Review

Liver and kidney preservation by perfusion

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The clinical boundaries of transplantation have been set in an era of simple cold storage. Research in organ preservation has led to the development of flush solutions that buffer the harsh molecular conditions which develop during ischaemia, and provide stored organs that are fit to sustain life after transplantation. Although simple and efficient, this method might be reaching its limit with respect to the duration, preservation, and the quality of organs that can be preserved. In addition, flush preservation does not allow for adequate viability assessment. There is good evidence that preservation times will be extended by the provision of continuous cellular substrate. Stimulation of in-vivo conditions by ex-vivo perfusion could also mean that marginal organs will be salvaged for transplantation. Perfusion will also allow for assessing the viability of organs before transplantation in a continuous fashion. The cumulative effect of these benefits would include expansion of the donor pool, less risk of primary non-function, and extension of the safe preservation period. Use of non-heart-beating donors, international organ sharing, and precise calculation of the risk of primary organ failure could become standard.

Developments in organ preservation methods over the past century mean that flushing the organ and storing it at low temperatures have become standard. Advances in perfusates have made this method efficacious and allowed organ transplantation to become a powerful life saving therapy around the world. However, clinical practice is currently restricted by the quality of donor organs, the number of donor organs available, and the inability to assess viability before transplantation. Delays in graft function are costly to the kidney recipient and likely to be fatal to the liver recipient. It is intuitive that better preservation can be accomplished by replicating the natural environment of an organ. Continuous perfusion provides a supply of metabolic substrates and removes byproducts, recreating the normal circulation. It is, therefore, a logical approach to organ preservation; indeed, some of the first experimental work on preservation involved use of continuous perfusion. Cold flush preservation, on the other hand, is simpler and cheaper, which is why it has become the standard means of organ preservation for clinical transplantation. However, since the potential for cold storage is approaching its limit, we need to reconsider continuous perfusion as a possible means to expand the horizons of organ preservation in the future. Here we review use of continuous perfusion as a method of organ preservation and discuss how this method might overcome some of the current clinical limitations.

History

During the first half of the 20th century, Alexis Carrel and Charles Lindbergh perfused organs with normothermic, oxygenated serum at supraphysiological volumes and showed gross viability for several days.[1] Carrel pioneered many types of preservation, and in his work, he notes the words of Le Gallois (1770–1814), "If one could substitute for the heart a kind of injection . . . of arterial blood, either natural or artificially made . . . one would succeed easily in maintaining alive indefinitely any part of the body". As stated, this is an ordinary, simple notion, which has proven to be extraordinarily difficult to apply.

In the early 1960s, as liver transplantation was approaching clinical reality, Sicular and Moore investigated various methods of cooling and preserving organs by measuring glucose metabolism and carbon dioxide production.[2] They reported surprisingly good maintenance of function in a liver perfused with acellular, oxygenated perfusate at 15°C. In 1963, attempting to apply the above stated principle of Le Gallois, Starzl's group used femoro-femoral, extracorporeal perfusion to preserve canine organs at 12–15°C with autologous blood.[3] Using this technique, function was limited to 6 h for the kidney and 2 h for the liver. It is important to note that in these experiments, perfusion was initiated after cessation of natural blood flow without flushing the organ in vivo; this created a period of warm ischaemia. In-vivo isolated perfusion of the liver with oxygenated blood at 10–18°C was used to preserve canine livers for up to 5 h before successful transplantation.[4] By 1967, the combination of continuous perfusion and hypothermic storage brought organ preservation to a new level. Using oxygenated plasma pumped in a pulsatile fashion at 8–12°C, canine kidneys were successfully preserved for 72 h as described by Belzer and colleagues in a landmark paper.[5]

The use of machine perfusion applied to the liver presents a logistical concern because of the dual blood supply. Pulsatile perfusion of the artery alone using balanced salt solution at 4°C and 4 atmospheres pressure of oxygen gave marginal results in canines in 1967.[6] Brettschneider and colleagues, working in Starzl's group, perfused both the portal vein and hepatic artery with a perfusate composed of half autologous blood and half

Search strategy and selection criteria

Data for this review were obtained with Pubmed searches for all articles describing perfusion in the context of organ preservation. Relevant materials from references in those articles not previously found or dating from earlier than the limit of the search engine were also retrieved. We included all relevant English-language articles. If the same message was contained in several studies, not all are necessarily cited here.

Lancet 2002; **359:** 604–13

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preservation solution under refrigerated, hyperbaric conditions. In this experiment in canines, the organ was flushed in vivo before being placed on the machine, and subsequently transplanted. All five animals survived with excellent hepatic function after 8–9 h of machine preservation. Three of five survived after 24 h preservation.[7] The method was successfully applied in seven human beings with periods of liver preservation from 4–7 h. All seven patients survived the first postoperative week.[8] This effective technique never gained widespread acceptance on account of logistical constraints, but it marked the first successful machine preservation of the liver and emphasised the importance of perfusing the portal vein and flushing the organ in vivo.

