



Supplemental oxygen during hypothermic kidney preservation: A systematic review



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ABSTRACT

We reviewed the evidence for ex-vivo Supplemental Oxygen during Hypothermic preservation (SOH) for deceased donor kidneys. Bibliographic databases were searched for human and animal studies of SOH in kidney transplantation reporting on patient or animal survival rate, discard rate, technical complications or renal function outcomes. We make special reference to a specific subgroup: supplemental oxygen applied during cold perfusion, referred to as Hypothermic Oxygenated Perfusion (HOP). Four human and 25 animal studies were identified. The data present conflicting results but suggest that the effects of oxygen on restoring kidney function during preservation may be of value for DCD kidneys and/or kidneys that have undergone a period of hypotension, warm ischemia or poor perfusion in the donor. There is very little information available from human or animal studies. This work highlights to the transplant community that far more high quality clinical studies are required to understand this technology and its role before widespread clinical introduction.

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1. Introduction

With changes to the donor pool in many countries, transplant professionals have had to re-consider kidneys from donation after circulatory death (DCD), as an additional supply of transplantable organs for their patients. Traditionally kidneys from DCD are considered to be less good because, unlike donation after brain death (DBD), kidneys from DCD are exposed to warm ischemic injury, which may result in higher rates of delayed graft function (DGF) associated with acute rejection and longer hospital stay [1]. More recent evidence however has shown that the long-term graft survival rate for kidneys from both donor types is equivalent [2].

The introduction of fluids specifically designed for organ preservation in the 1970s led to the rapid adoption of static cold storage preservation techniques over Hypothermic Machine Perfusion (HMP) [3,4]. A variety of preservation solutions have subsequently been used [5]. Recent studies using more advanced technology have shown benefits of machine perfusion [6]. Nevertheless, a recent systematic review and meta-analysis comparing machine perfusion with cold storage did not produce convincing evidence of the long term benefits [7].

The demand for improved preservation has led to continuing investigation of supplemental oxygenation in a variety of forms of machine perfusion and other preservation techniques. Methods of providing Supplemental Oxygen during Hypothermic preservation (SOH) include: oxygenated perfusate or perflurocarbon emulsion, hyperbaric oxygenation by the delivery of oxygen under increased atmospheric pressure, or retrograde persufflation of gaseous oxygen bubbled through the renal vasculature [4]. Under this bracket, we have specified a subgroup that we have called Hypothermic Oxygenated Perfusion (HOP), whereby additional oxygen is provided during cold ex-situ perfusion of the kidney. It is important to note that the methods so far used to deliver additional oxygen differ considerably in their technicalities and the results with one method are not necessarily transferable to another.

It is suggested that during hypothermic preservation, stores of adenosine triphosphate (ATP) are depleted, leading to a build-up of toxic substances and ultimately apoptosis and necrosis [4]. Additional oxygen may support the mitochondrial synthesis of ATP and in turn delay the injury process [4]. Some studies show that ATP could be restored to normal levels with the addition of oxygen during cold preservation [8].

To date there have been no systematic reviews comparing SOH with non-oxygenated preservation techniques. The primary aim of this study is to systematically review the evidence for SOH for kidney allografts. We reviewed the impact of SOH on patient and animal survival, and renal function of transplanted kidneys. This work is necessary due to

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the rapid development of SOH technology and its clinical adoption in some regions.

2. Materials and methods

The study was registered with the PROSPERO database of systematic review protocols on 12 August 2013 and can be accessed online (PROSPERO ID: CRD42013005170). The review was reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [9].

2.1. Eligibility criteria

Eligibility criteria included human (adult and pediatric) and animal kidney donors from whom the kidney underwent a period of ex-vivo preservation with supplemental oxygen (SOH) and were subsequently transplanted. We excluded studies of in situ preservation only in the donor. Hypothermic conditions were defined as temperatures $<35^{\circ}\text{C}$ or when the preservation method was described as 'hypothermic' in the study report. All study designs were included. We included direct comparisons of oxygenated preservation versus non-oxygenated preservation and studies investigating oxygenated preservation only. Studies had to report on at least one of the pre-specified outcomes. Primary outcomes included: recipient patient or animal survival, discard rate (defined as the number of organs not transplanted from suitable donors), technical complications, graft survival (defined as death censored or not death censored), creatinine clearance (peak serum creatinine within first week), estimated or calculated glomerular filtration rate (GFR), and delayed graft function and primary non-function (both as defined by the original study).

2.2. Information sources

The following databases were searched: Ovid's Medline (from 1948), Ovid's Embase (from 1974), the Transplant Library database from the Centre for Evidence in Transplantation, and Cochrane's CENTRAL database. The final literature searches were conducted on 19 April 2016. Searches consisted of medical subject headings and keywords combined with free text (Supplementary file 1). No language or date limits were applied.

2.3. Study selection and data extraction

Search results from each database were combined and duplicate references removed using Endnote X5 (Thomson Reuters, Philadelphia). References were screened independently by two authors (K.P. and J.O.C.) based on their title and abstract and the full text was obtained. Data was extracted independently and in duplicate by KP and LHMP. Discrepancies were resolved by discussion.

2.4. Risk of bias assessment

The two reviewers (KP and LHMP) assessed the risk of bias independently. Randomized controlled trials (RCTs) in humans were assessed by the Jadad scale [10] plus the description of allocation concealment and whether the analysis was based on the intention-to-treat (ITT) principle. Case series in humans were evaluated by a quality assessment tool using the modified Delphi technique [11]. Animal studies were assessed by a risk of bias tool developed by Krauth et al. [12]. The methodological quality of case reports was not assessed.

