Thesis

Comparison of Machine Perfusion Solutions in Liver, Kidney and Pancreas Transplantation: a Comprehensive Review

submitted by

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Graz, 09.12.2024 *Bohdana Oliinyk eh.*

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Zusammenfassung

Hintergrund: Die Organtransplantation hat sich traditionell auf die statische Kaltlagerung als Standardmethode zur Konservierung von Bauchorganen, einschließlich Leber, Niere und Pankreas, vor der Transplantation verlassen. Obwohl die Kaltlagerung zuverlässig ist, gibt es Einschränkungen hinsichtlich der Erhaltung der Organviabilität, insbesondere über längere Zeiträume. In den letzten Jahren hat die Maschinenperfusion als vielversprechende Alternative an Bedeutung gewonnen, da sie eine dynamische Konservierung ermöglicht, indem die Organe kontinuierlich perfundiert werden, um physiologische Bedingungen zu simulieren. Die Hauptperfusionsmodalitäten – hypotherme Maschinenperfusion (HMP), subnormotherme Maschinenperfusion (SNMP) und normotherme Maschinenperfusion (NMP) – bieten die Möglichkeit, die Konservierungsqualität zu verbessern, ischämische Schäden zu minimieren und den Pool an Spenderorganen zu erweitern, indem auch marginale Organe für die Transplantation geeignet gemacht werden. Trotz dieser Fortschritte bleibt die Wahl der Perfusionslösung ein kritischer Faktor, der den Erfolg der Organkonservierung und der Transplantationsergebnisse beeinflusst. Die Zusammensetzung und Eigenschaften von Perfusionslösungen können den zellulären Stoffwechsel, die Gewebeintegrität und die allgemeine Organfunktion beeinflussen. Daher ist es wichtig, verschiedene Perfusionslösungen systematisch zu untersuchen und zu bewerten, um ihre Wirksamkeit in verschiedenen Perfusionsmodalitäten zu bestimmen. Die Standardisierung der Verwendung der am besten geeigneten Perfusionslösungen könnte zu einer verbesserten Organviabilität, besseren Transplantationsergebnissen und einer erhöhten Verfügbarkeit von Spenderorganen führen.

Methoden: Die PubMed-Datenbank wurde nach Artikeln durchsucht, die Wortkombinationen zu Leber-, Nieren- und Bauchspeicheldrüsen-Maschinenperfusion betrafen. Primär wurden Studien, die verschiedene Perfusionslösungen und deren Modifikationen verglichen, in die Ergebnisse aufgenommen und kurz in einer Tabelle zusammengefasst, um einen besseren Überblick zu ermöglichen. Zusätzlich wurden relevante Artikel über den experimentellen und klinischen Einsatz bestimmter Perfusionslösungen, einschließlich ihrer Verwendung in randomisierten kontrollierten Studien (RCTs), ebenfalls berücksichtigt.

Ergebnisse: Es wurden insgesamt 53 Artikel gefunden, die einen direkten Vergleich von Perfusionslösungen und deren Modifikationen behandeln. Insgesamt konzentrierten sich 25 Studien auf die hypotherme Maschinenperfusion (HMP), davon 12 auf Leber-HMP und 13 auf Nieren-HMP. Vier Studien behandelten die subnormotherme Maschinenperfusion (SNMP),

aufgeteilt zwischen Leber und Niere. Zwanzig Studien verglichen Perfusionslösungen während der normothermen Maschinenperfusion (NMP), davon 11 auf Leber und 9 auf Niere. Zusätzlich wurden vier Studien zu Wiedererwärmungsverfahren behandelt (2 Leber, 2 Niere). Sieben Studien untersuchten Perfusionslösungen an menschlichen Transplantaten in experimentellen oder klinischen Transplantationseinstellungen. Es wurde nur eine Studie gefunden, die Perfusionslösungen während der Bauchspeicheldrüsen-Maschinenperfusion vergleicht. Relevante klinische Studien und RCTs wurden ebenfalls einbezogen.

Diskussion: Für die hypotherme Maschinenperfusion (HMP) bleibt die Belzer Maschinenperfusionlösung (MPS) die am weitesten verbreitete Lösung sowohl in der klinischen Anwendung als auch in randomisierten kontrollierten Studien (RCTs). Ihre weitreichende Nutzung unterstreicht ihre Zuverlässigkeit und Effektivität bei der Erhaltung der Organviabilität. Andere Perfusionslösungen zeigen jedoch ebenfalls vielversprechende Ergebnisse und könnten als alternative Optionen dienen. Dazu gehören HTK (Custodiol), IGL-2, Celsior und Vasosol sowie andere weniger erforschte vielversprechende Optionen, wie im Ergebnisteil beschrieben. Die ermutigenden Ergebnisse dieser Alternativen deuten darauf hin, dass weitere vergleichende Studien erforderlich sind, um ihre Wirksamkeit im Vergleich zu Belzer MPS und untereinander zu bewerten.

Bei der subnormothermen Maschinenperfusion (SNMP) scheint die Zugabe eines Sauerstoffträgers vorteilhaft für die Aufrechterhaltung des Organmetabolismus und der Viabilität zu sein. Obwohl die Forschung in diesem Bereich begrenzter ist, wurden beispielsweise auch Lösungen wie Celsior und Lifor ohne Zugabe eines Sauerstoffträgers getestet. Die Variabilität der Ergebnisse verdeutlicht jedoch die Notwendigkeit weiterer Untersuchungen, um die Zusammensetzung der Perfusionslösungen für SNMP zu optimieren und ihre potenziellen Vorteile zu bestätigen.

Die normotherme Maschinenperfusion (NMP) hat gezeigt, dass die Verwendung eines Sauerstoffträgers entscheidend ist, um physiologische Bedingungen zu simulieren und die Zellatmung zu unterstützen. Rote Blutkörperchen (RBCs) sind die am häufigsten verwendeten Sauerstoffträger in klinischen Settings und bieten eine effektive Sauerstoffversorgung der Organe. Hemopure, ein Sauerstoffträgerersatz, hat jedoch vielversprechende Ergebnisse gezeigt, was darauf hindeutet, dass es eine Alternative zu RBCs darstellen könnte, insbesondere in Situationen, in denen Blutprodukte nicht verfügbar oder unerwünscht sind. Auch andere alternative Sauerstoffträger zeigen Potenzial, und ihre Rolle in der NMP erfordert weitere

Erkundung, um die besten Formulierungen für die Erhaltung unterschiedlicher Bauchorgane zu bestimmen. Auch hinsichtlich des Mediums, das in Kombination mit dem Sauerstoffträger verwendet wird, wurde noch kein klarer Favorit gefunden, da verschiedene Optionen genutzt und untersucht werden.

Insgesamt hebt diese Übersicht die Notwendigkeit standardisierterer Forschungsbemühungen hervor, einschließlich gut geplanter RCTs, um die Effektivität verschiedener Perfusionslösungen sowie deren logistische und wirtschaftliche Vorteile direkt zu vergleichen. Solche Studien sollten nicht nur neue Lösungen mit Standardoptionen wie Belzer MPS und RBC-basierten Perfusaten vergleichen, sondern auch vielversprechende Alternativen untereinander bewerten. Auf diese Weise kann die effektivste Perfusionslösung für verschiedene Maschinenperfusion-Modalitäten identifiziert werden, was letztendlich die Organerhaltung, die Transplantationsergebnisse verbessert und den Pool an Spenderorganen erweitert.

Abstract

Background: Organ transplantation has traditionally relied on static cold storage as the standard method for preserving abdominal organs, including the liver, kidney, and pancreas, prior to transplantation. While cold storage is reliable, it has limitations in maintaining organ viability, particularly over extended periods. In recent years, machine perfusion has gained traction as a promising alternative, offering dynamic preservation by continuously perfusing organs to mimic physiological conditions. The primary perfusion modalities—hypothermic machine perfusion (HMP), subnormothermic machine perfusion (SNMP), and normothermic machine perfusion (NMP)—present opportunities to enhance preservation quality, minimize ischemic injury, and expand the donor organ pool by making marginal organs viable for transplantation. Despite these advancements, the choice of perfusion solution remains a critical factor that influences the success of organ preservation and transplantation outcomes. The composition and properties of perfusion solutions can impact cellular metabolism, tissue integrity, and overall organ function. Therefore, it is essential to explore and evaluate different perfusion solutions systematically to determine their efficacy across various perfusion modalities. Standardizing the use of the most suitable perfusion solutions could lead to improved organ viability, better transplantation outcomes, and increased availability of donor organs.

Methods: The PubMed database was searched for articles using word combinations, concerning liver, kidney and pancreas machine perfusion. Primarily, the studies comparing various perfusion solutions and their modifications were included in the results and summed up shortly in a table for easier overview. Additionally, relevant articles about experimental and clinical use of certain perfusion solutions, including their use in RCTs, were included as well.

Results: A total of 53 articles, where a direct comparison of perfusion solutions and its modifications were found. A total of 25 studies focused on hypothermic machine perfusion (HMP), with 12 on liver HMP and 13 on kidney HMP. Four studies addressed subnormothermic machine perfusion (SNMP), split between liver and kidney. Twenty studies compared perfusion solutions during normothermic machine perfusion (NMP), with 11 on liver and 9 on kidney. Additionally, four studies addressed rewarming procedures (2 liver, 2 kidney). Seven studies examined perfusion solutions on human grafts in experimental or clinical transplantation settings. Only one study comparing perfusion solutions during pancreas MP was found. Relevant clinical trials and RCTs were also included.

Discussion: For hypothermic machine perfusion (HMP), Belzer Machine Perfusion Solution (MPS) remains the most widely utilized in both clinical applications and randomized controlled trials (RCTs). Its widespread adoption underscores its reliability and effectiveness in preserving organ viability. However, other perfusion solutions also show promise and could serve as viable alternatives. These include HTK (Custodiol), IGL-2, Celsior and Vasosol, as well as other less-explored promising options, as described in the results section. The encouraging results from these alternatives suggest that more comparative studies are needed to assess their efficacy relative to Belzer MPS and each other.

In subnormothermic machine perfusion (SNMP), the inclusion of an oxygen carrier appears to be beneficial in maintaining organ metabolism and viability. Although research is more limited in this area, for exmaple, such solutions as Celsior and Lifor without an oxygen-carrier addition have also been tested. However, the variability in results highlights the need for further investigation to optimize the composition of perfusion solutions for SNMP and confirm their potential advantages.

Normothermic machine perfusion (NMP) has shown that the use of an oxygen carrier is crucial to mimic physiological conditions and support cellular respiration. Red blood cells (RBCs) are the most frequently used oxygen carriers in clinical settings, offering effective oxygenation of organs. However, Hemopure, an oxygen-carrying substitute, has demonstrated promising results, suggesting that it could serve as an alternative to RBCs, particularly in situations where blood products are unavailable or undesirable. Other alternative oxygen carriers also show potential, and their role in NMP warrants further exploration to determine the best formulations for preserving different types of abdominal organs. As for the medium used in combination with the oxygen carrier, there is also not a clear favorite found yet, with various options being utilized and studied.

Overall, this review highlights the need for more standardized research efforts, including well-designed RCTs, to directly compare the effectiveness of different perfusion solutions, as well as their logistic and economic advantages. Such studies should not only compare newer solutions against standard options like Belzer MPS and RBC-based perfusates but also evaluate promising alternatives against each other. By doing so, it will be possible to identify the most effective perfusion solutions for different machine perfusion modalities, ultimately improving organ preservation, transplantation outcomes, and expanding the donor organ pool.

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1. Introduction

Transplantation of abdominal organs is the gold standard for treating end-stage liver and kidney disease, as well as severe cases of diabetes (pancreas transplantation). It has been routinely performed for decades, with the first reports of human kidney and liver transplants dating back to the 1960s [1]. Yet, every year, many patients die while waiting for a transplant. According to the Council of Europe, 21 patients on waiting lists die every day without receiving a transplant, as of 2021. At the same time, many organs that could potentially be used for transplantation are being discarded. The Organ Procurement and Transplantation Network of the US reported a 20.1% non-utilization rate in 2019 [2]. Considering the fact that the supply of organs for transplantation still falls far short of meeting demand, new strategies are being developed to expand the donor pool by using marginal grafts or organs from extended criteria donors (ECD). There are different definitions of ECD, but some commonly mentioned factors include age (usually >60 years old), death from a cardiovascular event, prolonged warm ischemia time (WIT) and/or cold ischemia time (CIT), steatosis hepatis in liver transplantation, history of hypertension, and low creatinine clearance in kidney transplantation [3, 4]. Organs from such donors are more susceptible to various injuries and are generally considered to have a higher risk of graft failure.

