

PERSPECTIVE

HYPOTHERMIC ORGAN PERFUSION IN THE 2020'S: MIXING THE BENEFITS OF LOW TEMPERATURES AND DYNAMIC FLOW OUTSIDE THE BODY

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Abstract

The cold chain supply of donor organs for transplantation has been an integral part of the delivery of transplant clinical services over the past five decades. Within the technologies used for this, hypothermic machine perfusion (HMP) was a concept, which was attractive to maintain organs under optimal conditions outside the body, and many early research studies on HMP were reported. However, it took the arrival of important new concepts to ensure that HMP was logistically feasible and valuable from an organ physiology perspective within the clinical pathways. This review provides details of the current status of HMP across the range of organs transplanted in the clinic, and discusses what new areas might benefit from applying HMP in coming years. In conclusion, HMP is now being used more frequently for clinical organ preservation in a variety of settings. As new therapies such as cell or gene therapy become more common, HMP will continue to play an important facilitator role for optimising organs in the donor pathway.

Keywords: dynamic cold perfusion; hypothermic machine perfusion; organ perfusion systems; organ preservation.

Abbreviations: DCD – donors after cardiac death, DHOPE – dual hypothermic end-oxygenated perfusion, EVLP – *ex vivo* lung perfusion, HMP – hypothermic machine perfusion, HOPE – single hypothermic end-oxygenated perfusion, MPS – machine perfusion solution, SCS – static cold storage

INTRODUCTION

It is some 50 years since the late David Pegg, one of the founders of the Society for Low Temperature Biology, organised a symposium

on Organ Preservation (1). This was one of the first publications to focus on applying low temperature sciences to the growing field of organ transplantation, joining other pioneering reports from that period (2). It brought together

the small number of advocates in UK and Europe developing different strategies to sustain organs outside the body, including static cold storage (SCS), hyperbaric preservation, cryopreservation, and hypothermic machine perfusion (HMP). Since that time there have been many research studies on the topics, and of those, HMP gradually developed into a technology with global application, but in reality, this was only achieved in the 2000's, driven by differing needs of clinical services, crucial technical improvements, and a greater willingness to invest the scientific and surgical effort towards high quality, reliable and repeatable HMP. Now, we are moving into an era of application of HMP across a range of organs, and this review will seek to provide an understanding of these.

The processes of isolating organs for transplantation from the body circulation have become highly skilled and globally-practiced surgical procedures (3). These almost invariably require flushing of chilled sterile synthetic solutions into the vascular bed of the target organ to wash out blood, to start a cooling process and to mitigate against intra-organ thrombosis. At this point the vascular bed has been opened by the hydrostatic pressure of the flush solution (pragmatically delivered by a hydrostatic head of about 1 meter), which facilitates any application of HMP. The major inflow blood vessels (e.g., renal artery, liver portal vein) are amenable to cannulation for HPM, since these have been dissected free from tissue connections during the organ retrieval, although practiced manipulation must still be applied to avoid damage, which might later interfere with the surgical transplantation. The organ is then ready to be placed onto the HMP rig, often seated in a soft cradle arrangement to support organ weight and stabilise the position in relation to the inflow cannula. In the majority of cases, the outflow vessels are not cannulated, since early experiences indicated that this might increase resistance to outflow, potentially causing organ swelling and interstitial oedema (4-7).

Various iterations of HMP rigs have been developed over time, including the introduction of sterile disposable units for clinic use. It quickly became apparent that a recirculating system was needed to avoid wastage of large volumes of perfusate. The basic units comprise (A) sterile tubing circuit; (B) a reliable pump; (C) a cooling system, and monitoring for

