Oxygenated versus standard cold perfusion preservation in kidney transplantation (COMPARE): a randomised, double-blind, paired, phase 3 trial

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Summary

Background Deceased donor kidneys are preserved in cold hypoxic conditions. Providing oxygen during preservation might improve post-transplant outcomes, particularly for kidneys subjected to greater degrees of preservation injury. This study aimed to investigate whether supplemental oxygen during hypothermic machine perfusion (HMP) could improve the outcome of kidneys donated after circulatory death.

Methods This randomised, double-blind, paired, phase 3 trial was done in 19 European transplant centres. Kidney pairs from donors aged 50 years or older, donated after circulatory death, were eligible if both kidneys were transplanted into two different recipients. One kidney from each donor was randomly assigned using permuted blocks to oxygenated hypothermic machine perfusion ($HMPO_2$), the other to HMP without oxygenation. Perfusion was maintained from organ retrieval to implantation. The primary outcome was 12-month estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration equation in pairs of donated kidneys in which both transplanted kidneys were functioning at the end of follow-up. Safety outcomes were reported for all transplanted kidneys. Intention-to-treat analyses were done. This trial is registered with the ISRCTN Registry, ISRCTN32967929, and is now closed.

Findings Between March 15, 2015, and April 11, 2017, 197 kidney pairs were randomised with 106 pairs transplanted into eligible recipients. 23 kidney pairs were excluded from the primary analysis because of kidney failure or patient death. Mean eGFR at 12 months was 50·5 mL/min per 1·73 m² (SD 19·3) in the $HMPO_2$ group versus 46·7 mL/min per 1·73m² (17·1) in HMP (mean difference 3·7 mL/min per 1·73m², 95% CI –1·0 to 8·4; p=0·12). Fewer severe complications (Clavien-Dindo grade IIIb or more) were reported in the $HMPO_2$ group (46 of 417, 11%, 95% CI 8% to 14%) than in the HMP group (76 of 474, 16%, 13% to 20%; p=0·032). Graft failure was lower with $HMPO_2$ (three [3%] of 106) compared with HMP (11 [10%] of 106; hazard ratio 0·27, 95% CI 0·07 to 0·95; p=0·028).

Interpretation $HMPO_2$ of kidneys donated after circulatory death is safe and reduces post-transplant complications (grade IIIb or more). The 12-month difference in eGFR between the $HMPO_2$ and HMP groups was not significant when both kidneys from the same donor were still functioning 1-year post-transplant, but potential beneficial effects of $HMPO_2$ were suggested by analysis of secondary outcomes.

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Introduction

Worldwide, over 90 000 kidney transplantations took place in 2018.[1] Compared with dialysis, kidney transplantation improves survival and quality of life, making it the preferred treatment for end-stage renal disease, but many grafts fail prematurely. The introduction of effective immunosuppressants in the 1980s resulted in a substantial improvement in kidney graft survival, with current 1-year graft survival rates of 90% and higher.[1] However, the observed improvement in short-term graft survival has slowed down considerably since the year 2000, and overall graft failure rates remain high at approximately 5% per year after the first post-transplant year.[1]

Ischaemia-reperfusion injury, a universal consequence of the organ donation process, is an important non-immunological and modifiable contributor to kidney graft failure. Hypothermic preservation, the cornerstone of organ preservation, mitigates the detrimental effect of ischaemia by reducing cellular metabolism and oxygen demand of the donor organ. Two methods of hypothermic preservation—ie, static cold storage and hypothermic machine perfusion (HMP)—are used clinically. In static cold storage, the kidney is submerged in cold preservation solution and placed on melting ice in an ice box. During HMP, a device pumps cold preservation solution through the renal vasculature, which has been shown to improve post-transplant outcomes.[2–4]

Despite reduced metabolic needs, there is residual ongoing metabolism during hypothermic preservation. Hypoxia prevails in both cold storage and HMP because the preservation solution is not actively oxygenated. Animal experiments modelling organ donation after

Research in context

Evidence before this study

Although oxygen is vital to cellular survival, donor kidneys are preserved in cold hypoxic conditions because preservation solutions are currently not actively oxygenated. Animal studies from the past 10 years suggest that providing oxygen during kidney preservation using machine perfusion techniques might improve post-transplant outcomes by reducing the ischaemia-reperfusion injury cycle. A 2017 systematic review (registered in PROSPERO in 2013 with final searches carried out in 2016) of the evidence for supplemental oxygen during hypothermic preservation for deceased donor kidneys suggests that the effects of oxygen on restoring kidney function during preservation might be beneficial for kidneys donated after circulatory death and those that have undergone a period of hypotension, warm ischaemia, or poor perfusion in the donor. This review highlighted the need for high-quality clinical studies in this area.

In 2009, our randomised controlled trial comparing static cold storage of deceased donor kidneys with preservation using standard hypothermic machine perfusion (HMP) showed improved short-term kidney function. In 2012, a follow-up of this study showed that HMP also improved long-term graft survival in kidneys donated after brain death but not in kidneys donated after circulatory death. These findings have been supported by several subsequent meta-analyses; however, some controversy remains regarding the benefit of HMP in deceased donor kidneys that are donated after circulatory death, which is the fastest growing source of deceased donor organs. Thus, we aimed to investigate whether supplemental oxygen during HMP could improve the preservation of kidneys donated after circulatory death, especially because many centres have already introduced HMP in their clinical practice.