This literature review focuses on the period of time between the procurement of the organ from the donor and its implantation into the recipient. It also explores new approaches during this step to improve existing protocols and techniques that could potentially enable the use of grafts that would otherwise be discarded.

1.1 Organ preservation and its challenges

The period between explantation and implantation of the organ is a crucial step in transplantation. From the minute the blood supply to the graft is cut off until it is cooled or reoxygenated, the organ enters a state of warm ischemia. During this stage, the organ sustains significant damage because, although conditions still support physiological metabolism, the tissues do not receive enough oxygen to function properly. As a result, anaerobic glycolytic metabolic pathways are activated, leading to insufficient ATP production, calcium overload, mitochondrial dysfunction, accumulation of harmful substances such as reactive oxygen species (ROS), and ultimately, cell death [5]. Warm ischemia time exceeding 30 minutes is associated with an increased risk of graft loss [6]. This is especially relevant in cases of donation after

circulatory death (DCD), as these organs experience periods of hypo-/anoxia even before organ procurement begins, and are therefore often discarded.

To reduce ischemic damage during organ preservation, most strategies involve cooling, as it is known that cellular activity gradually slows with decreasing temperature, reducing the production of toxic metabolites. It is important to note, though, that at 4ºC, the most commonly used temperature, tissues still operate at 10% of their normal metabolic activity. The next type of injury the organ can suffer prior to implantation occurs during cold ischemia time (CIT). This phase must be carefully managed, as it includes the time needed for transporting the organ to the recipient and preparing for the procedure. CIT can often be prolonged, which is associated with a higher rate of complications, increased risk of delayed graft function (DGF), primary nonfunction, and longer ICU and hospital stays [7-11].

After a hypothermic ischemic organ is rewarmed and begins receiving oxygen again, it suffers additional damage, this time due to ischemia-reperfusion injury (IRI). IRI is a multifactorial condition caused by sterile inflammation, an immune response to tissues previously damaged by anoxic conditions, leading to further cell death. It is important to note that grafts from ECD are even more sensitive to this type of damage [12,13].

To mitigate these damaging conditions during organ preservation, different approaches are employed. First, organ preservation can be static, where the organ is simply stored in a preservation solution, or dynamic, where it is perfused, simulating the natural blood flow that occurs within the organ. Dynamic preservation is typically performed with the aid of specialized machines, a process known as machine perfusion. Additionally, the temperature at which the organ is preserved can vary: it can be kept under hypothermic (4ºC), subnormothermic (20-25ºC), or normothermic (around 37ºC) conditions, each with its own specific considerations. Dynamic preservation of organs is nowadays performed with help of specialised machines, thus called machine perfusion.

1.2 Static cold storage (SCS)

Static cold storage (SCS) is the most straightforward, easy-to-execute, effective, and cost-efficient method of organ preservation. This is why it has been the most commonly used method, despite more sophisticated alternatives being developed since Collins et al.'s success with it at the beginning of transplantation practice in the 1960s. An important step in

hypothermic organ preservation is the initial cooling of the organ, achieved by flushing cold preservation solution through the main vessel(s). This process rapidly decreases the temperature, quickly lowering metabolic activity and preventing further warm ischemia.

Naturally, SCS still has its limitations, such as cold ischemia damage, which cannot be ignored, especially with prolonged cold storage. For example, increased CIT in kidneys correlates with higher serum creatinine levels, lower creatinine clearance, increased urinary biomarkers of ischemic damage (Endothelin-1, Total nitric oxide), and poorer renal and tubular cell function [14]. These issues necessitate limiting the duration of this type of preservation, which varies depending on the organ in question. IRI is also an inevitable consequence in this setting. Additionally, SCS does not allow for the evaluation of the functional ability of the organ before implantation, and it limits therapeutic approaches during preservation due to decreased metabolic activity in cold conditions [15]. Therefore, SCS might not be the ideal method for preserving marginal organs or organs from ECD, which are being transplanted increasingly more often.

Nevertheless, the current gold standard for kidney preservation is SCS at 4ºC, with the organ submerged in a preservation solution on ice slush. However, as mentioned earlier, prolonged periods of SCS (>24h) are considered harmful to the organ due to high microvascular restriction, which increases IRI and, consequently, DGF and chronic graft failure [16]. Similarly, liver preservation by SCS has also been the standard, though CIT is usually limited to 16 hours and is associated with higher morbidity and longer hospital stays when cold storage exceeds 7 hours [17, 18]. SCS also remains the gold standard preservation method for the pancreas, with CIT of 12 hours or more being considered prolonged [19, 20].

1.2.1 SCS preservation solutions

Preservation solutions play a significant role in SCS, preventing or minimizing unfavorable effects of hypothermic conditions, such as interstitial and cell swelling, intracellular acidosis, and injury associated with increased ROS production. It is also important to provide the organ with substrates for the regeneration of energy compounds during reperfusion. To fulfill these functions, general components of most preservation solutions include:

- Colloids, such as hydroxyethyl starch (HES) or polyethylene glycol (PEG), used to control interstitial edema;
- Impermeant anions, that prevent cell swelling by controlling osmotic balance (glucose, mannitol, sucrose, raffinose, citrate);

- Buffers for pH control (bicarbonate, phosphate);
- Antioxidants and free-radical scavengers that reduce ROS production and help utilize them (allopurinol, glutathione);
- Energy substrates, such as adenine, adenosine, and amino acids, considering that even under hypothermic conditions, metabolism remains active to some extent [21].

The first solution developed for SCS was Collins solution, which allowed kidney preservation for up to 30 hours and was first used by Collins in 1969. The solution contained high potassium and low sodium levels, imitating intracellular conditions. High glucose concentration was used to control osmotic balance and edema. To decrease intracellular acidosis, a phosphate buffer was used. The original composition of Collins solution was widely used for over 10 years for kidney, lung, and liver preservation. Eventually, Collins C2 solution, with a higher glucose level, was developed to further decrease intracellular swelling. After magnesium phosphate precipitates were observed, magnesium sulfate was removed from the solution, creating a newer solution called Eurocollins (EC) [15, 21]. EC was successfully used in transplantation for many years and is not forgotten to this day, as recent articles have suggested its safe use, for example, for liver preservation in combination with University of Wisconsin solution (UW) as an effective and economic substitute [22]. Another study suggests effective use of EC for the procurement of marginal kidney grafts from DCD [23]. Nevertheless, EC is not commonly used anymore as it has been overshadowed by newer solutions. It has been shown in multiple randomized controlled trials (RCTs) that EC use results in a higher incidence of delayed graft function (DGF) in kidney transplantation compared to UW and histidine-tryptophan-ketoglutarate solutions [59]. Furthermore, EC has been reported to have less cooling power than Custodiol, Celsior, and Ringer lactate [24]. Another example is a study reporting EC's inferiority to Belzer solution in cold storage of rat pancreases [25].

Another solution developed in the 1970s was hyperosmolar citrate (HOC), also known as Marshall or Soltran solution. It was used for kidney preservation for up to 72 hours, mostly in Australia and the UK [26-28]. As it is more affordable than newer solutions and has comparable transplantation outcomes, it is still used in some hospitals [29].

A new era of preservation solutions began with the development of UW solution (also known as Belzer solution) by Belzer et al. in 1986. It shared some qualities, such as intracellular sodium-potassium balance and phosphate buffer, with EC, which was clinically used at that

time. The innovative composition of UW solution was initially discovered during a study aimed at reducing tissue edema in pancreas preservation, where lactobionate and raffinose were added instead of glucose. This was after the team proved that decreasing molecular weight correlates with increased edema [30]. Additionally, HES was added, raising oncotic pressure but making the solution viscous and somewhat harder to store. Allopurinol and glutathione were added as antioxidants, and adenosine was added as a source for replenishing energy compounds after reperfusion. UW solution has shown excellent results from the beginning, has been used in the clinical practice of kidney, liver, and pancreas transplantation for decades, and is still used today, although newer solutions attempt to address some of its shortcomings, such as high cost. Nevertheless, they are inevitably compared to the standard set by Belzer solution [21,31,32].

Since the use of HES in UW has raised concerns due to its high cost, viscosity, and possible biochemical interactions, dextran has been suggested as a replacement, showing good results in the 1990s [49-52]. However, no dextran-based solutions for abdominal organ preservation have appeared on the market.

Another widely used preservation solution is histidine-tryptophan-ketoglutarate solution (HTK), also known as Custodiol, which was initially used for cardioplegia and then heart transplantation in the 1980s. It contains intracellular sodium levels, low potassium levels, and no colloid, making it less viscous than UW. It also includes mannitol as an impermeable agent, α-ketoglutarate as an energy substrate, histidine as a buffer, and tryptophan as an antioxidant. Later, the solution was altered according to new knowledge, adding protective glycine, alanine, iron chelators (deferoxamine and LK-614), introducing sucrose, and removing mannitol. The new solution was called Custodiol-N [221]. Since the 1990s, HTK has been effectively used for the preservation of liver, kidney, and pancreas and is still routinely used today [21,31,33].

Celsior is another colloid-free solution developed in 1994, originally used in heart transplantation. It differs from earlier solutions with its extracellular sodium-potassium balance. Celsior contains mannitol, lactobionate, reduced glutathione, histidine, and glutamate. Its special focus on decreasing calcium overload is achieved by slight acidosis and high magnesium content [34]. Celsior has been successfully used in the preservation of liver, kidney, and pancreas, showing results comparable to UW and HTK, and remains viable today [35-37].

Institut-George-Lopez-1 (IGL-1) is another solution with extracellular sodium and potassium concentrations developed in the early 2000s. It is based on PEG, a large and flexible water-

soluble polymer that acts as an impermeable agent in IGL-1 and creates high osmotic pressure to prevent edema. Unlike HES, PEG is less likely to interact with biochemicals. It also makes the solution less viscous than UW but still more so than the non-viscous HTK. Other components of IGL-1 include lactobionate and raffinose (additional impermeables), phosphate buffer, glutathione and allopurinol as ROS scavengers, and adenosine as a nutrient [38-40]. IGL-1 is a popular preservation solution choice in Europe and has shown results comparable to, and in some aspects even superior to, UW and HTK for liver, kidney, and pancreas preservation [41-43].

Other SCS solutions worth mentioning include Solution de Conservation des Organes et des Tissus (SCOT) and hypertonic citrate adenine solution (HC-A). SCOT is another solution containing PEG, developed in the early 2000s in France. It has shown promising results in kidney and liver preservation; for example, a clinical study demonstrated that SCOT 15 decreases cytokine release from ischemia/reperfusion injury (IRI) and results in less acute kidney injury compared to UW [46-48].

HC-A, developed in the 1970s in China, has been the most common kidney preservation solution there for decades and is still widely used today. It is based on hyperosmolar citrate, containing adenine as a nutrient and mannitol for edema control [33,44]. Its successor, HC-A II, was improved with additional phosphate buffer, arginine, tryptophan, and ligustrazine as cell stabilizers and mitochondrial protectors, as well as reduced magnesium concentration and increased adenosine content [45]. Unfortunately, there is limited reporting on HC-A in English literature, despite its successful use in China.

Several studies have compared widely used preservation solutions for SCS, but no clear answer has emerged regarding the best fit, especially considering various settings such as single-organ or multi-organ retrieval, including thoracic organs, and durations of CIT. Some studies conclude that the most popular solutions have similar outcomes. For example, a multicenter French study comparing 4,928 liver transplantations from 2008 to 2013 found no significant differences in 1, 3, and 5-year patient and graft survival, or the length of ICU stay based on the type of preservation solution used (CS, IGL-1, UW, SCOT 15) [53]. Similarly, a recent meta-analysis found no significant difference in outcomes for UW, Celsior, HTK, and IGL-1 in liver transplantation [54]. Another study comparing UW and CS in kidney preservation showed no significant difference in creatinine levels at 1 and 12 months, as well as rates of DGF and graft

failure after 5 years between these two solutions [55]. For pancreas preservation, UW and HTK were compared in a smaller study, showing comparable serum fasting blood glucose, amylase levels, and short-term patient and graft survival rates at a mean CIT of 11 3 hours [56].

