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The effect on early renal function of various dynamic preservation strategies in a preclinical pig ischemia–reperfusion autotransplant model

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The aims of this study were to determine the most optimal timing to start machine perfusion during kidney preservation to improve early graft function and to evaluate the impact of temperature and oxygen supply during machine perfusion in a porcine ischemia–reperfusion autotransplant model. The left kidney of an approximately 40-kg female Belgian Landrace pig was exposed to 30 minutes of warm ischemia via vascular clamping and randomized to 1 of 6 study groups: (1) 22-hour static cold storage (SCS) (n = 6), (2) 22-hour hypothermic machine perfusion (HMP) (n = 6), (3) 22-hour oxygenated HMP (n = 7), (4) 20-hour HMP plus 2-hour normothermic perfusion (NP) (n = 6), (5) 20-hour SCS plus 2-hour oxygenated HMP (n = 7), and (6) 20-hour SCS plus 2-hour NP (n = 6). Graft recovery measured by serum creatinine level was significantly faster for continuous HMP preservation strategies compared with SCS alone and for all end-ischemic strategies. The active oxygenated 22-hour HMP group demonstrated a significantly faster recovery from early graft function compared with the 22-hour nonactive oxygenated HMP group. Active oxygenation was also found to be an important modulator of a faster increase in renal flow during HMP preservation. Continuous oxygenated HMP applied from the time of kidney procurement until transplant might be the best preservation strategy to improve early graft function.

KEYWORDS
animal models: porcine, delayed graft function (DGF), kidney (native) function/dysfunction, kidney transplantation/nephrology, organ perfusion and preservation, organ procurement and allocation, organ transplantation in general, translational research/science

1 | INTRODUCTION

Kidney transplant is the "gold standard" treatment for end-stage kidney disease, providing superior outcomes in quality of life and reduced morbidity and mortality compared with dialysis.[1-3] A major limitation is the limited number of deceased donor grafts.[4,5] In response to this organ shortage, kidney grafts from brain death extended criteria donors (ECD)[6] and from donation

Abbreviations: AST, aspartate aminotransaminase; AUC, area under the curve; DCD, donation after circulatory death; DGF, delayed graft function; ECD, extended criteria donors; HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; HOPE, hypothermic oxygenated perfusion; KPS-1, kidney perfusion solution-1; KT, kidney transplant; LDH, lactate dehydrogenase; LKT, LifePort Kidney Transporter; MMRM, mixed model for repeated measures; NP, normothermic perfusion; Po_2, partial oxygen pressure; ROS, reactive oxygen species; SCS, static cold storage.

after circulatory death (DCD)[7] are currently accepted for transplant. However, these suboptimal organs result in a higher rate of primary nonfunction, delayed graft function (DGF), and increased rates of early graft loss.[8-10] DGF is a well-known factor to trigger acute rejection and impairs long-term allograft survival.[11]

Static cold storage (SCS) alone is the worldwide "gold standard" for organ preservation. During the past 10-15 years, dynamic preservation strategies have been reintroduced as a method of organ preservation with the aim to recondition the kidney during preservation, decrease ischemia–reperfusion injury, and improve early and late graft function.[12,13] Continuous hypothermic machine perfusion (HMP) started immediately after procurement until implantation demonstrated better transplant outcomes compared with SCS alone[14-16] and reduced the risk of DGF regardless of donor type and cold ischemia time.[17] More recently, end-ischemic strategies have been shown to decrease the incidence of DGF by using HMP[18] or normothermic perfusion (NP)[19] after a period of SCS compared with SCS alone. Preclinical studies have demonstrated a beneficial effect on early graft function by adding oxygen during HMP.[20-23] Additionally, continuous NP was explored in a porcine kidney transplant model to evaluate feasibility[24,25] and demonstrated a positive effect on early graft function.[25]

Dynamic preservation strategies cover a large spectrum of preservation techniques and often are compared with only SCS alone. Three important variables to be chosen during machine perfusion are the temperature setting, the supplementation of oxygen, and the time to start machine perfusion during the period of the organ preservation. We designed a comparative preclinical study to evaluate the effect on early graft function of SCS and different dynamic preservation strategies in a pig ischemia–reperfusion autotransplant model. The aims were to determine the most optimal timing to start machine perfusion during kidney preservation to improve early graft function and to evaluate the impact of temperature and oxygen supply during machine perfusion.

2 | MATERIALS AND METHODS

2.1 | Animals

Female Belgian Landrace pigs of approximately 40 kg were used. All experiments were performed according to international guidelines, after approval by the local ethical committee for animal care. The animals were housed and cared according to Belgian laws on experimental animal welfare.

2.2 | Study design

A standardized ischemia–reperfusion pig kidney autotransplant model was used as illustrated in Figure 1.

2.3 | Anesthetic protocol

The anesthetic protocol was identical for the nephrectomy and transplant procedure. Anesthesia was induced via intramuscular injection