Subsequent introduction of Collins solution and successful preservation with simple cold storage moved the clinical focus away from machine perfusion.[9] Clinical priorities then moved towards the logistic advantages of simple flush preservation, which enabled rapid and economic distant procurement and transport of organs.

Belzer later focused his machine perfusion work on the liver using the porcine liver with the same cryoprecipitated plasma and hypothermic temperatures already used in kidney perfusion. This method involved continuous portal flow and pulsatile arterial flow. Four of five animals survived transplantation for 7 days after 8–10 h preservation, but only two of 12 survived beyond 12 h when the preservation period was extended to 24 h.[10] Similar results have been seen in canines.[11] The 24 h preservation barrier of machine-perfused livers was, somewhat controversially, achieved in 1973, by reports of 24 and 48 h preservations of the canine liver. The reported technique involved arterial and portal perfusion of hypothermic, oxygenated plasma with the addition of corticosteroids. 12 of 19 dogs survived 5 days in the 24 h group, and two of 19 survived in the 48 h group.[12,13] However, several animals were excluded from both groups secondary to technical difficulties.

In 1980, Kamada and colleagues perfused rat livers for 20–24 h investigating the addition of fluorocarbon emulsion as an oxygen carrier in the perfusate. The perfusions were carried out at 10°C and the control group was perfused with the same perfusate excluding the fluorocarbon emulsion. Long-term survival (88–370 days) was achieved in five of nine in the fluorocarbon emulsion group, while the longest survivor in the control group was 5 days after transplantation.[14] An advantage of machine perfusion over simple cold storage is the ability to provide a continuous supply of the substrates necessary for cellular functions. These results emphasised the need to enhance the benefits of continuous perfusion with the addition of oxygen.

The best results to date in liver preservation by machine perfusion were obtained by Belzer's group who used only the portal vein to perfuse canine livers for 72 h at 5°C.[15] This study showed the feasibility of use of machine perfusion to achieve substantially longer preservation periods than can be achieved with simple cold storage.

Physiological rationale

To understand the benefit of continuous perfusion during the preservation period, it is necessary to first understand the basic mechanisms of ischaemic injury and the rationale of cold storage.

Ischaemia-reperfusion injury

Oxygen is the fuel that drives all cellular activity by allowing the cell efficiently to regenerate adenosine triphosphate (ATP), the currency of cellular energy. Blood

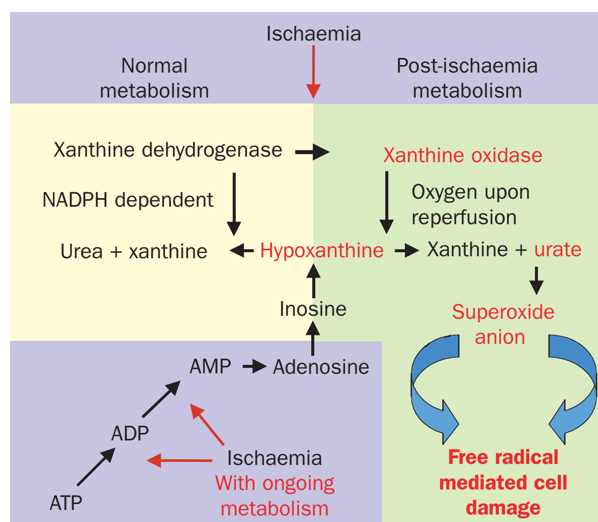

Figure 1: **Ischaemia leads to rapid breakdown of ATP**
Furthermore, ischaemia induces changes to the cellular mechanisms for handling the breakdown products of ATP, leading to free radical damage upon reperfusion.

flow is terminated during ischaemic preservation eliminating both the supply of oxygen, cofactors, nutrients, and also the means for disposal of waste and metabolic end products. This precipitates inefficient anaerobic metabolism, which leads to deprivation of high-energy phosphates and build up of acidic products (including lactic acid).

Sodium/potassium pumps, driven by ATP, become disarmed during ischaemia, creating a loss of electrolyte gradients and membrane integrity, which causes cellular oedema. Calcium then enters the cell uninhibited. In addition, the acidic environment promotes free calcium, which is bound to cellular proteins at physiological pH. Calcium activates phospholipases and triggers enzymatic cascades, which promote inflammation and degradation pathways leading to cell death. The phospholipases involved in calcium-mediated injury are thought to largely come from Kupffer and endothelial cells during ischaemia to the liver.[16] Phospholipase A_2 activation contributes to impairment of the electron transport chain, ATPases, adenine nucleotide translocase activities, and can also contribute to cellular oedema by altering the cytoskeleton through protease activation.[17]

Products of ATP breakdown are normally converted to urea by xanthine deydrogenase; however, in an ischaemic environment, xanthine deydrogenase is converted to xanthine oxidase. In the presence of oxygen (upon reperfusion) xanthine oxidase will convert the accumulated products into xanthine and the superoxide anion (free radical), which causes respiratory burst leading to lipid peroxidation and cellular destruction (figure 1).[18] This reaction is of particular importance in the liver, which holds the largest stores of ATP and xanthine dehydrogenase in the body.[19] Perfusion with an oxygen carrier during preservation will, at the very least, greatly attenuate this process by preventing total ischaemia.

