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Acellular Perfusate is an Adequate Alternative to Packed Red Blood Cells During Normothermic Human Kidney Perfusion

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Background. Brief normothermic machine perfusion is increasingly used to assess and recondition grafts before transplant. During normothermic machine perfusion, metabolic activity is typically maintained using red blood cell (RBC)–based solutions. However, the utilization of RBCs creates important logistical constraints. This study explored the feasibility of human kidney normothermic perfusion using William's E–based perfusate with no additional oxygen carrier. **Methods.** Sixteen human kidneys declined for transplant were perfused with a perfusion solution containing packed RBCs or William's E medium only for 6 h using a pressure-controlled system. The temperature was set at 37 °C. Renal artery resistance, oxygen extraction, metabolic activity, energy metabolism, and histological features were evaluated. **Results.** Baseline donor demographics were similar in both groups. Throughout perfusion, kidneys perfused with William's E exhibited improved renal flow ($P = 0.041$) but similar arterial resistance. Lactic acid levels remained higher in kidneys perfused with RBCs during the first 3 h of perfusion but were similar thereafter ($P = 0.95$ at 6 h). Throughout perfusion, kidneys from both groups exhibited comparable behavior regarding oxygen consumption ($P = 0.41$) and reconstitution of ATP tissue concentration ($P = 0.55$). Similarly, nicotinamide adenine dinucleotide levels were preserved during perfusion. There was no evidence of histological damage caused by either perfusate. **Conclusions.** In human kidneys, William's E medium provides a logistically convenient, off-the-shelf alternative to packed RBCs for up to 6 h of normothermic machine perfusion.

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Transplantation is the preferred treatment for end-stage kidney disease but faces severe shortage of available organs. Machine perfusion has emerged as an important alternative to static cold storage (SCS) to help expand the pool of usable organs.[1,2] Compared with SCS, hypothermic machine perfusion (HMP) without oxygen was shown to reduce delayed graft function (DGF) and improve 1-y and 3-y graft survival.[1] However, both static storage and oxygen-free perfusion at

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hypothermic storage diminish but do not entirely suspend cellular metabolism, resulting in a slow but inexorable consumption of cellular energy stores.[3] Although the addition of oxygen during hypothermic perfusion promotes mitochondrial ATP production,[3] the benefits in human remain uncertain.[4,5]

To reduce cold ischemia, normothermic (37 °C) machine perfusion (NMP), with "blood-mimicking perfusate," containing packed red blood cells (RBCs) with or without fresh frozen plasma, has shown great promise.[2,6] Although cold anoxic storage aims to arrest cell metabolism, NMP provides a continuous flow of warmed, oxygenated perfusate containing nutritional substrates, thereby maintaining the metabolic activity of the kidney.[7] In addition, NMP allows graft assessment, reconditioning, and repair.[8,9] In porcine preclinical models, NMP reduced graft injury and improved kidney function after kidney transplantation.[2,6] Consistently, in donated after circulatory death (DCD) pig kidneys, NMP improved early creatinine clearance and reduced apoptosis compared with SCS.[9] In a recent clinical study, short-term NMP after HMP appeared to reduce DGF rate, although no difference was observed at the 1-y follow-up.[10] Similarly, using a RBC-based, plasma-free solution, perfusion of marginal kidneys at 37 °C reduced the requirement for dialysis within the first 7 d (DGF) compared with SCS.[11]

Common clinical protocols use nutrient-enriched, RBC-based perfusate to deliver nutrients and oxygen during 1 h of perfusion.[12,13] However, perfusion of organs at 37 °C is limited by the availability and cost of a blood perfusion system, complex heating system, tight pH and glucose control, RBC hemolysis, and risk of infection and immunization.[14,15] In addition, failure of the perfusion machine would rapidly lead to irreversible intragraft thrombosis and, most importantly, add another ischemic insult. However, many of the above-mentioned concerns could be avoided using RBC-free perfusate. In that regard, we recently demonstrated that a 2:1 William's E media (WE):Hemopure (purified bovine hemoglobin) perfusate was a logistically more convenient, non-inferior, off-the-shelf alternative to RBCs during a 6-h normothermic human kidney perfusion.[16] Although effective, purified bovine hemoglobin also comes with several logistic impediments, including scarce availability and high utilization cost, and interferes with several assays used to determine graft viability. In addition, we often observed venous O_2 >400 mm Hg, suggesting that the addition of oxygen carrier might not be required to sustain kidney aerobic metabolism.

Altogether, further research is required to define the optimal perfusate at normothermia. Thus, in this study, we aimed to explore the viability and efficacy of an acellular WE perfusate during kidney NMP.

MATERIALS AND METHODS

Study Population

This study was approved and declared exempt by the Massachusetts General Hospital Institutional Review Board. Sixteen human kidneys were obtained from New England Donor Services after being declined for transplantation by all centers. No age or preservation time limits were applied. Kidneys DCD as well as kidneys donated after brain death (DBD) were accepted. Demographics of the kidney donors are shown in Table 1. Recorded information included donor age, gender, donor type (DBD/DCD), duration of cold ischemia time, duration of warm ischemic time if applicable, reason for discard, Kidney Donor Profile Index, and terminal creatinine. When applicable, DCD kidneys were preserved in the Belzer MPS UW solution on HMP pumps (Life Port Kidney Transporter from Organ Recovery Systems, Itasca, IL). Otherwise, organs were transported from their respective donor hospitals in SCS (Table 1).