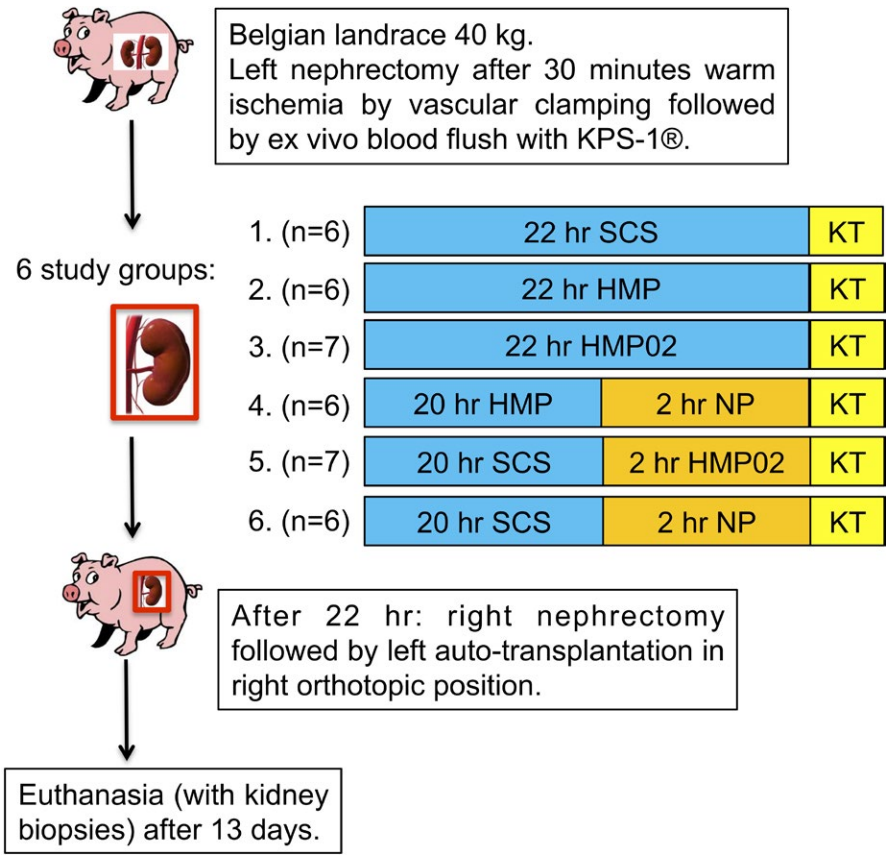

FIGURE 1 Study design of an ischemia–reperfusion pig kidney autotransplant model to evaluate the impact of various preservation strategies on renal function. HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; KT, kidney transplant; NP, normothermic perfusion; SCS, static cold storage [Color figure can be viewed at wileyonlinelibrary.com]

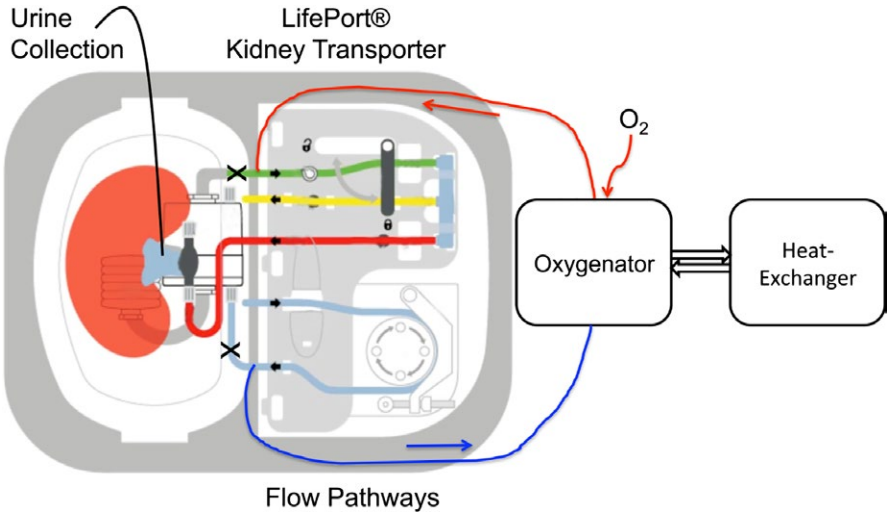

FIGURE 2 Schematic overview of the perfusion set up of the LifePort Kidney Transporter in all oxygenated machine perfusion study groups (courtesy of Organ Recovery Systems, Zaventem, Belgium). An external oxygenator and heat exchanger were added in bypass to the circuit. Only during normothermic perfusion was the ureter of the kidney cannulated for urine collection [Color figure can be viewed at wileyonlinelibrary.com]

of 6 mg/kg Tiletamine 50mg/ml and Zolazepam 50 mg/ml (Virbac, Leuven, Belgium) and 2 mg/kg Xylazine 2% (Bayer, Mechelen, Belgium) and maintained after intubation by 1%-1.5% Isoflurane (Zoetis, Louvain-La-Neuve, Belgium), air and oxygen. Atracurium (GlaxoSmithkline, Waver, Belgium) was administered intravenously as a bolus at 2 mg/kg at the start of the surgical procedure and repeated on indication at 1 mg/kg. Cefazolin (Biopharma, Rome, Italy) 2 g intravenous was given immediately after induction. Sodium chloride 0.9% (Baxter, Lessines, Belgium) at 40 mL/h was administered intravenously during both procedures.

2.4 | Surgical technique

2.4.1 | Left donor nephrectomy

The surgical procedure started with the positioning of a single-lumen vascular access catheter (ref ES-04730; Arrow International, Reading, PA) in the right internal jugular vein and tunneled behind the right ear. A blood transfusion bag containing CPDA (Fresenius Kali, Bad Homburg, Germany) was attached to the central jugular central, and approximately 250 mL of blood was collected and stored at 4°C. Colloid solution (20 mL/kg Geloplasma 3%, Fresenius Kabi, Schelle, Belgium) was used to replace the volume withdrawn in case of hypotension, followed by Hartmann solution 40 mL/kg.

The abdomen was opened through a midline incision. Thirty minutes of warm ischemia was induced by vascular clamping of the renal artery and vein before left kidney removal. An ex vivo blood flush of this kidney was obtained by 200 mL of kidney perfusion solution (KPS-1, Organ Recovery Systems) with 100 cm H_2O. The kidney was stored for a period of approximately 22 hours by 1 of the 6 studied preservation strategies: (a) 22-hour SCS (n = 6), (b) 22-hour HMP (n = 6), (c) 22-hour active oxygenated HMP (HMPO2) (n = 7), (d) 20-hour HMP plus 2-hour NP (n = 6), (e) 20-hour SCS plus 2-hour oxygenated HMP (n = 7), and (f) 20-hour SCS plus 2-hour NP (n = 6) (Figure 1). Abdominal closure was performed.

2.4.2 | Right nephrectomy and autotransplant of the left kidney

The midline wound was reopened, and a right nephrectomy was performed. The left kidney was then autotransplanted into the right renal bed by using the native right renal vein and artery. Furosemide 40 mg (Lasix, Sanofi, Diegem, Belgium) and mannitol 15% (132 mL) (Baxter, Lessines, Belgium) were administered at the start of the anastomosis. An end-to-end ureteroureteroneostomy was performed over a double-J stent. The abdomen was then reclosed.