At the same time, some studies find certain solutions superior in specific settings. For example, an experimental pancreas transplantation study suggests that Celsior is less effective in preventing pancreatic ischemia/reperfusion damage compared to UW [57]. A retrospective analysis of 380 pancreas transplantations also comparing preservation solutions stated that IGL-1 had the best graft survival rates, while HTK was associated with an increased pancreatitis rate [217]. As for liver preservation, experimental studies demonstrate that IGL-1 provides better protection for steatotic liver, resulting in less damage, higher autophagy induction, and less apoptosis compared to HTK [58]. IGL-1 also appears to preserve steatotic livers better compared to UW in a rat model [216]. Another study in clinical liver transplantation suggests IGL-1's superiority over HTK, showing lower enzyme peaks and better short-term survival rates [60]. However, some studies suggest HTK may have fewer biliary complications compared to UW and be more cost-effective [61]. A smaller study comparing Celsior to UW for livers from donors older than 80 years showed better long-term transplantation results, such as graft and donor survival at 3 and 12 months, for Celsior [62]. For renal SCS, the use of PEG in IGL-1 has previously shown better results in rat and pig kidney preservation compared to UW [39], and a study on pig kidneys later suggested SCOT (30 g/l PEG 20 kDa) was superior to IGL-1 (1 g/l PEG 35 kDa) and UW, showing less chronic fibrosis, epithelial-to-mesenchymal transition, and inflammation in the SCOT group [63]. A retrospective analysis of 917 human kidney transplantations using HTK, UW, or IGL-1 highlighted that IGL-1 had similar outcomes while including more DCD grafts in the group [42].

It is safe to say that no universal best preservation solution for SCS of abdominal organs has been established, and statistically, the main solutions used have similar clinical outcomes. However, many studies indicate that newer solutions show good results compared to the more expensive UW, which has been the gold standard. From a financial perspective, a US-based analysis concluded that transitioning from UW to HTK in kidney transplantation saved the hospital $548 per kidney donor [219].

1.3 Machine perfusion

The main purpose of this review is to showcase the literature on different solutions used in machine perfusion (MP) of abdominal organs and their comparison. The concept of extracorporeal organ perfusion dates back to the 19th century. Experimental work with dynamic preservation of different organs, including kidneys, was often carried out before SCS became prominent in the 1960s. SCS was more convenient and straightforward than perfusion, which, especially at that time, was challenging due to the need for complex and expensive equipment [15]. Today, MP is a method of dynamic preservation where cannulas are connected to the main vessels of the organ, delivering the solution into and out of the organ using a pump to create a circulating flow through the graft. The process is controlled and regulated via a device, with settings such as temperature, flow, and pressure being adjustable. The main temperature levels are typically 37ºC for normothermic MP, 20-25ºC for subnormothermic MP, and primarily 4ºC for hypothermic MP. Perfusion can also be oxygenated (particularly during NMP) or non-oxygenated, with the flow being either pulsatile or constant. MP can be used as a sole method of organ preservation or following SCS, and different types of MP can be combined (e.g., rewarming). There is no definitive answer as to which type of perfusion is best, and no universal protocol for MP exists, as it often varies based on center preference and experience with various procedures. Depending on the modality, some advantages of MP include the ability to monitor graft performance, deliver metabolic support, and, if needed, provide oxygen and nutrients. Additionally, MP allows for reconditioning or therapeutic approaches during perfusion. MP has recently gained increased attention in the context of marginal grafts or organs from ECD, as it is suggested to improve outcomes and enable more effective assessment of grafts prior to transplantation compared to SCS [64,65]. An additional possible advantage of MP is the ability to decontaminate the graft during perfusion [219]. Furthermore, a cost-effectiveness assessment has concluded that the introduction of MP for ECD kidney grafts is financially beneficial [220].

1.3.1 Hypothermic machine perfusion (HMP)

As mentioned above, HMP is a type of dynamic graft preservation set under hypothermic conditions, usually at 4ºC (ranging from 0ºC to 12ºC). This way, the metabolic activity of the organ is low due to the temperature, but at the same time, the organ can be better evaluated using pressure and data from perfusate injury markers, and possibly even treated via the circulating flow. HMP was a significant part of Belzer s work in the 1960s and was often used for preservation before convenient SCS with UW-solution became overwhelmingly popular.

Thanks to technological advancements, though, easy-to-use portable machine perfusion devices are commercially available today, making HMP accessible. [66]

According to many studies, HMP has been proven viable for liver, kidney, and pancreas preservation. [67-69] A recent meta-analysis of HMP of kidneys, which included data from 14 studies (2138 participants), showed that dynamic hypothermic preservation of kidneys, compared to SCS, reduces the risk of DGF and improves graft survival at 1 and 3 years, relevant for organs from both DCD and DBD donors. Surprisingly, the analysis also suggests that HMP is cost-effective compared to SCS at 1 year post-transplantation. [70]

As for liver HMP, a recent RCT has shown a lower risk of non-anastomotic biliary strictures, as well as less early allograft dysfunction, in comparison with SCS. [71] HMP of the pancreas is not yet clinically used, but there are preclinical models of animal and human pancreas HMP that prove it safe, feasible, and possibly beneficial, especially considering the fact that marginal human pancreata declined for transplantation could be successfully preserved by HMP with stable parameters. [72-74]

Considering the hypothermic conditions, there has been discussion about whether active oxygenation of the medium is necessary in an HMP setting, as well as the optimal oxygen saturation needed for oxygenation in a hypothermic environment. A study of HMP of rat livers explored different levels of oxygenation and possible toxic effects, such as the formation of ROS. The authors concluded that oxygenation could indeed be beneficial, showing higher ALT, AST, and LDH levels in the non-oxygenated group, as well as urea synthesis in oxygenated groups. The authors suggested that 21% oxygen saturation is superior to 95%, due to increased ROS formation at 95% oxygen saturation. [75] Another study on oxygenation during HMP used three groups of porcine kidneys, perfused with non-oxygenated solution, air-oxygenated solution, and 100% oxygen. The latter group showed the best results, such as better flow at reperfusion, doubled creatinine clearance, higher sodium excretion, and fewer signs of cell damage and tubular injury, compared to the non-oxygenated group. The air-oxygenated group showed results between the two groups. [76]

Therefore, hypothermic oxygenated perfusion (HOPE) is a particular modality of HMP, where the graft is (re-)oxygenated under hypothermic conditions. In the context of liver HOPE, it can be performed through the portal vein alone (single-HOPE) or through the portal vein and

hepatic artery; the latter method is called D-HOPE or dual-HOPE. Dual-HOPE is hypothetically beneficial, as it mimics the physiological perfusion of the organ, but the clinical benefit of D-HOPE is still debated. [95] Studies on HOPE claim that it reduces IRI by protecting mitochondrial function and prevents post-transplant cholangiopathy. [77-78] Additionally, a study on rat kidneys showed that HOPE reduces the immune response, demonstrating reduced cytokine release, T-cell, and macrophage activation, while non-oxygenated HMP did not have this effect. [79] Another interesting finding is that HOPE might decrease tumor recurrence, as a study shows up to 20% less tumor recurrence after the use of HOPE in liver transplantation, compared to unperfused liver grafts transplanted for HCC. [84] A recent multicenter randomized trial confirmed positive effects of HOPE, demonstrating a decrease in serum peak ALT, fewer cases of early allograft dysfunction, a reduction of 90-day post-transplant complications, and shorter ICU and hospital stays, compared to SCS in ECD-DBD liver transplantation. [80] Another multicenter RCT showed similar results between HOPE and SCS groups of DBD livers, though suggesting that HOPE decreases the number of liver-related graft complications. [81] For more sensitive DCD livers, a study on end-ischemic HOPE showed transplant outcomes similar to DBD livers and better than untreated DCD livers, again suggesting this method to be advantageous. [82] For steatotic liver grafts, studies on fatty rat livers have demonstrated that HOPE has protective effects against IRI, to which such liver grafts are especially sensitive, and showed generally better results compared to SCS. According to the authors, oxygenation seems to play a significant role in preserving such grafts. [99-100]

As for kidneys, an RCT found no benefits in using oxygenated end-hypothermic MP (short HOPE after SCS) over SCS only in ECD-DBD kidney transplantation at 1 year post-transplantation. However, the survival rates were very high in both groups. [85] A randomized trial comparing conventional HMP with oxygenated HMP of kidneys showed the latter to result in fewer severe complications and a slightly lower percentage of graft failure. [86] Similarly, a retrospective study comparing outcomes of kidney transplantations after oxygenated HMP versus non-oxygenated HMP showed no clear advantage of HOPE. [83]

A hypothesis of HMP improving outcomes of ECD-graft transplantations has been explored in many studies. For example, a randomized controlled study compared HMP and SCS of kidney pairs from DCDs, showing lower arterial resistances, higher urine output, remarkably lower serum creatinine levels, decreased incidence of DGF, and, lastly, higher 1- and 3-year graft survival rates for kidneys that underwent HMP. [87] On the other hand, an older UK multicenter

RCT showed no difference in DGF occurrence, renal function, patient and graft survival at 3 and 12 months between HMP and SCS of DCD kidneys. [88] An analysis of transplantations of kidneys from donors older than 65 years old, allocated in the Eurotransplant Senior Programme, showed that continuous pulsatile hypothermic MP is able to minimize the rate of never-functioning kidneys as well as improve the graft survival rate at 1 year after DGF. [89] An Italian clinical trial of 10 ECD-DBD kidneys and 10 ECD-DBD livers that underwent transplantation after HOPE showed fewer instances of graft dysfunction, as well as better graft survival (100%) at 1 year post-transplant compared to the SCS control group. Authors also noted a significantly lower median peak AST level in livers after HOPE. [90] Another study analyzed data of local ECD kidneys that underwent HMP directly after retrieval versus imported ECD kidney grafts that only underwent HMP shortly after a period of SCS, showing a lower rate of DGF and higher 1-year graft survival for local kidneys that were preserved via HMP the whole time from retrieval until implantation. [91] Lastly, a systematic review and meta-analysis including 608 transplanted livers in multiple studies and RCTs also concluded that HMP improves 1-year graft survival of ECD and DCD livers. [92]
Worth mentioning is a study observing possible contamination of the grafts during an HMP RCT, considering the complexity of the procedure. Here, the authors were able to demonstrate that no breach of sterility occurred during perfusion. [315]

An important advantage of HMP of ECD is the ability to better evaluate the marginal graft before making the decision to transplant it or discard it. For example, a study of ECD kidney grafts showed that there is a correlation between renal resistance trends during HMP and preimplantation biopsy scores, allowing for the use of resistance levels as a parameter to evaluate graft quality, theoretically even as a substitution for biopsy. [93] As for liver, a study using microdialysis concluded that glucose and lactate during dual-HOPE could serve as markers of hepatocellular injury in the viability assessment of liver grafts. [94]

Another topic of discussion concerning HMP is whether it should be used as a sole preservation method or following SCS, as well as its duration in a setting where it s applied after SCS. An experimental study on porcine kidneys compared various parameters in 2 groups of kidneys: perfused during the whole preservation time versus only for 2 hours after SCS. Results indicate that even a short-term reconditioning post-SCS of kidney grafts seems to have the same positive effects as HMP for the whole preservation duration. Worth mentioning is that in a clinical

setting, such HMP-reconditioning would also be logistically beneficial in comparison to full-time HMP, especially when the organ has to be transported first. [96] Another experimental study on porcine kidneys demonstrated that both graft groups that underwent HMP for either 1 hour or 4 hours after 18 hours of SCS showed similar results, both superior to SCS. The authors noted that the positive effects of HMP were already observed after 1 hour of perfusion. [97] Clinical trials of liver transplantations also showed that the application of HOPE for just 2 hours after SCS was beneficial compared to static preservation only. [71,80] At the same time, an observational cohort study concluded that a prolonged (>4 hours) period of HOPE did not have results inferior to livers transplanted after a shorter perfusion period, the prolonged perfusion being able to win time to overcome logistical hurdles. [98]