3. Results

The search identified 1942 unique references, which were screened for relevance, and subsequently 217 references were obtained for full

text review. From these 188 studies were excluded for the reasons presented in Fig. 1. A total of 29 studies (five RCTs [13–16], 14 cohort studies [17–30], eight case series [8,31–37] and two case reports [38,39] met our inclusion criteria. Four studies were in humans [14,31,32,39] and 25 were in animals. Of the 25 animal studies, all were auto-transplantation studies, eight were porcine, 15 canine, one in rats and one in rabbits. The methods of oxygenation in both human and animal studies included: ultra-barc oxygenation, membrane oxygenation, air pump oxygenation, oxygenated persufflation, oxygenated machine perfusion and hyperbaric oxygenation. Due to the study types identified, it was not possible to do a statistical summary analysis, so we present a narrative review of the results.

3.1. Risk of bias

The one human RCT by Rolles et al. [14] (Table S1) was quasi-randomized and described the patient withdrawal and dropout rates, but descriptions of randomization and blinding were not recorded. In addition, there was no description of allocation concealment or ITT analysis. The two human case series clearly reported the interventions, outcomes and follow up times but the study aim and information about the donor/recipient was not stated in the case series by Fuchinoue et al. [32]. The fourth human study was not scored as it was a report of two cases [39]. The risk of bias was assessed for all but one animal study, which was a case report [38] (Tables S3–S5); in all four RCTs the authors described the animal characteristics and a minimum of one week follow up (Table S3) [13,15,16,40]. The four RCTs did not report concealment of allocation, blinding of outcome assessment, ITT analysis, co-morbidities, inclusion/exclusion criteria or sample size calculation. All 14 animal cohort studies accounted for all animals, and presented a minimum of 1 week follow up (Table S4) [17–29]. All 6 animal case series accounted for all animals and presented a minimum of 1 week follow-up to report on creatinine clearance (Table S5) [8,33–37]. None of the case series reported sample size calculation. Overall, the animal studies presented the primary outcomes, accounted for attrition bias and described the animal characteristics but reported poorly on sample size calculation and co-morbidities.

4. Human study results

Four human studies were identified, which included one RCT [14], two case series [31,32] and one report of two cases [39] (Table 1). The RCT compared oxygenated persufflation with cold storage following a mean warm ischemia time (WIT) of 55 min (range 13–80 min) [14]. Patient survival at 15 days was 100% in both groups ($n = 20$ recipients). Renal function was measured by serum creatinine values at 15 days with no significant difference shown between the groups.

Oxygenated persufflation was also used in a human case series of four recipients [31]. Patient survival at 4 weeks was 75% and results showed that renal function may be maintained up to 1 month, following 2 hours of oxygenated persufflation. However, the study presented an unintentional period of increased persufflation time, and concluded that renal function may incur irreversible damage if persufflation exceeds 2 hours. Warm ischemic time ranged from 0 to 35 min. In the human studies of oxygenated persufflation the difference in survival rates (75–100%) may be attributed to differences in follow up time (15 days or 4 weeks) or WIT (0–35 min or 55 min) [14,31].

A human case series of 44 recipients reported on HOP using an oxygenated emulsion [32]. Patient survival rates were not presented, but graft survival was 80% at 12 months. Kidney function was well maintained up to 2 years post-transplant. A case study presented two kidneys that had undergone SOH in a hyperbaric chamber at either 3 or 5 atm of hyperbaric oxygen pressure [15]. The 2 recipients died with functioning grafts at eight and 16 days.

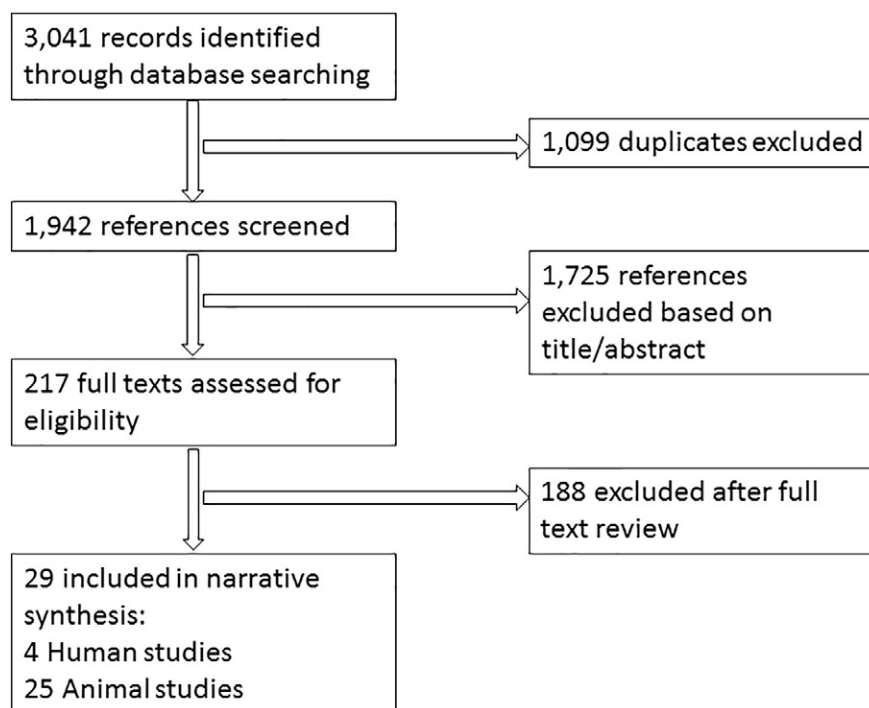


Fig. 1. PRISMA flow diagram of study selection and exclusion.

