REVIEW

Role of temperature in reconditioning and evaluation of cold preserved kidney and liver grafts

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Purpose of review
Organ shortage in transplantation medicine forces surgical research toward the development of more efficient approaches in organ preservation to enable the application of 'less than optimal' grafts. This review summarizes current techniques aiming to recondition cold-stored organ grafts prior to transplantation to reduce reperfusion-induced tissue injury and improve postimplantation graft function.

Recent findings
End-ischemic reconditioning has classically been attempted by cold oxygenated perfusion. By contrast, evaluation of graft performance prior to transplantation might be facilitated by perfusion at higher temperatures, ideally at normothermia. A drastic temperature shift from cold preservation to warm perfusion, however, has been incriminated to trigger a so-called rewarming injury associated with mitochondrial alterations. A controlled gradual warming up during machine perfusion could enhance the restitution of cellular homeostasis and improve functional outcome upon warm reperfusion.

Summary
Machine perfusion after conventional cold storage is beneficial for ulterior function after transplantation. Cold grafts should be initially perfused at low temperatures allowing for restitution of cellular homeostasis under protective hypothermic limitation of metabolic turnover. Delayed slow rewarming of the organ might further mitigate rewarming injury upon reperfusion and also increases the predictive power of evaluative measures, taken during pretransplant perfusion.

Keywords
controlled oxygenated rewarming, evaluation, machine perfusion, marginal graft, reconditioning

INTRODUCTION

Donor organ shortage still represents one of the major challenges in transplantation medicine and is likely to continue to be so in the future.

This implies increasing number of patients subjected to long times, waiting for an organ offer as well as an increasing mortality before actual organ transplantation [1–3].

As a consequence, the criteria for donor organ acceptance have been more and more extended, now including older donors and less than optimal grafts [4,5].

In most countries, organs retrieved after cardiac arrest of the donor have also become widely accepted as a clinical routine to expand the number of grafts available for transplantation [6–8].

However, although this proportion of 'less than optimal' organ grafts will most likely expand in the future, these grafts are often conflicted with a reduced functional reserve and, hence, less resilient against preservation and reperfusion injury.

It seems likely that the existing preservation technology should be further developed to comply with the future requirements in the evolving field of graft preservation and conditioning.

Recent advances are represented by the reappraisal and refinement of machine perfusion devices, allowing for easy transportation of kidney grafts as well as improved organ integrity upon transplantation [9,10]. Similar approaches are also under investigation for the liver [11].

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KEY POINTS

- Experimental studies have demonstrated that graft viability after simple cold storage can be improved by oxygenated perfusion in the cold.

- For evaluative purposes prior to transplantation, ex-vivo machine perfusion at higher temperatures might be desirable.

- After cold storage, perfusion at (sub)normothermia should be preceded by a period of adapted gradual warming up.

Classically, machine preservation has been performed in hypothermia, thus minimizing cell metabolism and technical requirements to guarantee for adequate metabolical environment during transportation.

Novel approaches foster the circumvention of cooling and rewarming of the graft by continuous normothermic machine perfusion (NMP) with diluted blood and nutritive enrichments. Pioneered by the group of Friend in liver preservation [12], the concept of maintaining a completely physiological environment by normothermic organ preservation has already proven effective in a first clinical pilot study [13▪]. Similar approaches showed encouraging results in experimental kidney preservation [14].

Dynamic organ preservation by continuous machine perfusion techniques, however, may not represent the end of the development.

Previous research indicated that structural integrity of the graft is most affected at the time of warm, oxygenated reperfusion, whereas only minor, reversible lesions actually manifest during vascular flush out and cold storage in modern preservation solution [15,16]. As the deleterious priming of cold-stored grafts is reversible and redox homeostasis can be re-established prior to transplantation and sanguineous reperfusion, graft dysfunction after implantation can potentially be abrogated. Based on this hypothesis, a brief period (1–3 h) of cold oxygenated perfusion prior to implantation has been experimentally shown to prevent cold storage-induced reperfusion injury in liver and kidney grafts. The protection provided by this technology applied after cold storage has been found to be at least equal to continuous normothermic or hypothermic graft perfusion during the whole preservation period [17▪,18].

In terms of practicability, such an approach appears to be very attractive. It precludes the necessity of perfusion devices being timely available at the retrieval site. Current procurement techniques do not need to be changed or made more complex.