A promising concept in HMP, apart from the positive effects of this preservation method itself, is the ability to include therapeutic substances into the circulating perfusate to protect and/or treat the graft during perfusion. An example of this is a study on rat kidneys, where mesenchymal stromal cells or extracellular vesicles (EV) derived from them were added to the preservation solution used in hypothermic perfusion, since these cells are known to reduce immune and inflammatory response as well as promote tissue repair. After studying the perfused kidneys, the authors concluded that such therapy could indeed help decrease ischemic and IRI damage. [101] The next step was to add EV from mesenchymal cells to HOPE of marginal human kidneys, deemed unsuited for transplantation. After analyzing various markers, reperfusing the kidneys under normothermic conditions, and comparing the results to kidneys perfused via HOPE without supplements, the authors concluded that the addition of EV was beneficial and could become a new strategy to increase the donor pool. [102] As the rate of liver steatosis increases, and many liver grafts are being declined because of that, numerous substances have been studied to find agents that could have a defatting effect on such organs during ex-situ perfusion. Some examples of such drugs with defatting mechanisms of action are forskolin, L-carnitine, scoparone, visfatin, rapamycin (mTOR), necrosulfonamide, and peroxisome proliferator-activated receptors. [103] A study on a fatty rat liver model has explored the efficiency of such a defatting cocktail during HOPE compared to HOPE without supplements as well as SCS. The results showed that the use of HOPE alone had a protective effect on fatty livers in comparison to SCS, and the defatting cocktail could additionally enhance the positive outcome, although it was unable to induce the decomposition of fat under hypothermic conditions. [104] Some other experimental therapeutic approaches in HMP with promising results include:

- Inhibitors of matrix metalloproteinases (play a role in ischemic injury) - MMP-2 siRNA and doxycycline in a rat kidney model [105, 113]
- Tissue plasminogen activator (alteplase) in human kidney grafts with extensive glomerular thrombosis, successful transplantation followed [106, 107]
- Heparin conjugate to protect vascular endothelium and reduce injury in a porcine kidney model [108,109]
- A novel cell-binding thrombin inhibitor thrombalexin in a porcine kidney model [110]
- Carbon monoxide-releasing molecules (CORM-401) in porcine kidneys, since carbon monoxide inhalation has been shown to reduce inflammation and cell death [111]
- Propofol as a cytoprotective agent and membrane-targeted antioxidant in porcine autotransplantation [112]

1.3.2 Subnormothermic machine perfusion (SNMP)

SNMP is a preservation method where the graft is perfused with a solution at temperatures below physiological levels to reduce ischemic injury, but not as low as in HMP, so a certain level of metabolic activity is present. The most commonly used temperature for SNMP is 21-22ºC. A study on porcine kidneys concluded that after 4 hours of SCS, perfusion with blood for 4 hours at 22ºC was superior to 15ºC and 37ºC. [114] According to some studies, organs functioning in such a reduced metabolic state might not necessarily need oxygen carriers to provide adequate oxygenation under these conditions. At the same time, the metabolism and functional activity are high enough to effectively test the viability of the organ. Among other things, this method is considered to prepare the graft, which had been cold-stored, for sudden normothermic conditions after implantation, serving as a preconditioning step between hypothermic and physiological states, where the temperature difference is quite large. [115] As for the type of perfusion flow, a study on pig kidneys demonstrated similar results for centrifugal perfusion, where the flow is steady and uniform, and for pulsatile perfusion. [131]

SNMP hasn t been clinically used yet, but its advantages have been investigated in numerous studies on subnormothermic perfusion of liver and kidneys, in animal models as well as human grafts, showing positive effects of this method. For example, in comparison to SCS, an experimental study on rat livers demonstrated that grafts preserved via SNMP had lower AST, ALT, biliary GGT, and LDH levels, as well as higher ATP levels, suggesting less damage in those livers. [116] As for human livers, discarded DCD-grafts that underwent subnormothermic perfusion had, in general, minimal injury according to biochemical and microscopic assessments, as well as higher bile production and ATP levels, and lower lactate levels after perfusion. [117] A recent study on pig livers after circulatory death also concluded that subnormothermic reperfusion has a preserving effect on mitochondrial function and prevents IRI injury, possibly activating protective genes against IRI as well as repair mechanisms, suggesting the method has the potential to be used in clinical liver transplantation. [118] A protocol for SNMP was presented by a team from Massachusetts General Hospital, which successfully perfused 22 human livers discarded for transplantation under subnormothermic conditions, claiming this method preserves the liver with minimal injury, as shown by histology and ALT levels, and supports the graft s functional recovery, reflected by ATP levels. [119]

Subnormothermic perfusion of the kidney has also been studied on animal models. For example, porcine DCD-kidney models were preserved for 7 hours with either SNMP, HOPE,

or SCS, showing superior results for the subnormothermic method, such as increased creatinine clearance, blood flow, and urine production, as well as the best structural integrity of all three groups. [120] Another study on porcine DCD-kidneys compared 4 hours of perfusion at either 4ºC or 22ºC and analyzed the grafts via 31P magnetic resonance spectroscopic imaging. The results demonstrated that subnormothermic perfusion increased ATP levels, cortical and medullary perfusion of the kidneys, while also showing fewer histological lesions compared to HMP. [121] Two studies have explored the positive effects of H2S supplementation in UW and blood during SNMP of porcine kidneys, using mitochondria-targeted synthetic H2S donors. Hydrogen sulfide is believed to mitigate mitochondrial dysfunction, oxidative stress, inflammation, and apoptosis, which are caused by IRI. Both studies reported good outcomes with high urine output, low tissue necrosis, and signs of activation of cytoprotective genes. [122,123]

Unfortunately, no data is yet available on pancreas subnormothermic preservation.

As for the duration of SNMP, there is no universal standard or limit, but a few studies have tried to address this question. In one study, authors analyzed rat livers after 15, 30, 60, and 90 minutes of oxygenated SNMP following 6 hours of SCS. Besides demonstrating overall positive effects of the preservation method, the authors concluded that even 30 minutes of SNMP are sufficient to reduce IRI injury. At the same time, no disadvantages were found in the usage of SNMP for 90 minutes. [124] A study on pig livers from DCDs, preserved via SCS for 2 hours and then undergoing SNMP for 120 minutes, demonstrated peak bile production at 90 minutes of perfusion, with hepatic sinusoidal space being wide at 90 minutes but narrowed at 120 minutes. Therefore, the results suggest that SNMP maintains graft condition optimally for up to 90 minutes, while longer perfusion could be counterproductive. [125]

A significant advantage of SNMP is that it allows for effective viability testing of the grafts, which is especially important for marginal grafts that could otherwise be discarded. During perfusion, produced bile as well as perfusate samples can be analyzed to measure the organ s function and markers of damage, such as lactate, in real time. Observation of flow rates also provides important information about the state of the graft. [119]
Some SNMP studies have been conducted on steatotic livers, considering that it is a widespread condition and often a limiting factor in transplantation, as well as because such grafts are more

sensitive to ischemia and reperfusion damage. The conclusions differed between studies. For example, in one experimental work, SNMP-perfused fatty rat livers demonstrated better results compared to SCS-preserved ones, with authors claiming that SNMP successfully protected macrosteatotic livers against preservation and reperfusion injury. [126] Further studies on fatty rat livers similarly claim that SNMP is superior to SCS, showing less damage to the biliary tree, decreased hepatocyte and sinusoidal cell apoptosis, as well as lower portal pressure in perfused grafts. [127-128] However, in a study on fourteen human steatotic livers discarded for transplantation and randomly assigned to SNMP and NMP, the results were not straightforward. While SNMP-livers had higher ATP levels due to lower ATP consumption under subnormothermic conditions, lower antioxidant capacity and high glutathione depletion were noted, with authors highlighting it as an unrecognized disadvantage of SNMP. [129] Another study on fatty rat livers preserved via SNMP with a defatting cocktail demonstrated that, even though lipid export seems to be possible under subnormothermic conditions, the necessary duration of perfusion would need to be much longer than for NMP. [130]

Since SNMP is a dynamic preservation method, it is possible to supplement the circulating perfusate with therapeutic substances, which is a promising concept for machine perfusion. Regarding this, some interesting studies have been conducted in the setting of SNMP. For example, protective effects of Nicorandil have been explored in a study on rat livers undergoing SNMP compared to SNMP and SCS without supplementation. The study proved the anti-ischemic qualities of Nicorandil, showing lower levels of liver enzymes, TNF-α, GLDH, and IL-1ß, as well as higher antioxidant enzyme activity, decreased apoptosis, and better-preserved parenchymal structure in treated livers. [132] The application of mesenchymal stem cells has also been studied in subnormothermic conditions (32ºC), showing possible activation of tissue regeneration in discarded human kidneys. Treated grafts demonstrated increased ATP and growth factors, as well as a greater number of cells undergoing mitosis and a decreased inflammatory response. [133] Other studies focused on immunosuppression, or better said on reducing the immunogenicity of the allograft during SNMP as preparation for implantation. Authors demonstrated that with the help of genetic and bioengineering novel supplements, such as RNA vectors or nanobarrier membranes, could be added during perfusion to reduce the need for systemic immunosuppressive therapies or even create immunologically invisible organs. [134, 135]

1.3.3 Normothermic machine perfusion (NMP)

Normothermic machine perfusion of allografts has been studied and clinically used more frequently in recent years. It is a method of dynamic preservation that simulates physiological conditions at temperatures ranging from 35.5 to 37.5ºC. In this setting, the graft requires continuous oxygen supply, so the perfusate is typically red blood cell-based or contains alternative oxygen carriers. Post-retrieval perfusion of liver grafts was first described in 1967 by Brettschneider et al., but it was overshadowed by the development of SCS strategies, which were more suitable at that time. NMP has gained relevance recently as a potential method for preserving marginal grafts due to the ongoing organ shortage crisis. [136]

Perfusion at normothermia not only preserves the graft optimally but also serves as a preconditioning tool before implantation after cold preservation. It replenishes ATP through near-physiological aerobic metabolism and protects the organ from IRI. Some studies also report recovery of grafts from warm and cold ischemia injury during NMP. Beyond ATP restoration, the reported possible mechanisms underlying the positive effects of NMP include protective cell responses due to activation of protective genes, release of stress proteins, and induction of cell proliferation. [137-138]

Another significant benefit of normothermic perfusion is the ability to perform a more accurate real-time assessment of graft viability pre-transplant, as the graft remains metabolically active. Although a universal marker to determine graft non-function has not been established, some variables used to evaluate the extent of graft injury and predict performance after transplantation have been studied extensively in both experimental and clinical settings. These include:

- Lactate levels in the perfusate
- Arterial and venous flows
- pH levels of the perfusate
- AST levels of the perfusate

Additionally, parameters such as urine output in kidneys, bile production, glucose levels, bile pH, and ATP levels in liver NMP, as well as biliary bicarbonate and LDH levels, can be used for viability testing. Inflammatory cytokines are also potential markers for assessing graft status during prolonged NMP. [138-141]

It is worth mentioning the ability to safely extend graft storage time using NMP, thereby avoiding additional cold ischemia, which is a risk factor for graft failure. This capability is important for overcoming logistical challenges such as limited operating room availability or complex recipient preparations. [136, 137, 142] Time limits for NMP have not been firmly established, but several studies have explored both shorter and longer durations. In a feasibility study, Vogel et al. demonstrated that it is possible to reliably preserve discarded human kidney grafts for 24 hours with crystalloid-diluted packed blood cells. [143] The authors also successfully transplanted porcine livers stored via NMP for 48 hours. [144] For kidney NMP duration, a study preserved porcine DCD grafts for 16 hours via SCS only, SCS with a short end-ischemic period of NMP, 8 hours SCS + 8 hours NMP, and solely NMP, followed by transplantation of all grafts. The results showed improved parameters and outcomes with increased NMP duration and decreased SCS duration, with the last group demonstrating the lowest peak serum creatinine and the best postoperative creatinine clearance, suggesting that such extended NMP durations are not only feasible but also beneficial. [145] The initial hypothermia during cold flush appears to have no negative effect on the graft when NMP is performed immediately afterward, as shown in a pig model of liver NMP. [222] However, studies on short end-ischemic NMP have shown variable results. The aforementioned study suggested that 1 hour of NMP might be insufficient for reconditioning, potentially causing negative effects due to rapid and frequent temperature changes that could increase oxygen demand and activate pro-inflammatory mechanisms without repairing effects. In contrast, Hosgood et al. demonstrated positive impacts of 1 hour of normothermic kidney perfusion following SCS. [146] Recently, the same authors reported severe IRI with infarction in post-transplant biopsy specimens and primary non-function after 3 months in 2 kidneys from uncontrolled DCD transplanted after 1 hour of post-SCS NMP, suggesting that short-term NMP may not be sufficient to predict the outcome of ECD kidneys. [147]

In recent years, commercially available perfusion machines have become increasingly common. For example, OrganOx Metra is a portable device designed in the UK for normothermic preservation and transportation of the liver, which has been proven to be cost-effective. [148] XVIVO is another manufacturer offering commercially available portable perfusion devices for the liver and kidney, such as LiverAssist™ and KidneyAssist™. [138] OCS™ Liver is another portable liver perfusion device developed by the US-based company TransMedics. [149] The use of these machines allows for continuous real-time control of

perfusion parameters and can be programmed to automatically deliver nutrition or therapeutic substances to the graft.

