# Ex-situ kidney perfusion: some like it hot others prefer to keep it cool

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## Abstract

**Purpose of review**

Machine perfusion technologies provide an opportunity for improved preservation, organ assessment and resuscitation of damaged kidneys. This review summaries the recent advancements in hypothermic and normothermic kidney machine perfusion technologies.

**Recent Findings**

Modifications to the perfusion conditions with the addition of oxygen during hypothermic machine perfusion can support a low level of metabolism which, in experimental settings, improves graft function. Normothermic machine perfusion technologies are evolving in different directions either for short resuscitation, more prolonged periods of perfusion, and the transition between hypothermic and normothermic conditions are being investigated. Clinical trials are ongoing in both hypothermic and normothermic settings.

Functional parameters can be used to assess kidney quality and although normothermic machine perfusion may hold an advantage over hypothermic machine perfusion, new metabolomics, proteomic, and genomic technologies may be applied in the future to both technologies to provide more rigorous information on kidney quality.

Promoting recovery by introducing an intervention during perfusion is an attractive area of research and therapies targeting the endothelium are a particular area of interest.

**Summary**

A great deal of research is still needed to optimise and logistically place hypothermic and normothermic perfusion technologies. In the future, we may progress towards organ-tailored preservation whereby high-risk kidneys can undergo assessment and repair before transplantation.

## Keywords

Hypothermic machine perfusion

Normothermic machine perfusion

Oxygenated machine perfusion

Graft viability

Graft resuscitation

## Introduction

*Ex-situ* kidney perfusion – also called machine perfusion (MP) – has been the topic of increased research over the past decade. Driven by the need to optimise preservation, assess graft viability, and repair damaged kidney grafts, a multitude of strategies for *ex-situ* perfusion have been developed. Hypothermic (HMP) and normothermic MP (NMP) have been studied quite intensively and are finding their way into clinical practice [1*].

We review recent developments and explore the future of these technologies.

## Preservation

*Ex-situ* perfusion instead of static cold storage (SCS) might improve preservation.

### Hypothermic

Data from randomised controlled trials and several meta-analyses have provided good evidence showing a reduction of delayed graft function (DGF) with HMP in brain dead donor kidneys [2-6*]. Although meta-analyses seem to support this finding in kidneys donated after circulatory death (DCD) [4], the two largest randomized controlled trials in DCDs contradict each other. One shows DGF reduction with HMP while the other does not [7,8]. Ongoing clinical trials might provide additional evidence [1*].

More data are needed focusing on the effect of HMP on long-term graft function and survival [1*,9*]. An improved 1 and 3-year graft survival after HMP, most pronounced in kidneys from expanded criteria donors (ECD) has been shown [10,11]. But this effect is not present in DCD kidneys [7,8,10]. Also, a clear benefit of HMP on relatively rare outcomes, such as primary non-function, has not been shown.

**Normothermic**

The clinical application of NMP has recently emerged. The report on NMP in a series of 17 ECD kidneys is the largest to date [12]. NMP showed a significant reduction in DGF compared to a matched cohort of SCS-kidneys (4% vs 36%, respectively). NMP is designed to resuscitate the kidney after SCS. NMP is carried out for 1h whilst the patient is being prepared for transplantation. After NMP, the kidney is flushed with cold preservation solution and placed back on ice until transplantation [13]. A multicentre randomized controlled trial in DCD category III/IV kidneys is ongoing in the UK (ISRCTN15821205). The trial will randomize kidneys to NMP (n=200) or SCS (n=200) and is due to end in 2020.

There is no survival data available on NMP.

## Refinement

Hypothermic conditions are designed to suppress metabolism and negate the need for oxygenation. Whereas, normothermic conditions restore cellular metabolism and therefore require oxygen.

***Hypothermia***

It is not known how HMP exerts its beneficial effects but perfusion likely helps maintain a healthy endothelium and replenish ATP; it might even alter the organ's immunogenicity [14,15*,16*]. Increased nitric oxide–dependent vasodilation and improved cortical microcirculation at reperfusion regulated through improved endothelial nitric oxide synthase phosphorylation has been demonstrated in HMP-preserved DCD porcine kidneys [17]. Moreover, a degree of vascular shear stress, critical for normal vascular function, is maintained by the flow during HMP. This could have an anti-inflammatory effect through activation of flow-dependent genes [18,19].