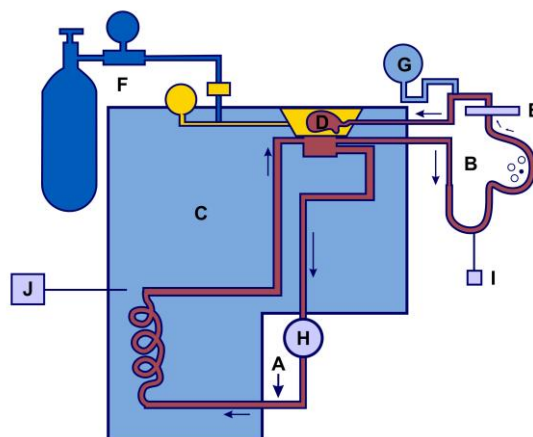


Figure 1. A stylised HMP circuit. The main tubing circuits (A) and organ pump (B) are the main components with cooling provided by environment (C). The perfusate flows in the direction of arrows to the organ (D) through the filter (E) and pressure monitor (G). If oxygen is supplied (F) the gas tubing also contains filters and pressure/flow monitors. (Older systems sometimes used a secondary cooling pump (H) to improve temperature control but this is not common now). Perfusate flow rate monitoring (I) and tracking of the system cooling (J) may be additional.

perfusion pressure and temperature; (D) organ perfused with synthetic cell-free solution in a sterile chamber; (E) filtration system for the recirculating perfusate; (F) a supply of oxygenation (not in all cases) and in line pressure monitor (G); – see below Figure.1) Further details of the essential components for HMP can be found in other reviews (8-11).

For the purposes of this review, it is just worth making comments on a few of these aspects.

The application of optimal perfusion pressure by the pump is an area of importance to avoid injury to the organ vascular bed. Because the perfusate is an aqueous solution containing multiple solutes, and used at cool temperatures, viscosities differ from what the organ would experience *in vivo*. Thus, optimised perfusate flow rates have been found to be about 25% of what the organ would experience in the body (12, 13). However, since metabolic rates will also be depressed by cooling these low flows are pragmatically accepted as effective. Nevertheless, HMP is also supporting oxidative metabolism, which has posed the question about active supply of oxygen to the perfusate. This is a complex question since the cool aqueous

perfusate will carry some dissolved oxygen, which may be sufficient for a particular organ (14). This debate continues even now, but there appears to be a growing appetite for active oxygenation (14, 15). The composition of the synthetic perfusates has changed over time and with the organ of choice on HMP. However, as a generalisation, the solutions contain mixes of ionic compounds, buffers, oncotic agents and energy substrates (often carbohydrates), reflecting both alignment with plasma solutes but also seeking to enhance organ preservation at cool temperatures (9, 10,16). Finally, the nature of the applied pressures delivered by the pump has been debated, with some proponents arguing that a pulsatile pressure wave form better protects the organ vascular bed (12, 17), (as an observation, most peristaltic pumps used in HMP produce some form of pulsatile pressure waves).

CURRENT CLINICAL AND TRANSLATIONAL APPLICATIONS OF HMP

Kidney HMP

Renal HMP can be considered in some ways as the leading area for the development of machines and protocols delivering *ex vivo* low temperature for organ perfusion, following many studies in the middle years of the previous century (18-20). However, the size and complexity of the equipment available at the time meant that only a few centres (21-23) continued with HMP.

The main advance came in the 2000's with the development of a new generation of portable kidney HMP machines, such as the Lifeport module (Organ Recovery Systems, Illinois, USA), Kidney Assist machine (Organ Assist, Groningen, Netherlands) or Waters 3M device (Minnesota, USA). Kidneys can be perfused via disposable tubing sets and organ placement cradles, with pressure-controlled peristaltic pumps, in line bubble traps and filters, and maintained at between 4 – 10 °C, usually by surrounding but separated from melting ice. In the majority of cases, Belzer KPS1 solution is the chosen perfusate. The availability of these reliable machines drove a number of clinical trials, which showed that, especially in the case of organs from extended criteria donors, improvements in post-transplant renal function could be demonstrated for organs undergoing HMP versus SCS (24-26). Throughout the

2010's, many more centres engaged in renal HMP with high-quality systematic reviews continuing to show the value of cold perfusion versus static storage (27, 28). Particularly for kidneys, which have experienced hypoxia during retrieval, the active addition of oxygen to the perfusate was found to be beneficial to outcomes and could be provided by simple bubble or surface pass of gas to the cold perfusate (29, 30).