Regarding oxygenation during NMP, it is clear that under normothermia, tissues require an adequate amount of oxygen, as metabolism is near-physiological. Therefore, most protocols use high levels of O_2: either 100% or a mixture of 95% oxygen with 5% CO_2, although this is not a universal strategy and requires further study. Normally, the partial pressure of oxygen during perfusion exceeds physiological levels—70-75 kPa. However, some authors describe the use of lower pO_2 levels, closer to physiological levels, around 26 kPa. [138] At the same time, some studies show that kidneys can tolerate a broad range of oxygen concentrations without significant injury or changes in renal function. [150-151] As for liver NMP, although most protocols describe the use of high O_2 concentrations, a study comparing two groups of human liver grafts perfused with either high O_2 tension or air (21% O_2) suggested that lower oxygen tension might be beneficial over hyperoxia by preventing post-reperfusion syndrome and vasoplegia. [152]

Liver NMP has been used clinically in many centers worldwide, with numerous RCTs demonstrating its viability. For example, a UK multi-center trial involving 334 liver grafts randomly assigned to either SCS or NMP demonstrated a lower discard rate in the NMP group, longer mean preservation duration, and lower levels of graft injury. [153] Another RCT, including 293 liver transplantations from multiple US centers, compared grafts that underwent NMP using the portable OCS Liver device with those stored via SCS. Preservation by NMP with the OCS Liver resulted in higher use of grafts from DCDs, reduced histopathologic evidence of IRI, and fewer ischemic biliary complications at 6 and 12 months. [149] A clinical trial involving 31 livers that met high-risk criteria and were discarded by multiple centers demonstrated that after undergoing NMP, 22 of them achieved viability for transplantation. The transplantation outcomes of these livers after a median perfusion time of 18 hours were positive, with 100% 90-day survival. Only 4 patients (18%) developed biliary complications and required re-transplantation at follow-up after 542 days. [154] At a 5-year follow-up, patient and graft survival rates were 82% and 72%, respectively, while the 4 patients who died all had functioning grafts. [155] Another study similarly demonstrated recovery of 15 out of 21 discarded livers, with successful transplantation following NMP of 4-10 hours. Seven of the transplanted livers developed early graft dysfunction but recovered quickly, and only one required biliary stents due to ischemic cholangiopathy after 4 months. No complications were

detected at the 14-month follow-up. [156] A different trial compared NMP and SCS of 20 livers from older (>70 years old) donors, showing histological evidence of reduced IRI after NMP but failing to demonstrate clinical benefit after transplantation, possibly due to small sample sizes. [157]

For kidney NMP, fewer large clinical trials have been performed, but it has been shown to be safe and suitable for clinical use. An RCT by Hosgood et al. compared results of the preservation of 338 DCD kidneys stored via SCS alone or with 1 hour of NMP following SCS. Out of these, 277 kidneys were transplanted, showing no difference in DGF between the two groups, likely due to the short duration of end-ischemic NMP. On the other hand, the addition of NMP was not associated with an increase in complications. [158] The first North American results of 13 kidney transplantations after NMP for 1-3 hours following anoxic HMP, compared to 26 grafts preserved by anoxic HMP alone, showed the first group to have slightly lower DGF rates (30.8% versus 46.2%) but no differences in patient and graft survival during a 1-year follow-up. [159] A further study on human kidneys explored the endocrine function of grafts perfused under normothermia for 2 hours following preservation via HMP. Compared to grafts only preserved via HMP, the fifteen kidneys that underwent 2 hours of post-ischemic NMP demonstrated significantly higher levels of prorenin, renin, erythropoietin, and active vitamin D, with 12 of them producing urine during NMP. No correlation was found between hormone levels and DGF, likely due to the small group size. [160]

As for pancreas NMP, it has not yet been used clinically due to the organ's high susceptibility to injury and edema. However, some experimental studies on animal models and discarded human grafts have shown promising results. After a previous study demonstrated low-pressure perfusion to be favorable for the pancreas [161], Mazilescu et al. developed a pancreas NMP technique using an arterial pressure of 25 mm Hg and incorporating a dialysis circuit. Porcine pancreata underwent this perfusion for 6 hours, developing only minimal edema, with some grafts being autotransplanted after 3 hours of NMP. Histological assessment showed intact islet cells and mild injury, and transplanted grafts had immediate function post-transplantation, with glucose levels in the normal range. [162] In a recent feasibility study by the same group, 6 discarded human pancreata, including one from a DCD, successfully underwent NMP for 4 hours. During perfusion, glucose and lactate levels decreased, while insulin levels increased, and at the end of perfusion, the grafts had minimal injury and edema, demonstrating the feasibility and safety of pancreas NMP. [163] Although there is potential for pancreas NMP to

be integrated into clinical transplantation practice, it must be studied more extensively pre-clinically, for example, to identify biomarkers for graft assessment.

Similarly to previously described perfusion modalities, NMP has a high potential for therapeutic approaches during perfusion, such as perfusate supplementation. These strategies could be even more successful in the case of NMP, considering the high metabolic activity of the graft under normothermia. Some therapeutic directions that have been explored in the NMP setting include:

- Multipotent Adult Progenitor Cells, which possess potent immunomodulatory properties aimed at minimizing IRI. In one study, 5 pairs of human kidneys were simultaneously perfused by NMP for 7 hours, demonstrating positive changes in injury markers and clinically relevant variables for the treated group [164].
- Bone Marrow-Derived Mesenchymal Stromal Cells used during rat liver NMP in multiple studies by the Tianjin group showed lower levels of transaminases in perfusate, increased bile production, and better histological outcomes [173].
- Multipotent Adult Progenitor Cells in NMP of discarded human livers exhibited anti-inflammatory and immunomodulatory benefits [174].
- Human Liver Stem Cell-Derived EVs in rat liver NMP resulted in lower AST and LDH levels, reduced histological damage, and apoptosis [175].
- Anti-IRI Agents: CD47-blocking antibody, soluble complement receptor 1, and recombinant thrombomodulin were tested in a rat model. Subsequently, the CD47-blocking antibody, being the most effective of the three, was tested on a pig model, resulting in better renal perfusion/flow and a reduction of oxidative stress and histologic damage, although without any impact on the functional parameters of the graft [165].
- Human Adipose-Tissue-Derived Mesenchymal Stromal Cells in a porcine kidney model undergoing 7 hours of NMP showed lower levels of injury markers and the release of immunomodulatory cytokines [166].
- Nanoparticles serving as delivery agents to the kidney endothelium during NMP, with depots for long-term drug release [167].
- Plasminogen and Tissue Plasminogen Activator during human kidney NMP to lyse microvascular obstructions caused by prolonged SCS, reducing renal injury and improving function [168].

- Nitric Oxide Donor Sodium Nitroprusside and Carbon Monoxide-Releasing Molecule as vasodilatory agents aimed at minimizing IRI in a porcine NMP model, resulting in better flow and creatinine clearance [169].
- Erythropoietin as a renoprotector in porcine kidney models that underwent 17 hours of SCS followed by 2 hours of NMP demonstrated a reduction in inflammation and tissue remodeling through caspase-3 and IL-1β [170].
- Nonerythropoiesis Cyclic Helix B Peptide Derived from Erythropoietin in a similar model (18 hours SCS, 3 hours NMP) showed increased renal blood flow and urine output, as well as decreased tissue damage [171].
- Pre- and Postconditioning of rat and porcine kidneys with metformin as a renoprotector resulted in reduced proteinuria and injury markers, decreased tubular necrosis and vacuolation, although there was no effect on creatinine clearance [172].
- Cholangiocyte Organoids in mouse and human liver NMP for regenerating bile ducts after injury demonstrated proof of concept, with increased bile production and higher pH [176].

A topic that needs to be highlighted separately is the potential of defatting strategies for steatotic livers during NMP. This condition is common and often turns out to be a limiting factor, leading to the discard of many liver grafts that could potentially become viable after treatment. Some animal studies show evidence that NMP alone could have a defatting effect on liver grafts. For example, a study on steatotic porcine livers undergoing prolonged NMP demonstrated histological evidence of defatting from approximately 28% steatosis to 15% and proved that NMP can successfully preserve such grafts [177]. Conversely, a study on steatotic human livers showed no defatting effect after 24 hours of NMP [178]. Multiple studies have also focused on adding defatting pharmaceutical substances during NMP, showing promising results. Nagrath et al. achieved a 50% defatting after 3 hours in a rat model using such a defatting cocktail [179]. Similarly, a study on discarded human livers demonstrated a reduction of tissue triglycerides by 38% and macrovesicular steatosis by 40% after 6 hours of NMP with defatting supplements [180]. Although NMP appears to be the most suitable preservation modality for treating steatotic livers, an important point of discussion is the possible toxicity of the components of these therapies, which needs further investigation. Another relative downside is the likely long duration of defatting NMP required to achieve a reduction in steatosis [181].

NMP is a complex preservation technique, and it remains a topic of discussion whether it is worth the resources it requires or in which situations it should be used. Compared to SCS, the previously mentioned liver NMP RCTs show significant advantages of normothermic perfusion, such as the possibility of longer preservation duration, lower graft discard rates, higher use of organs from DCDs, reduced graft injury including IRI damage, and a lower occurrence of ischemic biliary complications [149, 153, 157]. A comparative study by Fodor et al. also highlighted the prolonged organ preservation (21 hours vs. 7 hours) as well as reduced rates of ischemic-type bile duct lesions for NMP-assigned grafts compared to those that underwent SCS [182]. Additionally, a cost-utility analysis in the Canadian setting declared the addition of NMP to SCS cost-effective compared to SCS alone [183].

Multiple meta-analyses have compared large NMP and HMP studies with SCS in liver preservation, concluding that these machine perfusion modalities are indeed advantageous. The most recent analysis, including 7 RCTs (4 on HOPE and 3 on NMP), claimed that grafts preserved by either of these two methods had lower rates of early allograft dysfunction, non-anastomotic strictures, and biliary complications compared to SCS. However, such positive outcomes as significantly reduced major complications, fewer graft losses, and re-transplantations" were only highlighted for the HOPE groups [184]. Another similar meta-analysis outlined the reduction of total biliary complications and ischemic cholangiopathy for HMP but not for NMP [185]. A further analysis also concluded that only HMP, and not NMP, had a positive effect on biliary complications. Additionally, significantly better graft and patient survival was found only in HOPE studies and not in NMP studies [186].

As mentioned previously, when directly compared to sole kidney HMP, added NMP has shown better results in the context of DGF with no differences in patient and graft survival [159]. Other than that, there is not much data available on the direct comparison of the two machine perfusion modalities. A logistically important difference is that reconditioning by HMP is successful even with a short perfusion duration of 1 hour, while NMP is considered to be more successful in a setting of multiple-hour perfusion.

SNMP in a DCD rat liver perfusion study performed similarly to NMP, reducing IRI [187]. A previously mentioned study that examined sub- and normothermic perfusion of discarded human fatty livers highlighted possible disadvantages of SNMP compared to NMP. While SNMP increased ATP levels more than NMP due to lower ATP consumption under

subnormothermic conditions, it resulted in reduced glutathione and decreased anti-oxidative capacity, which could possibly lead to oxidative injury at reperfusion [129]. A study on rat livers also demonstrated that lactate and ammonia metabolism was higher during NMP compared to SNMP and concluded that normothermia is the most optimal preservation temperature for the liver [188].