Added value of this study

To our knowledge, this phase 3, randomised, double-blind, paired design trial is the first to investigate the value of supplemental oxygen during hypothermic organ preservation. In this trial we randomised kidney pairs from donors following circulatory death who were aged 50 years or older, to compare standard non-actively oxygenated HMP with oxygenated HMP ($HMPO_2$). The results show that $HMPO_2$ is feasible, safe, and easy to administer, and leads to fewer severe postoperative complications. There was no difference between groups for the primary outcome of estimated glomerular filtration rate (eGFR), which is an established predictor of long-term graft survival, for kidney pairs where both transplanted kidneys were functioning at the end of follow-up. However, when the beneficial effect of $HMPO_2$ on graft survival was considered, $HMPO_2$ was associated with improved 1-year graft function as measured by eGFR. We also found a significant decrease in acute rejection rates shown by a biopsy in the first year post-transplant. Exploratory analysis suggests that reduced rejection might be the underlying mechanism of the observed beneficial effect of oxygen.

Implications of all the available evidence

Given that $HMPO_2$ is simple and would be a minimal cost extension to the current preservation strategies, it has the potential for quick implementation in clinical practice with a direct beneficial effect of improving outcomes for many patients. The findings of this study underpin increasing evidence suggesting a close link between hypoxia, and innate and adaptive immunity that lead to chronic scarring and loss of kidney function, which needs further investigation. Furthermore, the beneficial mechanisms are probably similar in other organs, and future studies investigating the effect of supplemental oxygen during hypothermic organ preservation might want to include organ rejection and any confounding factors in their design.

circulatory death suggest that active oxygenation during hypothermia is essential to reduce oxidative stress and improve cellular energy status.[5–10] Oxygenated HMP ($HMPO_2$) leads to improved early kidney function and reduced fibrosis in porcine models.[5–10] To date, to our knowledge, there are no well-designed clinical studies assessing the effect of $HMPO_2$ in kidney transplantation.[11]

This study aimed to assess the effect of oxygen delivery in the setting of kidney donation after circulatory death from donors aged 50 years or older. This donor population was chosen because it represents the fastest growing source of donor kidneys (appendix p 6). Kidneys donated after circulatory death are more susceptible to the ischaemia-reperfusion injury cascade than are kidneys donated after brain death, resulting in higher post-transplant complication rates.[12] The challenge is to increase the number of organs that can be used for transplants and transplant kidneys without compromising organ function and survival. To overcome this challenge, considerable efforts should focus on further optimisation of kidney preservation and HMP. Indeed, although HMP is frequently used to preserve donated kidneys after circulatory death,[13,14] and meta-analyses have shown that HMP reduces the risk of delayed graft function in all types of deceased donor kidneys when compared with static cold storage, evidence showing that HMP improves long-term graft function or survival of kidneys donated after circulatory death is scarce.

See Online for appendix

Methods

Study design

This investigator-driven, international, randomised, double-blind, paired, controlled, phase 3 trial involved 12 organ procurement teams and their associated

hospitals in Belgium, the Netherlands and the south of the UK. Kidneys were transplanted in 19 kidney transplant centres in the same countries (appendix p 2). The EU-funded Consortium for Organ Preservation in Europe (COPE) set up and coordinated this trial. Approval was obtained by the institutional review boards or independent ethics committees in each trial region. This trial is reported in accordance with the CONSORT guidelines. One major amendment was made to the trial design after the start of recruitment and is detailed in the outcomes section.

Participants

Inclusion was limited to kidney pairs procured by controlled donation after circulatory death from donors aged 50 years or older, when both kidneys were deemed transplantable by the donor surgeon. This donor group was deliberately chosen because it represents the fastest growing source of deceased donor kidneys (appendix p 6). Additionally, organs from such donors are more susceptible to ischaemia-reperfusion and preservation injury.[12,15] Informed consent was obtained from the donor's relatives when required by national law. The study adhered to the Declaration of Istanbul. Recipients were eligible if they were aged 18 years or older and listed for only a kidney transplant in one of the participating centres. Donor kidneys from Belgium and the Netherlands were allocated by Eurotransplant, and donor kidneys from the UK were offered by NHS Blood and Transplant services. Randomisation took place early in the donation process and recipients were informed that the kidney they had been offered was included in this trial (appendix p 2). All participants gave written consent to use follow-up data stored in a coded way in a secure online database established by the COPE Consortium. The consent also included collection and storage of biological samples. Organ allocation was done following rules established by Eurotransplant and NHS Blood and Transplant.

Randomisation and masking

Using a computer-generated randomisation scheme with permuted blocks, stratified by organ allocation region, one kidney from each donor pair was randomly assigned to $HMPO_2$ and the contralateral kidney to standard HMP without oxygenation (appendix pp 2–3). Randomisation took place after the donor surgeon had confirmed transplantability of both donor kidneys. All clinical decisions made thereafter, including graft suitability, were made independently from the trial team. Donors, organ transport, and recipients were managed according to local protocols. For standardised protocol purposes, trained technicians were involved and responded when a potential donor was announced. Technicians transported the perfusion device to the donor hospital, randomised kidney pairs, supported surgeons with connecting the kidney to the device, controlled oxygenation, and collected baseline, donation, and transplantation data. Clinicians were masked to treatment allocation by use of empty dummy oxygen bottles in the control standard HMP group. Follow-up data were collected by the transplant centres. The unit of randomisation was the donor kidney pairs and analyses are reported for transplant recipients. All health-care professionals and patients receiving a kidney transplant, involved the trial, were masked to any perfusion characteristics, such as flow and resistance, that are displayed by the device to avoid any influence of these characteristics on organ acceptance.