Modifying HMP conditions towards supporting metabolism by adding oxygen is compelling. Oxygenation during HMP restores ATP content in the kidney [20,21*]. In the liver, oxygenated HMP reversibly suppresses mitochondrial oxidative metabolism after SCS. Mitochondrial release of

reactive oxygen species at reperfusion is decreased with deactivation of numerous intracellular and extracellular pathways, including the inflammatory response [14,22]. There is some evidence to support a similar hypothesis in kidneys [21*]. Preclinical studies have shown improvement of graft function after oxygenated HMP, particularly in DCD kidneys [20,23-26]. The effect of oxygenated HMP is being investigated in two randomized clinical trials, one in DCD (ISRCTN32967929), the other in ECD (ISRCTN63852508) which will finish in 2018 [1*].

***Normothermia***

ATP replenishment, which prevents further breakdown of metabolites and restores cellular function, is likely to be the key component of protection during NMP [27]. NMP also upregulates protective mechanisms (e.g. heat shock protein 70) that aid regeneration and repair [27]. Restoration of circulation at a near-physiological pressure is likely to be beneficial to the endothelium maintaining critical shear stress levels

There is little information on the optimal NMP perfusate and apart from the acellular perfusate described by Brasile *et al* [28,29], recent techniques use a packed red blood cell based solution [12,30*,31**]. The red cells can be suspended in crystalloid [12] or Steen solution [30*].

NMP systems typically use a super-physiological concentration of oxygen (95%) balanced with carbon dioxide to ensure optimal oxygenation and acid based balance. Nonetheless, in a highly oxygenated environment there is the danger of promoting reactive oxygen species production and inducing oxidative damage. Kron *et al*, demonstrated that an increased release of injury markers (8-OHdG, HMGB-1) into the perfusate and more TLR-4 positive cells suggesting more oxidative stress during NMP in a rodent model [21]. NMP of liver report the application of air balanced with physiological levels of oxygen to maintain acid base homeostasis [32*]. Research in the kidney to determine the optimal oxygen concentration is underway.

## Assessment

The ability to assess a kidney and predict the outcome has been a major area of research. *Ex-situ* perfusion gives information on flow and renal resistance characteristics. Sampling of the perfusate allows the measurement of injury or function which could be informative.

### *Hypothermia*

There is no good evidence to suggest that accurate assessment of kidney viability during HMP is possible. Previous work has shown that an association between perfusion characteristics – such as flow and renal resistance – exists. Higher resistance is an independent risk factor for the development of DGF [33] and primary non function [34]. Importantly, however, the predictive value of the renal resistance is too low for it to be used as a single and reliable viability measure. Similar evidence exists for commonly used injury markers such as glutathione S-transferase, lactate dehydrogynase, heart-type fatty acid binding protein, redox-active iron, IL-18, and neutrophil gelatinase-associated lipocalin (NGAL) [35]. Despite being an independent risk factor for DGF [36] and primary non-function [37], the associations and predictive capacity of any determined cut-off were too low to be of any help at the individual kidney-recipient level. Recent work from the US adds to the evidence that perfusion dynamics and currently identified injury markers are not useful diagnostic tools when considered on their own. In a large prospective study Parikh *et al* studied the relationship between several perfusate markers (NGAL, liver-type fatty acid binding protein, IL-18, and kidney injury molecule-1), renal resistance and flow, and outcome in 671 kidneys preserved by HMP. Perfusate NGAL and liver-type fatty acid binding protein measured near the end of HMP as well as resistance and flow were only modestly associated with 6 month estimated glomerular filtration rate (eGFR) [38*].

With the emergence of new technologies such as metabolomics, proteomics, and genomics it might be that in the future a (set of) viability markers might still be identified. As an example, Guy *et al*

have recently shown that 28 different metabolites varied in concentration throughout HMP. Leucine, inosine, gluconate, and glucose predicted DGF, with areas under the curve above 0.70 [39*]. Additional evidence of ongoing metabolism during HMP has been shown in porcine kidneys [40**]. Perhaps focusing on metabolic activity and potential to recuperate function instead of trying to quantify the extent on injury will prove more successful as a viability tool.