2.5 | Machine perfusion

The LifePort Kidney Transporter (LKT) (Organ Recovery Systems) was used for all machine perfusion groups. An external oxygenator (Dideco Perfusion Tubing Systems, Sorin Group, Mirandola, Italy) and a heat exchanger were added to the circuit of the LKT (Figure 2). The LKT pumps the perfusate through the kidney vasculature in a pulsatile and pressure-controlled way. The pressure can vary from 0 to 100 mm Hg. The LKT continuously registers renal resistance and flow measures.

2.5.1 | Hypothermic machine perfusion

The KPS-1 solution was pumped through the kidney at a pressure of 30 mm Hg. Oxygen could be added to the perfusate as a function of the study groups (95% O_2/5% CO_2, FIO_2 at 30%) providing a perfusate partial pressure of oxygen (PO_2) of 220-240 mm Hg. Perfusate and kidney were cooled to 3°C.

2.5.2 | Normothermic machine perfusion

Prior to the start NP, the circuit was primed with 250 mL of Ringer solution; 250 mL of autologous whole blood, which was depleted of leukocytes by using a white cell filter (LeukoGuard RS, Haemonetics, Braintree, MA), and heparin 5000 IU. The kidney was connected to the LKT, and the NP solution was pumped through the kidney while adding oxygen to the perfusate (95% O_2/5% CO_2, FIO_2 at 30%) at a

pressure of 30 mm Hg at the start and heat exchanger at 37°C. As soon as the infusion temperature reached 28°C, the pressure was increased to 50 mm Hg and from 32°C to 37°C, the pressure was further increased to 70 mm Hg. The temperature was maintained at 37°C.

Several substances were added to the perfusate as previously described in detail by Nicholson and Hosgood.[19]

2.6 | Postoperative care

Buprenorphine hydrochloride 0.01 mg/kg intravenous was administered after left nephrectomy and up to 48 hours after transplant for postoperative analgesia, Hartmann solution (40 mL/kg/24 h) during the first 2 days after transplant and long-acting oxytetracycline 20 mg/kg intramuscular 2 days after the transplant. Animals were allowed free access to water immediately, and food was introduced on the first day after transplant.

The surviving animals were killed on the 13th day after transplant by a lethal intravenous injection of T61 (Intervet International, MDS Animal Health, Boxmeer, Netherlands) under general anesthesia.

2.7 | Outcome measures

The primary endpoint was early graft function (measured as daily serum creatinine). The secondary endpoints were renal flow and resistance during machine perfusion and perfusate and serum biomarkers.

2.8 | Statistical methods

The trial followed a parallel design with 6 active groups. In total, 46 animals were included in this analysis. No explicit imputation was foreseen, so all analyses were conducted on the observed cases only.

The primary endpoint of early graft function was assessed through analysis of serum creatinine over time by using GLM techniques. Serum creatinine values were normalized to 40 kg animal body weight before use in the analysis to account for differences due to animal/organ size. Serum creatinine was analyzed by using a longitudinal mixed model for repeated measurements (MMRM) with main factors of treatment, time-point, and interaction term to test for overall difference in graft function over time between treatment groups. Individual comparison of groups were conducted using contrast analyses within the main MMRM model testing the null hypothesis that the difference in least-squares means between 2 groups under investigation was 0 (no mean difference between groups). For the contrast analysis, *P* values (based on *t* test of least squares means) were reported along with a graphic mean (SE) plot where deemed appropriate. All secondary endpoint variables were similarly modeled to the primary endpoint. In case of variables without longitudinal structure (eg, area under the curve [AUC]), the MMRM model was reduced to a basis ANOVA model with factor treatment. As this was a preclinical exploratory trial, no formal sample size calculations were performed and no correction for multiplicity was foreseen. All analyses were performed by using SAS 9.2 or SAS 9.4 (SAS Institute, Cary, NC).

3 | RESULTS

3.1 | Operative data, major complications, and animal survival

Operative data are shown in Table 1. Forty-six autotransplants were performed. Eight were excluded from the analysis for death in relation to complications (sudden death [n = 3], bleeding from central line damage [n = 1], necrotizing anal prolapse [n = 1], arterial thrombosis [n = 1], intestinal volvulus [n = 1], and massive bleeding after reperfusion [n = 1]).

3.2 | Graft function

3.2.1 | Impact of timing to start machine perfusion

An area under the curve (AUC) analysis of the serum creatinine from day 1 until day 13 after transplant is shown in Figure 3A.

TABLE 1 Operative data for 38 pigs in an ischemia–reperfusion autotransplant model according to the study group

Variable	22 h SCS (n = 6)	22 h HMP (n = 6)	22 h HMPO2 (n = 7)	20 h HMP +2 h NP (n = 6)	20 h SCS +2 h HMPO2 (n = 7)	20 h SCS +2 h NP (n = 6)
Weight, kg	40.40 ± 5.57	39.23 ± 2.00	43.26 ± 3.14	41.96 ± 6.91	39.96 ± 5.94	41.30 ± 3.43
Left nephrectomy procedure time, h:min	3:41 ± 0:46	3:16 ± 0:31	3:27 ± 0:44	3:16 ± 0:43	3:37 ± 0:23	3:49 ± 0:13
Right nephrectomy and autotransplant procedure time, h:min	3:04 ± 0:38	3:52 ± 0:54	3:36 ± 0:58	3:07 ± 0:42	3:38 ± 0:45	3:22 ± 0:34
Cold ischemic time, h:min	21:56 ± 0:37	22:40 ± 0:36	22:23 ± 0:24	20:51 ± 0:57	22:14 ± 0:24	20:50 ± 0:52
Anastomotic time, min	30 ± 5	32 ± 4	30 ± 6	26 ± 7	28 ± 4	30 ± 7
Total ischemic time, h:min	22:56 ± 0:35	23:42 ± 0:35	23:23 ± 0:26	21:47 ± 0:51	23:21 ± 0:29	21:48 ± 0:50

HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; NP, normothermic perfusion; SCS, static cold storage. Data are given as the mean ± SD.