For more on the **COPE** see http://www.cope-eu.org

Procedures

Immediately following removal from the donor, the kidney was connected to a Kidney Assist Transporter device (Organ Assist BV, Groningen, Netherlands) to be perfused during the entire preservation period, until the kidney was removed from the device to be prepared for transplant, using University of Wisconsin Machine Preservation Solution (Bridge to Life, DC, USA) that was either actively oxygenated ($HMPO_2$ group) or non-actively oxygenated (HMP group) at 1–4°C, with a fixed mean perfusion pressure of 25 mm Hg. No changes to perfusion settings were made and all involved were masked to perfusion characteristics. Oxygen (100%) was given at 100 mL/min, resulting in perfusate partial oxygen tension of around 600 mm Hg in $HMPO_2$ (appendix pp 3–4). Donor blood and urine, recipient blood, and a kidney tissue biopsy were collected at prespecified time-points (appendix p 4) from each donor, kidney, and recipient in the study when consent was in place. Additionally, samples of perfusate fluid were collected from every kidney. Samples were stored in a central biobank established by the COPE Consortium for ongoing mechanistic studies. No patient identifiable data were associated with the sample.

Outcomes

The primary endpoint was renal function at 12 months after kidney transplantation, which is independently associated with an increased risk of graft failure.[16,17] We planned to obtain creatinine clearance calculated from a 24 h urine collection at 12 months post-transplant to measure renal function. During recruitment, while data were still masked, we observed that a high proportion of creatinine clearance values were missing, as many centres had abandoned this method of assessing renal function in favour of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Therefore, the data monitoring committee, investigators, and the trial statistician jointly decided to change the way that renal function was measured. The primary endpoint changed from creatinine clearance to an estimated glomerular filtration rate (eGFR) using the CKD-EPI equation[18] at 12 months post-transplant, which was originally a secondary endpoint (appendix p 4).

The first secondary endpoint was survival of the graft and the patient for up to 12 months post-transplant. Graft

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failure was defined as a return to chronic dialysis or pre-emptive re-transplantation. The second was short-term outcomes as determined by primary permanent non-function of the graft from time of transplantation (resulting in re-institution of chronic dialysis), delayed graft function (dialysis during the first week post-transplant with eventual return of kidney function), and functional delayed graft function (absence of a decrease in serum creatinine by a minimum of 10% per day during 3 consecutive days in the first postoperative week, excluding those with acute rejection of calcineurin inhibitor toxicity). The third secondary endpoint was renal function measured using the CKD-EPI equation at 3 months and 6 months, and by the four variable Modification of Diet in Renal Disease (MDRD) equation[18] at 3, 6, and 12 months. The fourth secondary endpoint was also renal function but, measured by creatinine clearance from a 24 h urine collection at 12 months. The fifth secondary endpoint was acute rejection up to 12 months shown by a biopsy. Lastly, safety events were recorded as a secondary endpoint. Reporting of adverse events was in accordance with the Medical Devices (MEDDEV) guidelines.[19] Following trial completion, adverse events were reviewed by two clinicians masked to treatment and graded according to the Clavien-Dindo system.[20] The proportion of adverse events graded IIIb or more was compared between groups. Safety and adverse events are reported for all randomised kidneys.

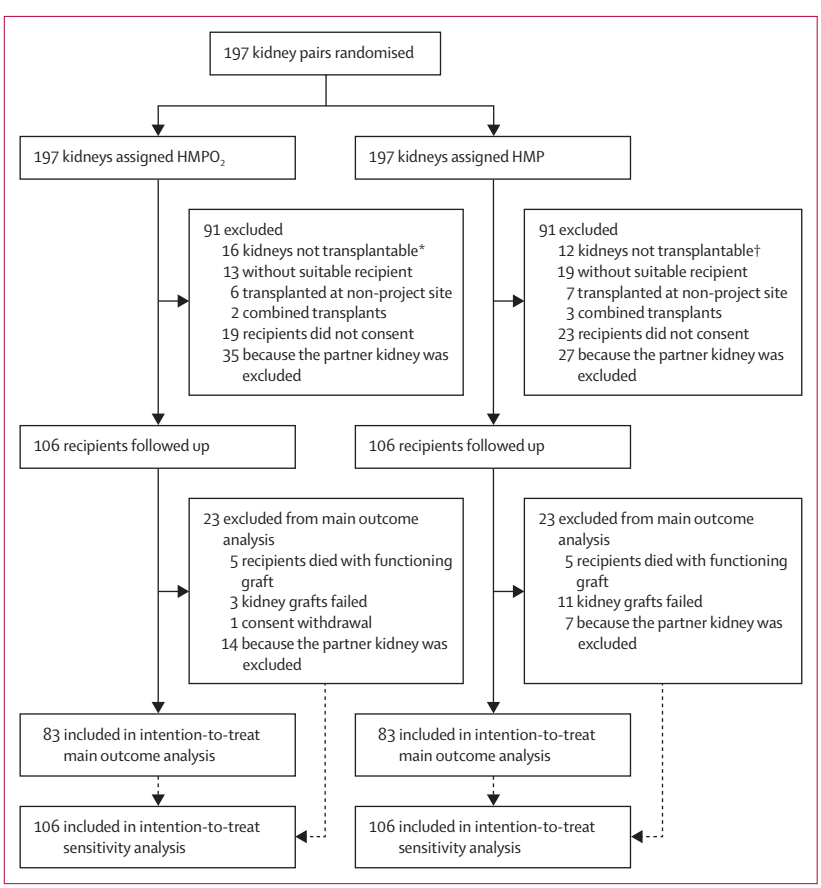

Figure: **Trial profile**
The drop-out rate between randomisation and final analysis is the direct consequence of the paired design of the trial and matches the predicted drop-out rate. HMP=hypothermic machine perfusion. $HMPO_2$=oxygenated hypothermic machine perfusion. *Seven kidneys at a donor centre and nine at recipient centre. †Six at a donor centre and six at recipient centre.