Graft Preparation

After arrival at our laboratory, extrarenal fat was cleaned off. Next, both the renal artery and ureter were dissected and cannulated using 10- or 12-French cannula as appropriate. Before perfusion, the kidney was flushed with 500 mL of 4 °C Lactated Ringer's solution as published.[16]

Ex Vivo NMP

NMP was performed for 6 h using a modified pressure-controlled commercial perfusion system (Organ Assist, Groningen, The Netherlands) as previously published.[16] Pressure was set at 70 mm Hg, and flow was adapted accordingly.

TABLE 1.

Donor demographics

	Blood (N = 5)	WE (N = 11)	*P*
Age, y, median (IQR)	59.0 (58.0–66.0)	58.0 (49.0–63.5)	0.408
Gender, n (%)			
Female	2 (40.0)	5 (45.5)	1
Male	3 (60.0)	6 (54.5)	
Donor type, n (%)			
DBD	3 (60.0)	7 (63.6)	1
DCD	2 (40.0)	4 (36.4)	
Cold ischemia time, h, median (IQR)	19.1 (16.8–19.5)	19.1 (15.9–24.6)	0.849
Warm ischemia time, min, median (IQR)	61.5 (56.3–66.8)	50.0 (25.3–73.5)	0.532
KDPI %, median (IQR)	94.0 (85.0–99.0)	90.0 (80.5–97.0)	0.570
Storage, n (%)			
SCS	2 (40.0)	8 (72.7)	0.299
HMP	3 (60.0)	3 (27.3)	
Terminal creatinine, median (IQR)	1.36 (0.930–2.43)	2.14 (1.47–3.24)	0.534

DBD, donated after brain death; DCD, donated after circulatory death; HMP, hypothermic machine perfusion; IQR, interquartile range; KDPI, Kidney Donor Profile Index; SCS, static cold storage; WE, William's E medium.

The temperature was set at 37 °C. pH and electrolyte were kept within the physiological range (see below).[16] In both groups, the total volume of the perfusate was 2000 mL. The RBC perfusate consisted of 2 units (500 mL) of packed RBCs (type-specific or O Rh-negative blood, irradiated and leukocyte-depleted) in 1500 mL of WE with glucose (2 g/L) and glutamine (50 mg/L). The WE group consisted of 2000 mL of WE with glucose (2 g/L) and glutamine (50 mg/L). Rationale for using WE is that it provides sufficient amino acids and is buffered using HCO_3.[17] In addition, both groups were supplemented with dexamethasone (16 mg), insulin (20 UI), heparin (10 000 UI), furosemide (100 mg), creatinine (200 mg), nitroprusside (25 mg/h) as well as sodium bicarbonate 8.4% (titrated as needed to reach pH 7.35–7.45) and calcium chloride (titrated as needed to ionize calcium of 1.1–1.4 mmol/L). The perfusate was oxygenated with a carbogen mixture of 95% O_2/5% CO_2, achieving maximum partial oxygen pressure of >500 mm Hg and undepleted oxygen outflow (>200 mm Hg). Glucose levels were maintained at 100 to 200 mg/dL. The urine was not recirculated. Fresh media was used to replace urine (1:1).

Perfusion Assessment

One milliliter samples of perfusion media were collected at baseline, at 5 and 30 min every hour from the arterial inflow cannula and venous outflow (taken directly from the vein(s)). Samples were analyzed for pH, partial pressure of oxygen, partial pressure of carbon dioxide, oxygen saturation, electrolytes, glucose and creatinine levels, and analyzed as published.[16] Briefly, oxygen consumption (mL O_2/min per g) was calculated as follows: (oxygen solubility coefficient [mL O_2/mm HgO_2 per mL] × arterial partial oxygen pressure [mm Hg] × renal artery flow [mL/min] – venous partial oxygen pressure [mm Hg] × renal artery flow [mL/min] + hemoglobin concentration [g/dL]/100 × hemoglobin oxygen-binding capacity [mL O_2/g] × arterial oxygen saturation [%]/100 × renal artery flow [mL/min] – venous oxygen saturation [%]/100 × renal artery flow [mL/min])/kidney weight. Oxygen extraction was obtained by dividing oxygen consumption by oxygen delivery. Oxygen delivery was calculated as follow: hemoglobin concentration [g/dL] × hemoglobin oxygen-binding capacity [mL O_2/g] × arterial oxygen saturation [%]/100 + oxygen solubility coefficient [mL O_2/mm HgO_2 per mL] × arterial partial oxygen pressure [mm Hg] × renal artery flow [mL/min]/ kidney weight. The calculations took into consideration the differences in hemoglobin-based oxygen carriers (HBOCs) and RBCs, hemoglobin content, and O_2 transport properties.