***Normothermia***

With the restoration of metabolism and function, assessment during NMP has a major advantage over hypothermic techniques. Measures of function (renal blood flow and urine output) in combination with the macroscopic appearance during NMP can be used to formulate a simple scoring system [41*]. Preliminary results showed that DGF was more frequent and eGFR at 12 months lower in kidneys with a higher injury score [41*]. To test these criteria we have set up a research study to assess and transplant kidneys that have been declined for transplantation by all UK centers. So far, 3 kidneys have been successfully transplanted as part of this program [42**].

Changes potassium and lactate in the perfusate and urinary biomarkers such as NGAL and endothelin-1 appear to reflect damage but their prediction on outcome remains to be determined [43*]. As with HMP, metabolomics, proteomics, and genomics approaches may also prove informative in the future.

## Targeted treatment and Repair

An appealing quality of *ex-situ* perfusion is the administration of therapeutic agents solely to the organ pre-transplantation. This avoids unwanted systemic effects from administering these agents to the patient and obviates problems with targeting organ specific cells. The applications could include use of stem cell and gene therapy to target complex issues such as rejection and fibrosis.

***Hypothermia***

Hypothermia reduces active metabolism substantially – and intentionally – thereby likely minimising any potential effect of drugs targeted to repair damage that has already happened. Nevertheless, a recent porcine study showed that thrombalexin – a conjugate peptide of the direct thrombin inhibitor – adheres to the endothelium when given during HMP. Kidneys treated with thrombalexin had increased blood flow during whole blood normothermic reperfusion which mimics transplantation. Although no markers of kidney function or injury were measured and additional research is needed, this study shows that targeted treatment under hypothermic conditions might be possible [44*].

***Normothermia***

NMP may provide a more obvious and beneficial platform for therapy delivery as the kidney is in a functioning state. This allows close monitoring of the effects and the isolated study of mechanisms and therapeutic actions of treatment agents.

Using human kidneys, Brasile *et al* demonstrated effective transfection of a recombinant adenovirus with an encoded GFP reporter protein delivered during NMP [45]. Gene-silencing techniques can also be used to promote cell survival [46]. In collaboration with Yale University, preliminary work is underway by our group to assess the uptake of endothelium targeted nanoparticles using NMP in declined human kidneys.

Another growing area of interest is the use of mesenchymal stem cells (MSCs). Although MSCs are notable for their differentiation properties, which modulate tissue repair and regeneration, their immunomodulatory and paracrine properties, such as anti-apoptotic and anti-fibrotic effects, make them attractive in the transplant setting [47*]. Experimental studies using different models of kidney injury with MSCs have demonstrated amelioration in kidney function reduced tubular injury, and prolonged survival following ischemia-reperfusion injury.

## Implementation

Successful implementation of any technology relies on the ability to accommodate the logistics of transportation of the organ from donor to recipient center. This has led to several questions concerning the timing of HMP and NMP techniques. Should perfusion be carried out continuously or at the beginning, middle, or the end of the preservation interval (Fig. 1).

***Hypothermia***

The contradicting results of HMP in DCD kidneys from the most recent two randomized controlled trials might be related to the setting in which HMP is used. In the Eurotransplant MP-Trial, kidneys were placed on the HMP device at the donor center, immediately after retrieval [7]. In the UK trial, those kidneys retrieved away from the transplant center were cold stored during transfer after which HMP was started [8]. As such, it could be that HMP needs to be used in a continuous setting to achieve a benefit.

***Normothermic***

The preliminary results of a short resuscitation period using NMP are encouraging. Nonetheless, more prolonged periods of perfusion may be advantageous. In a DCD porcine kidney model 16h of NMP resulted in improved function compared to 16h SCS or 15h SCS followed by 1h NMP or 8h SCS and 8h NMP [31**].