Statistical analysis

Previous data from the University Hospitals in Leuven, Belgium (appendix p 5) show a correlation coefficient of 0·4 for the function of two kidneys from the same donor, a SD of 12 mL/min per 1·73m², and a mean eGFR of 46 mL/min per 1·73 m² at 12 months post-transplant (appendix p 5). This study took into consideration the minimal clinically important difference and was powered to detect an 8 mL/min per 1·73 m² difference in eGFR (using the CKD-EPI equation)[18] with a 90% power at 5% significance, which required 81 kidney pairs for analysis of the primary endpoint (appendix p 5).

The use of eGFR as a primary endpoint needed careful consideration because eGFR overestimates true renal function when the graft fails, and patients return to dialysis. Also, eGFR at 12 months is not available when a patient dies with a functioning graft before that time. For the primary analysis, only kidney pairs for which both grafts were still functioning at 12 months post-transplant were considered. Because this criterion could introduce undesired bias towards either group, a prespecified sensitivity analysis of the primary endpoint was carried out to account for kidneys that failed before follow-up at 12 months. In this sensitivity analysis, kidneys with graft failure, with the patient receiving chronic dialysis treatment, were given a nominal eGFR value of 10 mL/min per 1·73 m², matching the start of chronic dialysis in patients with end-stage renal failure during the IDEAL trial.[21] Guidelines promote dialysis initiation when patients have symptoms or signs of advanced chronic kidney disease that are most likely to occur with eGFR values ranging from 5 mL/min per 1·73 m² to 10 mL/min per 1·73 m². When the patient died with a functioning graft, the last available eGFR measurement was carried forward and used.

Primary analyses were done according to the intention-to-treat principle, with a prespecified per-protocol analysis done as a sensitivity analysis. All reported p values are two-sided and unadjusted for multiple testing. We considered p values of less than 0·05 to indicate statistical significance and reported 95% CIs. For the primary endpoint we used a paired *t* test. For secondary endpoints, we used paired *t* tests or Wilcoxon rank-sum tests for continuous variables and McNemar's test for discrete variables. Time to graft failure and patient death were compared with Kaplan-Meier curves and log-rank methods. Multivariate analyses were done using generalised estimating equation models with either a binomial or Gaussian distribution. No interim analyses of study

endpoints were carried out. Percentages in this study might not precisely reflect the absolute figures because of rounding to whole numbers. Analyses were done with SAS (version 9.4) and STATA (version 15). At regular intervals, an independent data monitoring committee reviewed confidential reports covering recruitment, safety parameters, and endpoint data. This trial was preregistered with ISRCTN, 32967929.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 15, 2015, and April 11, 2017, 197 kidney pairs were randomised, with one kidney from each donor randomly assigned to either $HMPO_2$ or HMP without oxygenation, and 91 kidney pairs subsequently excluded (figure). Of 394 total kidneys, 16 (4%) were considered not transplantable by the retrieving donor surgeon after randomisation (eight [4%], 95% CI 2–8% in each group) and 12 (3%) considered not transplantable by the transplant surgeon after perfusion (eight [4%], 95% CI 2–8% in $HMPO_2$ group; four [2%], 1–5% in HMP group). Because of graft failure or patient death, only 83 kidney pairs were available for primary outcome analysis, with 106 available for sensitivity analysis and secondary outcome analyses. The allocation groups were balanced as shown by the donor and recipient baseline characteristics (tables 1, 2). 11 (5%) of 212 kidneys were cold-stored because machine perfusion was not possible (appendix p 6) and for nine (5%) of 201 machine-perfused kidneys randomised allocation was accidently switched (appendix p 7); these organs were included in

	$HMPO_2$ (n=106)	HMP (n=106)
Donor characteristics		
Age, years	58·0 (54·0–63·0)	58·0 (54·0–63·0)
Female	40 (38%)	40 (38%)
Male	66 (62%)	66 (62%)
Body-mass index, kg/m²	25 (23–28)	25 (23–28)
Condition leading to death		
Trauma	16 (15%)	16 (15%)
Cerebrovascular event	42 (40%)	42 (40%)
Hypoxia	39 (37%)	39 (37%)
Other	9 (9%)	9 (9%)
Cytomegalovirus status		
Positive	44 (42%)	44 (42%)
Negative	61 (58%)	61 (58%)
Unknown	1 (1%)	1 (1%)
Arterial hypertension*	29 (27%)	29 (27%)
Last creatinine, mg/dL	0·7 (0·6–0·9)	0·7 (0·6–0·9)
Donor warm ischaemic time, min	28·5 (22–36)	28·5 (22–36)

Data are n (%) or median (IQR). HMP=hypothermic machine perfusion. $HMPO_2$=oxygenated hypothermic machine perfusion. *Six records missing.