5. Animal studies

In total 25 animal studies were identified, of which eight were comparative (Table 2) and 17 evaluated SOH without comparison to HMP (Table 3). We present results from these animal studies grouped by species.

5.1. Studies comparing SOH to HMP in porcine models (Table 2)

One RCT used a *LifePort® Kidney Transporter* (Organ Recovery Systems, Chicago, USA) in an auto-transplant model of heart beating donation to compare HOP with HMP by using a membrane oxygenator [40]. After 1 week, there was no significant difference in renal function between the groups [40]. Another comparative study of HOP versus HMP used the *Kidney Assist®* (Organ Assist, Groningen, The Netherlands) in model of DCD (60 min WIT). There was no significant difference in

peak serum creatinine after surgery [19]. Retrograde oxygenated persufflation was compared to HMP in a model of DCD (60 min WIT) [15]. After 7 days, serum creatinine returned to normal in the persufflation group, but remained significantly elevated in the kidneys that were machine perfused with air. There was no significant difference in survival at the short follow-up of 7 days.

5.2. Studies comparing SOH to HMP in other animal models (Table 2)

One RCT in canines presented results from kidneys that were preserved in the *LifePort® Kidney Transporter* (Organ Recovery Systems, Chicago USA) without oxygen or in the *RM3® Kidney Perfusion System* (Waters Medical LLC, Rochester, USA) which has a membrane oxygenator [13]. All kidneys underwent 45 min WIT and supplemental oxygen made no difference in animal survival or kidney function.

Table 1
Included human studies of supplemental oxygenation during hypothermic preservation.

Author (year), Study type	Interventions	Perfusion parameters	Follow up and Patient survival	Patient serum creatinine (μmol/L)
Rolles (1989), RCT	1) Cold perfusion followed by oxygen persufflation (n = 10) 2) Storage in RME solution (n = 10)	Temperature NR Duration NR pO ₂ was 13–15 mmHg	15 days 1) 100% 2) 100%	Mean at day 15: 1) 457 ± 136 μmol/L 2) 826 ± 162 μmol/L (p = NS)
Fuchinoue (1986), case series	Cold machine perfusion with Oxypherol emulsion (n = 30)	5–8 °C 7.5 h 100 mL/min	1 year	Mean at 1 month: 194 ± 80 μmol/L
Flatmark (1975), case series	Continuous machine perfusion and oxygenated persufflation. The oxygenator gassed the perfusate with a mixture of 33% O ₂ , 66% N ₂ and 1% CO ₂ (n = 4).	8–10 °C 1–2 h 60 mmHg perfusion pressure; 280 ml/min O ₂ flow	Not reported 1 month 75%	Mean at 1 month: 194 μmol/L
Manax (1965), case report	1) Cold perfusion followed by placement in a hyperbaric chamber at 3 atm pO ₂ (n = 1) 2) Cold perfused followed by placement in a hyperbaric chamber at 5 atm pO ₂ (n = 1)	2 °C Duration NR 3 atm pO ₂ or 5 atm pO ₂	7 months Both died with functioning grafts at 8 and 16 days	NR

RME = Ross, Marshall and Escott's solution; NR = Not Reported. P-values are as calculated in the original reports.

Table 2

Included animal studies of Supplemental Oxygenated Hypothermic (SOH) preservation techniques compared to Hypothermic Machine Perfusion (HMP) (n = 8).

