored ATP levels might have contributed to our oxHMP kidneys superior oxygen consumption during NMP.

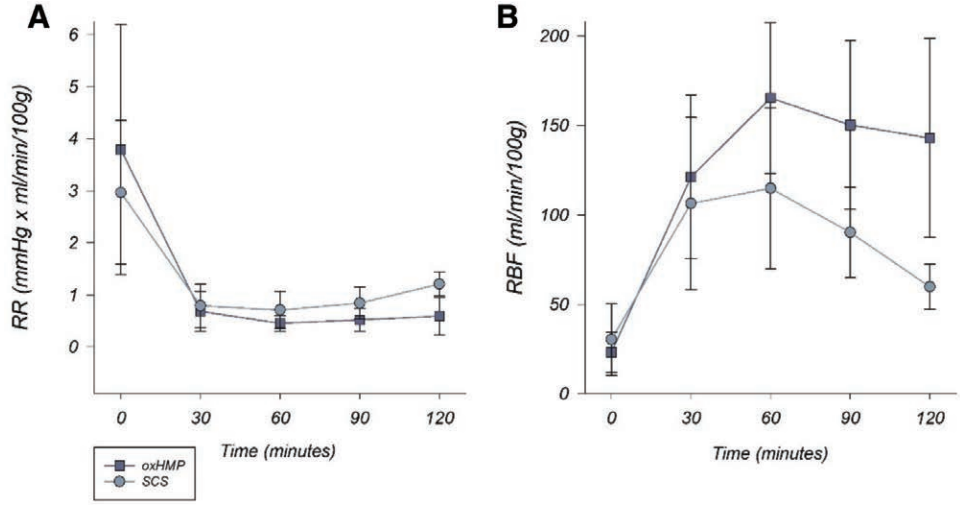

FIGURE 3. Renal hemodynamical parameters. A, Renal resistance (RR) and (B) renal blood flow (RBF), during 120 min of NMP corrected for 100 g of kidney weight, presented as mean with SD. During NMP, there was a significant difference in the progression of both RR and RBF between groups, $P < 0.001$ (RR) and $P < 0.001$ (RBF). NMP, normothermic machine perfusion; oxHMP, oxygenated hypothermic machine perfusion; SCS, static cold storage.