Cold storage

Keeping ATP depletion to a minimum is critical for control of the cascades of ischaemic injury. An important underlying principle of hypothermic preservation is to slow those processes that require ATP and those that accumulate injury after ATP depletion occurs. Metabolism is slowed 1·5–2 fold for every 10°C drop in temperature, but there is still considerable activity at 1°C.[16] Therefore,

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even when organs are stored at ice temperature, ATP concentrations drop substantially and, thus, the stage is set for reperfusion injury: cellular ATP drops by 84% in the rabbit liver after 6 h of cold storage in acellular perfusate solution.[20] Hypothermia does abrogate some of the harmful effects of ATP depletion—eg, rat hepatocytes show little histological change on electron microscopy after cold storage.[21] However, in the same study, endothelial cells showed progressive deterioration, and Kupffer cells showed activation with increasing time of cold storage, suggesting these could be the mechanisms that underlie graft failure after storage injury.

In addition to ATP depletion, acute hypothermia induces calcium influx into most parenchymal, endothelial, and Kupffer cells, which will precipitate tissue destruction as described above.[22] Structurally, sinusoidal cells have been shown to undergo degeneration and partial disappearance after just 4 h of cold storage and almost total disappearance after 8 h.[19]

Preservation by perfusion

Preventing ATP loss and altogether avoiding the injurious ischaemic cascades that are set into motion after ATP depletion requires perfusion with an oxygenated solution.[23–26] Perfusion preservation of rat liver has been noted to suppress completely the loss of ATP seen in cold storage, even at 48 h of perfusion.[25] An investigation into incremental cold ischaemia times followed by 45 min of ischaemic rewarming in rats showed the progressive rise in liver enzymes and the decrease in ATP with increasing cold ischaemia times.[27] Remarkably, 30 min of oxygenated perfusion before rewarming brings the ischaemic organs into close approximation to the non-ischaemic controls on all variables. This finding suggests that ATP concentrations can be reset by perfusion in reversibly injured organs, which might be highly relevant to clinical practice.

The ischaemic-rewarming period that occurs during transplantation is a cause for energy depletion, tissue injury, and, if sustained, leads to graft failure.[28,29] In human beings, adenine nucleotide content has been shown to decrease rapidly during ischaemic rewarming and the level of recovery to be inversely proportional to warm ischaemia time.[30] Glycogen depletion in porcine livers during the rewarming period has been shown to be significantly dependent on the glycogen stores at the beginning of the period.[31] Therefore, by using a perfusion system to restore energy levels before transplantation, livers with extended cold storage times could possibly be transplanted as effectively as if there had been no preservation period. This might lead to a decrease in primary non-function rate and improved early graft function.

Perfusate

The ideal perfusate for continuous perfusion of an organ has not yet been defined. However, the perfusate should deliver oxygen to the organ by using an oxygen carrier for the full benefit of perfusion to be realised.[7,14,32] Simple oxygenated buffer solutions require higher flows for adequate oxygen delivery and create degenerative changes in the perfused tissues that are not seen when red blood cells are used as the oxygen carrier.[33–36] In addition to tissue damage, high flows can decrease first-pass hepatic clearance.[37] Similarly, high pressures cause hepatic barotrauma and enlarge sinusoidal fenestrations.[38] A haematocrit of 20% has been suggested to provide the optimum combination of haemodynamics and oxygen-carrying capacity.[39] Also, red blood cells can attenuate sinusoidal damage by scavenging xanthine-oxidase-dependent radicals in perfused rat livers.[40] The red blood cells in this study were washed and leukocyte depleted. Similarly, in the porcine liver non-heart-beating donor model, better ATP replenishment and less sinusoidal damage were noted when washed red blood cells in Krebs-Henseleit solution were used as the perfusate compared with whole blood.[41] Fluorocarbon compounds as the oxygen carrier have been shown to provide similar support of metabolic liver function and preservation outcomes as blood.[42]

In addition to an oxygen carrier, any of the known free-radical scavengers, vasoactive substances, inflammatory mediators, calcium-channel blockers, insulin, antibiotics, metabolic precursors, bile salts, and nutrition can be added to the perfusate to provide a continuous cellular supply, an advantage not offered by cold storage.