Author (year), design, number of donors (n)	Preservation technique including method of oxygenation (number of organs transplanted)	Perfusion parameters	Follow up and Animal survival	Animal serum creatinine ($\mu\text{mol/L}$)/ creatinine clearance
PORCINE				
Gallinat (2012), RCT, auto-transplantation	1) Continuous perfusion in the LifePort with a membrane oxygenator (5) 2) Non-oxygenated perfusion as above (5)	21 h $pO_2 > 500$ mmHg perfusion pressure was 30 mmHg	1 week NR	Peak creatinine clearance within the first week: 1) 33 ml/min 2) 31 ml/min p = NS
Treckman (2009), RCT, auto-transplantation	Following 60 min WIT: 1) Continuous retrograde oxygenated persufflation where gaseous oxygen was administered through the renal vein (7) 2) Cold storage (7) 3) Pulsatile perfusion with room air inside a perfusion box (7)	5 °C 4 h $pO_2 = 18$ mmHg perfusion pressure was 40–50 mmHg	7 days 1) 100% 2) 57% 3) 60%	Creatinine clearance within 7 days post transplantation: 1) 186.5 ± 66.3 $\mu\text{mol/L}$ 2) 301.4 ± 228.9 $\mu\text{mol/L}$ 3) 424.3 ± 251.9 $\mu\text{mol/L}$ mg/dL Groups 1 v 2, p ≤ 0.05
Thuillier (2013), cohort study, auto-transplantation, n = 10	Following 60 min WIT: 1) Continuous machine perfusion with oxygen using the Kidney Assist (4) 2) Continuous machine perfusion without oxygen using the Kidney Assist (4)	4 °C 22 h pO_2 unclear perfusion pressure was 25 mmHg	3 months NR	Serum creatinine 2 weeks post transplantation: 1) 800 $\mu\text{mol/L}$ 2) 1200 $\mu\text{mol/L}$ Not statistically tested
CANINE				
Lindell (2013), RCT, auto-transplantation, n = 20	Following 45 min WIT: 1) Continuous perfusion in the RM3 and oxygenated by sweeping air over the membrane oxygenator (8) 2) Pulsatile perfusion in LifePort (8) 3) Non-pulsatile perfusion in LifePort (4)	6–8 °C 24 h perfusion pressure was 45 mmHg, O_2 flow was 2–4 L/min	7 days 1) 100% 2) 100% 3) 50%	Serum creatinine at 7 days: 1) 424.3 $\mu\text{mol/L}$ 2) 380.1 $\mu\text{mol/L}$ 3) 795.6 $\mu\text{mol/L}$ Groups 2 v 3, p < 0.05
Quin (1973), cohort study, auto-transplantation	Following 40 min WIT: 1) Continuous perfusion in Vickers unit using Phenoxybenzamine, clear solution (6) 2) Continuous perfusion in Vickers unit using Phenoxybenzamine, cloudy solution (6) 3) Continuous perfusion in Vickers unit without phenoxybenzamine (6) 4) Cold storage (7) 5) Non-oxygenated perfusion using the Brunius and Gelin technique (6) 6) Non-oxygenated perfusion using Modified Dextran 40 solution followed by Perfudex (10)	5 °C Perfused for 4 h, followed by storage under hypothermia and hyperbaria for 20 h 2 mL/min, 3 atm O_2	NR 1) 83% 2) 0% 3) 33% 4) 14% 5) 33% 6) 30%	Serum creatinine values ² : 1) 415.5 $\mu\text{mol/L}$ 2) - 3) 472.9 $\mu\text{mol/L}$ 4) 733.7 $\mu\text{mol/L}$ 5) 592.3 $\mu\text{mol/L}$ 6) 680.7 $\mu\text{mol/L}$ Not statistically tested
Rolles (1984), cohort study, auto-transplantation	1) 30 min WIT, then 24 h continuous oxygenated persufflation using the following pretreatment and washout fluids: 1a) Ringer's/mannitol pretreatment, flushed with RME (5) 1b) Ringer's/mannitol pretreatment, flushed with CC (5) 1c) Frusemide/saline pretreatment, flushed with JF (6) 1d) Frusemide/saline pretreatment, flushed with RME (5) 2) 60 min WIT then continuous 24 h preservation, pretreatment with mannitol/Ringer's and flush with RME and the following persufflation gases: 2a) No persufflation (5) 2b) Oxygen persufflation (5) 2c) Air persufflation (6) 2d) Nitrogen persufflation (5) 2e) Helium persufflation (5) 3) 30 min WIT then continuous 48 h preservation, all received mannitol/Ringer's pretreatment and flush with RME 3a) No persufflation (5) 3b) Oxygen persufflation (5)	0 °C 24 h or 48 h O_2 flow was 30–300 ml/min	3 months 1a) 100% 1b) 100% 1c) 0% 1d) 80% 2a) 0% 2b) 60% 2c) 50% 2d) 0% 2e) 0% 3a) 20% 3b) 80%	Peak serum creatinine: 1a) 550 ± 180 $\mu\text{mol/L}$ 1b) 283 ± 48 mmol/L $\mu\text{mol/L}$ 1010 ± 140 mmol/L 1d) 668 ± 210 $\mu\text{mol/L}$ 2a) >1300 mmol/L 2b) 946 ± 211 $\mu\text{mol/L}$ 2c) 1240 ± 260 $\mu\text{mol/L}$ 2d) 1450 ± 130 $\mu\text{mol/L}$ 2e) >1300 $\mu\text{mol/L}$ 3a) 1150 ± 150 $\mu\text{mol/L}$ 3b) 815 ± 99 $\mu\text{mol/L}$ Groups 2c v 2b, p < 0.05
Brasile (1994), cohort study, auto-transplantation	1) Continuous pulsatile perfusion with O_2 supplemented perfusate at 32 °C for 4 h (1) 2) Continuous pulsatile perfusion with O_2 supplemented perfusate at 32 °C for 7 h (1) 3) Continuous pulsatile perfusion with O_2 in saline at 32 °C for 4 h (1) 4) Cold storage in an airtight container filled with O_2 supplemented perfusate at 25 °C for 4 h (1) 5) Pulsatile perfusion with a basal perfusate without O_2 (1)	25–32 °C 4 h or 7 h perfusion pressure was 62 mmHg, O_2 flow 60–100 ml/min	9 days Euthanized days 2–9	Peak serum creatinine: 1) 353.5 $\mu\text{mol/L}$ 2) 707.2 $\mu\text{mol/L}$ 3) Not transplanted 4) 442 $\mu\text{mol/L}$ 5) 707.2 $\mu\text{mol/L}$ Not statistically tested
LEPORINE				
Pegg (1974) cohort study, auto-transplantation	1) Continuous oxygenated perfusion with a filter and a bubble trap to the arterial cannula. Kidneys were perfused with 95% O_2 and 5% CO_2 (10) 2) Kidneys were perfused with 95% air and 5% CO_2 (10) 3) Kidneys were perfused with 95% nitrogen and 5% CO_2 (10)	5 °C 24 h perfusion pressure 40 mmHg	3 months 1) 80% 2) 80% 3) 60%	Peak serum creatinine: 1) 813.3 ± 212.2 $\mu\text{mol/L}$ 2) 795.6 ± 132.6 $\mu\text{mol/L}$ 3) 813.3 ± 159.1 $\mu\text{mol/L}$ P = NS