Table 1: **Baseline donor characteristics in the intention-to-treat population**

	$HMPO_2$ (n=106)	HMP (n=106)	p value*
Recipient characteristics			
Age, years	60 (53–68)	61 (51–65)	0·30
Female	37 (35%)	39 (37%)	0·12
Male	69 (65%)	67 (63%)	..
Previous transplant	4 (4%)	4 (4%)	0·99
Panel-reactive antibody			
0–10%	90 (85%)	89 (84%)	0·80
11–84%	8 (8%)	9 (9%)	..
≥85%	2 (2%)†	0 (0%)	..
Missing	6 (5·7%)	8 (8%)	..
Cytomegalovirus status			
Positive	61 (58%)	64 (61%)	0·79
Negative	42 (40%)	36 (34%)	..
Unknown	2 (2%)	1 (1%)	..
Missing	1 (1%)	5 (5%)	..
Immunosuppressive drugs			
Prednisolone	100 (94%)	98 (93%)	0·34
Cyclosporine	2 (2%)	1 (1%)	..
Tacrolimus	103 (97%)	104 (98%)	0·65
Azathioprine	1 (1%)	1 (1%)	..
Mycophenolate mofetil	104 (98%)	103 (97%)	0·94
Antithymocyte globulin	9 (9%)	14 (13%)	0·13
Interleukin-2 receptor antagonists	52 (49%)	56 (53%)	0·34
Human leucocyte antigen mismatches‡			
0	5 (5%)	5 (5%)	..
1	8 (8%)	5 (5%)	..
2	20 (19%)	20 (19%)	..
3	32 (30%)	34 (32%)	..
4	29 (27%)	33 (31%)	..
5	7 (7%)	5 (5%)	..
6	5 (5%)	3 (3%)	..
Cytomegalovirus mismatch			
Yes	53 (50%)	50 (47%)	0·89
No	49 (46%)	49 (46%)	..
Missing	4 (4%)	7 (7%)	..
Cold ischaemia time,§ h	11·0 (8·7–13·7)	10·3 (8·9–14·0)	0·41
Perfusion time,¶ h	6·85 (4·5–9·1)	7·40 (4·8–9·9)	0·21

Data are n (%) or median (IQR). HMP=hypothermic machine perfusion. $HMPO_2$=oxygenated hypothermic machine perfusion. *p values comparing groups between $HMPO_2$ and HMP were calculated with a paired *t* test or Wilcoxon rank-sum test for continuous variables and McNemar's test for discrete variables. The unit of randomisation was donor kidney pairs and not recipients. †These recipients did not develop acute rejection shown by a biopsy. ‡One record missing. §Four records missing. ¶21 records missing.

Table 2: **Baseline recipient and transplant characteristics in the intention-to-treat population**

	$HMPO_2$ mean	HMP mean	Mean or risk difference*	p value†
Primary endpoint‡				
Primary comparison (n=83)	50·5 (19·3)	46·7 (17·1)	3·7 (–1·0 to 8·4)	0·12
Sensitivity analysis (n=106)	47·6 (20·1)	42·6 (20·3)	5·0 (0·4 to 9·7)	0·035
Secondary endpoints				
Primary non-function (n=106)	3 (3%)	5 (5%)	–2 (–7 to 3)	0·48
Delayed graft function (n=106)	38 (36%)	38 (36%)	0 (–14 to 14)	0·99
Functional delayed graft function (n=106)	76 (72%)	76 (72%)	0 (–13 to 11)	0·99
Acute rejection shown by a biopsy (n=106)	15 (14%)	27 (26%)	–11 (–22 to –0·01)	0·040
Renal function post-transplant				
GFR at 3 months (mL/min per 1·73 m^2)				
CKD-EPI equation (n=88)	46·5 (18·2)	45·0 (16·9)	1·5 (–3·2 to 6·3)	0·53
MDRD equation (n=89)	44·8 (15·7)	44·3 (23·8)	0·5 (–5·2 to 6·1)	0·87
GFR at 6 months (mL/min per 1·73 m^2)				
CKD-EPI equation (n=83)	50·1 (18·5)	47·1 (19·6)	3·0 (–1·8 to 7·7)	0·22
MDRD equation (n=85)	48·1 (17·7)	44·7 (17·9)	3·4 (–1·2 to 8·0)	0·15
GFR at 12 months (mL/min per 1·73 m^2)				
MDRD equation (mL/min per 1·73m^2) primary comparison (n=83)	48·8 (19·5)	44·4 (15·4)	4·4 (–0·2 to 9·1)	0·062
MDRD equation (mL/min per 1·73 m^2) sensitivity analysis (n=106)	46·1 (19·9)	40·7 (18·8)	5·4 (0·8 to 10·0)	0·021
Creatinine clearance in 24 h urine collection (mL/min) (n=77)	58·2 (21·4)	51·1 (21·9)	7·1 (1·1 to 13·0)	0·021

Data are n (%), mean (SD), mean difference (95% CI), or risk difference (95% CI). CKD-EPI=Chronic Kidney Disease Epidemiology Collaboration. GFR=glomerular filtration rate. HMP=hypothermic machine perfusion. $HMPO_2$=oxygenated hypothermic machine perfusion. MDRD=Modification of Diet in Renal Disease. *Risk difference instead of mean difference is shown only for secondary endpoints. †p values were calculated with the use of a paired *t* test or Wilcoxon rank-sum test for continuous variables and the McNemar's test for discrete variables. ‡GFR at 12 months post-transplant measured using the CKD-EPI equation.[22]

Table 3: **Univariable differences between $HMPO_2$ and HMP groups**

	$HMPO_2$ survival probability (95% CI)	HMP survival probability (95% CI)
Graft survival		
7 days	0·98 (0·93–0·99)	0·95 (0·89–0·98)
3 months	0·97 (0·91–0·99)	0·92 (0·85–0·96)
6 months	0·97 (0·91–0·99)	0·91 (0·83–0·95)
12 months	0·97 (0·91–0·99)	0·89 (0·82–0·94)
Patient survival		
7 days	1·00	0·99 (0·94–0·99)
3 months	0·94 (0·88–0·97)	0·95 (0·89–0·98)
6 months	0·94 (0·88–0·97)	0·95 (0·89–0·98)
12 months	0·93 (0·87–0·97)	0·93 (0·83–0·96)

HMP=hypothermic machine perfusion. $HMPO_2$=oxygenated hypothermic machine perfusion.