Sample analysis was performed using an i-STAT Blood Analyzer (Abbott Point of Care, Princeton, NJ). Oxygen consumption (mL O_2/min/g), extraction (%), and arterial resistance were calculated as described.[16] The calculations considered the differences in RBCs, hemoglobin content, and O_2 transport properties. Kidney weight was recorded before and after perfusion. The ureteral outflow was drained in a collection tube, and urine production was assessed every hour.

Histological Evaluation

Time zero wedge biopsies were collected on arrival to the perfusion laboratory (before ex vivo perfusion) and after 3 and 6 h of NMP. Biopsies were fixed in 10% formalin, stained with hematoxylin and eosin to characterize changes associated with NMP in both groups as described previously,[16] and were evaluated by 2 blinded investigators.

Energy and Redox Measurements

Polar metabolite profiling was performed as described previously.[16] Briefly, human kidney wedge biopsies were homogenized on dry ice in 80% methanol and kept at –80 °C overnight. Debris were pelleted, and the methanol suspension was dried under nitrogen. The resulting pellet was resuspended in water, and metabolites were measured using targeted tandem mass spectrometry with polarity switching and selected reaction monitoring with a Sciex TripleTOF 6600 Quadrupole Time-Of-Flight (AB Sciex, Foster City, CA) as described.[16] Energy charge (EC) was calculated using the following formula: EC = [2ATP + ADP]/ATP + ADP + AMP and normalized to tissue volume.

Statistical Analysis

Data are presented as median ± IQR or mean ± SEM when appropriate. Differences are considered significant when a P value of <0.05. Statistical significance was assessed in GraphPad Prism version 9.1.1 (GraphPad Software, San Diego, CA) using the Student t test, 1-way ANOVA, or 2-way ANOVA unless otherwise specified.

RESULTS

Donor Characteristics

Donor demographics are presented in Table 1. Briefly, 16 discarded human kidneys were included in this study from 12 different donors. Seven DBD (63.6%) and 4 DCD (36.4%) were perfused with WE solution without packed RBCs (WE). In addition, 3 DBD (60.0%), and 2 DCD (40.0%) were perfused with packed RBCs/WE perfusate (RBC group). Median donor age was 58 (49–64) and 59 (58–66) y in the WE and RBC groups, respectively (P = 0.408). In the WE group, there were 54.5% men (n = 6), and in the RBC group, there were 60% men (n = 3) (P = 1.0). The median cold ischemia time and warm ischemia time in the WE and RBC groups were 19.1 (15.9–24.6) versus 19.1 (16.8–19.5, P = 0.849) h and 50.0 (25.3–73.5) and 61.5 (56.3–66.8, P = 0.532) h, respectively. Three (27.3%) kidneys perfused with WE and 3 of those perfused with RBCs (60%) underwent HMP by the organ procurement organization before being declined for transplant and offered for research (P = 0.299). Other donor characteristics, including the Kidney Donor Profile Index and serum creatinine levels, were similar among the 2 treatment groups (Table 1).

Kidney Function During Perfusion

Renal artery flow improved over time, reaching means of 448 (130) mL/min at 6 h compared with 357 (136) mL/min in the RBC group (P > 0.05; Figure 1A). Similarly, vascular resistance decreased over time, which was initially more pronounced in the WE group but reached similar steady levels (0.3–0.1 Hg min/mL) by 2 h of perfusion (P > 0.05; Figure 1B). The median total urine output was 183.0 (25.0–333.5) mL in the WE group versus 7.3 (2.5–208.4) mL in the RBC group (P = 0.22; Figure 2A). Similarly, although urine output tended to be superior during WE perfusion, this did not reach statistical significance at any given time (Figure 2A). There was also no difference in creatinine clearance (Figure 2B). Interestingly,

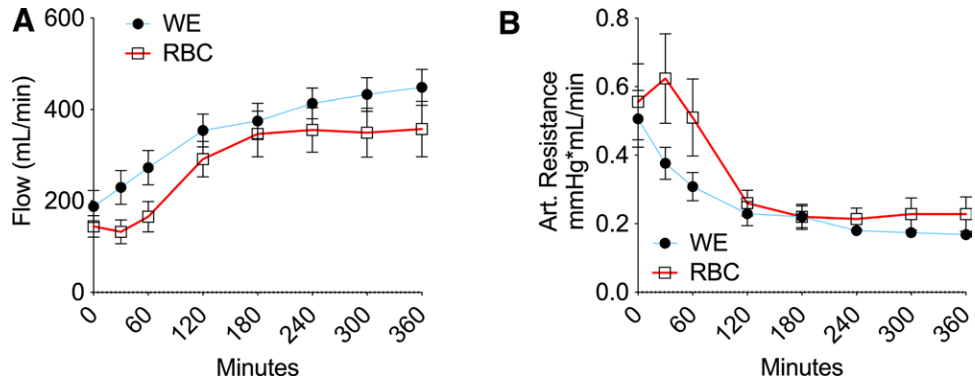

FIGURE 1. Renal flow and resistance during normothermic perfusion. A, Renal artery flow (mL/min) and renal artery resistance (B) in human kidneys perfused with WE or packed RBCs. Bars indicate mean ± SEM. N = 5–11 per group. RBC, packed red blood cell; WE, William's E medium.