Studies are grouped by type and experimental animal, in reverse chronological order. WIT = warm ischemia time; RME = Ross Marshall and Escott's solution; CC = Cambridge isotonic citrate; JF = Johnson's flush solution, NR = Not Reported. Kidney Assist = Lifeport = LifePort® Kidney Transporter (Organ Recovery Systems, Chicago USA), Kidney Assist® (Organ Assist, Groningen, The Netherlands), RM3 = RM3® Kidney Perfusion System (Waters Medical LLC, Rochester, USA). P-values are as calculated in the original reports.

One canine cohort study compared a variety of persufflation regimens and gases [18]. Kidneys showed better renal function at 3 months when oxygen was used for persufflation rather than another gas, or no persufflation at all [18].

One case series in 20 rabbits found better 2 month survival for kidneys preserved with oxygen persufflation (80%) compared with nitrogen persufflation (60%) [28]. There was no significant difference in renal function at 3 months, whether rabbit kidneys were preserved with air, oxygen or nitrogen in this study.

One cohort study in canines comparing hyperbaric perfusion against HMP or cold storage following 40 min WIT showed a wide range of overlapping survival rates (0%–83% and 14–33%) [17]. Serum creatinine values returned to normal at a slower rate when the kidney was preserved without oxygen. Following transplantation of canine kidneys preserved by oxygenated emulsion, elevated serum creatinine levels returned to preoperative levels in, however they continued to rise in the non-oxygenated group [29].

5.3. Other porcine studies of SOH (Table 3)

One RCT compared retrograde oxygenated persufflation versus cold storage following a range of WITs [16]. Survival was 100% ($n = 6$) for persufflation preceded by 60 min WIT, 71% ($n = 7$) for persufflation preceded by 90 min WIT and 33% ($n = 3$) for persufflation preceded by 120 min WIT. In kidneys with 60 min WIT, serum creatinine levels were significantly lower following HOP than cold storage.

One recent animal study by Minor et al. 2015 used HOP with either KPS-1 or Custodiol-N supplemented with dextran 40 as the preservation fluid in the LifePort® Kidney Transporter [30]. Renal function normalized in both groups within a few days.

Two cohort studies compared cold storage in UW with HOP (membrane oxygenation in polysol solution) [20,21]. Survival rates were lowest when kidneys were preserved by HOP at a relatively high pressure of 60/40 mmHg (60%, $n = 5$ recipients) [21]. The survival rate of kidneys preserved by all other regimens, including non-oxygenated cold storage, was 100% [20,21]. However, renal function was significantly better in the groups preserved by membrane oxygenation at regular and low pressures compared to cold storage.

One case series presented 100% porcine survival at 7 days with kidneys preserved by oxygenated machine perfusion with HTK or Belzer solution, versus 80% when kidneys were cold stored (numbers unclear) [8]. The report describes serum creatinine levels returning to normal levels significantly faster when kidneys were preserved by HOP compared to cold storage.

5.4. SOH in other animal studies (Table 3)

Seven canine studies presented survival rates following a period of oxygenated preservation within a hyperbaric chamber [24–26,33–36]. A higher rate of survival was presented when perfusion was administered at the beginning of the hyperbaric preservation for at least four hours (54–100%) [25,26,34] or throughout the entire hyperbaric preservation period (27%–100%) [33,35]. Kidneys that underwent a period of hyperbaric storage and perfusion for at least four hours presented better rates of survival than those kidneys that were not perfused [25,33,35]. Renal function was significantly better in kidneys that were perfused throughout the hyperbaric preservation period compared to those kidneys that were not perfused at all [33].

There was one study of persufflation in canines [22]. Rates of 100% survival were seen in the oxygenated persufflation groups ($n = 13$) compared with 38–50% survival ($n = 20$) in kidneys that were preserved by the other methods, (bubbled oxygenation of the solution surrounding the kidney, flushing with oxygenated solution, and cold storage). Renal function was significantly better in kidneys preserved by HOP at low pressure (12 mmHg) compared with cold storage, with 21 days follow-up. There was no difference in renal function between

kidneys preserved by oxygenated persufflation at high pressure (60 mmHg) or when surrounded by bubbled oxygen, flushed or cold stored.

In one study in rats, kidneys that underwent cold storage or SOH with retrograde oxygenated persufflation showed low survival rates of 0% and 30% respectively (following 30 min WIT) [27]. Renal function did not differ between these two groups.

6. Discussion

This review has examined the evidence for supplemental oxygenation during ex-vivo hypothermic preservation of kidneys. There are limited results available from studies in humans and these are restricted to data published before 1990. It is likely that the limited number of human studies in this review correlates with the adoption of static cold storage preservation as the dominant method in recent decades. Results from animal studies are inconsistent and conflicting and no firm conclusion can be drawn from them given the study qualities, era and subsequent technological developments, which mean that the reality of SOH is now quite different.