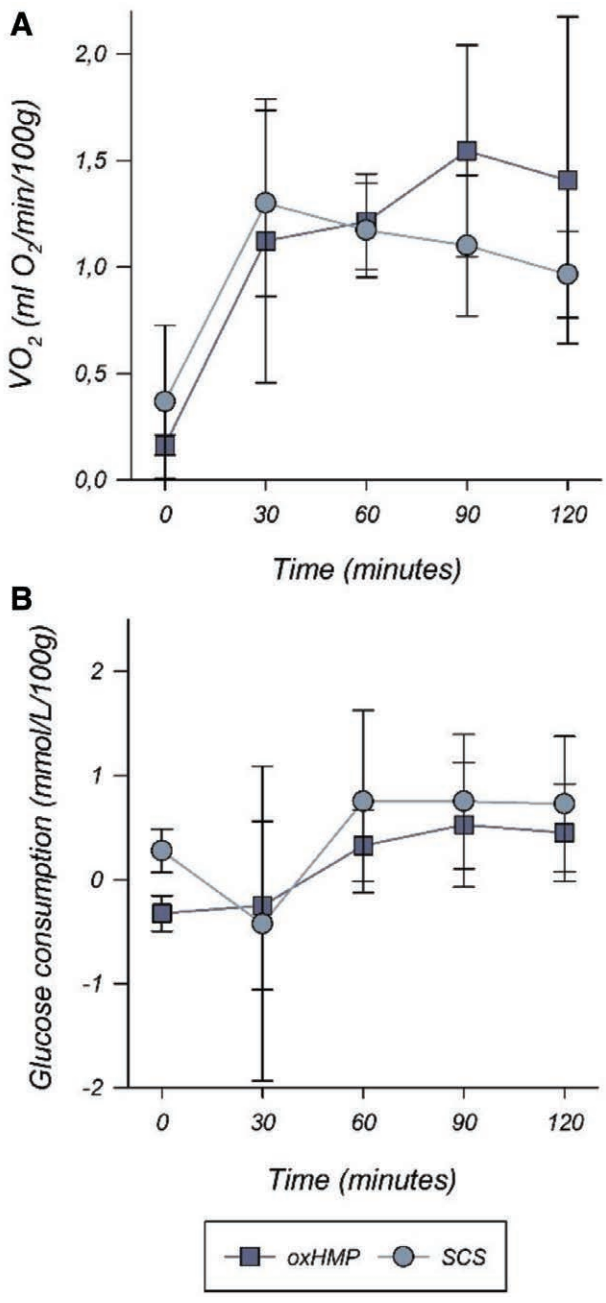

FIGURE 4. Renal metabolism. A, Oxygen consumption (VO_2) and (B) glucose consumption, during 120 min of NMP corrected for 100 g of kidney weight, presented as mean with SD. During NMP, there was a significant difference in the progression of oxygen consumption between groups, $P < 0.0001$. There were similar levels of glucose consumption between groups, $P = 0.350$. NMP, normothermic machine perfusion; oxHMP, oxygenated hypothermic machine perfusion; SCS, static cold storage.

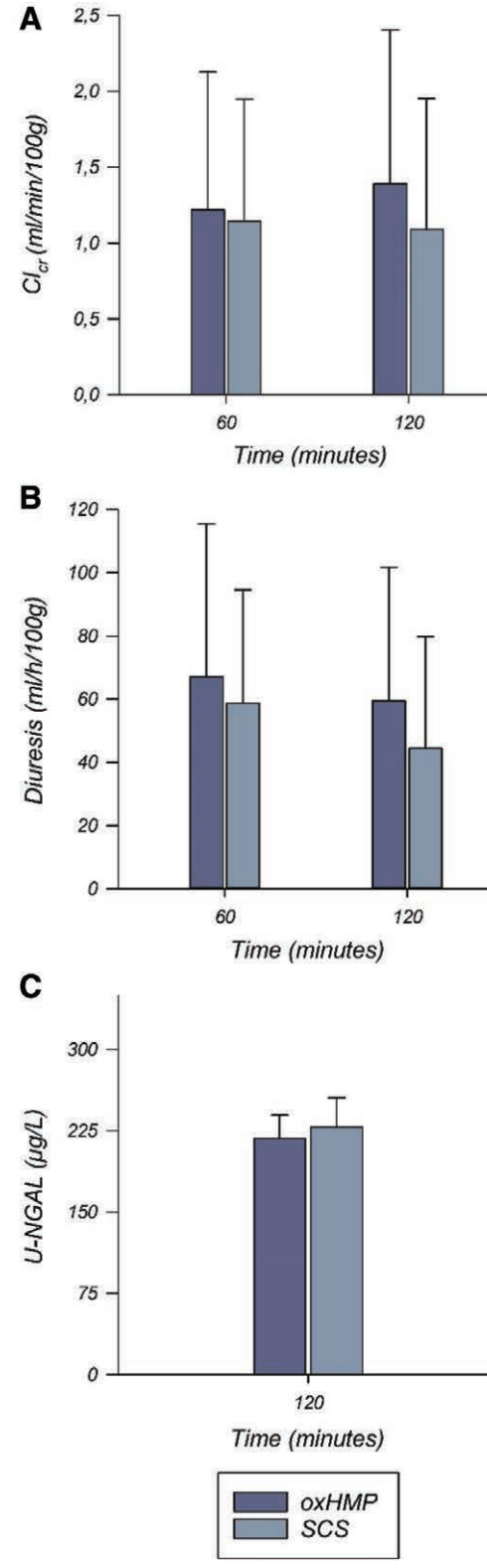

FIGURE 5. Renal function and injury. A, Creatinine clearance (Cl_{cr}), (B) diuresis during 120 min of NMP corrected for 100 g of kidney weight, and (C) urinary NGAL (U-NGAL) presented as mean with SD. NGAL, neutrophil gelatinase-associated lipocalin; NMP, normothermic machine perfusion; oxHMP, oxygenated hypothermic machine perfusion; SCS, static cold storage.