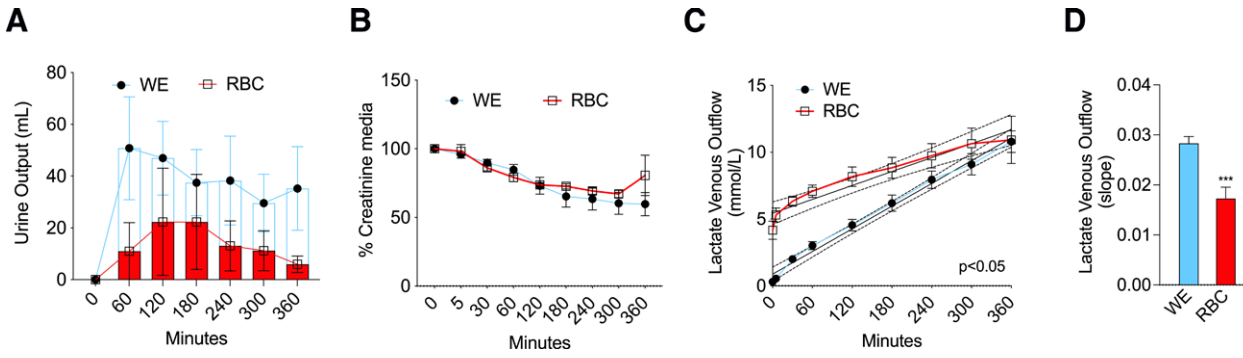

FIGURE 2. Kidney function during ex vivo perfusion. A, Urine output at the indicated time during NMP with WE or packed RBCs. B, Percentage of creatinine remaining in the perfusate over time in both groups as indicated. Lactate levels at each time point (C) and slope (D) measured in the venous outflow of kidneys perfused with WE or RBCs. Black lines indicate simple linear regression and 95% CI. Bars indicate mean ± SEM, and asterisks indicate the significance of the difference between perfusion methods by Mann-Whitney tests. ***$P < 0.001$. n = 5–11 per group. CI, confidence interval; RBC, packed red blood cell; WE, William's E medium.

lactic acid levels over time were significantly higher in kidneys perfused with RBCs up to 3 h of perfusion (Figure 2C) but because the rate of rise was faster in kidneys perfused with WE (0.028–0.001 versus 0.017–0.002 mmol/L; Figure 2D), the lactate levels were equivalent by 6 h. Although oxygen consumption was similar in both groups ($P = 0.41$; Figure 3A), renal oxygen extraction was increased in the absence of RBC (at 3 h, WE: 31.7% 22.7, RBC 4.8 3.5 mL O_2/min per g tissue, $P = 0.04$; Figure 3B). Of importance, both groups had a sustained oxygen extraction reflecting active metabolism throughout the entire duration of perfusion.

Renal Energy Metabolism

Tissue concentration of ATP was used as an indicator of the energy status of the grafts. The overall renal EC was similar in both groups ($P = 0.57$ at 6 h; Figure 4A). Interestingly, total ATP ($P = 0.55$; Figure 4B), ATP:ADP ratio ($P = 0.18$; Figure 4C), and ATP:AMP ratio ($P = 0.30$; Figure 4D) initially dropped on RBC kidney perfusion, whereas it increased during WE perfusion after the first hour. In both groups, these metabolites rapidly reached similar steady-state levels ($P > 0.05$). At 6-h perfusion, the mean ATP content was 5.08 ± 5.42 µg/mL in the WE group and 3.80 ± 2.95 µg/mL in

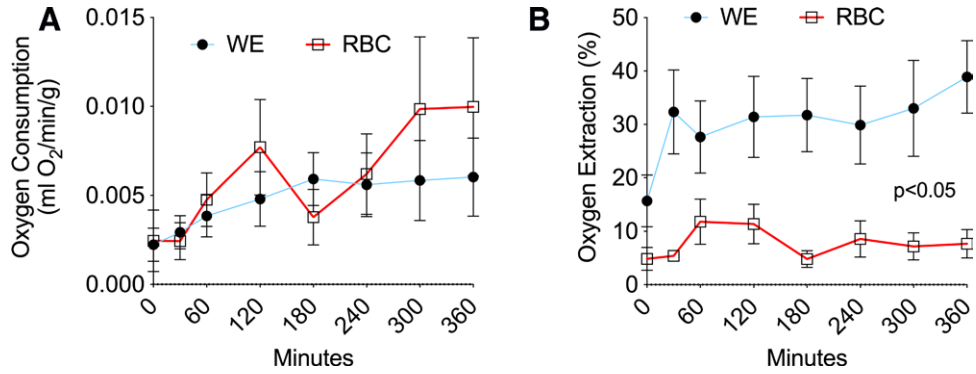

FIGURE 3. Oxygen consumption during the 6 h normothermic ex vivo kidney perfusion. Kidney (A) oxygen consumption (mL O_2/min per g tissue) and (B) oxygen extraction percentage. Bars indicate mean ± SEM. n = 5–11 per group. RBC, red blood cell; WE, William's E medium.

the RBC group ($P = 0.55$; Figure 4). Of interest and central to energy and redox metabolism, both oxidized nicotinamide adenine dinucleotide (Figure 5A) and its reduced form (Figure 5B) levels remained stable during the perfusion in both groups.