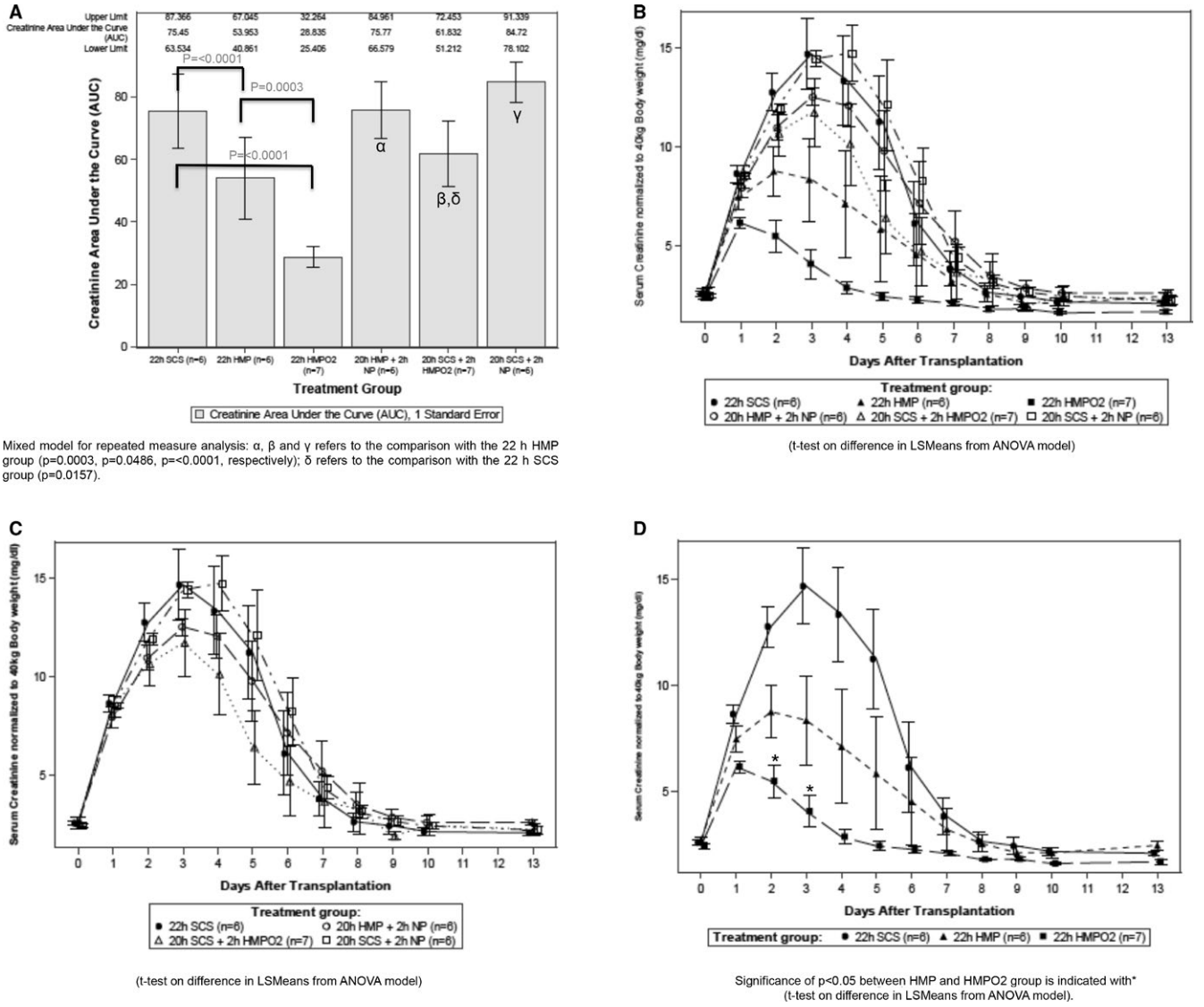

FIGURE 3 Area under the curve analysis of serum creatinine from day 1 to day 13 after transplant according to the study group demonstrating that continuous HMP with or without O_2 significantly improved early graft function compared with SCS alone and all end-ischemic machine perfusion strategies (A). Evolution of daily postoperative serum creatinine in all study groups (B), in end-ischemic machine perfusion groups (C), and continuous HMP groups with or without oxygen compared with the SCS group (D). HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; NP, normothermic perfusion; SCS, static cold storage

Comparison of the study groups demonstrated a significant difference in favor of both 22-hour continuous HMP groups, independent of the supplementation of oxygen, compared with SCS alone ($P < .0001$) and compared with all end-ischemic machine perfusion groups.

The daily evolution of serum creatinine after transplant is shown in Figure 3B. Peak serum creatinine levels in end-ischemic strategies (including NP and hypothermic oxygenated perfusion [HOPE]) did not significantly differ from SCS alone. Continuous HMP with or without active oxygen supply demonstrated a more effective strategy to decrease serum creatinine during the first 7 days after transplant compared with SCS alone and compared with all end-ischemic strategies.

3.2.2 | Impact of oxygen and temperature on end-ischemic machine perfusion strategies

Impact of temperature: normothermic perfusion after SCS or HMP: Both study groups using end-ischemic NP did not demonstrate a significant difference in serum creatinine AUC (Figure 3A) or in daily serum creatinine (Figure 3C) compared with the SCS-alone group.