Pulsatile versus non-pulsatile perfusion

The most frequently used method of perfusing kidneys and livers simulates normal physiology by using pulsatile flow for the renal or hepatic artery and non-pulsatile flow for the portal vein. Some studies have shown better kidney function and microcirculation by using pulsatile perfusion.[43,44] However, non-pulsatile flow is used clinically in Japan with similar results to pulsatile preservation.[45,46] Data from studies on rat livers has suggested better maintenance of energy charge and decreased lipid peroxidation in organs preserved with pulsatile as opposed to continuous perfusion.[47] In this study, both methods of perfusion were clearly better than cold storage. Non-pulsatile perfusion of an artery induces a 15% increase in vascular resistance thus requiring increased perfusion pressure to maintain equal flow.[48] No such differences are seen in portal vein pressures whether pulsatile or non-pulsatile perfusion is applied.[47]

Normothermic versus hypothermic perfusion

Hypothermia slows metabolism, reduces oxygen requirements, and is clearly essential for simple storage. However, if continuous cellular substrate is provided, this is no longer an absolute requirement. Hypothermia during perfusion allows for less oxygenation and lower flow rates, but is also associated with increased vascular resistance and decreased oxygen delivery. Thus, an oxygen carrier might not be necessary to achieve adequate oxygen delivery during hypothermic preservation.[15,25] Conversely, normothermic perfusion without adequate oxygenation creates inferior results to simple cold storage.[49] Although there is a lack of comparative data, there is evidence that hypothermia itself induces injury through several pathways; it is therefore logical to postulate that normothermic, or near normothermic perfusion providing oxygen and other physiological substrates will be the ideal method to maintain organs in a viable state for sustained periods.

Clinical application

Continuous perfusion preservation is only clinically used for the kidney and only at a few centres. The balance of evidence suggests that this method provides better kidney preservation. Nevertheless, there remains a major controversy regarding the place of machine perfusion preservation in renal transplantation. Figure 2 depicts an example of a commercially available kidney perfusion machine.

Early reports based on retrospective comparisons of machine perfusion and cold storage showed no significant differences in allograft function after transplantation.[50–55] However, these studies were probably biased in favour of

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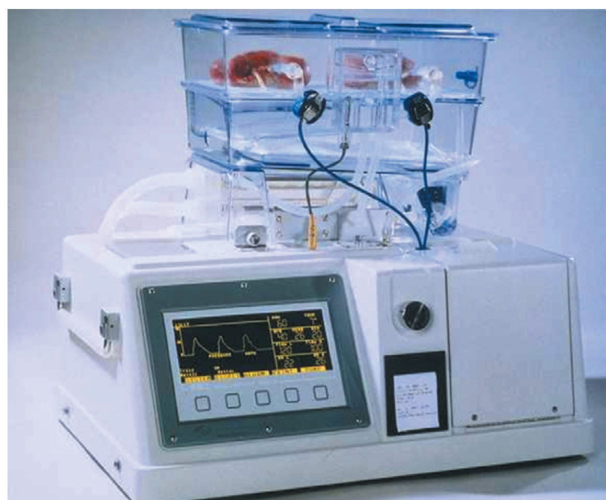

Figure 2: **Standard kidney perfusion machine used clinically**
Waters RM3 Renal Preservation System (Waters Medical Systems, Minneapolis, MN, USA).

cold storage, because the practice of the time was to use machine perfusion for much longer cold ischaemia times.[56,57] In addition, little information was included about the recipient patients and the institutions where they were treated.[57] A prospective randomised trial on kidneys from the same donor was done to eliminate donor factors as variables.[58] There was no statistical difference in the rate of delayed graft function in 27 pairs of matched kidneys. However, when delayed graft function did occur, machine-perfused kidneys showed a significant earlier recovery and a significantly higher 1-year graft survival. A similar study comparing 26 donor pairs suggested a beneficial effect of the machine preserved kidney, but this difference was not significant.[59] A comparison of 51 donor pairs showed no significant difference in postoperative dialysis requirements.[60] In a larger study of 96 matched pairs, no difference was detected in delayed function, dialysis requirements, or 3-month or 1-year graft survival.[61] However, an increased rate of acute tubular necrosis was shown in the cold stored organs when the preservation period exceeded 24 h. The rate of acute tubular necrosis in the machine-perfused group was independent of time, a finding that had been noted previously.[52,62] Similarly, a retrospective analysis from Kuwait, studying donor kidneys with cold ischaemia times of 30–76 h, showed a significantly increased need for dialysis in the cold stored group, although the difference in graft function at 2 years was not significant.[63]

Several other trials have concluded that kidneys preserved by machine perfusion show better early function than preservation by cold storage.[64–67] This includes a study of 29 matched pairs, which showed dialysis requirements in 17% of the perfused group and 63% for the cold stored allografts.[64] The importance of early function has been reinforced by several studies that show a correlation between initial graft dysfunction and long-term graft survival.[68–72] A 6-year, single institution review showed better early and long-term renal function in kidneys preserved with machine perfusion.[73] These results were obtained with hypothermic pulsatile perfusion with Belzer-MPS solution. Even applying perfusion to donor kidneys that have been initially cold stored has been shown to confer similar advantages.[74]

It is well established that machine perfusion can provide longer successful preservation periods. Successful kidney preservation has been achieved by machine perfusion for up to 5–7 days.[75–77] The clinical advantages of machine perfusion have been shown using both Belzer-MPS and simple acellular perfusate solution as the perfusate.[78,79]

Two points should be made regarding these clinical comparisons. First, most of the clinical trial data relate to short-term preservation of good donor kidneys; this is not the area where the major advantages of preservation will be manifest. Second, the method used at most institutions does not incorporate an oxygen carrier, thereby at least partly nullifying the advantage of continuous perfusion.