Worth mentioning are the possible technical risks due to the complexity of NMP. For example, a Canadian clinical trial on NMP of human livers reported the discard of one graft due to a technical failure of portal cannulation [189]. Furthermore, a study on human kidney NMP demonstrated proof of microbial contamination during perfusion, although no related complications were detected at 6-15 months follow-up [190].

1.3.4 Normothermic regional perfusion (NRP)

Normothermic regional perfusion (NRP) is, in contrast to NMP, not a graft preservation method but a technique used for the procurement of organs from DCDs, followed by SCS or MP. During this approach, the organ is perfused in situ with its own blood via extracorporeal membrane oxygenation (ECMO) through cannulas in the main vessels, which are clamped to achieve perfusion exclusively within the abdominal compartment. NRP is started immediately following circulatory death or after the mandatory hands-off warm ischemia time, if required by law, and is then performed for 1-4 hours. This technique has recently been successfully used as a go-to method for liver and/or kidney procurement after DCDs in some European countries, such as Norway, France, and Italy, and there are reports of its use in China and the US [191-194].

Since HOPE is also a viable strategy for DCDs, a multi-center study compared the results of liver transplantations of DCD grafts after NRP procurement to immediate HOPE treatment, demonstrating similar results for both strategies, with a slight favor towards HOPE, comparable to DBD transplantation [195]. An experimental study on rodent livers, on the other hand, has shown that HOPE could be beneficial for high-risk DCD livers compared to NRP [196]. A combination of these two techniques, meaning HOPE following NRP, has also been used in liver and kidney DCDs. Studies on liver transplantations using both methods have shown outcomes comparable to DBD transplantations and better results compared to the use of HOPE alone [193, 197, 198]. A study comparing transplantations after DCDs using NRP with subsequent SCS versus NMP following short SCS has shown similar results for both strategies, comparable to DBD [199]. There have also been reports of the successful sequential use of NRP and NMP, or even combining NRP with both HMP and NMP [200, 201].

1.3.5 Controlled oxygenated rewarming (COR)

Controlled oxygenated rewarming (COR) is a preservation technique where the previously described machine perfusion modalities are, so to speak, used sequentially. The graft is perfused under gradually increasing temperatures until subnormothermia or normothermia. Naturally, with the rising temperature, there is a need for oxygen supply to the organ, so the perfusion must be oxygenated. The drastic change in temperature that occurs during the end-ischemic NMP strategy can potentially cause further injury, referred to as rewarming injury" [202], which could be mitigated by the COR technique. This method might be optimal for resuscitating grafts preserved via SCS, gently" preparing the organ for the change of conditions at implantation.

A preclinical study divided porcine kidneys that underwent SCS for 20 hours into two groups. One group underwent 2 hours of NMP straight after SCS, while the other was slowly rewarmed to 35°C for 90 minutes and then perfused at normothermia for the remaining 30 minutes. The control group was only cold-stored. At the end, all the kidneys were implanted. Post-implant results demonstrated only a slight improvement in renal function for the NMP group, whereas the COR group showed a 2-3 fold improvement. The authors concluded that gradual rewarming supports the recovery of mitochondrial function and improves oxygen utilization efficiency, resulting in overall better recovery of function, likely due to a more mild and adapted increase in cellular metabolism [203]. Another study using a porcine kidney model compared 2 hours of COR following 6 hours of SCS to 8 hours of NMP or SCS. The results showed similar renal function at implantation for NMP and COR groups, which improved compared to SCS. Additionally, COR-treated kidneys demonstrated reduced proinflammatory upregulation. This study also highlighted the logistical convenience of COR after SCS compared to preservation by NMP alone [204]. In another study, immediate rewarming of porcine grafts after SCS was compared to rewarming following a period of hypothermic perfusion after SCS. Both groups showed significantly better results at reperfusion compared to SCS alone, and the different start of rewarming seemed to play no significant role [205].

Preclinical studies on animal livers have also shown positive results. For example, rat livers demonstrated higher energy levels, better bile flow, lower ALT levels, and better histopathology at reperfusion after COR to 20 and 35°C, compared to SCS. Interestingly, no significant differences were detected between grafts rewarmed to 20°C or 35°C [206]. In a

study on pig livers that underwent 18 hours of SCS, COR to 20°C for 60 minutes with subsequent perfusion at 20°C for 2 hours was compared to 3 hours of NMP. The results demonstrated better energetic recovery, lower enzyme levels, and increased bile production in favor of COR [207]. Liver COR has already been used clinically, showing great results in a RCT where 40 livers were assigned either to be solely cold-stored or to undergo 90 minutes of machine perfusion with COR to 20°C after SCS. The grafts in the COR group demonstrated decreased AST levels, better function, including increased production of coagulation factor V, and fewer severe complications [208]. A further clinical study reported the successful resuscitation of 11 of 16 declined livers from DCDs via a combination of HOPE for 1 hour, followed by 1 hour of COR and NMP for viability assessment. The revived" livers were successfully transplanted with 100% patient and graft survival at 3 and 6 months. This study demonstrated the importance of COR and MP in general, with authors highlighting a 20% increase in viable liver grafts due to the described strategy [209]. Another study examined 4 discarded liver grafts after 10 hours of SCS that were either perfused for 6 hours by NMP or underwent subnormothermic COR with the same perfusion time. As a result, one graft (50%) from each group successfully recovered and could have been transplanted [210].

A pilot study of clinical kidney COR also demonstrated good results. In six ECD kidney pairs, one graft from each pair was subjected to SCS while the other underwent COR to 35°C for 90 minutes with subsequent perfusion for 30 more minutes. COR-treated kidneys showed significant improvement in early allograft function, as demonstrated by twofold higher creatinine clearance compared to the control group and sodium excretion in the normal range, while elevated in grafts that underwent SCS. The authors also noted the correlation of graft function post-implantation to parameters and their dynamics measured during MP [211].

COR has not yet been tried for pancreas grafts, although it could be an exciting approach for pancreas preservation as well.

1.3.6 Ischemia-free transplantation

Another interesting pre-transplant strategy is ischemia-free transplantation. This method, which originated in China, allows for continuous oxygenated NMP of the organ starting from procurement until implantation into the recipient, without any cooling. NMP begins immediately after donor circulation is cut off and continues until the anastomoses in the recipient are completed under NMP. This approach avoids both cold and warm ischemia times as well as IRI. During preservation, the graft undergoing NMP can be biopsied for morphological assessment, and its functional activity can be evaluated via biochemical analysis of the perfusate. The use of this technique for ECD grafts could lead to increased organ utilization and further expand the donor pool due to its particularly gentle" approach [212].

The first case of ischemia-free human transplantation was reported in 2018, when a steatotic liver was successfully procured, preserved, and transplanted without ever stopping the blood supply to the organ. No complications were noted; graft injury was described as minimal, the studied IRI pathways were not activated after implantation, and the level of inflammatory cytokines was much lower compared to conventional preservation [213]. Recently, results of the first RCT of ischemia-free liver transplantation were published. Out of a total of 65 patients, 32 received grafts that underwent the ischemia-free approach, while the rest received conventionally cold-stored grafts. The first group demonstrated a significantly lower occurrence of early allograft dysfunction and post-reperfusion syndrome. Additionally, the rate of non-anastomotic biliary strictures at 12 months was much lower in the first group [214].

Not long after the first ischemia-free liver transplantation, this approach was successfully applied to kidney transplantation. The donor kidney functioned well throughout the procedure, and the recipient had an uneventful recovery. It is worth noting, however, that the graft came from a young donor who had suffered brain death [215]. It remains to be seen how ischemia-free kidney transplantation performs with grafts from ECDs..

Table 1. Summary of MP modalities

	HMP	SNMP	NMP	Rewarming
Temperature	4°C (0°-12°C)	21-22°C	35.5-37.5ºC	4°-22°/37°C
Need of oxygenation	+/-	+	+	+
Need of an oxygen carrier	-	+/-	+	+/-
Complexity	Low	Medium	High	High
Abdominal organs (with available experimental or clinical data)	Liver, kidney, pancreas	Liver and kidney	Liver, kidney, pancreas	Liver and kidney
Graft evaluation	Limited	Somewhat limited	Broad	Somewhat limited to broad
Available clinical studies	Liver, kidney	-	Liver, kidney	Kidney

2. Methods

PubMed database was searched using word combinations „perfusion solution liver“, „perfusion solution kidney“, „liver perfusion blood“, „kidney perfusion blood“, „liver perfusion RBC“, „kidney perfusion RBC“ „pancreas machine perfusion“ from the beginning of record until 09.06.2024. Articles, that directly compared perfusion solutions as well as most relevant articles, describing or mentioning certain perfusion solutions for certain MP modalities were included and described in „Results under respective sections. A table (Table 1) roughly describing included literature was created and displayed in „Results“ for better overview.

3. Results

Out of hundreds of search results in the PubMed database, that come up after using the aforementioned word combinations, 53 articles that described the usage of different solutions during the same MP approach (Table 3), were included and described in the results under respective sections. Among these, 12 articles described liver HMP, 13 articles — kidney HMP, which summed up to 26 studies on HMP. Next, 4 articles on SNMP were found, including 2 on liver SNMP and 2 on kidney SNMP. A total of 20 articles comparing perfusion solution approaches during NMP were found, among which 11 articles on liver NMP and 9 articles kidney NMP. Furthermore 2 articles focusing on liver rewarming procedure and 2 articles on kidney rewarming were also included. Out of all these, 7 described the usage of perfusion solutions on human grafts in experimental setting or followed by clinical transplantation. One article comparing different perfusion solutions during pancreas MP, more specifically HMP, was found. Additionally, a number of further relevant articles, including clinical trials and RCTs, which describe the usage of certain perfusion solutions during liver, kidney or pancreas MP, were also included in the results.

Table 2. Results of the PubMed database search

	HMP	SNMP	NMP	Rewarming
Total number of results after searching the word combinations	1618	58	791	162
Total number of articles on abdominal organs with comparison of perfusion solutions	26	4	20	4
Articles comparing perfusion solutions of liver	12	2	11	2
Articles comparing perfusion solutions of kidney	13	2	9	2
Articles comparing perfusion solutions of pancreas	1	0	0	0

Articles on human grafts comparing the solutions (experimental or clinical)	2 (kidney)	0	4 (2 liver, 2 kidney)	1 (liver)

Table 3. Articles on MP, comparing perfusion solutions				
Authors and year of publishment	Perfusion procedure	Organ	Solutions used during perfusion	Outcome
Kasperk R, Füzesi L, Müller M, Schumpelick V in 1994 [223]	Continuous hypothermic perfusion	Rat liver	Eurocollins vs UW solutuion (gluconate substituted for lactobionate)	Superiority of UW solution (less cell swelling, better maintenance of tissue architecture and microcirculation)
Olschewski P, Tolba R, Akbar S, Minor T in 2003 [277]	24 h HMP, reperfusion	Rat liver (DCD)	HTK vs Belzer MPS	Similar resistance, edema formation, glutamate-pyruvate transaminase levels, oxygen consumption and bile production. HTK: higher glutamate-dehydrogenase levels.
Stegemann J, Hirner A, Rauen U, Minor T in 2010 [224]	18 h HMP	Rat liver	HTK (Custodiol) vs Custodiol-N vs Custodiol-N + 25 microM deferoxamine + 2.5 microM iron chelator LK 614 vs Custodiol-N + 25 microM deferoxamine + 7.5 microM iron chelator LK 614	Superiority of Custodiol-N over HTK (lower LDH and ALT), best results with addition of 25 microM deferoxamine + 2.5 microM iron chelator LK 614
Jia JJ, Zhang J, Li JH et al in 2015 [225]	6 h HMP	Porcine liver	Saline vs UW vs HTK	Similar ALT, AST, LDH and malondialdehyde between UW and HTK; HTK: lower portal vein resistance, higher ATP; UW: less edema
Iwata H, Matsuno N, Ishii D et al in 2023 [226]	4 h HMP, reperfusion	Porcine liver (split-liver grafts)	UW-gluconate vs HTK	HTK: lower portal vein and hepatic artery resistance and histologically better protection against IRI