There is also some evidence that an intermediate period of NMP may be beneficial. We reported a single case whereby the kidney underwent 1h NMP after SCS. Normally the second cold ischemic period after NMP is short but due to adverse circumstances the kidney was placed back on ice for 5 hours [48]. Nevertheless, it was transplanted successfully with no adverse effects and immediate graft function.

***Hypothermia to normothermic***

The transition from hypothermia to normothermia has been a topic of interest in the last few years. Minor *et al*, used controlled oxygenated rewarming [49*]. After a period of SCS porcine kidneys were gradually rewarmed to 20°C over a period of 90 minutes. At reperfusion kidney function was improved, oxidative damage and inflammatory gene expression were lower. This appears to be a promising move forward in the combined use of HMP and NMP techniques.

## Future

For any new technology to become widely implemented it would not only need to show benefit but it would also need to be cost-beneficial. *Ex-situ* kidney perfusion as a strategy should therefore be affordable and the cost balanced against the expected benefits and the number needed to treat to achieve those benefits. In addition to the machines, disposables, perfusate, and personnel time are needed. The cost of these vary depending on the type of *ex-situ* perfusion used. Invariably those of NMP will be higher as it relies on more complicated equipment and – at least in its current setting – needs continuous supervision.

The adaptability of *ex-situ* kidney perfusion seems endless. Not only can we vary temperature and perfusate but also the timing (Fig. 1) and length of perfusion can be changed as well as the addition of numerous potential additives to either improve preservation or elicit repair. Furthermore, there are literality multiple combinations of the 'basic' *ex-situ* perfusion techniques possible (e.g. HMP followed by a short period of NMP for assessment purposes). In this quickly advancing field it will become increasingly important for the transplant community to retain oversight of the progress and developments and to identify the next important questions to answer. With only a finite number of transplants performed each year and large numbers needed to conduct appropriately powered trials as well as a number of organ preservation strategies that could 'compete' with each other, national and international collaboration and perhaps prioritisation has never been more important or challenging.

We envision the use of *ex-situ* kidney perfusion as an organ-tailored preservation, diagnostic, and treatment technology where the temperature or combination of temperatures depends on the risk estimate of a particular kidney (Fig. 2). The identification of what defines a high-risk and injured kidney and which parameters specific to *ex-situ* preservation can provide additional information should be a priority. Next, the ideal settings for assessment and repair need to be defined. These settings might be different for different types of kidneys – as is becoming clear from the evidence on HMP where the effects seem different depending on organ type. Perhaps that HMP (or oxygenated HMP) will provide a 'preservation mode' before the kidney goes on to 'assessment mode' during a short period of NMP. If repair is needed, it could go on to 'repair mode' targeting specific areas of the kidney depending on the injury perceived to be present (e.g. in kidneys with acute tubular necrosis in the donor this might be mainly tubular damage, in DCDs it might be endothelial activation, …).

## Conclusion

*Ex-situ* perfusion opens many doors to advance kidney preservation but a lot of questions remain unanswered and even unidentified. We need to assemble the evidence, target key questions in unified international efforts and identify the populations that would benefit most from the different possible *ex-situ* preservation settings or combination of these settings.

## Key points

- Hypothermic machine perfusion reduces the risk of delayed graft function and might improve outcome in a subgroup of donor kidneys
- There is no quality evidence to support the use of hypothermic machine perfusion characteristics or perfusate injury markers as stand-alone tools to assess kidney viability
- Normothermic machine perfusion shows promising results as an assessment and resuscitation tool
- Organ tailored preservation – using *ex situ* perfusion in a variety of modes – is the future
- Efforts to improve preservation should be directed and unified international efforts are needed to advance the field

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1. Acknowledgements

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   IJ holds a Named Chair at the KU Leuven from the Centrale Afdeling voor Fractionering

   SH None

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## Figure legends

Figure 1. Different applications of hypothermic and normothermic kidney perfusion technologies

1: Hypothermic or normothermic perfusion (MP) for the entire preservation interval

2: A short period of MP at the donor hospital followed by static cold storage (SSCS) for transportation to the recipient center.

3: End MP at the recipient center.

4: An intermittent period of MP which may be in an organ hub or at the recipient center. After perfusion kidneys are placed back in SSCS.

Figure 2. Tailored preservation protocol.