Trafficking of machines or continuous surveillance of the transported graft, as required for continuous normothermic perfusion techniques, are not necessary.

End-ischemic reconditioning of the organ will be possible 'in house' in a controlled setting following arrival at the implantation center.

We imagine a future focus of preservation techniques centered on evaluation, reconditioning and putatively personalized treatment options in a setting of 'in house' machine perfusion approaches.

The purpose of this review is to compile recent insights into techniques that could be used as ex-post procedure to recondition cold-preserved donor grafts after arriving in the transplantation clinic and on the emerging challenge to evaluate organ quality prior to actual implantation.

HYPOTHERMIC RECONDITIONING

End-ischemic reconditioning of grafts has classically been performed in deep hypothermia. More than 1 decade has been spent on thorough experimental investigation of underlying mechanisms and most appropriate modalities of this technique.

It has been found that only 1–2 h of tissue oxygenation in the cold, immediately prior to transplantation, should be sufficient to improve organ function after transplantation of livers [19,20] or kidneys [21,22].

A pivotal role for aerobic cell signaling even in the cold has been established in systematic experimental studies, showing nonoxygenated perfusion to be inferior to perfusion with 100% oxygenated perfusates in kidneys [23,24] as well as in livers [25,26]. Thereby, the importance of high oxygen delivery seems to increase with the degree of graft injury prior to subjection to the reconditioning protocol [16].

Moreover, one study reporting negative results of hypothermic machine perfusion (HMP) after preceding cold storage in an isolated pig kidney model has been performed without active oxygenation during perfusion [27].

Warm ischemic tissue alteration in donation after cardiac death (DCD) grafts and, to a lesser extent, even prolonged ischemic preservation leads to a depletion of energy stores and perturbations in cellular signal homeostasis [16].

In contrast to initial warm reperfusion, hypothermic tissue oxygenation provides the opportunity to re-establish mitochondrial redox activity and replenish cellular energy status at a slow rate while metabolism is still dampened by hypothermia [25,28]. Thus, the trigger for the exacerbation of a majority of pathways leading to structural tissue

injury associated with warm reoxygenation/reperfusion of the dyshomeostatic cell could be circumvented.

For instance, the depletion of the mitochondrial heat shock protein 70 (mtHSP70) during preservation and reperfusion could be mitigated by 90 min of cold, oxygenated machine perfusion immediately prior to warm reperfusion [28]. The mtHSP70 is known as chaperone that protects the mitochondrial electron transport chain during pathophysiological situations and stabilizes respiratory function [29].

Likewise, 2 h of machine perfusion after cold storage effectively reoxygenated the cytochrome c oxidase as evidenced by transhepatic spectroscopy [30].

As a consequence, early resumption of mitochondrial respiration upon warm reperfusion is improved and oxygen-free radical-mediated tissue injury will be reduced.

Moreover, cellular liberation of so-called danger-associated patterns, known triggers for the upregulation of innate immune and inflammatory responses, can be mitigated by end-ischemic hypothermic oxygenation [17■,19].

Free high-mobility group protein 1 as well as consecutive signal expression of INFß were found significantly reduced after rat or pig liver transplantation, when hypothermic oxygenation was effectuated at the end of cold preservation.

At the same time, hypothermic reconditioning prevents activation of Kupffer cells [25] and lessens neutrophil and platelet activation upon reperfusion [15].

On a functional level, Schlegel *et al.* [31] could show in a rodent DCD liver transplant model that end-ischemic hypothermic oxygenated perfusion protects from biliary injury, which represents a key issue for successful graft function.

These observations have recently been confirmed by Westerkamp *et al.* [32], who were able to use human livers that had been declined for liver transplantation in an isolated sanguineous reperfusion model, and similar results were also observed clinically after more extended periods (3–7 h) of HMP [11].

Another therapeutic effect of HMP could be attributed to the maintenance or restoration of a physiological phenotype of the vascular endothelium by pulsatile stimulation of endothelial mechanoreceptors (reviewed in [16]). Therefore, HMP should be performed in a pulsatile rather than in a continuous pattern.

It had been shown in the kidney that even a short end-ischemic perfusion period sufficiently restores intracellular expression of the Krüppel-like factor 2 [33], which orchestrates a plethora of gene regulation pathways in favor of a physiological endothelial phenotype that prevents inflammatory reactions, upregulation of adhesion molecules and pathological vasoconstriction. In consequence, initial posttransplant tissue perfusion is improved, facilitating for example adequate oxygen delivery during the critical period of warm reperfusion and resulting in improved renal function within 1 week after experimental transplantation in the pigs [34].