Currently, renal HMP is being practiced in many centres worldwide on a routine basis, using well-established techniques with good transplant outcomes. Several approaches have proposed organ assessment during renal HMP (29, 31), an area requiring further research.

Another research topic is whether stored kidneys respond equally successfully to a period (often 2 – 6h) of single inflow hypothermic end-oxygenated perfusion (HOPE) as they do to continuous renal HMP (29, 31), which if proven could simplify logistics of the application further. Since HOPE is delivered just before the recipient operation, it avoids a need for transport of sensitive equipment over long distances. Also, questions about the optimal temperatures used for *in vitro* perfusion (hypothermic, sub-normothermic or normothermic) remain to be answered, particularly in novel areas such as targeted cell or gene therapy whilst the organ is being perfused (29). Future work will need to evaluate the cost/benefit ratios for normothermic perfusion techniques (32, 33) against HMP for routine clinical practice.

Liver HMP

Liver HMP may now be second only to the kidney in clinical application over the past few years, but whilst there was clear academic interest in the technique back to the 1970's (34-36), widespread uptake awaited clinical trials, which identified its' safety of efficacy (37, 38). It is also the organ system where the value of HOPE has had significant focus over the past decade (39-41), with evidence of regeneration of energy supplies. Limiting HMP to the end of preservation after the livers have been retrieved and stored by SCS simplifies the logistics of perfusion. The organ anatomy (with 2 major vascular inflow sites at portal vein and hepatic artery has also required developments of HMP equipment capable of delivery both simultaneously and generated debate about the relative merits of these approaches (42).

The prospective evaluation of liver HMP is building strongly, and the multicentre dual

inflow HOPE (DHOPE) trial used donors after cardiac death (DCD) has recently reported outcomes (43). Dual inflow perfusion was carried out for a minimum of 2h at 10 °C at low pressures and pulsatile wave form, using the Organ Assist device, with Belzer Machine Perfusion Solution (MPS), and 100% oxygen supplied to the oxygenator. A clear statistical benefit was seen in the DHOPE group compared to SCS organs when the development of post-transplant biliary complications (biliary strictures related to organ preservation can be life-threatening, and were reduced by a factor of 4 by using the HMP regime). A similar HMP protocol was applied again using Organ Assist for extended criteria liver grafts and either single (portal vein) inflow alone, or dual inflow, and 2h oxygenated perfusion at 12 °C in a single centre trial (44). Early indices of liver function were improved by HMP, but the trial was not large enough to differentiate between single or dual perfusion benefits. In another single centre study using similar HOPE protocols, the majority of 25 perfusions were performed with dual inflow and oxygenation (45). HMP was associated with

a reduction in post-reperfusion syndrome (an indicator of poor preservation) and in acute kidney injury, (which is also a patient complication resulting from poor liver preservation) compared to SCS. The value of HMP in improving vascular resistance in the livers was also noted. Technologies continue to be developed and Figure 2 shows VitaSmart, which is another HOPE machine.

Further trial protocols for liver HMP continue to be reported and there will be more information accumulating in future years. As with other HMP, there will also be evaluation of HMP against normothermic liver perfusion, which is also being trialled, perhaps providing indications for where one or other technique may be most beneficial.

Heart HMP

HMP of heart has also been of interest for several decades (46), but clinical translation has been slow so far. In the intervening period, there were many small animal studies but HMP of human hearts was only rarely reported, attributed in part to a lack of commercial interest in developing suitable equipment (47). The Steen group is one, which has worked on heart HMP consistently over a number of years (48). In most cases, the equipment was developed to provide retrograde aortic perfusion of the heart at low flow and perfusion pressures (47, 48). In a porcine model, Collins et al (47) used a modified UW solution in which the potassium concentration was reduced to 5 mmol/L to avoid problems of vasoconstriction. Perfusion was undertaken using a modified renal HMP device for periods of 4 hours at 4 - 6 °C. Perfusion related oedema of the heart was noted but considered to be without long-term detrimental effects on tissue viability. A small number of human hearts donated for research were preserved on the HMP equipment with good recovery of functional indices when the organs were subjected to *in vitro* warm blood reperfusion.