Jain S, Lee SH, Korneszczuk K, Culberson CR et al in 2008 [276]	5 h HMP, reperfusion	Rat liver (DCD)	UW-solution vs UW supplemented with antioxidants, calcium chloride, thromboxane A2 inhibitor and anaplerotic substrates	Supplemented UW: higher ATP, lower LDH levels, increased bile production, less edema formation, histologically good tissue preservation
Asong-Fontem N, Panisello-Rosello A, Sebagh M et al in 2022 [227]	2 h HOPE, reperfusion	Steatotic rat liver	IGL-2 vs Belzer-MPS	IGL-2: histologically less damage, lower ALT, AST, no weight gain, biomarkers: better glycocalyx preservation, lower apoptosis and inflammation
Muller X, Rossignol G, Couillerot J et al in 2024 [228]	2h HOPE, reperfusion	Porcine liver	IGL-2 vs Belzer-MPS	No differences in transaminase levels, lactate clearance, histological signs of IRI, bile production and inflammatory reaction
Vekemans K, Liu Q, Heedfeld V et al in 2009 [230]	24 h HMP -> transplantation	Porcine liver	Aqix RS-1 vs KPS-1 with deferoxamine, ROS-inhibitors and nutrients	Similar morphological integrity and AST levels; Aqix RS-1: lower TNF-α levels; KPS-1: better hyaluronic acid clearance
Bae C, Pichardo EM, Huang H, Henry SD in 2014 [278]	8 h HMP, reperfusion	Rat liver (DCD)	KPS-1 vs Vasosol vs Vasosol+vitamin E	Vasosol groups: decreased ALT levels, inflammatory and apoptotic markers, with further reduced rates in the vitamin E group.
Bessems M, Doorschodt BM, van Vliet AK et al in 2005 [231]	24 h HMP, reperfusion	Rat liver	Polysol vs UW-gluconate	Similar pH, flow, lactate levels, dry/wet ratios, histological findings Polysol: slightly less liver enzyme release and higher bile production

Bessems M, Doorschodt BM, Hooijschuur O et al in 2005 [232]	24 h HMP, reperfusion	Rat liver	Polysol + HES vs Polysol + dextran vs Polysol + PEG vs UW-G	All Polysol groups: higher oxygen consumption, less hepatocellular damage, better flow and increased bile production Slightly better results in Polysol+PEG, worst results in Polysol-HES
Lledó-Garcia E, Hernández-Fernández C, Díez-Cordero JM, García-Barreno P et al in 2003 [234]	3 h HMP	Porcine kidney	Eurocollins vs Belzer solution	Eurocollins: increased weight gain, worse histopathological findings
Lindell SL, Compagnon P, Mangino MJ, Southard JH in 2005 [235]	60 min or 75 min WIT, followed by 72 h or 24 h of HMP	Canine kidney	Belzer-MPS vs UW solution (Viaspan)	Better survival rates in the 75 min WIT UW group, compared to 75 min WIT Belzer-MPS group
Guarrera JV, Polyak MM, Arrington B, Boykin J et al in 2004 [236]	HMP (CIT around 26h), followed by clinical transplantation	Human kidney	Belzer-MPS vs Belzer II albumin gluconate vs Belzer-MPS modified with nitroglycerin, prostaglandin E1 and polyethylene glycol-superoxide dismutase	Reduced DGF and lower creatinine levels at 6 months after use of the modified Belzer-MPS; Unmodified Belzer-MPS slightly better results than Belzer II albumin gluconate

Vaziri N, Thuillier R, Favreau FD, Eugene M et al in 2011 [237]	24 h HMP, autotransplantation	Porcine kidney (DCD)	KPS-1 vs UW (Viaspan)	Superiority of KPS-1 over Viaspan: less resistance, better flow, faster diuresis regain, lower creatinine and urinary enzyme levels, lower oxidative stress and immune response activation, better histology Simialar animal survival rate at 4 months
Manekeller S, Leuvenink H, Sitzia M, Minor T in 2005 [238]	18 h HMP, autotransplantation	Porcine kidney (DCD)	Non-colloidal HTK vs Belzer-MPS	No significant differences
Gallinat A, Lüer B, Swoboda S, Rauen U et al in 2013 [239]	20 h HMP, reperfusion	Porcine kidney	Custodiol-N (with dextran 40) vs KPS-1	Custodiol-N group: higher flow rates, oxygen consumption, urine production and creatinine clearance
Minor T, Paul A, Efferz P, Wohlschlaeger J et al in 2015 [240]	21 h HMP, autotransplantation	Porcine kidney	Custodiol-N (with dextran 40) vs KPS-1	Custodiol-N group: higher creatinine clearance, lower urea levels KPS-1 group: higher free-radical induced tissue damage and tubular cell injury (via biomarkers), higher activation of TNF-alpha and some endothelial markers, histopathologically more epithelial vacuolization
Thuillier R, Codas R, Marchand E, Lathelize H et al in 2012 [241]	60 min WIT, followed by 22 h HMP, autotransplantation	Porcine kidney	IGL-1 vs Belzer-MPS	Belzer-MPS: slightly less histological injury at 30 days post-transplant; IGL-1: weaker immune response at 3 months post-transplant

Maio R, Costa P, Figueiredo N, Santos I in 2007 [242]	6 h HMP	Porcine kidney	Celsior vs Celsior with addition of 30 mg/l PEG 20.000 D vs Belzer-MPS	Celsior + PEG: lower infrarenal resistance, better flow, lower histological injury score and lesser weight gain; Celsior without PEG: the worst results
Baicu SC, Taylor MJ, Brockbank KG in 2006 [245]	72 h HMP	Porcine kidney	Unisol (UHK) vs Belzer-MPS	More stable pH-levels in the Unicol group, compared to Belzer.MPS group
Baicu SC, Taylor MJ, Brockbank KG in 2007 [244]	72 h HMP	Porcine kidney	Unisol (UHK) vs Belzer-MPS	More pronounced tubular atrophy and weight gain, but higher metabolic activity in Unisol group
Hamaoui K, Aftab A, Gowers S, Boutelle M et al in 2017 [246]	10 h HMP, reperfusion	Porcine kidney (DCD)	Adenosine and lidocaine solution with bovine serum albumin vs UW solution	Modified adenosine and lidocaine solution: higher weight gain during perfusion, lower weight gain during reperfusion; better buffering capacity and lower lactate levels; UW: slightly higher urine output, histologically higher tubular dilation
Le Meur Y, Badet L, Essig M, Thierry A et al in 2020 [247]	HMP (mean CIT 12 h), clinical transplantation	Human kidneys	Belzer-MPS modified with M101 vs simple Belzer-MPS	Significantly lower DGF rates at 3 months in M101 group.
Ogbemudia AE, Hakim G, Dengu F, El-Gilani F et al in 2021 [299]	6 h HMP, reperfusion	Porcine pancreas	UW vs IGL-2	UW: higher amylase, lipase and lactate levels during perfusion; glucoselevel increase and insulin secretion induction at reperfusion only in the UW group

Shonaka T, Matsuno N, Obara H, Yoshikawa R et al in 2018 [248]	4 h SNMP, reperfusion	Porcine liver (DCD)	UW solution + human-derived hemoglobin-containing vesicles vs simple UW solution	UW solution with human-derived hemoglobin-containing vesicles demonstrated significantly higher oxygen consumption during SNMP
Shonaka T, Matsuno N, Obara H, Yoshikawa R et al in 2019 [249]	4 h SNMP, reperfusion	Porcine liver (DCD)	UW solution + human-derived hemoglobin-containing vesicles vs simple UW solution	UW solution with human-derived hemoglobin-containing vesicles: lower ASK and LDH levels, pH less acidotic, histologically less bile duct damage and disorder around the central veins
Bhattacharjee RN, Patel SVB, Sun Q, Jiang L et al in 2020 [250]	4 h post-ischemic SNMP, reperfusion	Porcine kidney	HBOC-201 (Hemopure) vs autologous blood, both mixed 1:2 with an isotonic crystalloid solution	Similar oxygen saturation; HBOC group: higher flow and urine output, slightly lower damage and inflammation marker levels.
Gage F, Leeser DB, Porterfield NK, Graybill JC et al in 2009 [251]	24 h SNMP	Porcine kidney (DCD)	Lifor vs Belzer-MPS	Lifor: stable high flow and low resistance rates; Belzer MPS: highest flow rates, that declined after 15 hours; high pro-inflammatory cytokine levels
Motoyama S, Saito S, Inaba H, Kitamura M et al in 2000 [252]	NMP	Rat liver	Perfusate containing RBCs vs without RBCs	Without RBCs: increased H2O2 production, injury and apoptosis in the sinusoidal endothelial cells, H2O2 occurrence in hepatocytes
Hafez TS, Habib MM, Seifalian AM, Fuller BJ et al in 2004 [253]	3 h NMP	Rabbit liver	Modified Krebs-Henseleit solution with vs without rabbit RBCs	RBC group: higher mitochondrial cytochrome oxidase content (marker of oxygen availability)

Dondossola D, Santini A, Lonati C, Zanella A et al in 2019 [254]	2,5 h NMP	Rat liver	Dulbecco-Modified-Eagle-Medium with vs without human RBCs	RBC group: higher levels of oxygen delivery and consumption, increased lactate clearance, glucose and potassium uptake, bile production and ATP levels; higher portal vein pressure and resistance; histologically less edema.
Yoshimoto S, Ohara M, Torai S, Kasamatsu H, Ishikawa J et al in 2021 [255]	3 h NMP	Porcine liver (DCD)	Whole blood vs diluted with pig serum and Leibovitz's Medium (10% blood)	Whole blood: stable pH levels (acidic in the diluted blood group), lower guanosine (marker of IRI) and lactate levels, choline metabolism closer to physiological
Starnes HF Jr, Tewari A, Flokas K, Kosek JC et al in 1991 [256]	2 h NMP	Rat liver	1) Krebs-Ringer-bicarbonate solution with 20% of washed human RBC and bovine serum albumin 2) Krebs-Ringer-bicarbonate with purified human hemoglobin 3) Krebs-Ringer-bicarbonate with bovine serum albumin 4) perfluorochemical emulsion FC 43	1 and 2: less weight gain in perfused livers, lower pH levels, as well as stabilized glucose and lactate levels, no major alterations in liver morphology 1: highest levels of glycogen and ATP, lowest potassium outflow
Laing RW, Bhogal RH, Wallace L, Boteon Y et al in 2017 [257]	6 h NMP	Human liver	Hemopure-based vs RBC based medium	Significantly higher oxygen extraction in the HBOC-group; otherwise no significant differences

Matton APM, Burlage LC, van Rijn R, de Vries Y et al in 2018 [258]	6 h NMP	Human liver	RBCs with FFP (control historical cohort) vs Hemopure with FFP vs Hemopure with Gelofusine	Hemopure groups: higher portal and arterial flow rates, higher ATP content, bile production, increased oxygen extraction, smaller ALT-increase Hemopure + Gelofusine: highest ATP levels
Jennings H, Carlson KN, Little C, Verhagen JC et al in 2022 [259]	4 h NMP	Rat liver	Oxyglobin with William's E medium vs human or rat RBCs with William's E medium	Higher AST levels in human RBC group; the least pronounced activation of liver-resident immune cells (mediators of rejection) in the Oxyglobin group
Liu Q, Nassar A, Farias K, Buccini L et al in 2016 [262]	10 h NMP, reperfusion	Porcine liver (DCD)	Steen vs Steen + RBCs vs whole blood	Steen: increased AST, ALT and LDH, worst bile production and bilirubin levels, lower VO2 and higher lactate levels, worst histological findings Whole blood: the best results
Linares-Cervantes I, Kollmann D, Goto T, Echeverri J et al in 2019 [263]	5 h NMP, transplantation	Porcine liver (DCD)	Steen + RBCs vs Gelofusine + RBCs vs whole blood	Steen: higher flow, lower resistance, better hyaluronic acid clearance, after transplantation: lower AST, AP and bilirubin levels, lowest INR rates and lactate levels, best maintained endothelial integrity Whole blood: higher AST, higher bile production with less acidotic bile pH Gelofusine: most weight gain
Chen M, Chen X, Wang J, Ren H et al in 2022 [260]	24 h NMP	Porcine liver (DCD)	Novel oxygen carrying perfusate based on perfluoronaphthalene-albumin nanoparticles vs whole blood	Lower resistance rates in the nanoparticle group, otherwise similar lactate clearance, AST, ALT, bile production and histological findings