Table 4: **Survival probability at follow-up**

the intention-to-treat analysis. Because this was a paired trial, some kidneys that were randomised were not eligible for primary outcome analysis because their partner kidney had been excluded (35 in $HMPO_2$ group and 27 in HMP group).

At 12 months post-transplant, the eGFR was higher in the $HMPO_2$ group than in the HMP group (mean difference 3·7 mL/min per 1·73 m², 95% CI –1·0 to 8·4; p=0·12) for those pairs of which both donor kidneys were still functioning. The sensitivity analysis of the main outcome, which accounts for donor kidneys that failed before 12 months follow-up and patient death, showed a mean difference of 5·0 mL/min per 1·73 m² (95% CI 0·35 to 9·68; p=0·035) in favour of $HMPO_2$ (table 3). Post-hoc sensitivity analyses using different imputation methods supported these findings (appendix p 7). Multivariate regression analyses, including clinically relevant baseline variables, showed that donor age was the only independent predictor of eGFR at 12 months (appendix p 8).

Graft failure at 12-months post-transplant was significantly lower in the $HMPO_2$ group (three [3%] of 106) than in the HMP group (11 [10%], HR 0·27, 95% CI 0·07–0·95; p=0·028). Table 4 shows the graft survival probability at each follow-up visit and the Kaplan-Meier curve in the appendix (p 12) shows time to graft failure. Two grafts failed because of preservation injury, three from immunological reasons, three from arterial thrombosis, one from venous thrombosis, and five from other reasons (appendix p 8). After 3 months post-transplant there were no graft failures in the $HMPO_2$ group, whereas there were four (36%) graft failures in the HMP group. There was no significant difference in patient survival; seven patients died over 12 months in the $HMPO_2$ group and eight patients died in the HMP group (7% *vs* 8%, HR 0·88, 95% CI 0·32–2·41; p=0·80; table 4; appendix p 13). Five patients died from infection, four from cardiovascular disease, one from a cerebrovascular event, one from cancer, one from multiple organ failure, and three from unknown causes (appendix p 8). In each group, five patients died with a functioning graft. Rates of primary non-function, delayed graft function, and functional delayed graft function were similar between the $HMPO_2$ and HMP groups (tables 3; appendix p 8). Renal function improved over time and at all time-points the renal function was better in the $HMPO_2$ group (table 3; appendix pp 13–14). CKD stages are shown in the appendix (pp 8–9). Creatinine clearance from a 24 h urine collection was significantly higher in the $HMPO_2$ group than in the HMP group at 12 months (table 3).

The relative risk reduction of acute rejection shown by a biopsy was 44% (relative risk ratio 0·56, 95% CI 0·31–0·98) in the $HMPO_2$ group (14%) compared with the HMP group (26%, absolute risk difference –11%, –22 to –0·01; p=0·040; table 3). Rates of acute rejection occurring after 3 months post-transplant were higher in the HMP group (appendix pp 9,14). Recipients in both groups were well matched for induction therapy and maintenance immunosuppression (table 2; appendix p 9). Post-hoc analysis of patients receiving induction therapy showed a similar reduction in rejection rates

between both groups (appendix p 10). Exploratory analysis showed no difference in Banff grading and no difference in response to steroid pulse treatment (appendix p 10). Preclinical findings suggested a link between $HMPO_2$ and acute rejection.[23] Exploratory multivariate analysis was done in this study to look at determinants of acute rejection shown by a biopsy. These findings showed that $HMPO_2$ was the only independent factor protecting against acute rejection suggesting that the effect of $HMPO_2$ on eGFR might be mediated through a reduction in acute rejection shown by biopsy. The adjusted odds of acute rejection occurring in the $HMPO_2$ group was approximately 55% lower (OR 0·45, 95% CI 0·20–0·99) than in the HMP group (appendix p 10). Results from the per-protocol analysis, which included 88 kidney pairs, supported the intention-to-treat analysis, although the findings were not statistically different except for a lower graft failure rate seen in the $HMPO_2$ group (appendix p 10).

The proportion of recipients with reported adverse events was similar in the two groups (26% [95% CI 19–34%] in the $HMPO_2$ group *vs* 28% [20–36%] in the HMP group). Table 5 shows adverse events for all randomised kidneys according to the perfusion type received. Of 891 adverse events and serious adverse events reported for recipients (417 in $HMPO_2$, 474 in HMP), fewer severe (Clavien–Dindo grade ≥IIIb) complications[20] were reported in the $HMPO_2$ group versus the HMP group (46 [11%] of 417, 95% CI 8–14% *vs* 76 [16%] of 474, 13–20%; p=0·032). One kidney that underwent $HMPO_2$ was not transplanted following a technical issue with leakage of perfusion fluid. Modifications to the device were made to avoid re-occurrence (appendix p 5).