Despite evidence that the current techniques in machine perfusion provide a higher quality graft and allow longer preservation periods, the standard practice today continues to involve flushing the organ with acellular perfusate or Collins solution and storage at 4°C for the minimum time necessary before transplantation. A valid argument in favour of this practice is that acceptable results are obtained with a simpler and cheaper method of preservation than machine perfusion, which requires an expensive, cumbersome device and additional personnel to run it. This includes extra surgical steps to secure perfusion cannula within the vessels and taking them down again at the end of preservation. The device also requires more delicate transportation. However, supporters of machine perfusion point to the expense involved in treating patients with delayed graft function.[80] Data from Johns Hopkins Hospital in Baltimore, MD, USA, show that the chance of needing dialysis after transplantation is 2·33 times higher when simple cold storage is used in donors over 55 years of age than machine-perfused kidneys in the same donor group.[81] It was calculated that the financial effect of using the more expensive machine perfusion method in all donors older than 55 years would be a net saving of US$14 700 per patient.

Non-heart-beating donors

The benefits of machine perfusion are most clearly seen in organs from marginal donors. A particularly important pool of marginal donor organs, and the focus of considerable attention, is the non-heart-beating donor. In the past decade, the UK, like other countries, has experienced a plateau in the number of donors available and cadaveric transplantations done, although the waiting list continues to grow (figure 3). Organs from donors with a non-beating heart present an untapped source of organs

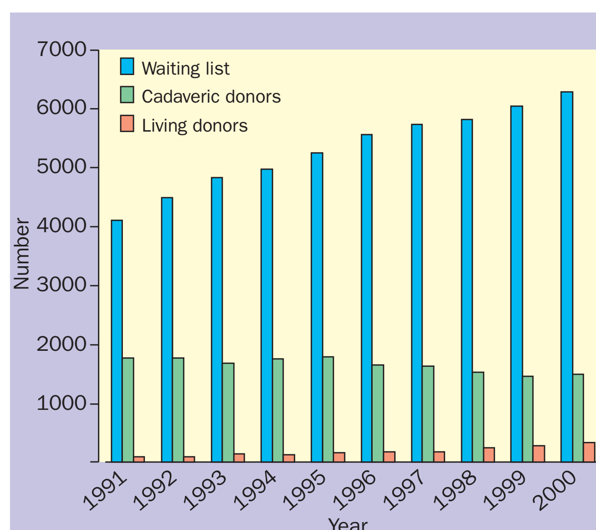

Figure 3: **Waiting list and donor data for renal transplantation in the UK from 1991–2000**
According to UKTSSA data updated on the website.

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that could have an important impact on the large discrepancy that exists between supply and demand.[82] To make optimum use of this potential organ supply, the ischaemic injury that occurs after a period of warm ischaemia needs to be reversed. Preservation by perfusion can achieve this goal, whereas cold storage can lead to sequential injury as described above and frequently leaves the organ unfit for transplantation.[83] The cold storage of marginal organs comprises four consecutive injuries: preretrieval injury, cold storage ischaemia, ischaemic rewarming (warm ischaemia), and reperfusion. Perfusion can break this cycle by maintaining or replenishing cellular substrates during the preservation period. This perfusion can be done before storage or before reimplantation.

Liver

The clinical experience of using liver from non-heart-beating donors is currently limited.[84-86] Under controlled circumstances, when the warm ischaemia time is restricted to 20–30 min, the results are acceptable. In the uncontrolled setting, when cardiac arrest occurs outside the operating room, results have been poor with a high rate of primary non-function.[85] Most of the successful cases reported from this group use continuous in-vivo perfusion with cardiopulmonary bypass or chest compressions with oxygenation. It is likely that this provides some recovery of cellular energy stores before cold storage.