A transportable heart HMP system was developed by Steen and colleagues (48) and used to preserve porcine hearts, which were subsequently transplanted. Hearts were perfused for 24h at 8 °C in a model of organ donation after brain death. The equipment included automatic control systems for pressure and flow during perfusion, automatic gas exchange and a leucocyte filter. An intermittent perfusion profile (15 min every hour) was imposed with a



Figure 2. An example of liver HMP for clinical DHOPE (Vitasmart). The main console houses the pump, filter, oxygenator and control panel, with a display panel for continuous readout. The liver is housed in the ice-cooled bowl covered by the sterile drape. (Image provided by Bridge to Life).

pressure of about 25mm Hg aimed to provide flows of about 100 ml/min. A customised perfusion solution (48) was used with a low K⁺ concentration (23 mmol/L) and range of pharmacological and hormonal supplements and albumin (75g/L). Using this HMP system, good stable cardiac function was observed over 24h of transplantation. The same system was used more recently in a continuous perfusion mode for 8h HMP and subsequent assessment by heterotopic xenografting of hearts obtained from genetically modified pigs (49). Good and stable cardiac function after HMP was demonstrated and there was clear evidence of an additional benefit of the HMP with indices of passenger donor leucocyte depletion and reduction of inflammatory markers during the preservation perfusion. Later, genetically modified porcine heart, which was for the first time in transplantation history transplanted to a human patient, was preserved under HMP with Steen machine and solution being used (50). (Initial cardiac function was good but unfortunately, the recipient died from complications two months later).

The place of cold heart HMP in the overall strategies for heart preservation will need further in-depth analyses. For example, in a porcine model, 4h heart HMP using HTK solution at 4 °C and a pressure of 15mm Hg had inferior outcomes to organs maintained at 37 °C with perfusion of hormone supplemented autologous blood at 60 mm Hg pressure (51). It may well be that further optimisation of perfusate composition and flow/pressure relationships during heart HMP will be required to obtain maximum benefit from the HMP strategy.

Lung HMP

Lungs are another organ system where HMP has been only infrequently studied. Early on, Toledo-Pereyra's group (52) reported on HMP of a lung-heart anatomical bloc during 24h at 4 °C in a canine model using a hyperosmolar colloid solution containing dextrose, sucrose and ATP-MgCl₂ plus oxygen free radical scavengers. Post-HMP *in vitro* blood perfusion for 3h demonstrated successful gas exchange and patency for the lungs. Over the past decade, normothermic perfusion of lung grafts has become more popular (53-55), but is out with the scope of this review. However, a study in a canine transplant model of lung donation after cardiac death employed a 2h end-ischemic period of HMP to recondition the lungs (56). Lung perfusion was carried out at 10 °C using

Steen solution. Dynamic pulmonary compliance was stable during HMP, and biopsies demonstrated better washout of microthrombi and white cells compared with lungs subjected to SCS. The authors commented on the fact that HMP techniques were potentially less demanding than those carried out at higher temperatures. More recently, extended (24h) *ex vivo* lung perfusion (EVLP) was performed on human donor lungs donated for research, which had been preserved by SCS for up to 24 h (57). Whilst benefits for the EVLP system were reported, once again the perfusion temperature was run at 37 °C. Thus, at present, the future of lung HMP is under question and may need identification of novel technologies to make it attractive when compared to EVLP run at normothermia.