	$HMPO_2$ (N=141)	HMP (N=133)
Any adverse event during donor procedure	20	7
Damaged polar artery	8	2
Massive atherosclerosis preventing safe connection to the device	5	1
Multiple renal arteries and no appropriate patch holder available	1	1
Device issue preventing correct set-up	6	3
Any adverse event during organ preservation	6	8
Oxygen (not) administered erroneously	5	7
Perfusate leakage	1*	1
Any serious adverse event in recipients	213	209
Cardiovascular		
Cardiac failure	8	2
Myocardial infarction	3	3
Diarrhoea or vomiting	28	12
Electrolyte disturbances	5	8
Infection		
Abdomen	3	2
Chest	16	7
CMV infection or reactivation	5	8
Sepsis	13	10
Urinary tract	34	20
Wound	4	2
Kidney dysfunction	57	59
Malaise	7	10
Permanent graft failure	7	13
Related to surgery		
Arterial stenosis	2	4
Arterial thrombosis	0	2
Bleeding	4	9
Lymphocele	0	3
Ureteral stenosis	7	11
Ureteral necrosis	3	4
Venous thrombosis	1	1
Seroma	2	1
Surgical revision within 12 months	4	18
Respiratory failure	4	9
Suspicion of rejection	32	32
Transfusion	11	11
Deaths and cause of death		
Cardiac event	3	1
Infection leading to sepsis	2	4
Cerebrovascular event	0	1
Cancer	2	0
Multiple organ failure	0	1
Death from unknown cause	1	3

(Table 5 continues in next column)

	$HMPO_2$ (N=141)	HMP (N=133)
(Continued from previous column)		
Any adverse event in recipients	204	265
Diarrhea or vomiting	8	10
Electrolyte disturbances	9	12
Infection		
Abdomen	2	0
Chest	3	5
CMV infection or reactivation	4	9
Urinary tract	15	11
Wound	5	4
Kidney dysfunction	11	22
Lymphocele	0	4
Malaise	4	8
Seroma	1	1
Suspicion of rejection	7	12
Transfusion	1	2
Ureteral stenosis	0	1
Other	139	160

CMV=cytomegalovirus. HMP=hypothermic machine perfusion. $HMPO_2$=oxygenated hypothermic machine perfusion. *Leading to kidney discard (see appendix for further details).

Table 5: **Adverse events and Serious Adverse Events according to the MEDDEV guidelines.**

Discussion

To our knowledge, this international, double-blind, paired, multicentre, randomised controlled trial is the first to test the effect of oxygenation during hypothermic kidney preservation. This trial was embedded in the standard practice of organ donation and allocation and included 106 paired donor kidneys from donors aged 50 years and older after circulatory death, with one kidney preserved by $HMPO_2$ and the other by standard HMP. The results showed that $HMPO_2$ is feasible, safe, and easy to deliver. Severe post-transplant complications were reduced by $HMPO_2$ compared with HMP. When both kidneys from the same donor were still functioning at 12 months post-transplant, $HMPO_2$ did not show significantly improved eGFR. When considering the beneficial effect of $HMPO_2$ versus HMP on graft survival, $HMPO_2$ shows a significant improvement in renal function at 12 months post-transplant. A significant relative risk reduction of acute rejection was observed after $HMPO_2$ compared with preservation with standard HMP.

The clinical benefits observed in this trial are consistent with results from previous animal studies,[8,10,24] in which active oxygenation during preservation improves kidney function and reduces fibrosis and affects long-term graft survival. These effects appear to be mediated through a reduction in the immune response to ischaemia-reperfusion injury. Innate and adaptive immunity are activated upon reperfusion when a superoxide burst from the mitochondrial respiratory chain induces tissue injury and damage-associated molecular patterns.[25] A sterile inflammatory response with a maladaptive injury repair initiates alloreactive T-cell and B-cell responses, priming the organ for rejection and fibrosis.[26–28] $HMPO_2$ reduces damage-associated molecular patterns, prevents mitochondrial superoxide production, and reduces endothelial, macrophage, and T-cell activation after reperfusion. Oxygen is essential to obtain these beneficial effects, for which supra-physiological oxygen tensions are required under hypothermic conditions in the absence of oxygen carriers.[8,9] Concern has been expressed about the possible increase in oxidative stress with $HMPO_2$, due to supra-physiological oxygen levels and because higher levels of lipid peroxidation have been reported after oxygenated perfusion.[24] However, we found no evidence that oxygenation at these levels increased post-transplant morbidity. In fact, the incidence of severe post-transplantation complications was significantly lower in the $HMPO_2$ group than in the HMP group. Only one adverse event was attributed to the device, with effective measures taken to prevent further events. Mortality rates at 12 months were acceptable for this older patient population, with no differences observed between treatment groups.

In the $HMPO_2$ group, the observed 44% relative risk reduction in acute rejection is in line with a previous study[23] showing a reduction in acute rejection rates in rodent kidney transplants. $HMPO_2$ was also the only independent predictor of acute rejection when adjusting for other known risk factors such as human leucocyte antigen mismatches and the use of induction therapy. These results taken together suggest that the effect of $HMPO_2$ on eGFR might be mediated through a reduction in acute rejection. Further research is needed to unravel the effects of $HMPO_2$ on kidney immunogenicity and the immunological mechanisms of rejection. Uncontrolled confounders might have influenced the observed reduction in acute rejection as immunosuppressive regimens were followed according to standard practice but were not always fully identical. Also, detailed information on trough levels of calcineurin and donor-specific antibody formation was not recorded. Reduced immunogenicity with a dampened inflammatory response leading to lower rejection rates might be the reason for improved graft survival in these donor kidneys.

Persistent inflammation in scarred areas after T-cell mediated rejection has been associated with chronic scarring and fibrosis due to maladaptive injury responses, which are important risk factors for long-term graft failure.[27] Over a third (36%) of graft failures in the HMP group occurred after 3 months post-transplant, suggesting that immunological factors were most likely at play. All graft failures in the $HMPO_2$ group occurred in the first 3 months post-transplant. Although the 12 month graft failure of 10% in the control HMP group might appear high, a thorough analysis showed that this rate matches graft failure rates in similar kidney transplant cohorts (appendix p 6). Because of the small number of graft failures, it was not possible to establish whether organ rejection was an independent determinant of graft failure in this cohort. A post-hoc analysis of biopsies showed no difference in Banff severity grading. Biopsies were scored as part of clinical routine; thus, we cannot exclude that interobserver variability might have masked any differences.