Impact of oxygen: use of HOPE end- ischemic: Serum creatinine AUC using HMPO2 after SCS was significant lower compared with the SCS alone group ($P = .0157$) (Figure 3A). No significant

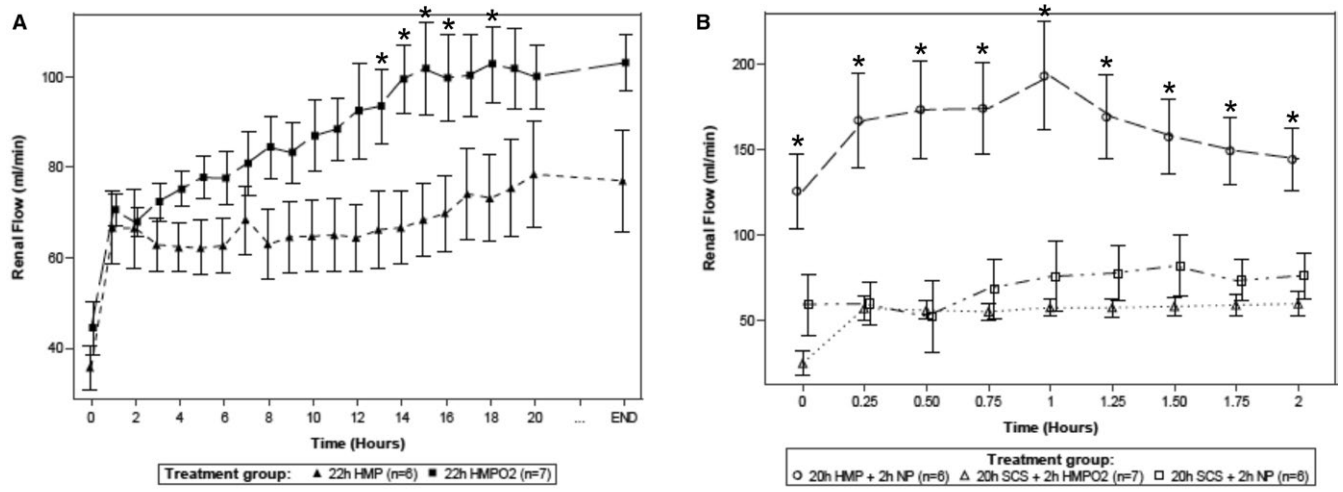

FIGURE 4 Influence of oxygen supply on ex vivo renal flow during continuous hypothermic machine perfusion with or without active oxygenation (A) and during end-ischemic machine perfusion groups (B). HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; NP, normothermic perfusion; SCS, static cold storage

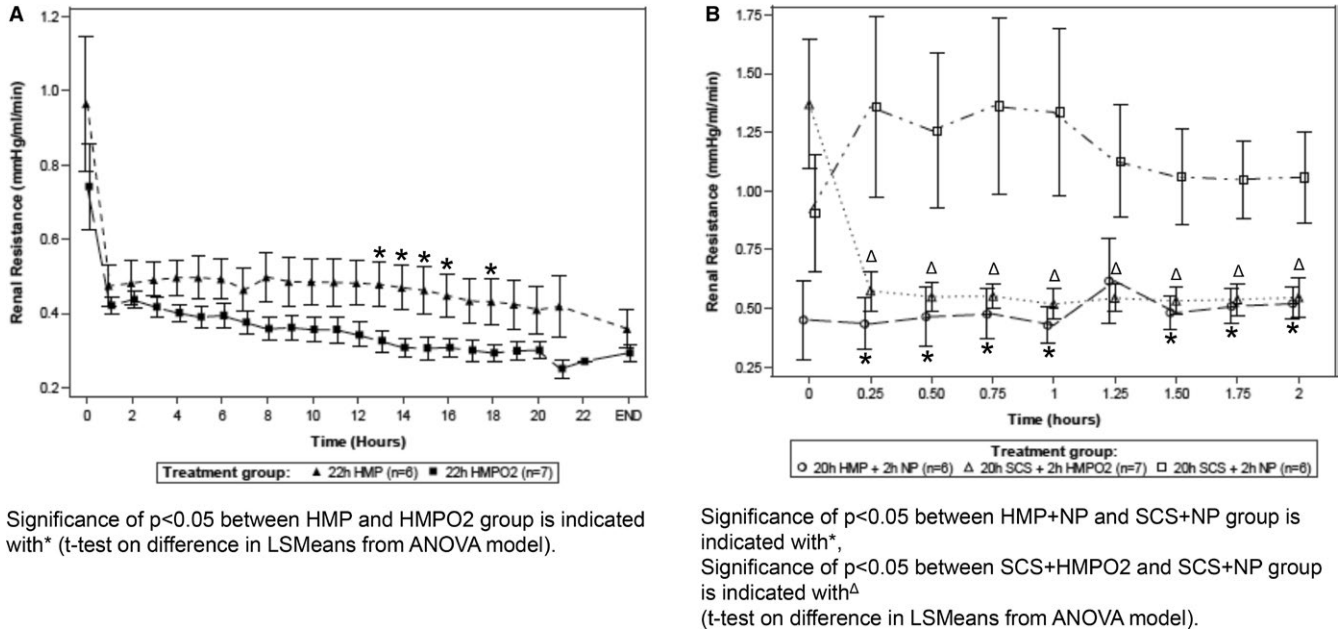

FIGURE 5 Evolution of renal resistance in the continuous hypothermic machine perfusion groups with or without oxygenation (A) and in the 3 end-ischemic machine perfusion groups (B). HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; NP, normothermic perfusion; SCS, static cold storage

difference in daily serum creatinine was observed at days 1, 2, 3, 7, and 13 after transplant between both study groups (Figure 3C).

3.2.3 | Impact of oxygen during continuous HMP

Comparison of the study groups demonstrated a significant difference of serum creatinine AUC in favor of the continuous HMPO2 group compared with both SCS alone ($P < .0001$) and nonactive oxygenated HMP groups ($P = .0003$) overall (Figure 3A).