At present, there is no clear consensus among the research community about the need for oxygen supply during hypothermic machine perfusion. The supposed benefits of supplemental oxygen have been assessed through the evaluation of several pathways that are surrogates for clinical outcomes. These have included cellular ATP, histological assessment of fibrosis and edema, cytokine release and maintenance of the renal microcirculation. Rolles et al. showed significantly better renal function of kidneys preserved with HOP (persufflation with oxygen versus air) [18] but were unable to demonstrate any re-synthesis of ATP or adenosine diphosphate. In comparison, a more recent study by Minor et al. has shown an eight fold increase in tissue ATP in kidneys that underwent HOP compared to HNOP [8].

The development of tissue fibrosis due to anoxic metabolism is one indicator of ischemic damage and has been used as a surrogate outcome of renal function in studies of preservation methods [19]. However, in one study, the development of tissue fibrosis in renal samples was equivalent in both groups [19]. Macrophages and monocytes as indicators of both innate and adaptive immune responses were also measured, with no significant difference between the groups.

Doorschodt et al. presented results of the histological examination of kidneys preserved by HOP, which showed that less tissue edema developed in HOP kidneys compared to kidneys that were cold stored [20]. A cohort study by Maathuis et al. describes how endothelial cells are critically sensitive to anoxia, which can lead to endothelial swelling and no-reflow (when the kidney fails to re-perfuse following ischemic injury) [21]. They showed that oxygen during preservation minimizes pro-inflammatory cytokine production. Low perfusion pressure in combination with high oxygen availability may improve renal microcirculatory parameters to reduce the formation of reactive oxygen species after reperfusion. Results of the study by Maathuis et al. highlight that damage from perfusion can be avoided at lower pressures, and that oxygen supplementation may improve microcirculation during the preservation and post-transplant period [21].

The more clinically relevant outcomes from the studies included in this review are at times conflicting and overall do not provide convincing evidence for routine introduction of HOP. Two RCTs using porcine and canine kidneys did not find any beneficial effect of HOP on renal function whether the WIT was 0 or 45 min [13,40]. In the RCT by Lindell et al. the authors suggested that the lack of impact on renal function was caused by the lower temperature of the non-oxygenated system compared to the oxygenated RM3 machine, although the difference in temperature was very small (5.5 versus 2.2 degrees centigrade respectively). The study concludes that at very low temperatures active or passive oxygenation is not necessary for DCD kidneys [13]. In the RCT by Gallinat et al. the kidney was immediately preserved without the preceding tissue damage

Table 3

Included animal studies of Supplemental Oxygenated Hypothermic (SOH) preservation not compared to Hypothermic Machine Perfusion (HMP) (n = 17).