Pancreas HMP

Pancreas transplantation has the potential for high clinical utilisation for amelioration of diabetes and metabolic syndrome, but utilisation of donated organs has been limited by concerns about graft ischaemic injury and coagulation. In addition, the organ is friable with complex vascular inflow resulting in technical challenges for developing equipment. Pancreas HMP has been infrequently studied since early work from Florack and colleagues (58) who showed in a canine segmental autotransplant model that a filtered plasma perfusate for pulsatile HMP was successful only in about 60% of grafts with oedema and technical factors as complications. A decade ago, Papalois group used a porcine pancreas model to investigate HMP at between 4 – 10 °C using Waters RM3 perfusion machine with perfusate passive oxygenation and inflow cannulation via an aortic conduit. Using Belzer MPS, pulsatile perfusion pressures were in the range 15 – 23 mm Hg (giving total flow volumes of about 150 ml min⁻¹) over 5h (59). In organs, which were subjected to 3h ischaemia prior to HMP, the histology of pancreas injury was significantly reduced by the HMP period. More recently the same group used human pancreata donated for research, which underwent HMP in the same system followed by warm blood perfusion *in vitro* (60). Results demonstrated good preservation of both exocrine and endocrine functions of the pancreas, along with a strong insulin release in response to an added bolus of glucose. The challenges of the complex pancreatic inflow anatomy were met by Leuvenink and colleagues who used a dual

pump perfusion circuit in human research organs (61). A customised dual arterial perfusion system was constructed with two pumps and pulsatile flow into the two main arterial conduits for the pancreas, with oxygenation and pressure controls. Low perfusion pressures (25 mm Hg) were maintained at 5-7 °C for 6h using Belzer MPS. Ten organs were perfused (5 after donation via brain death, 5 after cardiac death with longer ischemic times), in all cases HMP stimulated ATP production to good levels compared to starting points and there was no gross oedema. As another index of viability, two organs underwent experimental isolations of Islet of Langerhans, which in turn had good viabilities and function.

The role of pancreas HMP is also of potential interest in organs to be used specifically for Islet isolations, where subsequent warm perfusion with digestive enzymes is needed. Taylor and colleagues (62) used a porcine model to perform 24h HMP at low pressure (10 mm Hg) using Kidney Preservation Solution 1 on a Lifeport kidney perfusion machine and dual cannulation. Greater purity and yields of Islets (x3) were obtained compared with those from static cold stored organs. More recently the Leuvenink group investigated the dual cannulation, pressure controlled HMP system previously described (61) to subject research donor pancreata to 6h perfusion before islet isolation and assessment by xenografting Islets into mice (63). Results indicated that HMP allowed isolation of Islets with high viabilities and good glucose tolerance testing after implantation.

In common with other organ systems, the debates continue surrounding the choice of HMP or normothermic perfusion for the pancreas (64), and this will need further clarification in the next few years.

HMP and organ viability assessments

Whilst the use of low temperatures in HMP might be thought to preclude the measurement of viability, there have in fact been a number of developments, which defy this point of view. The improvement in vascular flow and reduction of a high resistance over the course of HMP has been linked to organs, which function well after transplantation (29, 65). The release of intracellular enzymes can be measured in the perfusate (37, 66) but often does not provide clear-cut differentiation between well or poorly preserved organs. More recently, focus on

release of mitochondrial component (Flavin mononucleotide, FMN) have become a focus for organ viability (67, 68) with encouraging results. These and other molecular assays will increase the utility of HMP further.

CONCLUSIONS

The next few years will be a very interesting time for HMP. The technique will be critically evaluated against other perfusion modalities such as normothermic organ perfusion, but the new developments in HMP such as improved machines, novel viability markers and favourable service cost analyses will likely mean that HMP will be an invaluable technology in transplantation well into the future. In addition, organs which have been functionally challenged prior to preservation (such as those from DCD) may benefit from metabolic repair during HMP and so increase the number of transplantable organs. HMP will also be a potentially crucial contributor in other areas of novel cryo-biotechnology, such as shallow sub-zero organ preservation for medium-term extended storage (69) or assisting the delivery of stem cells for organ conditioning (70). These will undoubtedly drive innovation and application.

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