Unlike in animal studies, this trial did not show a difference in early renal function, which was assessed by the presence or absence of delayed graft function. An association between delayed graft function and acute rejection has been previously reported, therefore the absence of a difference in delayed graft function between both groups observed in this trial is intriguing and we can only speculate on the reasons for this observation. This association has been mostly described for kidneys donated after brain death. A Canadian study[29] showed that delayed graft function is an important risk factor for acute rejection in a contemporary cohort of kidney transplant recipients. The association was less pronounced in recipients who were older (≥60), diabetic, unsensitised, and received donor kidneys with expanded criteria.[29] The population of recipients in the Canadian trial is similar to the population in our trial and might explain the absence of an association. In addition, there is a physiological difference of delayed graft function in kidneys donated after circulatory death compared with those donated after

brain death, with selective activation of resilience-associated pathways in grafts donated after circulatory death.[15] The pathways leading to delayed graft function and acute rejection might also differ, which might explain the observed difference in acute rejection despite similar delayed graft function rates.

This preservation trial in kidney transplantation has limitations. Because, to our knowledge, no preclinical studies had investigated the potential effect of $HMPO_2$ on acute rejection at the time of this trial design, information on calcineurin inhibitor trough levels, donor specific antibody titres, proteinuria, and independent scoring of biopsies was not collected. Future trials investigating oxygenation in organ preservation should consider collecting the necessary data to allow in-depth analysis of the effect on acute rejection. We suggest that using eGFR as a primary endpoint in kidney transplantation trials requires careful consideration. In this preservation trial, the primary endpoint should be interpreted with the sensitivity analysis. Indeed, reporting eGFR only in pairs of donated kidneys in which both transplanted kidneys were functioning at 12 months post-transplant excludes informative dropouts, which could be relevant and associated with the trial intervention (and were considered in the sensitivity analysis). It could be argued that the sensitivity analysis of the primary outcome should have been the primary outcome from the start, although, because an effect on graft survival was not anticipated, underlying assumptions would have weakened the sample size calculation.

Although initially we wanted to measure renal function from a 24 h urinary creatinine clearance, this primary endpoint had to be changed to eGFR, which was originally a secondary endpoint. eGFR at 12 months is independently associated with longer-term graft survival in all donor types (after circulatory death, brain death, and in living donors).[17] Also, eGFR presents a clinically important outcome measure already integrated in daily practice and was reproducibly attainable in all participating centres. The CKD-EPI equation was therefore chosen to replace the 24 h urinary creatinine clearance to estimate GFR because it reflects the true GFR of the transplanted kidney better than other calculations of GFR or serum creatinine values.[16] Our findings are supported by the analysis of the original primary endpoint that showed improved 24 h urinary creatinine clearance at 12 months post-transplant in the $HMPO_2$ group in 77 of 106 kidney pairs.

This trial focused on older kidneys donated after circulatory death; therefore, future studies should focus on the extent to which $HMPO_2$ would benefit kidneys donated after brain death, and whether oxygenation during the entire preservation period is necessary. Research comparing $HMPO_2$ with other emerging perfusion strategies such as normothermic perfusion, will be important. For $HMPO_2$ to be supported by health-care funders, a health–economic analysis is needed. Adding oxygen to current standard HMP would be a low additional cost and the cost-effectiveness of HMP has already been shown.[22] Furthermore, our results suggest that considerable benefits will accrue, not only from reduced severe complications but also from reduced diagnostic procedures and hospital readmissions associated with acute rejection, and most importantly from improved graft survival, reducing the cost of chronic dialysis.

In this international, multicentre trial in kidney preservation we have shown that $HMPO_2$ confers a clinically relevant benefit compared with standard HMP. $HMPO_2$ improved renal function while taking improved graft survival into account and reduced severe post-operative complications and kidney rejection after transplantation of kidneys donated after circulatory death. Given that the cost for additional oxygen is low and the benefits for patients appear considerable, this new and rather simple extension to the current preservation strategy has the potential for quick implementation in clinical practice to improve patient outcomes and reduce health-care costs.

Contributors

IJ, HGDL, SRK, JP, and RJP designed this study with help from other authors. RJP is the chief investigator of the COPE Consortium. IJ, AB, HSH, FEMvdL, JP, and RJP oversaw the data collection. IJ, HSH, and RJP were responsible for the clinical conduct of the study in the respective trial regions. IJ, LD, and SRK were responsible for the statistical design and analysis. SRK provided governance oversight to ensure the study adhered to all regulatory and ethical requirements. IJ wrote the manuscript with input from all authors. All authors reviewed the manuscript. JP and RJP contributed equally to this work.

Declaration of interests

AB and HGDL report support from the European Commission 7th Framework Programme, during the conduct of the study. HGDL is board member of Dutch Transplantation Society and member of the implementation group for Machine Perfusion in Netherlands. SRK reports personal fees from OrganOx, outside the submitted work. All other authors declare no competing interests. Organ Assist provided the perfusion device and disposables, and was not involved in study design, conduct, data analysis, or manuscript preparation.

Data sharing

The COPE consortium supports wider dissemination of information from the research it conducts to increase cooperation between investigators. The de-identified individual participant data and data dictionary will be made available to researchers on request as detailed in the appendix (p 15).

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