Author (year), design, number of donors (n)	Preservation technique including method of oxygenation (number of organs transplanted)	Perfusion parameters	Follow up and Animal survival	Animal serum creatinine $\mu\text{mol/L}$ / creatinine clearance
PORCINE				
Minor (2015), cohort study, auto-transplantation	1) Continuous oxygenated perfusion with Custodiol-N and Dextran 40 in with membrane oxygenator (6) 2) Continuous oxygenated perfusion with MPS in Lifeport with membrane oxygenator (6)	Temperature NR 21 h $pO_2 > 500$ mmHg	7 days NR	No significant difference reported
Doorschodt (2009), cohort study, auto-transplantation	1) Continuous oxygenated perfusion with Airdrive system using polysol and a membrane oxygenator (7) 2) Cold storage with polysol (7) 3) Cold storage with UW (7)	2–6 °C 20 h 25 mmHg	7 days 1) 100% 2) 100% 3) 100%	Creatinine clearance at 7 days: 1) 33.3 ± 4.9 ml/min 2) 33 ± 6.1 ml/min 3) 11.2 ± 19.6 ml/min Groups 1 v 3, $p < 0.01$
Treckman (2006), RCT, auto-transplantation	1) 60 min WIT, continuous retrograde oxygenated persufflation (6) 2) 90 min WIT, continuous retrograde oxygenated persufflation (7) 3) 120 min WIT, continuous retrograde oxygenated persufflation (3) 4) 60 min WIT, cold storage (7) 5) 90 min WIT, cold storage (6) 6) 120 min WIT, cold storage (3)	4 °C 4 h 18 mmHg	7 days 1) 100% 2) 71% 3) 33% 4) 57% 5) 83% 6) 33%	Peak serum creatinine: 1) $536.4 \mu\text{mol/L}$ 2) $1281.8 \mu\text{mol/L}$ 3) $1149.2 \mu\text{mol/L}$ 4) $972.4 \mu\text{mol/L}$ 5) $1060.8 \mu\text{mol/L}$ 6) $1547 \mu\text{mol/L}$ Groups 1 v 2, $p \leq 0.05$
Maathuis (2007), cohort study, auto-transplantation	1a) Continuous pulsatile perfusion with membrane oxygenator at 30/20 mmHg (5) 1b) Continuous pulsatile perfusion with membrane oxygenator at 60/40 mmHg (5) 2) Cold storage (5)	Temperature NR 20 h O_2 flow was 100 mL/min; intrarenal pressures were 30/20 mmHg and 60/40 mmHg HOP: 6–8 °C 18 h $pO_2 > 500$ mmHg	7 days 1a) 100% 1b) 60% 2) 100% 7 days	Peak serum creatinine within: 1a) $463 \pm 127 \mu\text{mol/L}$ 1b) $428 \pm 129 \mu\text{mol/L}$ 2) $940 \pm 90 \mu\text{mol/L}$ Groups 1 and 2 v 3, $p < 0.05$ Median serum creatinine: 1) $1100 \mu\text{mol/L}$ 2) $375 \mu\text{mol/L}$ 3) $375 \mu\text{mol/L}$ Groups 1 and 2 v 3, $p < 0.05$
Minor (2005), case series, auto-transplantation	1) Continuous pulsatile perfusion with a membrane oxygenator using HTK (NR) 2) Continuous pulsatile perfusion with a membrane oxygenator using Belzer Solution (NR) 3) Cold flush and static storage (5)	6–8 °C 18 h $pO_2 > 500$ mmHg	7 days 1) 100% 2) 100% 3) 80%	Median serum creatinine: 1) $1100 \mu\text{mol/L}$ 2) $375 \mu\text{mol/L}$ 3) $375 \mu\text{mol/L}$ Groups 1 and 2 v 3, $p < 0.05$
CANINE				
Ross (1979), cohort study, auto-transplantation	Following 30 min WIT: 1a) Continuous oxygenated persufflation via the renal vein (7) 1b) Continuous oxygenated persufflation via the renal artery (6) 2) Flush with oxygenated solution, and perfusate and storage solution were oxygenated. Renal vessels were not perfused (5) 3) Flush with oxygenated solution only but no further oxygenation (8) 4) Flushed and stored in cold hypertonic citrate solution (7)	NR 24 h 10–12 mmHg or 60 mmHg, 150 ml/min	21 days 1a) 100% 1b) 100% 2) 40% 3) 38% 4) 50%	Mean maximum creatinine: 1a) $450 \mu\text{mol/L}$ 1b) $810 \mu\text{mol/L}$ 2) $1180 \mu\text{mol/L}$ 3) $780 \mu\text{mol/L}$ 4) $1270 \mu\text{mol/L}$ Group 1a results significantly better than other groups
Grundmann (1975), cohort study, auto-transplantation	All had continuous oxygenated perfusion with human albumin and supplemental oxygen at different pressures: 1a) 15 mmHg (10) 1b) 20 mmHg (8) 1c) 40 mmHg (4) 1d) 50 mmHg (10) 1e) 60 mmHg (4)	7 °C 72 h Perfusion pressure as described pO_2 was 140 mmHg	1 week NR	Mean serum creatinine, all groups: $92.8 \mu\text{mol/L}$
Snell (1974) cohort study, auto-transplantation	All preserved in the Vickers hyperbaric oxygen perfusion unit. 1) 45 min WIT followed by hyperbaric storage and perfusion (4) 2) 60 min WIT followed by hyperbaric storage and perfusion (13) 3) Hyperbaric oxygenation with perfusion (8) 4) Hyperbaric oxygenation, no perfusion (5) 5) Hyperbaric oxygenation, 1 h perfusion (5)	5 °C Perfusion rate was 2 mL/min; pO_2 was 45 psi	28 days 1) 75% 2) 54% 3) 63% 4) 20% 5) 20%	Peak mean serum creatinine: 1) $388.9 \pm 150.3 \mu\text{mol/L}$ 2) $477.4 \pm 353.6 \mu\text{mol/L}$ Mean creatinine at 28 days: 3) $97.2 \pm 8.8 \mu\text{mol/L}$ 4) $371.3 \mu\text{mol/L}$ 5) $212.2 \mu\text{mol/L}$ Not statistically tested
Snell (1972), cohort study, auto-transplantation	Kidneys were stored in the Vickers hyperbaric oxygen perfusion unit 1) WIT 45 min (4) 2) WIT 60 min (6)	5 °C 24 h preservation beginning with 4 h perfusion Perfusion rate was 2 mL/min; pO_2 was 310 kN/m^2	28 days 1) 75% 2) 67%	Mean serum creatinine: 1) $380.1 \mu\text{mol/L}$ 2) $636.5 \mu\text{mol/L}$ Not statistically tested
Rudolf (1967), cohort study, auto-transplantation	1) & 2) hyperbaric oxygen and hypothermia at 3 atm (11) - subgroup extended preservation for 48 h at 8 or 15 atm (9) 3) Supercooling (–3 °C) and hyperbaric oxygenation 4) Intermittent perfusion, hypothermia and hyperbaric oxygenation (5) 5) No preservation, immediate re-implant(5)	2 °C 24 h Perfusion pressure was 40–60 mmHg and flow was 10–40 mL/min; pO_2 was 3 atm,	1 year 1) and 2) 45% and 0% for the subgroup 3) 40% 4) 0% 5) NR	Average creatinine clearance at 6 months: 1) & 2) 1.7 mL/min 3) 3.5 mL/min 4) – 5) – Not statistically tested
Stowe (1986), case series, auto-transplantation	Oxygenated persufflation via the renal vein: 1) With desferoxamine and Collins's solution (4) 2) No desferoxamine, Ringer's solution (4) 3) With desferoxamine and Ringer's solution (3)	4–5 °C 48 h O_2 flow rate was 150 mL/min; pO_2 was 7–10 mmHg.	22 days 1) 25% 2) 25% 3) 100%	Mean peak serum creatinine: 1) $884 \mu\text{mol/L}$ 2) $1060.8 \mu\text{mol/L}$ 3) $618.8 \mu\text{mol/L}$ Not statistically tested
Hopkinson (1972), case series, auto-transplantation	Continuous hypothermic perfusion in which oxygen pressurizes the preservation chamber and oxygenates the	5 °C 4 h	1 month	Serum creatinine at 3 days: $3.306 \pm 115.8 \mu\text{mol/L}$