Histological Evaluation

Next, we analyzed cortical biopsies stained with hematoxylin and eosin taken at baseline (prior perfusion T0) and after 3 and 6 h of machine perfusion with WE and RBCs. There was no difference between experimental groups and over time in tubular integrity/dilatation, epithelial vacuolization/desquamation (Figure 6).

DISCUSSION

Supplementing perfusates with RBCs or artificial oxygen carriers has been considered a prerequisite for successful NMP of clinical organ grafts. In this capacity, we reported in human kidneys that RBC could be substituted by a synthetic HBOC.[16] However, renal perfusions with RBC-free artificial buffer have a long-standing history in physiologic research and are recognized to be suitable if colloidal support and higher oxygen partial pressures are provided.[18] Here, we aimed to understand the role of oxygen carriers through direct comparison of an acellular, WE-based media in human kidney grafts. In this study, kidney perfusion from both groups exhibited comparable behavior regarding vascular flow, oxygen

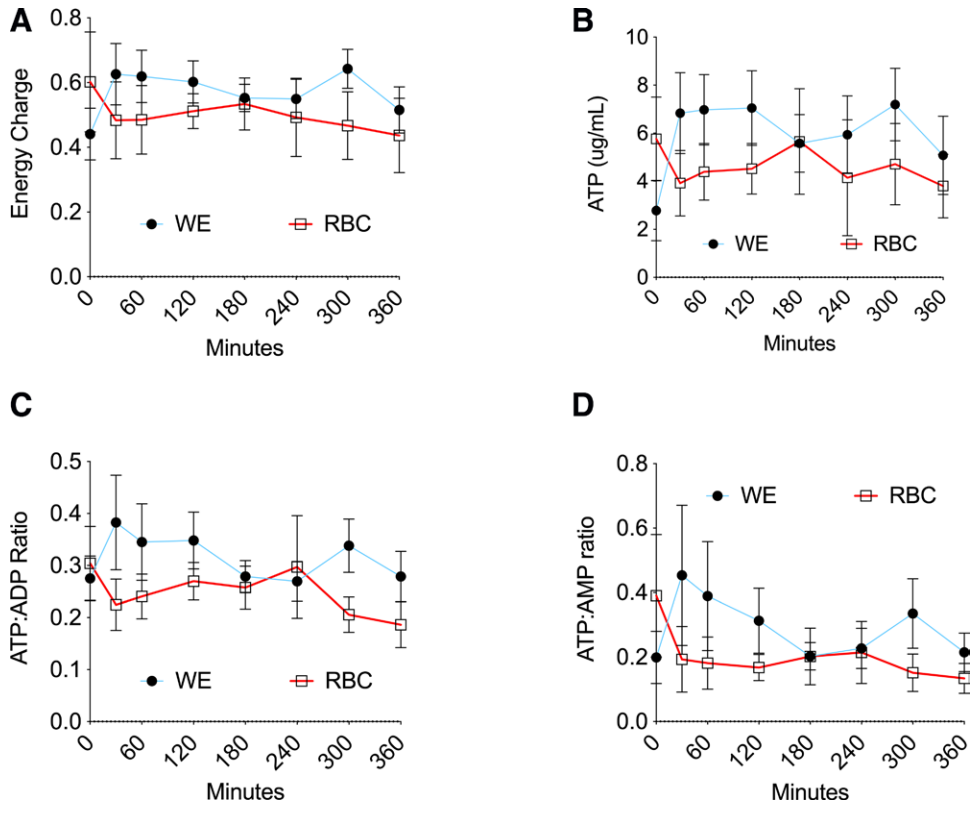

FIGURE 4. Kidney energy change with WE and RBC perfusate. (A) Overall energy charge (calculated as follow: [2ATP+ADP]/ATP+ADP+AMP) in kidney biopsies over time during the indicated perfusion. B–D, Total ATP (A), ATP/ADP ratio (B), and ATP/AMP ratio (C) in kidneys perfused with WE or RBCs. RBC, red blood cell; WE, William's E medium. Bars indicate mean ± SEM. n = 5–11 per group.