The daily evolution of serum creatinine after transplant is shown in Figure 3D for the continuous dynamic preservation strategies compared with SCS. The serum creatinine was significantly lower at day 1 after transplant using continuous HMPO2 compared with SCS alone ($P = .0005$). No difference was observed at day 1 between the nonoxygenated HMP group and the SCS alone group ($P = .0903$), nor between the nonactive oxygenated and the HMPO2 group ($P = .0507$). However, at days 2 and 3 after transplant, the serum creatinine was significantly lower in both the 22-hour oxygenated group (day 2:

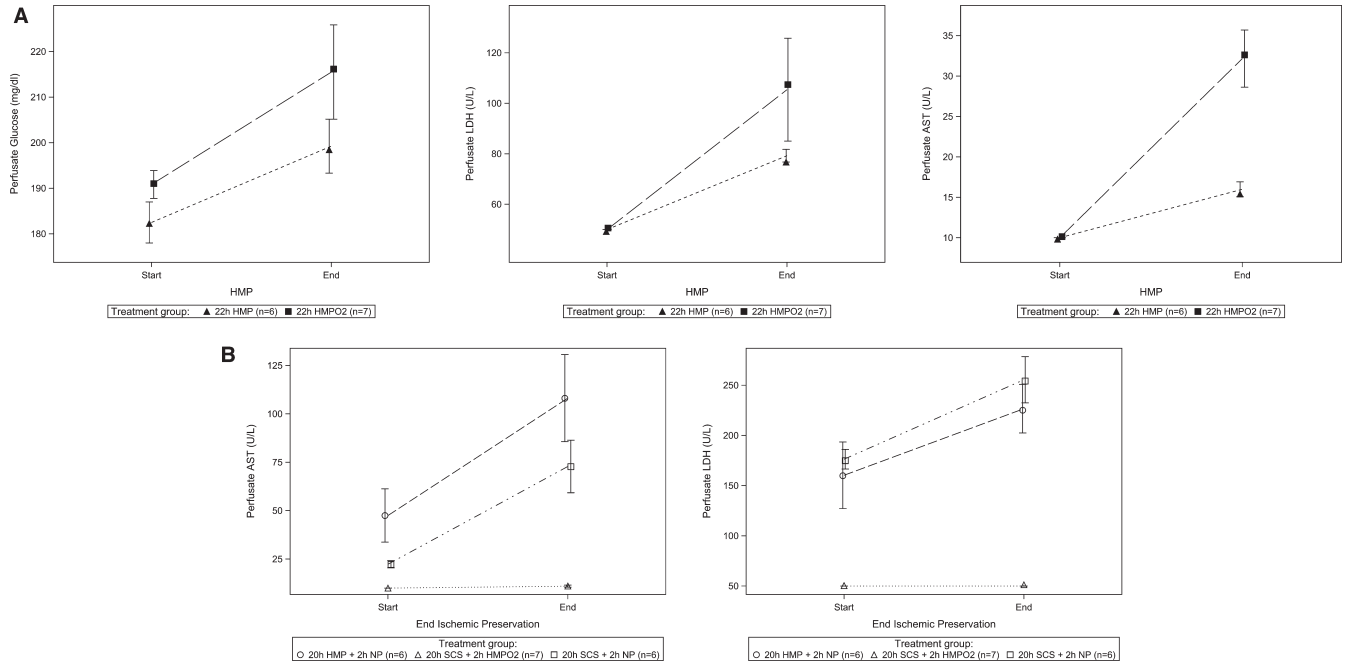

FIGURE 6 Evolution of perfusate glucose, LDH and AST during continuous hypothermic machine perfusion with and without active oxygenation (A) and evolution of perfusate LDH and AST in the 3 end-ischemic machine perfusion groups (B). AST, aspartate aminotransaminase; HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; LDH, lactate dehydrogenase; NP, normothermic perfusion; SCS, static cold storage

$P < .0001$; day 3: $P < .0001$) and the nonoxygenated HMP group (day2: $P = .0045$; day 3: $P = .0033$) group compared with SCS alone.

At days 2 and 3, a significant difference was observed in serum creatinine in favor of the HMPO2 group compared with the nonoxygenated HMP group ($P = .0138$ and $P = .0334$ at days 2 and 3, respectively). From day 7, no difference was observed between the study groups.

3.3 | Impact of oxygen on renal flow and resistance during machine perfusion

3.3.1 | Impact of oxygen during continuous HMP

Active oxygenation during continuous HMPO2 resulted in faster increase in renal flow compared with nonactive oxygenated continuous HMP (Figure 4A). The difference in renal flow was significantly higher in favor of the oxygenated HMP group starting at 13 hours of HMP ($P = .0469$) until 18 hours of machine perfusion ($P = .0433$). Thereafter, no significant differences in renal flow were observed between both groups ($P = .0600$) at the end of the machine perfusion. The same observation was noted for renal resistance in favor of the HMP02 group (Figure 5A).

3.3.2 | Impact of oxygen during end-ischemic strategies

End-ischemic NP after previous HMP resulted in a significantly higher renal flow from the start until the end of the machine perfusion compared with NP after SCS ($P \leq .0001$) (Figure 4B). No difference was observed in renal flow between end-ischemic NP and HMPO2 after 20 hours of SCS ($P = .3024$). The same findings were also observed for renal resistance in favor of the end-ischemic NP after previous HMP (Figure 5B).

3.4 | Perfusate parameters

No difference was found in perfusate glucose, lactate dehydrogenase (LDH), and aspartate aminotransaminase (AST) at start and at the end between the oxygenated and nonoxygenated continuous HMP group (Figure 6A).

End-ischemic HMPO2 after a period of SCS did not influence perfusate AST and LDH (Figure 6B). Perfusate AST at start of NP was more pronounced after preceding HMP compared with preceding SCS ($P = .0134$) but did not reach statistical significance at the end of NP ($P = .0500$). In contrast, perfusate LDH was higher at the start and end of NP after preceding SCS compared with preceding HMP but did not reach statistical significance.

3.5 | Evolution of postoperative serum LDH and AST

A postoperative LDH/AST peak was observed at day 1 but dropped significantly after day 3. No significant difference was observed in the postoperative LDH/AST profile from day 1 to 13 between all groups (Figure 7A-D).