The logistically attractive concept of in-house HMP could recently be validated in a clinical pilot study in DCD livers [35]. In comparison with a matched group of normally preserved liver grafts, 1–2 h hypothermic oxygenated machine perfusion via the portal vein immediately prior to transplantation resulted in significantly less biliary complications and lower postoperative transaminase release in the recipient.

As for the kidney, a European multicenter study is under way to validate the preclinical data on preimplantation-oxygenated HMP reconditioning after cold storage [21] in a clinical trial on extended criteria donor kidneys (POMP Study ISRCTN no.: 32967929).

Growing interest in evaluating organ integrity or the success of reconditioning, respectively, prior to engraftment has driven newer investigations on the value of perfusion parameters to this purpose. Vascular perfusion resistance is an easily and continuously available parameter during HMP and therefore has often raised hope to be useful for predicting graft function after transplantation.

Indeed, vascular perfusion characteristics during HMP have successfully been linked to the development of delayed graft function [9], but poor individual predictive accuracy precludes its use in pretransplant decision-making. Gómez *et al.* [36] have recently shown that there was no agreement between vascular resistance measured during HMP and later ultrasonographic perfusion data in the transplanted kidney.

Although metabolic activities are severely dampened in hypothermia, they are not completely stopped. Thus, oxygenated HMP can be used to calculate oxygen consumption, which Bunegin *et al.* [37] could show to correlate with glomerular filtration rate of isolated perfused rat kidneys. In his model, the correlation with ulterior renal filtration was much better for oxygen consumption ($r^2 = 0.87$) than for renal vascular resistance ($r^2 = 0.258$) during HMP.

Focusing on structural rather than functional evaluation parameters, a variety of biomarkers in the perfusate of machine-perfused organs has been put under investigation. In the liver, cytosolic hepatic enzymes found in the machine perfusate

correlated with maximal serum concentrations after transplantation [38].

Larger clinical studies, investigating several different molecular markers (e.g. lactate dehydrogenase, aspartate aminotransferase, gluthation-*S*-transferase, alanine aminotransferase, *n*-acetyl-ß-glucosaminidase or fatty acid binding protein), have found weak correlations with delayed graft function after transplantation and concluded that perfusate biomarkers do not qualify for isolated use in discarding decision-making [39,40].

Technically much more demanding, microdialysis has also been proposed as a tool for assessing organ viability during HMP, but still awaits confirmation in an actual reperfusion model [41].

NORMOTHERMIC PERFUSION

Recently, the idea of preserving organ grafts by continuous normothermic perfusion with complex machine perfusion apparatus has found its way into clinical application in several experienced centers [13▪,42]. The apparent success of this technique partly relies on the concept to avoid extended periods of static cold storage that may trigger hypothermia-associated cell injury.

However, addressing organ grafts arriving only after longer periods of conventional cold storage, it seems less evident to argue in favor of normothermic reconditioning.

Indeed, NMP had repeatedly been shown to display much more beneficial potential for the preserved organ, if started in temporal proximity to graft retrieval and continued throughout the whole preservation period than as postponed NMP starting after extended times of cold storage [43,44].

Nonetheless, NMP has also been tried for postpreservation reconditioning and proven superior to static cold storage alone in kidneys [45,46]. Some clinical reports are suggesting NMP to be helpful as well after cold storage of the liver [47,48], whereas others' results seem to be more controversial [42].

A possible underlying mechanism may lie in the mitigation of initial inflammatory reactions upon warm reperfusion by using perfusates void of leukocytes and thrombocytes. Thus, cellular interactions fostering innate immune reactions or proinflammatory priming could be postponed to a later phase, when the graft has genuine protective pathways that might have already been restored to a certain extent.

Another valuable benefit of NMP after static cold storage can be seen in its potential for viability testing prior to engraftment as proposed by the group of Porte using discarded human donor livers [49,50]. Later, actual clinical transplantation of suboptimal donor livers was done by others after having

shown satisfying performance upon preimplant NMP [47,48].