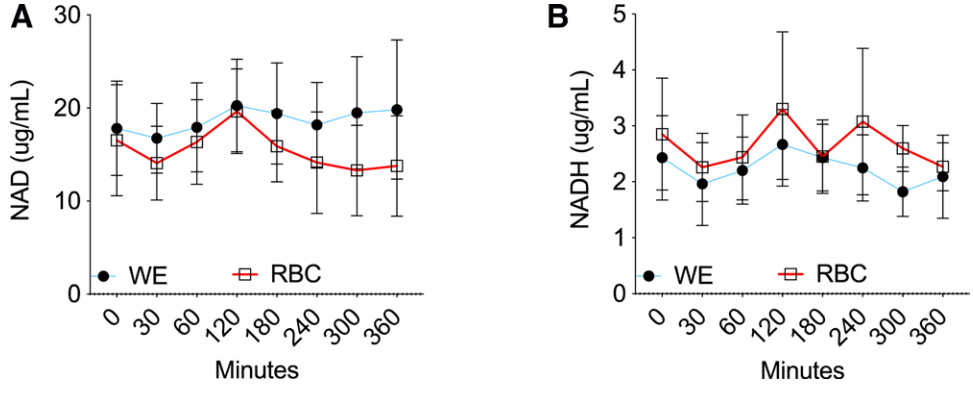

FIGURE 5. Effect of perfusion on NAD. (A and B), Quantification of (A) oxidized NAD (NAD^+), (B) reduced form (NADH) and in kidney biopsies at indicated time during perfusion with WE and RBCs. Bars indicate mean ± SEM. N = 5–11 per group. NAD, nicotinamide adenine dinucleotide; RBC, red blood cell; WE, William's E medium.

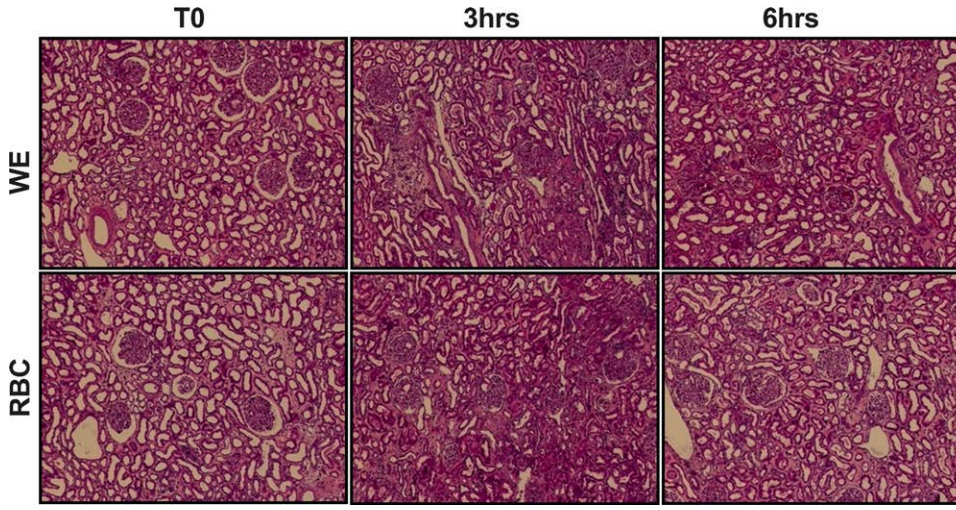

FIGURE 6. Histological assessment of human kidney. Photomicrographs (×10) of cortical biopsies fixed in 10% formalin and stained with hematoxylin and eosin taken at baseline (prior perfusion T0) and after 3 and 6 h of machine perfusion with WE and RBCs as indicated. Histology reveals regular glomerular without debris and intact epithelial brush border and tubules. n = 5–1 per group. RBC, red blood cell; WE, William's E medium.

consumption, and reconstitution of tissue ATP. Similarly, we found that tissue ATP and oxygen consumption were similar between RBCs and acellular media (WE), thereby suggesting that perfusion of human kidney grafts at 37 °C for 6 h with an acellular WE is noninferior to RBCs.

Interestingly, oxygen extraction was increased in the kidneys perfused with WE. In fact, oxygen delivery (arterial oxygen content × arterial flow/kidney weight) was reduced in the absence of RBCs, thus leading to higher oxygen extraction (the ratio of oxygen consumption to oxygen delivery) to support aerobic metabolism. Somewhat consistently, previous studies comparing HBOC- versus RBC-based solutions during human liver NMP reported higher oxygen extraction using HBOCs compared with RBCs.[19,20] Of interest, lactic acids were higher in the first 3 h of perfusion with RBCs, which is comparable with our previous study comparing RBCs with HBOCs.[16] However, lactate production rate was higher, reaching similar levels at 6 h of perfusion. The latter suggests that 6 h might be the longest perfusion duration possible without an oxygen carrier. Of importance, lactate levels were higher in kidneys perfused with RBCs at the initiation of the perfusion, likely due to the RBCs themselves and not because the grafts were producing more lactate. The rate of lactate increase might be more relevant here, and it is still possible that there is some low level of ischemia in the acellular perfusion.