The concept of ameliorating ischaemic injury by normothermic or hypothermic perfusion after the ischaemic period has been documented in the non-transplant model using perfluorocarbon in the perfusate.[87] In the porcine transplant model, using cardiopulmonary bypass to flush the donor with oxygenated Euro-Collins solution with simultaneous core cooling after 10 min of arrest, tissue energy charge was slightly restored and all the recipient animals survived.[88] In this study, organs from the control group were flushed and stored by standard technique after 10 min of arrest with no recipient survivors. Sanguineous perfusion of isolated porcine livers at body temperature for 3 h has been shown to resuscitate basic liver function after 75 min of arrest.[89] Similar studies in rats have suggested liver recoverability after 30 min of arrest.[90] Total body reperfusion with cardiopulmonary bypass after circulatory arrest has been shown to revive total ATP content in porcine livers and kidneys.[91] Conditioning the porcine liver with 30 min of normothermic isolated perfusion in vivo with whole blood can increase mitochondrial ATP content after 10 min of circulatory arrest.[92] In the same model, better ATP replenishment and less sinusoidal damage were reported when washed red blood cells in Krebs-Henseleit solution were used as the perfusate.[41] Cardiopulmonary bypass for only 10 min after 30 min of arrest provided functional recovery of the heart, liver, and kidney in canines.[93]

In a porcine study from Barcelona, animals underwent circulatory arrest followed by cardiopulmonary bypass to perfuse the livers in vivo for 30 min before cold storage and transplantation.[94] The control group did not undergo bypass before cold storage. Transplant survival was significantly related to warm ischaemia time. However, even after 40 min of arrest, 50% survived in the bypass group, whereas there were no survivors in the control group after only 20 min of arrest. There was evidence that bypass was associated with regeneration of tissue energy charge and that maintenance of these energy stores after arrest correlated with post-transplant survival.[95,96] This work has drawn attention to the concept of restoring cellular energy stores after warm ischaemic injury, but before preservation.

Delivery of oxygen in various ways during the preservation period has also shown encouraging results in non-heart-beating donors. Intravascular persufflation of oxygen during cold storage in acellular perfusate solution was noted to produce 100% survival in transplanted pigs after 45 min of donor circulatory arrest, although all the controls died without persufflation.[97] Machine perfusion of the pig liver through the hepatic artery with acellular perfusate-gluconate was associated with 80% survival after 90 min of hypotension and a brief period of arrest; this contrasted with 25% survival with cold storage.[98] In this study, perfusion through the artery alone was shown to be better than infusing through the portal vein alone.

A group from Berlin showed impressive experimental data with perfusion preservation to salvage livers from non-heart-beating donors.[99] After 1 h of warm ischaemia before harvest, porcine livers were preserved for 4 h using either cold storage in acellular perfusate solution or normothermic extracorporeal perfusion with whole blood perfusate. Excellent post-transplant function was seen in all the animals that received perfused livers, whereas there were no survivors among those that received cold-stored livers.

From a variety of sources, there is good evidence that perfusing the liver at some point during the preservation period might be the best way to access the potential of the non-heart-beating donor pool for liver transplantation.

Kidney

The consequences of ischaemic injury are less critical in kidney than in liver transplantation; patients receiving kidneys with delayed function can be sustained with dialysis while the organ recovers. For this reason, the clinical use of non-heart-beating donors has predominantly centred on kidney transplantation. There are convincing data that machine perfusion is better than cold storage in this group[46,100–105] showing better early function, lower dialysis requirement, and improved 1-month survival in machine-perfused kidneys than cold stored kidneys in matched pairs.[104,105] Cases have also been reported of salvaging kidneys by machine perfusion from non-heart-beating donors with over 2 h of warm ischaemia time.[98] The advantage of machine perfusion in non-heart-beating donor kidneys could be sufficiently great that it would be costeffective to use it universally in this situation.[106]

Assessment of viability

An important limitation of preserving organs by cold storage is the lack of a method for assessing whether the organ will function properly after transplantation. Perfusion offers the attractive feature of providing a means to assess organ viability before transplantation; this can be done by studying the perfusion characteristics (haemodynamics) of the organ and by analysing the perfusate. Although this might not be necessary for organs harvested under ideal circumstances—these are almost certain to function—it becomes critically important for marginal organs that are more likely to fail. At present, organs are rejected if the chance of functioning is judged to be low. However, because there is no definitive measure of function, it is inevitable that some viable organs are rejected. The use of continuous perfusion would enable the viability of organs to be assessed during the preservation period, which would expand the number of usable organs and simultaneously keep risk to the recipient to a minimum.

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Kidney
Delayed graft function does not have the same disastrous consequence after kidney transplantation as following liver transplantation. Indeed, dialysis provides the luxury of allowing the recipients to survive a period of graft non-function while the organ recovers and sustains those patients who receive grafts that never function. However, the cost of delayed graft function, both financially and emotionally, is considerable.[70,71] Therefore, to be able to select those organs that will function well, particularly separating out those that will never function, would be an enormous benefit. Measurement of serum markers, perfusion flow dynamics, and ATP content are all potential ways by which this could be done.