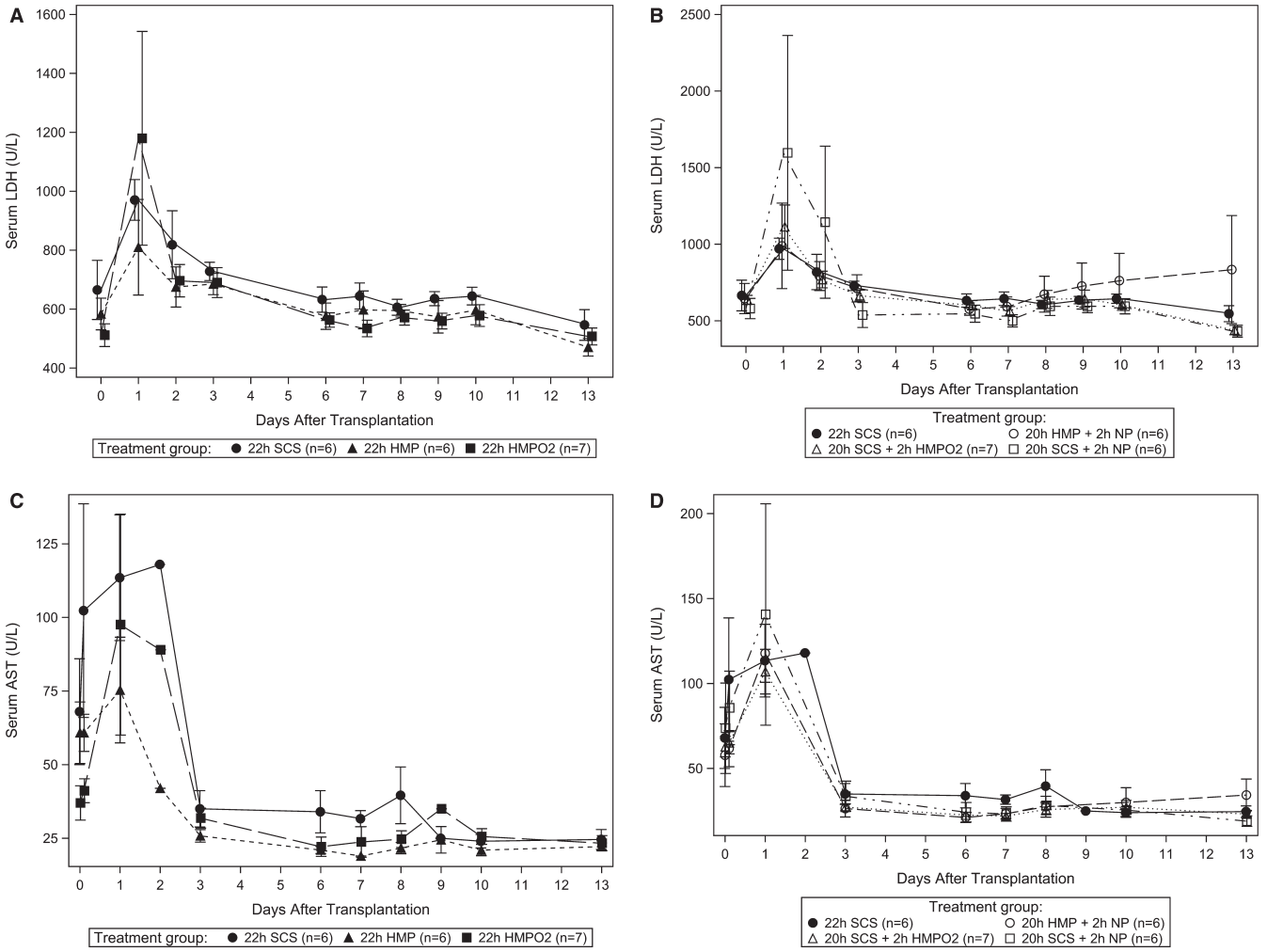

FIGURE 7 Evolution of posttransplant serum LDH and AST in the continuous HMP groups with or without oxygen and the SCS group (A and C, respectively) and in the 3 end-ischemic machine perfusion and SCS groups ([B] and [C], respectively). AST, aspartate aminotransaminase; HMP, hypothermic machine perfusion; HMPO2, oxygenated hypothermic machine perfusion; LDH, lactate dehydrogenase; NP, normothermic perfusion; SCS, static cold storage

4 | DISCUSSION

This ischemia–reperfusion porcine kidney autotransplant model is the first animal study on early renal function that compares different continuous, end-ischemic, and SCS preservation strategies with the aim to determine the machine perfusion strategy that offers the best graft outcome. Hypothermic perfusion strategies applied from time of kidney procurement until transplant and regardless of supplemental oxygenation led to a significantly positive effect on early graft function compared with SCS alone and compared with different end-ischemic strategies (including HOPE and NP). The overall treatment effect on posttransplant serum creatinine of each preservation strategy demonstrated a significant difference in favor of the continuously oxygenated HMP group compared with the nonactive oxygenated HMP group.

The active oxygenated 22-hour HMP group demonstrated a significantly faster recovery from early graft function compared with the 22-hour nonactive oxygenated HMP group. Continuous active oxygenated HMP is probably the most convenient to apply in clinical setting for organ sharing. Active oxygenation seems an important modulator for a faster increase in renal flow during HMP preservation. This effect on early graft function was obtained during continuous HMP but not when applied as an end-ischemic strategy. This result is in contrast to a recent study in a rat model demonstrating a benefit of end-ischemic oxygenated HMP after SCS.[23] However, our results are in line with a preclinical study that demonstrated the benefit of active oxygen supplementation during HMP in a porcine model with a partial oxygen pressure of approximately 760-800 mm Hg.[21]

End-ischemic NP demonstrated no positive effect on early graft function in this study. In 2013, the first clinical study, applying end-ischemic NP after SCS (using kidney grafts originated from ECD) demonstrated a reduction of the DGF rate in favor of the NP group (5.6% vs 36.2%, $P = .014$).[19] Despite using the same perfusate composition, we observed a higher serum creatinine at day 3 after transplant compared with SCS alone, illustrating that NP after SCS could lead to an even more substantial ischemia–reperfusion injury.