Interestingly, we found that ATP levels and EC were stable over the 6-h perfusion duration in both WE and RBC groups. Previously, we reported that kidney ATP levels were biomarkers of kidney injury during perfusion in porcine DCD grafts.[3] This is consistent with the hypothesis that the kidneys are functionally and metabolically active in the presence of sufficient oxygen during the perfusion period. Thus, cells can use ATP to sustain metabolic processes that protect them from ischemic damage. Several studies demonstrated that ATP levels correlate with ischemic injury of the kidney[21] and liver.[22] Moreover, ATP is often used as a marker of viability during ischemia.[23] In humans, ATP level in liver tissue is an independent predictor of initial graft function.[24] Notably, ATP levels measured after transplantation were inversely related to warm ischemia time.[25] Similarly, low ATP levels were significantly associated with primary liver graft nonfunction.[26] Moreover, in kidney, the gene expression profile of NMP kidneys resembled no storage kidneys and was enriched for pathways related to fatty acid metabolism and oxidative phosphorylation.[7]

Glomerular function, evaluated by renal clearance of creatinine, did not differ significantly between the groups. This is consistent with another study[27] in which perfusion of porcine kidneys with an acellular media (Aqix RS I) supplemented with 40 g/L of bovine serum albumin resulted in similar creatine clearance compared to perfusion with RBCs. However, this differs from another study[28] using porcine kidneys subjected to 30 min of warm ischemia, HMP for 3 h, and NMP for 4 h. In the latter, perfusion of DCD pig kidneys with diluted blood during NMP reduced renal injury, improved Na reabsorption and creatine clearance, and was associated with increased tissue ATP levels. Of interest, Von Horn et al[27] also demonstrated that creatinine clearance and oxygen consumption were reduced when the perfusate oxygenation was diminished from >500 mm Hg (similar to our study and supraphysiologic) to 200 mm Hg, at a flow rate of ~0.5 L/min. This suggests that the requirement for an O_2 carrier (RBCs or synthetic) depends on the amount of dissolved O_2 provided.

Here, the perfusion pressure was kept constant during NMP. Thus, low renal blood flow indicates a high intrarenal resistance, underlying vascular injury, or interstitial edema. Both renal artery flow and resistance were similar in both experimental conditions. None of the groups demonstrated significant vacuolization or histological injury. In our study, flow during NMP was between 145 and 450 mL/min. The flow was consistent with our previous findings,[16] and within the range of DCD pig kidneys[28] and DBD, healthy porcine grafts. Consistently, lower flow and higher resistance were observed during HMP with[29] or without[30] oxygen. Notably, low renal arterial flow and high resistance were associated with allograft dysfunction during NMP[8] and HMP.[29]

Limitations need to be acknowledged. The number of samples was relatively small (n = 11 WE, 5 blood), thus increasing the risk of type II error. We further appreciate that most of the kidneys used in this study were deemed marginal

(Kidney Donor Profile Index >90%). The utilization here of both DBD and DCD organs should improve the external validity of our study. Although the broader utility of these perfusion media should be tested in all donor types, we predict that the outcome would be similar in a younger, less morbid population. In addition, we did not correlate perfusion parameters or ATP levels with kidney function in vivo or after transplantation. Although our group[3,16] and others[8,27] have used a combination of EC, kidney metabolism, perfusion parameters, urine output, macroscopic and microscopic assessment, and gene-associated damage to provide an overall measure of kidney quality during NMP, it remains to be established whether these interventions have an impact on both short-term (eg, DGF)[1] and long-term kidney graft function.[31] However, early human data suggest that NMP reduces DGF compared with SCS.[10,11] In DCD kidney, transplant of a 1-h period of NMP at the end of SCS did not reduce the rate of DGF compared to SCS alone.[32] The efficacy of NMP for a longer period or continuous (initiated at the donor hospital) NMP needs to be tested for future trials.

In conclusion, this study demonstrates that human kidneys undergoing NMP for 6 h with WE are feasible and do not result in inferior outcomes compared with perfusion with RBCs. Furthermore, we show that the functional and histological integrity of kidney grafts during perfusion at 37 °C is maintained for up to 6 h. These findings using an acellular perfusate provide a simple, effective alternative that can potentially eliminate the risk of thrombosis and graft loss during NMP of human kidney grafts.