Serum markers—In the early days of clinical transplantation, several groups were investigating the prediction of viability in preserved kidneys. Some of these early studies involved measuring lactate dehydrogenase in the venous effluent of flushed kidneys.[107,108] No correlation with lactate dehydrogenase and warm ischaemia was reported. However, Koostra's group in the Netherlands reported that lactate dehydrogenase was significantly higher in the machine perfusate of failed grafts than those that functioned.[109] Lactate dehydrogenase was subsequently recorded as higher in the implanted, non-functioning grafts than in the functioning group, but this difference was not significant. Early studies also measured ligandin in the perfusate.[110,111] Ligandin has since been defined as glutathione-S-transferase, a lysosomal enzyme from proximal tubular cells with three isoforms.[112] Koostra's group has shown α-glutathione-S-transferase concentrations to be significantly different between the functioning and non-functioning groups in a study of 28 kidneys from non-heart-beating donors.[113] Correlation was shown between α-glutathione-S-transferase and warm ischaemia time, suggesting this enzyme to be a promising candidate as a viability marker. In Newcastle, UK, after achieving only 45·5% 1-year graft survival using cold storage on uncontrolled kidneys from non-heart-beating donors, machine perfusion was implemented and total glutathione-S-transferase measurement used to accept or reject organs.[114] This resulted in a doubling of the 1-year graft survival (to 88·4%) in this high-risk group ($p=0·023$).

Perfusion flow dynamics—Flow dynamics can easily be monitored during perfusion, although it has been suggested that pressure-flow relations reveal injury that has already taken place, whereas enzymes in the perfusate show dynamic changes and reperfusion injury.[115] However, perfusion flow has been shown to correlate inversely with both primary non-function and total ischaemia time[116] and directly with early graft function.[104,117] An inverse correlation between perfusion resistance and early graft function has been established.[104] Similarly, renal vascular resistance during perfusion has been reported to be significantly higher in non-functioning grafts.[118] The flow, pressure, and resistance patterns of kidneys from non-heart-beating donors undergoing non-pulsatile machine perfusion are being applied clinically for predicting function.[119] The combination of a flow of more than 0·4 mL/min per g, and a vascular resistance of less than 80 mm Hg/mL $min^{-1}g^{-1}$, and with no increase in perfusion pressure during the perfusion have been shown to be highly predictive of good function. Furthermore, these criteria can be used to define those kidneys that need more urgent transplantation.[120]

ATP content—Experimental approaches to assess viability have focused on quantification of ischaemic damage. As depletion of ATP is the cornerstone of ischaemic changes, it is logical to use this as a marker of ischaemic change. However, data suggest that simply measuring adenine nucleotide in the perfusate during machine perfusion is not an adequate determinant of viability in organs sustaining ischaemic injury.[39] Measuring phosphomonoester to inorganic phosphate ratio by phosphorus-31 magnetic resonance spectroscopy has been shown to correlate with viability in human recipients.[121] This technology can be applied to machine-perfused or cold-stored organs. Intrarenal flow distribution determined by radioactive xenon-133 and a technetium-labelled, non-polar carrier during machine perfusion has been suggested as a good measure of ischaemic damage in canines.[103]

Liver
The prediction of outcome of preserved livers is currently not possible, and the practice of clinical liver transplantation depends on the donor history and liver appearance; there are no validated means of predicting outcome on the basis of biochemical or other tests. Thus, the vital decision as to whether to accept or decline a donor organ is made on subjective criteria. Since the consequences of primary non-function are so severe, there is a tendency to reject organs where there is serious doubt about viability; thereby inevitably wasting a proportion of viable organs.

If an organ were maintained in a functioning state on a perfusion circuit before transplantation, it would enable objective evaluation of function after organ retrieval but before committing the patient to the transplant. This would make it possible to study livers that would normally be rejected on the basis of history alone allowing for the use of marginal donors in a way that is not currently possible. Isolated perfusion has been shown by several groups to enable the assessment of liver viability, essentially by replicating the in-vivo state.[122–125] It therefore becomes important to define those variables that correlate with survival, and there are several variables that can be tested on a circuit.

ATP content—In a study comparing the effect of progressive warm ischaemia time in the porcine liver from non-heart-beating donors, ATP content was noted to decrease with increasing warm ischaemia time. These livers were then cold stored and transplanted without prior perfusion; and a further decrease in ATP concentration occurred during cold preservation. Postoperatively, ATP concentrations had returned to near normal after 1 h in survivors whereas the non-survivors had little recovery of ATP content and died from primary graft failure.[83] Similar results have been seen in rats.[126] These data suggest that a graft could be tested on a circuit for as little as 1 h to determine its viability using ATP content.

Serum markers—Hepatocellular enzymes have long been the focus of viability testing. AST (aspartate aminotransferase), ALT (alanine aminotransferase), and lactate dehydrogenase were shown to correlate well with ischaemic time in canine perfusion studies.[127] Using the isolated rat liver perfusion model, acid phosphatase, ALT, and AST were noted to correlate inversely with liver function during perfusion and were proposed as a means of monitoring the adequacy of preservation.[128] In a similar model, livers were perfused with blood for 3 h before transplantation in association with a variety of

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preservation times in both hypothermic and normothermic environments.[129] AST and lactate dehydrogenase were noted to predict survival within and across preservation group, whereas bile flow, bile salt secretion, and ALT predicted survival within preservation groups only.