During NMP, the urinary output and creatinine clearance were similar between groups with a tendency of diminishing urine production at the end of perfusion. In literature, there is no evidence that indicates diuresis as a sufficient measure of renal function during NMP, creatinine clearance being better, although this biomarker is limited as well. Previous experience indicates that renal clearance during NMP is <10 mL/min/100 g even if kidneys have only minor ischemic injury, and 1–2 mL/min/100 g when the warm ischemia increased,[29,30] which is in accordance with our results. In this small study, we showed an insignificant creatinine clearance increase in the oxHMP group in accordance with improved glomerular

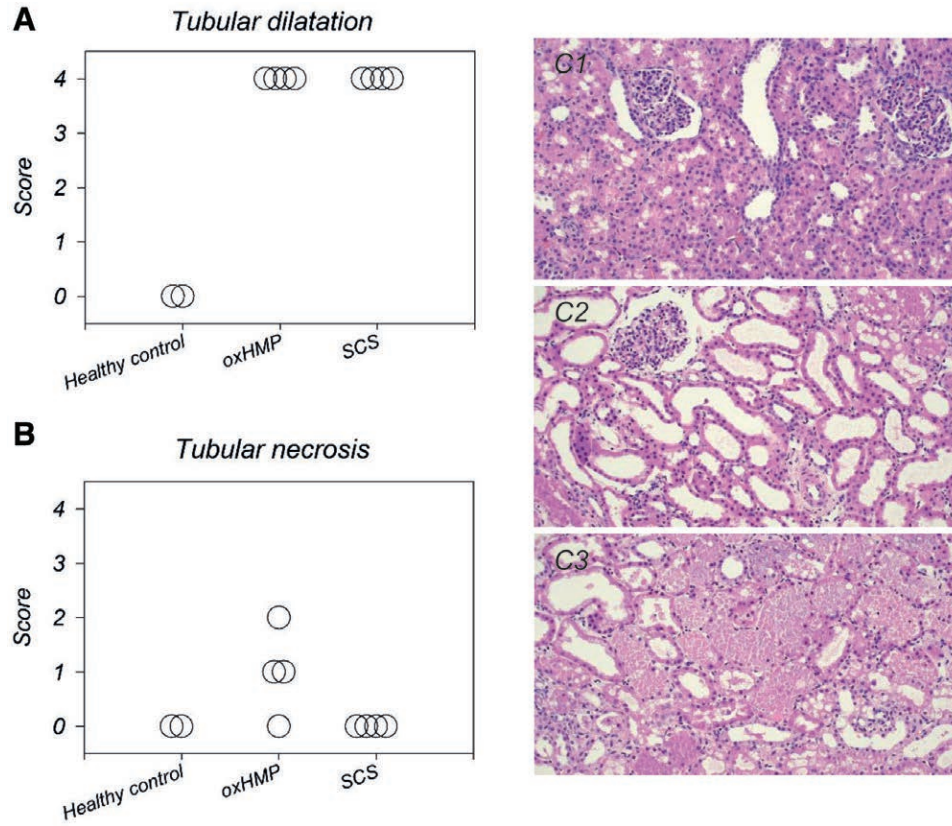

FIGURE 6. Histological examination. A and B, Scores presented with individual scores. Healthy control kidneys were not exposed to warm ischemia, oxHMP, or NMP. C1, Healthy kidney tissue. C2, Tubular dilatation with intraluminal debris. C3, Example of tubular necrosis. C1–C3, Magnification ×200. NMP, normothermic machine perfusion; oxHMP, oxygenated hypothermic machine perfusion; SCS, static cold storage.

filtration suggested by others.[6,24,27] We found similar urinary NGAL levels and histology between groups. Urinary NGAL is a sensitive early biomarker of kidney injury,[31-33] and higher levels have shown some association with posttransplant delayed graft function; however, deciding to transplant or discard a kidney merely based on NGAL is infeasible.

A general limitation of NMP is the lack of a biomarker predicting posttransplant outcome, which has been discussed earlier[34] and not even histological findings are necessarily associated to later kidney function.[35] A previous study have described ATP improvements during NMP as a supplement to reported hemodynamics[27,36]; however, concomitant kidney function improvements were not reported, and most studies do not include the transplantation outcome parameter leaving it still questionable whether ATP levels are prognostic.

Posttransplant outcomes are still the paramount measurements in preservation and organ optimization research. Solid predictive biomarkers have not been proven, and evaluation of kidney quality during NMP are so far based on several parameters with hemodynamics as the back bone supplemented with selected markers of function and injury. To our knowledge, there is no proven correlation between any NMP biomarker and posttransplant kidney function, and the next step for evaluating machine perfusion protocols will be preclinical transplantation studies.