Likewise, a combination of urinary biomarkers like neutrophil gelatinase-associated lipocalin, together with perfusion parameters upon NMP of kidneys that had been declined for transplantation, has been suggested to be an informative measure of kidney graft quality [51]. Validation of those data, however, has been done only by correlation with renal parameters in the donor and not with ulterior function upon reperfusion.

More systematic studies might be useful in the future to establish correlations of machine perfusion data to functional recovery of marginal or optimal organ grafts upon subsequent reperfusion. From a theoretical point of view, NMP, that allows analyzing graft performance under most physiological conditions, may represent a superior approach compared with evaluation at lower temperatures. However, comparative studies on the respective potential of hypothermic or normothermic perfusion as tool to predict graft recovery after transplantation are missing so far, and superior predictive power of the normothermic approach remains to be established.

CONTROLLED OXYGENATED REWARMING

Experimental data are suggesting that longer periods of exposure to hypothermia render the tissue vulnerable to an abrupt rise in temperature,
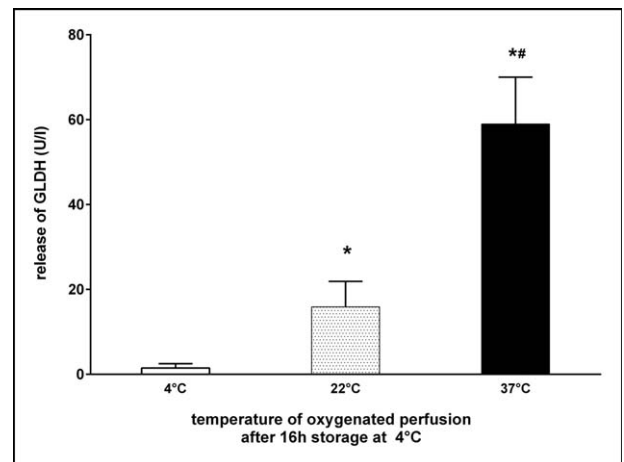

FIGURE 1. Impact of reperfusion temperature on enzyme release after cold preservation. Rat livers retrieved 30 min after cardiac standstill and cold stored for 18 h in HTK solution. Reperfusion was performed with fully oxygenated Williams-E solution at the indicated temperatures, and cumulative release of mitochondrial glutamate dehydrogenase was recorded during 2 h. Values are mean ± SD from n = 5 per group; *, **$P < 0.05$ vs. 4 and 22 °C, respectively, by Tukey–Kramer multiple comparisons test. HTK, histidine-tryptophan-ketoglutarate solution.

irrespective of the adequate fulfillment of nutritional or energetic requirements [16,52,53].

Thus, initial reperfusion temperature after cold preservation seems to be notably interrelated with the accompanying cellular injury (Fig. 1), and 'rewarming injury' should be considered as a genuine pathomechanism contributing to graft dysfunction after transplantation. Cell culture experiments are indicating that the threshold to trigger this kind of injury lies at about 13–16 °C, as longer periods of incubation below this temperature range could provoke cell injury upon rewarming [52].

Systematic investigations of the influence of temperature during reconditioning of cold-shipped organ grafts on functional recovery upon later sanguineous reperfusion have been performed by our group [54▪].

Using isolated pig livers that were cold preserved for 18 h, we could corroborate the beneficial effect of a brief HMP prior to warm sanguineous reperfusion, whereas little evidence was found for a therapeutic effect of a reconditioning perfusion at constant 20 °C.

By contrast, a gradual warming up of the graft during the reconditioning perfusion, starting with an extended period in hypothermia and then slowly rising up to room temperature (RT), disclosed significant advantages, even in comparison with the other reconditioning protocols, and also maintained cellular autophagy [54▪], which has been previously pointed out to be important for regenerative processes [55].

It is felt that this procedure, termed 'controlled oxygenated rewarming' (COR) facilitates/improves restitution of cellular homeostasis and mitigates rewarming injury by adapted increase of temperature and metabolism.

Similar results were then described by Matsuno *et al.* [56], evaluating the release of transaminases after gradual rewarming perfusion in a porcine liver transplantation model. Further corroboration in favor of a slowed down rewarming process after cold storage comes from Banan *et al.* [57], showing significantly reduced Kupffer cell activation upon isolated reperfusion of cold-stored porcine livers after gradual rewarming.