In the porcine non-heart-beating donor, there was a significant difference in lactate dehydrogenase between survivors and non-survivors at 1 and 4 h after transplantation.[83] AST and lactate dehydrogenase measured in the effluent from the vena cava in cold-stored human livers have been inversely correlated with 1-month graft survival.[130] The correlation was stronger with AST than lactate dehydrogenase in this study. In another, similar study, AST, ALT, and lactate dehydrogenase in the effluent all predicted non-function.[131] All enzymes released into serum are readily applicable to a perfused organ during preservation, and it might be possible to define the concentrations of AST, ALT, or lactate dehydrogenase that predict non-function.

Thrombomodulin has been measured clinically from blood samples taken immediately after reperfusion.[132] This marker was shown to correlate with subsequent transaminase elevation and the amount of intrasinusoidal granulocytes seen on biopsy 1 h after reperfusion. Increased thrombomodulin concentrations indicate endothelial injury, which often exceeds hepatocellular injury; it has been claimed by some that this does not correlate with graft function as well as hepatocellular injury.[29,130,131] However, endothelial injury has been shown to be a key initiating event in ischaemic injury during preservation and reperfusion.[19] Furthermore, in this study, rises in thrombomodulin concentrations did correlate with subsequent rises in transaminase. It is likely that hepatocellular injury occurs subsequent to endothelial activation and injury. This implies that markers of hepatocellular injury (transaminases) are more specific in predicting graft failure, whereas markers of endothelial injury (thrombomodulin) are more sensitive. In this latter study, measurements were taken 3 min after reperfusion, suggesting that this could provide rapid assessment of viability during perfusion.

Data from the Barcelona non-heart-beating donor study suggested that hyaluronic acid and glutathione-S-transferase could be used as viability markers since these correlated inversely with survival.[95] While measurement of these enzymes did clearly distinguish between the 20-min and 40-min cardiac arrest groups, it did not separate the 30-min and 40-min cardiac arrest groups, implying poor sensitivity. Similarly, measurement of hyaluronic acid in the effluent of cold-stored human livers before transplantation has been shown to be unhelpful.[133]

Bile—Bile production, an important indicator of a functioning liver after transplantation, can easily be monitored on perfusion circuit. In rats, bile flow after transplantation predicted survival and was correlated with ATP content, which also separated survivors from non-survivors.[126] Clinical studies using T tubes to monitor bile after transplantation have concluded that it is possible to detect grafts destined for primary non-function by low bile acid concentration and high lecithin concentration.[134] Serum total bile acids have been shown to rise in the human transplant recipient during the anhepatic phase and correct rapidly upon hepatic reperfusion.[135] Graft failure has been shown to occur when this fall in serum total bile acids did not occur.[136] Thus, the provision of a fixed amount of bile acid to a perfusion circuit, allowing the rate of clearance to be measured, might be useful as a rapid means to predict viability in a short time; this has not been tested experimentally.

Aminoacid clearance—Hepatic clearance rate of amino acids was shown to predict survivors very clearly in a study of progressive ischaemia in canines.[137] When subjecting dogs to 30, 60, or 90 min of hepatic ischaemia, prediction of function by aminoacid clearance was better than with transaminases, total flow, arterial ketone body ratio, tissue oxygen pressure, and oxygen consumption. Clearance can be calculated over a short time span and, thus, this might be of value as a means of assessment of a perfused liver before transplantation.

Venous oxygen saturation—Hepatic venous oxygen saturation in the porcine transplant model was reported to be significantly higher in functioning grafts than in their non-functioning cohorts.[138] Although not clinically helpful because the data are collected after transplantation, it would be an easy variable to track on a perfusion circuit giving continuous, real-time feedback of graft viability.

Perfusion flow dynamics—As in the case of perfused kidneys, the flow characteristics of livers during perfusion indicate the degree of injury sustained. In the Barcelona study, total and portal flow was shown to correlate with warm ischaemia time.[139] By applying a specific cut-off point for total flow at 1·7 L/min per m^2, prediction of post-operative survival was possible with specificity of 87·5%, and sensitivity of 82%. In addition, the hepatic oxygen extraction ratio was noted to be higher with longer warm ischaemia times and to be significantly higher in the livers that did not survive. There is a comprehensive array of physiological variables that might be useful in predicting the function of a liver before transplantation. However, most are of limited clinical application due to the fact that they are monitored after transplantation. For this reason, a review of experimental data relating to markers of synthetic function, drug metabolism, bile secretion, energy metabolism, microvascular injury, and other variables concluded that none of these methods was better than conventional liver function tests and liver appearance.[140] However, use of perfusion during preservation would render such measurements clinically applicable by testing the perfusate. With increasing clinical experience with perfusion, the safe limits could be defined that would readily distinguish a viable organ from one destined to fail.

Conflict of interest statement
None declared.

Acknowledgments
C Imber was funded by the Hanson Trust. The authors are also funded by Roche Organ Transplant Research Foundation. The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

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