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REFERENCES

1. Moers C, Smits JM, Maathuis MH, et al. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med*. 2009;360:7–19.
2. Agius T, Songeon J, Klauser A, et al. Subnormothermic ex vivo porcine kidney perfusion improves energy metabolism: analysis using. *Transplant Direct*. 2022;8:e1354.
3. Longchamp A, Klauser A, Songeon J, et al. Ex vivo analysis of kidney graft viability using 31P magnetic resonance imaging spectroscopy. *Transplantation*. 2020;104:1825–1831.
4. Jochmans I, Brat A, Davies L, et al; COMPARE Trial Collaboration and Consortium for Organ Preservation in Europe (COPE). Oxygenated versus standard cold perfusion preservation in kidney transplantation (COMPARE): a randomised, double-blind, paired, phase 3 trial. *Lancet*. 2020;396:1653–1662.
5. Husen P, Boffa C, Jochmans I, et al. Oxygenated end-hypothermic machine perfusion in expanded criteria donor kidney transplant: a randomized clinical trial. *JAMA Surg*. 2021;156:517–525.
6. Urbanellis P, Hamar M, Kaths JM, et al. Normothermic ex vivo kidney perfusion improves early DCD graft function compared with hypothermic machine perfusion and static cold storage. *Transplantation*. 2020;104:947–955.
7. Urbanellis P, McEvoy CM, Škrtić M, et al. Transcriptome analysis of kidney grafts subjected to normothermic ex vivo perfusion demonstrates an enrichment of mitochondrial metabolism genes. *Transplant Direct*. 2021;7:e719.
8. Hosgood SA, Barlow AD, Hunter JP, et al. Ex vivo normothermic perfusion for quality assessment of marginal donor kidney transplants. *Br J Surg*. 2015;102:1433–1440.
9. Kaths JM, Hamar M, Echeverri J, et al. Normothermic ex vivo kidney perfusion for graft quality assessment prior to transplantation. *Am J Transplant*. 2018;18:580–589.
10. Mazilescu LI, Urbanellis P, Kim SJ, et al. Normothermic ex vivo kidney perfusion for human kidney transplantation: first North American results. *Transplantation*. 2022;106:1852–1859.
11. Nicholson ML, Hosgood SA. Renal transplantation after ex vivo normothermic perfusion: the first clinical study. *Am J Transplant*. 2013;13:1246–1252.
12. Hosgood SA, Nicholson ML. First in man renal transplantation after ex vivo normothermic perfusion. *Transplantation*. 2011;92:735–738.
13. Hosgood SA, Saeb-Parsy K, Hamed MO, et al. Successful transplantation of human kidneys deemed untransplantable but resuscitated by ex vivo normothermic machine perfusion. *Am J Transplant*. 2016;16:3282–3285.
14. Parente A, Osei-Bordom DC, Ronca V, et al. Organ restoration with normothermic machine perfusion and immune reaction. *Front Immunol*. 2020;11:565616.
15. Eshmuminov D, Becker D, Bautista Borrego L, et al. An integrated perfusion machine preserves injured human livers for 1 week. *Nat Biotechnol*. 2020;38:189–198.
16. Aburawi MM, Fontan FM, Karimian N, et al. Synthetic hemoglobin-based oxygen carriers are an acceptable alternative for packed red blood cells in normothermic kidney perfusion. *Am J Transplant*. 2019;19:2814–2824.
17. Elliott TR, Nicholson ML, Hosgood SA. Normothermic kidney perfusion: an overview of protocols and strategies. *Am J Transplant*. 2021;21:1382–1390.
18. Maack T. Physiological evaluation of the isolated perfused rat kidney. *Am J Physiol*. 1980;238:F71–F78.
19. Laing RW, Bhogal RH, Wallace L, et al. The use of an acellular oxygen carrier in a human liver model of normothermic machine perfusion. *Transplantation*. 2017;101:2746–2756.
20. Matton APM, Burlage LC, van Rijn R, et al. Normothermic machine perfusion of donor livers without the need for human blood products. *Liver Transpl*. 2018;24:528–538.
21. Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. *J Renal Inj Prev*. 2015;4:20–27.
22. Berendsen TA, Izamis ML, Xu H, et al. Hepatocyte viability and adenosine triphosphate content decrease linearly over time during conventional cold storage of rat liver grafts. *Transplant Proc*. 2011;43:1484–1488.
23. Longchamp A, Mirabella T, Arduini A, et al. Amino acid restriction triggers angiogenesis via GCN2/ATF4 regulation of VEGF and H. *Cell*. 2018;173:117–129.e14.
24. González FX, Rimola A, Grande L, et al. Predictive factors of early postoperative graft function in human liver transplantation. *Hepatology*. 1994;20:565–573.
25. Kamiike W, Burdelski M, Steinhoff G, et al. Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation. *Transplantation*. 1988;45:138–143.
26. Hamamoto I, Takaya S, Todo S, et al. Can adenine nucleotides predict primary nonfunction of the human liver homograft? *Transpl Int*. 1994;7:89–95.
27. von Horn C, Zlatev H, Lüer B, et al. The impact of oxygen supply and erythrocytes during normothermic kidney perfusion. *Sci Rep*. 2023;13:2021.
28. Venema LH, van Leeuwen LL, Posma RA, et al; COPE Consortium. Impact of red blood cells on function and metabolism of porcine deceased donor kidneys during normothermic machine perfusion. *Transplantation*. 2022;106:1170–1179.
29. Meister FA, Czigany Z, Rietzler K, et al. Decrease of renal resistance during hypothermic oxygenated machine perfusion is associated with early allograft function in extended criteria donation kidney transplantation. *Sci Rep*. 2020;10:17726.
30. Weberskirch S, Katou S, Reuter S, et al. Dynamic parameters of hypothermic machine perfusion—an image of initial graft function in adult kidney transplantation? *J Clin Med*. 2022;11:5698.
31. Tingle SJ, Figueiredo RS, Moir JA, et al. Machine perfusion preservation versus static cold storage for deceased donor kidney transplantation. *Cochrane Database Syst Rev*. 2019;3:CD011671.
32. Hosgood SA, Callaghan CJ, Wilson CH, et al. Normothermic machine perfusion versus static cold storage in donation after circulatory death kidney transplantation: a randomized controlled trial. *Nat Med*. 2023;29:1511–1519.