It could be speculated that the kidneys used in the clinical trial were more damaged organs and therefore may benefit more from the normothermic reconditioning than the kidneys used in our model, which originated from healthy pigs with 30 minutes of warm ischemia by vascular clamping. In line with previous published work by Hosgood, our study demonstrates that the use of NP after a period of HMP did not improve early graft recovery.[26] We agree that NP remains an interesting tool to evaluate transplantability of discarded kidneys[27,28] and more insights toward the precise role of NP will be obtained by an ongoing multicenter randomized clinical trial.[29]

Kaths et al demonstrates that intermediate (8 hours) or prolonged (16 hours) NP following SCS is superior to brief preimplantation NP in a pig kidney transplant model.[25,30] They suggest that 1 hour of NP is insufficient time for effective repair mechanisms and results in an upregulation of proinflammatory signaling by rapid warming. These results are in line with our results but in contrast with the results from Hosgood and colleagues.[19,31] Underlying mechanisms of NP are not fully understood. Different perfusion systems in combination with differences in perfusate composition, use of vasodilator, pressure settings, flow rates and the duration of the machine perfusion are probably an explanation for these contrasting results. We are aware that our study lacks a continuous NP group; however, because of its complexity, and the fact that the NP protocol used in our study was not designed for long periods, we did not include this in our study design.

Our study demonstrated a significantly faster recovery from early graft function using end-ischemic HMPO2 after SCS compared with SCS alone. This strategy could be an interesting tool to decrease the DGF rate, especially in transplant programs dealing with a high incidence of DGF. Consistently, the strategy without active oxygenation has been recently confirmed in clinics by the Essen Group demonstrating a faster recovery from early graft function using end-ischemic nonactive oxygenated HMP for at least 2 hours after SCS compared with SCS alone.[18] In a rodent model reported by Kron et al, the end-ischemic HMPO2 compared with other clinically (SCS alone and end-ischemic normothermic perfusion) used preservation approaches was demonstrated to be a superior technique.[23] However, it should be stressed that in the latter study, the perfusate oxygen concentration was higher than in our protocol (450-600 vs 220-240 mm Hg).

Historically, mitochondrial reactive oxygen species (ROS) production during ischemia–reperfusion injury has been considered to be a nonspecific response to reperfusion. However, a recent metabolomic analysis by Chouchani suggests that selective accumulation of the citric acid cycle intermediate succinate is a universal metabolic signature of ischemia in a range of tissues and is responsible for mitochondrial ROS production during reperfusion.[32,33] On reperfusion, the accumulated succinate is rapidly re-oxidized by succinate dehydrogenase, driving extensive ROS generation by reverse electron transport at mitochondrial complex I. Superoxide generation by reverse electron transport upon reperfusion is the major source of ischemia–reperfusion injury. Kron et al demonstrated in a rodent kidney transplant model that end-ischemic hypothermic oxygenated perfusion did not lead to a relevant ROS release, in contrast to end-ischemic NP.[23] The proposed working mechanism of oxygenated HMP is situated on 3 levels. First, active oxygenation during cold perfusion influences the metabolic status. It improves the function of the mitochondria enzyme complexes probably leading to forward instead of reverse electron flow therefore uploading adenine nucleotides (ATP/ADP) during cold oxygenation. Cellular redox state changes from reduced to oxidized and also succinate is also removed. Second, it affects the endothelium by cleaning the glycocalix and upregulation of shear stress–sensitive genes. Third, it will lead to activation of protective pathways (eg, hypoxia inducible factors, heat shock protein 70, etc.). These changes during HMPO2 will lead to less initial ROS, and the amount of released danger-associated molecular patterns will be subsequently decreased, resulting in less inflammation and a decreased ischemic injury after reperfusion.

Currently, perfusion characteristics during HMP, such as renal resistance, have a limited value for prediction of delayed graft function.[17,34] Few data are available regarding the impact of oxygen on perfusion characteristics during HMP. Our data demonstrate a faster increase in renal perfusion flow and a lower renal resistance when adding active oxygen to the perfusate during long periods of HMP compared with nonactive oxygenated HMP. A possible explanation might be the crucial role of nitric oxide as a potent vasodilator during perfusion. The synthesis of nitric oxide is the result from an oxidation process and this complex reaction requires the presence of co-substrate oxygen.[35] Because the variance is much lower for renal resistance compared with renal flow during machine perfusion we believe that renal resistance is a more reliable parameter than renal flow. These results are in line with those of Hoyer et al, which also demonstrate a faster increase in perfusion flow comparing 21-hour HMP with air and with 100% oxygen.[21] Most studies, however, compare only the perfusion characteristics at the start and at the end but not the trend during machine perfusion.[36] Further research is needed to examine their value in graft quality assessment and the determination of optimal perfusion time.

Based on our preclinical results, kidney preservation and reconditioning should be started immediately after ex vivo blood flush and maintained on HMP conditions during the whole period of organ preservation. Active oxygenation during HMP seems to be an extremely important factor for a faster increase in ex vivo renal flow during machine perfusion and to have a positive influence on early graft function. The influence of different partial oxygen pressures and optimal timing to start active perfusate oxygenation during HMP (initial at donor site, continuous, or end-ischemic at receptor site) should be confirmed by clinical studies in the near future.

Translation of our results into the clinical setting must be done with caution. We chose 30 minutes of preceding warm ischemia before procurement in this autotransplant model. First, 30 minutes of warm ischemia time is similar to the mean first warm ischemia time (time from switch of ventilation to start cold perfusion) in most clinical controlled DCD programs worldwide. Second, 30 minutes is frequently used in preclinical models.[21,25,26] Third, longer warm ischemia time results in a higher degree of renal insufficiency after

autotransplant and was badly tolerated by the pigs and therefore declined because of ethical reasons. Because most extended deceased donor kidneys have an inferior quality compared with our studied pig kidneys, there might be even more benefit from the reconditioning of continuous active oxygenated HMP. In contrast to the cardiopulmonary bypass (flow-driven) technology used by Hosgood,[19] the LKT has a roller pump (pressure-driven), originally designed to perfuse kidneys in hypothermic conditions. To reduce hemolysis of the diluted blood, NP in our model was limited to 2 hours. Therefore, we consider it unlikely that this could have influenced our primary endpoint, but comparison with other NP types must be done with caution.

In conclusion, continuous oxygenated HMP applied from the time of kidney procurement until transplant might be the best preservation strategy to improve early graft function compared with SCS alone or continuous nonactive oxygenated HMP and compared with different end-ischemic preservation strategies.

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DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. The LifePort Kidney Transporter, the Perfusion Circuits and the oxygenators were supplied by Organ Recovery Systems (Zaventem, Belgium).

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