(continued on next page)

Table 3 (continued)

Author (year), design, number of donors (n)	Preservation technique including method of oxygenation (number of organs transplanted)	Perfusion parameters	Follow up and Animal survival	Animal serum creatinine $\mu\text{mol/L}$ / creatinine clearance
Hendry (1968), case series, auto-transplantation	perfusate by a membrane oxygenator (5). 1) Cold storage (5) 2) Hyperbaric oxygenation but no perfusion at 3 atm O ₂ (5) 3) Kidneys were stored in the Vickers hyperbaric oxygen perfusion unit at 1 atm O ₂ (5) 4) Kidneys were stored in the Vickers hyperbaric oxygen perfusion unit at 3 atm O ₂ (5)	2 ml/min, 310 kN/m ² 10 °C 24 h Perfusion pressure was 30 mmHg; chamber pO ₂ was either 1 or 3 atm	100% 14 months 1) 100% 2) 80% 3) 20% 4) 40%	Peak serum creatinine: 1) 134.4 $\mu\text{mol/L}$ 2) 100.8 $\mu\text{mol/L}$ 3) 203.3 $\mu\text{mol/L}$ 4) 118.5 $\mu\text{mol/L}$
Lempert (1965), case series, auto-transplantation	Continuous, hypothermic, hyperbaric perfusion. 1) Perfusion for 4–7 h (9) 2) Perfusion for 18–24 h (18)	4 °C 4–7 h or 18–24 h 25 ml/min	1 year 1) 77% 2) 27%	NR
Makin (1965), case series, auto-transplantation	Cold perfusion followed by perfusion in a hypothermic, hyperbaric pressure chamber. 1a) 3 atm (7) 1b) 5 atm (4)	Temperature NR 24 h (total preservation time) 3 atm or 5 atm	Up to 8 months 9% (1/11)	NR
Booth (1966), case report, auto-transplantation	Ultrabarc oxygenation where kidneys were connected to a source of high pressure oxygen (2).	23 °C 2 h decreased from 1950 lb./in ² to 1450 lb./in ²	39 days Euthanized day 39	Creatinine at day 1: 238.7 $\mu\text{mol/L}$
MURINE				
Yin (1996), cohort study, auto-transplantation	1) 30 min WIT followed by continuous retrograde oxygen persufflation (10) 2) 30 min WIT followed by cold storage in UW (10)	4 °C 24 h O ₂ flow rate was 20 ml/min; pO ₂ was 15–25 mmHg	14 days 1) 30% 2) 0%	Mean peak serum creatinine at 2 days: 1) 515 $\mu\text{mol/L}$ 2) 582 $\mu\text{mol/L}$ P = 0.69

Studies are grouped by type and experimental animal, in reverse chronological order. WIT = warm ischemia time; HTK = Histidine-tryptophan-ketoglutarate; UW = University of Wisconsin; MPS = Belzer machine perfusion solution; OHP = atmospheres of hyperbaric oxygen pressure; NR = not reported. Airdrive = Airdrive® Portable Organ Perfusion System (QRS, Randmeer, The Netherlands), Lifeport = LifePort® Kidney Transporter (Organ Recovery Systems, Chicago USA), P-values are as calculated in the original reports.

(following zero ischemia time) therefore the authors concluded that following zero WIT there was no need for supplemental oxygen [40].

It may be possible that porcine kidneys are more tolerant of WIT than human kidneys due to a greater tolerance for ischemia [16]. In addition, most of the animal studies included were of auto-transplantation, which reduces the immunological implications drastically and may impact upon the relative effect of HOP.

The primary criticism of this review may be the low level of evidence included, however this is a key finding of the review and highlights the need for good quality clinic RCTs. An assessment of all the available evidence is crucial in determining the current state of evidence on a particular topic. The methodological quality and reporting of the human studies was poor with only one study testing the statistical significance of observed differences. The included studies were small and not powered to identify anything other than very large differences in outcomes. The short-term follow-up of most studies also means that no conclusions can be drawn about long term outcomes of HOP. In addition, several studies provided very little information about the preservation method, for example the Waters RM3 unit can be used with supplemental oxygen but this is usually operated using just air. Many studies compared HOP to static cold storage when the correct comparator would have been HMP. The degree to which the perfusion fluid in HMP systems is oxygenated is not known. Given the study mix, summary statistics would not have been an appropriate way to bring together the results of all studies, or even groups of studies.

In summary, this narrative systematic review has shown that oxygen supplementation during hypothermic preservation of kidneys presents varying results, and the method of delivery has been adapted over several decades. Some animal studies show that HOP may improve renal function for kidneys that have undergone a period of warm ischemia. The evidence from clinical studies is very limited and RCTs in humans using new technology are needed to validate whether oxygenated perfusion improves outcomes before the widespread clinical introduction of new oxygenation techniques. This work shows how little is known about SOH and far more high quality work is required to understand and develop this technology. We therefore await with interest the results of two ongoing RCTs in this area; one which will compare HOP with HMP in DCD kidneys throughout the preservation period (COMPARE Trial,

ISRCTN32967929), and one in ECD kidneys using pre-implantation HOP or HMP (COPE- POMP Trial, ISRCTN63852508).

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