Two important methodological settings in the present study need to be discussed: the temperature during NMP and the choice of oxygen carrier. Normothermia within NMP is not well-defined. However, 2 recent reviews state normothermia as 35°C to 37°C degrees.[37,38] Our group chose to perform NMP at the lower end of this spectrum since in cardiopulmonary bypass technology, this is done to lower core body temperature by approximately 2 degrees C° to let mild hypothermia act cytoprotective.

Previous porcine NMP models have mainly used perfusion solutions based on autologous RBC, either as modified whole blood solutions or as packed RBC mixed with a plasma substitute solution.[14,39-43] To enable evolvement of our model to a full porcine autotransplantation model,[15] we used stored cross-matched allogeneic RBC as oxygen carrier as used in clinical transfusion practice. Allogeneic RBC implies addition of incompatibility testing since allogeneic blood transfusion in pigs is known to cause adverse immunologic reactions.[44] In a review by Smith et al,[45] knowledge of porcine blood types was thoroughly described and the authors highlighted the importance of using cross-matched porcine blood products in experimental research. When using washed allogeneic RBC in NMP, the risk of adverse immunologic reactions on the pump must be considered negligible, due to lack of immunologic components. Nevertheless, when transplanting the kidney to a foreign immunologically active environment, there is a theoretical risk of AO incompatibility if RBC washout after NMP is insufficient. The present study was designed with the intention of subsequent expansion to a full autotransplantation model. With this in mind, and since blood group diversity in research pigs is well known, we chose to implement RBC-typing, a method previously described by Martinez-Alarcon et al.[46] This was followed by immediate cross-matching to ensure compatibility. The above-described management has, to our knowledge, not been described in previous NMP work.

Still, limitations are worth mentioning, as we did not perform NMP experiments with deliberately noncompatible RBC, and the actual risk of adverse immunologic impact is therefore unknown.

A wide variety of NMP durations are used in NMP research. Our objective was not to investigate the possible beneficial effects of prolonged NMP but to investigate initial hemodynamics, and we used 2 hours of NMP. Moreover, a short period of NMP is clinically applicable and may have potential for actual implementation.[40,47,48] The precise role of a brief NMP resuscitation in clinical use has not been solidly established yet, and we await the results of the multicenter randomized clinical kidney trial in the United Kingdom.[49] Alternatively, in a different experimental NMP model, Fabry et al[50] recently demonstrated good outcomes when avoiding cold preflush before NMP, and thus going directly from nephrectomy to NMP. These results are interesting, yet controversial, as removal of blood and organ cooling is clinical practice and is thought to be fundamental in organ preservation. Due to logistic difficulties in implementing such a protocol in clinics, we believe that the cold preservation strategy as presented in our study is in the near future, a more realistic clinical implementation strategy. The many possible approaches before NMP indicate that pre-NMP management is a field that needs to be further elucidated. Our study is a contribution to this pre-NMP management.

Some limitations of the study need to be enlightened. Due to animal ethics, a sample size of only 4 research animals was used in each group. Consequently, we cannot exclude the possible risk of underpowering, but nevertheless, we demonstrated significant difference in hemodynamics. Some of the perfusate ingredients may have limited the study. The perfusate contained sodium nitroprusside as vasodilator and Nutriflex, which were omitted in subsequent studies as they may have contributed to elevated lactate levels (data not shown). Nutriflex may contribute to nitric oxide and sodium nitroprusside can cause cyanide toxicity with methemoglobinemia causing metabolic lactic acidosis. Lactate was lower in later experiment when abandoning the use of nitroprusside.

A supplemental control group of HMP without oxygenation was not conducted and might be considered a study design limitation. At our facility, SCS was standard cold preservation, and while other studies had shown beneficial effect of oxygenation during HMP versus no oxygenation,[6,10,27] we did not find it necessary to conduct an extra control group.

In conclusion, oxHMP offers the best pre-NMP preservation strategy compared to SCS to improve the initial NMP hemodynamic parameters of ischemically injured kidneys; however, no functional improvement was demonstrated. The allogenic RBC management described in this study is applicable in experimental porcine NMP studies.

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