The principle of COR also applies to other organs, as could be evidenced in a porcine renal reperfusion model [58]. Showing that COR not only serves for revitalizing critically preserved donor organs but also improves well preserved grafts after HMP, Schopp *et al.* could further support that 'rewarming injury' represents a genuine phenomenon that may occur even after optimal cold preservation, for example by continuous HMP.

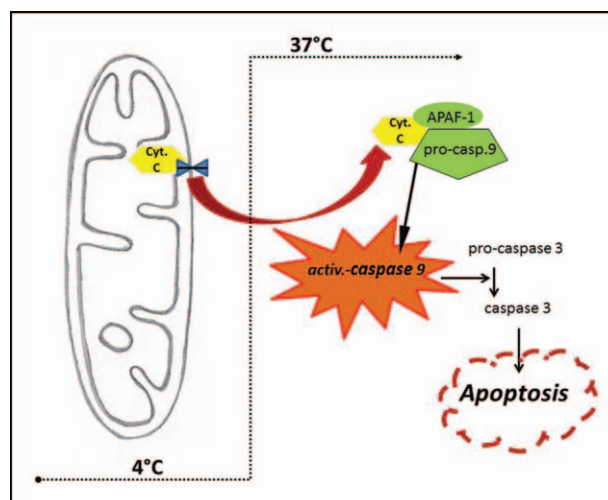Likewise, in isolated rat kidneys, Mahboub *et al.* [59] have shown a reduction of renal tubular injury

FIGURE 2. Schematic illustration of mitochondrial rewarming injury.

and better endothelial preservation, if warm reperfusion after 24 h of cold storage was preceded by gradual rewarming, although the reported temperature steps were rather coarse in this study.

When directly compared with a normothermic reconditioning protocol, COR showed superior functional outcome of pig livers upon reperfusion, which seemed to be mediated through better protection of mitochondrial function, improved energetic recovery and a mitigation of mitochondrial induction of cellular apoptosis [60].

Mitochondrial protection by COR was also seen in the kidney, in which gentle warming up prevented the activation of caspase 9 and significant reduced mitochondrial loss of nicotinamide adenine dinucleotide [58].

A schematic representation of these data as constituents of a mitochondrial rewarming injury is given in Fig. 2.

Meanwhile, COR has already been confirmed as useful means in clinical practice [61]. In a series of six patients who received liver grafts treated by COR prior to implantation, no complications were observed, and early allograft dysfunction [62] was found significantly reduced when compared with a matched historical control group.

An open question remains that whether warming up should or could be pursued up to temperatures above 20 °C. Pilot experiments addressing this issue have been undertaken on isolated rat livers. The rewarming machine perfusion has been performed with Aqix RS-I solution (Aqix Ltd, London, United Kingdom), a serum-like new preservation solution with stable pH characteristics over the whole temperature range from 8 to 35 °C.

It was found that no therapeutic benefit was achieved in warming up to more than 20 °C (vHorn,

unpublished observations) but these results remain to be confirmed in large-size animals.

Notwithstanding this, the period of constant perfusion at the end of the rewarming protocol can and should be used also for evaluative purposes, and these might benefit from normothermia, even if no additional therapeutic effect would be expected.

So far, encouraging results have been disclosed using mid-thermic perfusion parameters at the end of COR as predictive tool for later graft function upon warm reperfusion. Thus, a good correlation has been observed between hepatic net release of glucose during ex-vivo perfusion, as measured in real time using an acid–base laboratory, and ulterior liver integrity after clinical transplantation [61].

In the kidney, functional data during perfusion at $20\,^{\circ}C$ significantly outweighed the weaker predictive power of markers obtained during hypothermic perfusion [63■] and showed very well correlations with ulterior renal function upon warm reperfusion.

It might be speculated that further warming up to near normothermia would possibly represent an even better prognostic tool than perfusion at RT, and the results on this issue are awaited.

CONCLUSION

Machine perfusion after conventional cold storage of organ graft is possible and beneficial for ulterior function after transplantation. For optimal therapeutic reconditioning and mitigation of reperfusion injury, the graft should be initially perfused at low temperatures allowing for restitution of cellular homeostasis under protective hypothermic limitation of metabolic turnover. Further, controlled rewarming of the organ might improve functional and structural integrity after transplantation by mitigating the so-called rearming injury upon reperfusion and also increases the predictive power of evaluative measures taken during pretransplant perfusion.

Conflicts of interest
There are no conflicts of interest.

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