De Beule J, Keppens D, Korf H, Jochmans I in 2022 [264]	4 h NMP either right after explantation, after 22 h of SCS or 60 min of WIT	Porcine kidney	RBCs vs whole blood	Similar creatinine clearance, oxygen consumption and AST levels, no significant differences in pro- and anti-inflammatory cytokine levels
Pool MBF, Hamelink TL, van Goor H, van den Heuvel MC et al in 2021 [265]	7 h NMP	Porcine kidney	Autologous RBCs with: 1) William's E medium + bovine albumin 2) 200 ml Albuman with saline 3) Ringers lactate with saline, adenine, glucose and mannitol (SAG-M) 4) 100 ml Albuman with saline	1) highest weight gain, increased activity of Bax-gene (pro-apoptic) 2) lowest urine output, lowest creatinine clearance, but least weight gain, lower injury marker and less glomerular dilatation 3) highest urine output; pH started higher, but ended lower; highest creatinine clearance, unstable Na and K levels; high AST and LDH levels 4) highest AST and LDH levels
von Horn C, Zlatev H, Lüer B, Malkus L et al in 2023 [266]	6 h NMP	Porcine kidney	Aqix RS-I with 500mmHg pO2 vs Aqix RS-I + RBCs with 500mmHg pO2 vs Aqix RS-I + RBCs with 200mmHg pO2	Acellular group: slightly higher flow, RBC groups: creatinine clearance declined slightly after 4 h, increased HMGB1 200mmHg group: increased tenascin C Otherwise similar

Venema LH, van Leeuwen LL, Posma RA, van Goor H et al in 2022 [267]	3h of HMP (Belzer MPS) + 4h of NMP	Porcine kidney	1) Aqix + dextran 40 2) Aqix + RBCs + dextran 40 3) Aqix + bovine albumin 4) Aqix + RBCs + bovine albumin 5) diluted blood	RBC-containing groups: higher oxygen consumption, less sodium excretion, better sodium reabsorption, higher ATP (best results in the blood group); Acellular groups: more damage (histopathology); 1-4) higher lactate levels; 4) lowest sodium excretion and best reabsorption, compared to other Aqix groups; 2) better ATP content, compared to other Aqix groups, lowest creatinine clearance. In general, Aqix + RBCs + bovine albumin better results, compared to other Aqix groups, but blood perfusion remains superior.
Longchamp A, Fontan FM, Aburawi MM, Eymard C et al in 2024 [268]	6 h NMP	Human kidney	William's E medium with or without RBC addition	Acellular group: higher flow and lower resistance, higher ATP levels, but higher lactate increase
Zarnitz L, Doorschodt BM, Ernst L, Hosseinnejad A et al in 2023 [272]	6 h NMP	Porcine kidney	Ecosol **vs** RBCs + Ringer (+mannitol)	Ecosol: more stable pH, but acidotic, HIF1-α slightly higher (oxidative stress), lower LDH and urine protein, more weight gain, higher Caspase 3, more prominent tubular injury; gel-like formation due to wrong PEG molecule — possibly negative effect on the results; RBC group: higher tubular vacuolization

Aburawi MM, Fontan FM, Karimian N, Eymard C et al in 2019 [269]	6 h NMP	Human kidney	Hemopure+ William's E medium **vs** RBCs + William's E medium	RBC group: higher lactate levels, more weight gain, increased loss of tubular integrity, epithelial vacuolization and coagulation necrosis
Edgworth E, Ernst L, Czigany Z, Saritas T et al in 2022 [270]	6 h NMP	Porcine kidney	Supplemented Ringer as base + either Oxyglobin or RBCs + with or without addition of ascorbic acid	Oxyglobin: increase in resistance, decrease in flow; lower creatinine clearance and urine production; higher salt excretion, less stable pH, higher lactate levels, higher LDH levels, high methemoglobin levels without significant difference related to vitamin C supplementation
Jägers J, Kirsch M, Cantore M, Karaman O, Ferenz KB in 2022 [271]	2 h NMP	Rat kidney	Base solution consisting Ringer lactate + bovine serum albumin alone or with addition of 2%, 4% or 8% of albumin-derived perfluorocarbon-based artificial oxygen carrier	Only the group containing 4% albumin-derived perfluorocarbon-based artificial oxygen carrier could improve the results, compared to unsupplemented perfusion.
von Horn C, Lüer B, Malkus L, Minor T in 2024 [273]	60 min rewarming (8°-35°) -> 60 min NMP, reperfusion	Rat liver	Steen vs Belzer MPS	Steen: higher ALT rates, higher apoptosis and inflammatory markers,
an Leeuwen OB, Bodewes SB, Lantinga VA, Haring MPD et al in 2022 [261]	1 h HOPE, 1 h rewarming and 2,5h NMP, viable grafts transplanted	Human liver	Gelofusine with albumin + either Hemopure or RBCs	Simpler procedure without the need to switch solutions in Hemopure group, otherwise similar results during perfusion and up to 12 months post-transplant

von Horn C, Wilde B, Rauen U, Paul A, Minor T in 2021 [274]	Post-ischemic rewarming (8°-20°) for 60 min and SNMP for 30 min, reperfusion	Porcine kidney	Custodiol-MP vs Belzer MPS	Custodiol-MP showed slightly higher creatinine clearance rates; otherwise similar results.
Mahboub P, Aburawi M, Karimian N, Lin F et al in 2020 [275]	60 min rewarming (10°-37°), 30 min NMP, reperfusion	Rat kidney (DCD)	William's E medium with or without Hemopure addition	Hemopure group: ultrafiltrate production, GFR and sodium reabsorption higher during reperfusion; MetHb up to 7% at perfusion and up to 20% at reperfusion

3.1 Hypothermic perfusion solutions

3.1.1 Literature on perfusion solutions for liver HMP

Starting with literature that includes comparisons of perfusion solutions for HMP of the liver, the Eurocollins solution is worth mentioning. It is an improved version of the first commercially available preservation solution for SCS, the Collins solution. It is not used much anymore, having been overshadowed by newer mediums that show better results. Besides being used for simple cold preservation, this medium has also been questioned as a solution for continuous perfusion, which was less common than SCS at the time the Eurocollins solution was relevant. Nevertheless, in 1994, a study on isolated rat livers compared perfusion with Eurocollins to its successor, the UW solution, which has been the gold standard for preservation of various organs and different strategies for a long time. As expected, UW showed better results compared to Eurocollins, causing less cell swelling, keeping the liver sinusoids open, and thus preserving the architecture of hepatocytes and maintaining microcirculation better than the other solution [223].

The next solution to challenge UW and considered equivalent to it in kidney and liver preservation is the colloid-free HTK (Custodiol). In 2003, it was compared to Belzer MPS during 24 hours of oxygenated HMP of DCD rat livers. After initial perfusion, the livers also underwent reperfusion with oxygenated Krebs-Henseleit buffer. During perfusion, the two groups demonstrated similar resistance parameters. The extent of tissue edema after perfusion was also similar between the groups. After 45 minutes of reperfusion, the two groups showed similar oxygen utilization, glutamate-pyruvate transaminase concentrations, and bile production. The only significant difference was the higher content of glutamate-dehydrogenase in the HTK group. Nevertheless, the authors described perfusion with HTK as equally effective as Belzer MPS [277]. Later, the modified HTK, Custodiol-N, was also tested in the context of liver HMP. A study on rat livers compared standard HTK to the newer Custodiol-N. Additionally, the authors investigated the benefits of adding iron chelators, comparing Custodiol-N without the addition of deferoxamine and LK 614 to the same solution with these additives. Two different concentrations of LK 614 were used: 2.5 μM versus the normally used 7.5 μM. Livers underwent oxygenated HMP for 18 hours and were evaluated after normothermic reperfusion in vitro with Krebs-Henseleit buffer. The base Custodiol-N solution proved superior to HTK, showing lower ALT and LDH levels, while the best results were

achieved with the addition of deferoxamine and 2.5 μM of LK 614. The authors highlighted that even though 7.5 μM could be the standard concentration for cold preservation, a lower dose of LK 614 seems to work better for perfusion [224]. Later, a study on porcine livers compared saline, HTK, and UW for SCS as well as HMP for 6 hours. Regardless of the perfusate, HMP proved superior to SCS. As for perfusion solutions, HTK and UW showed similar results upon histopathological assessment and similar levels of ALT, AST, and LDH. The malondialdehyde levels, tested as a marker of oxidative ROS-induced damage, were also similar in all HMP groups. The study demonstrated that the use of HTK resulted in the lowest portal vein resistance and much higher ATP levels, while UW seemed to prevent edema better. The authors concluded that HTK could be the better solution for MP, citing its lower viscosity and absence of HES [225]. Similar results were observed in another study comparing UW and HTK for liver HMP. Here, porcine split-liver grafts underwent 4 hours of HMP with either UW-gluconate solution or HTK as the perfusate and isolated reperfusion. In this study, as well as in the previously described one, perfusion with HTK resulted in lower portal vein resistance. Additionally, a lower hepatic artery resistance was detected, but only during the perfusion phase. Furthermore, after a thorough analysis of liver tissue, the authors mentioned that perfusion with HTK seemed to better protect the graft from IRI [226].

Regarding UW itself, since its composition is relatively simple and straightforward, modifications of the solution have been investigated over time with the aim of improving its protective qualities. This is particularly relevant in cases of extended warm ischemia time (WIT) during DCD. In a study on rat livers that underwent 60 minutes of WIT, authors aimed to protect grafts from excessive injury and enhance energy recovery through supplementation of the perfusate with antioxidants, calcium chloride, thromboxane A2 inhibitor, and important anaplerotic substrates. This was tested during 5 hours of HMP with either standard or supplemented UW solution, followed by reperfusion with Krebs-Henseleit solution. The groups perfused with supplemented UW demonstrated higher ATP levels and lower LDH levels, as well as increased bile production and less edema formation, compared to the standard UW group. Additionally, good preservation of liver tissue was observed in the supplemented group. This study suggests that such supplementation of UW is feasible and could be beneficial, particularly for perfusion of DCD livers. [276]

A newer preservation solution that has also gained popularity is the IGL-1 preservation solution. Recently, it has also been modified for perfusion purposes, improving vasodilatation and antioxidant ability by increasing PEG35 and glutathione concentrations. The new perfusion

solution is called IGL-2. It had to, of course, be compared to the gold standard, the UW solution. The latter, primarily a SCS solution, has been modified for perfusion as well, becoming known as Belzer-MPS. In two recent preclinical studies, IGL-2 has been compared to Belzer-MPS. Firstly, both solutions were used on fatty rat livers for HOPE, followed by normothermic reperfusion. Here, all livers were cold-stored for 24 hours in either UW or IGL-2, with half of them additionally undergoing 2 hours of HOPE with either IGL-2 or Belzer-MPS. Lastly, all grafts were reperfused under normothermia for 2 hours. The group that underwent HOPE with IGL-2 demonstrated lower damage grade scores during histological evaluation and lower ALT and AST perfusate levels. By analyzing different biomarkers, authors demonstrated better glycocalyx preservation as well as lower apoptosis and inflammation occurrence. The weight of livers was measured throughout the experiment, with weight gain indicating possible edema formation. The IGL-2 HOPE group, in contrast to the Belzer-MPS HOPE group, showed weight loss, further signaling its superiority. Additionally, authors mentioned the universality of IGL-2, as it can be used both during SCS and HOPE, while UW must be replaced with Belzer-MPS for perfusion. [227] The most recent study used a porcine liver model to compare SCS and HOPE with IGL-2 versus SCS with UW followed by HOPE with Belzer-MPS. After 6 hours of SCS, all grafts underwent 2 hours of HOPE and then 2 hours of normothermic reperfusion. In this study, authors did not detect any significant differences in the results using these solutions. Transaminase levels, lactate clearance, histological signs of IRI, bile production, and inflammatory reactions were all similar between both groups. Nevertheless, it was once again highlighted that IGL-2 is a viable solution for both SCS and HOPE of the liver, which is logistically advantageous compared to UW and Belzer-MPS. [228]

A further solution tested for HMP of the liver in comparison to UW is Aqix RS-1, which was created as a universal solution for the transportation and/or storage of any tissue or organ at any temperature. Its composition is similar to serum and utilizes sodium bicarbonate as one buffer, with BES as the other buffer, differing from those in other perfusion solutions. [229] Aqix RS-1 was tested against a UW-like perfusate, KPS-1, which is a standard kidney perfusion solution enriched with deferoxamine, ROS-inhibitors, and nutrients, and has the same composition as UW-MPS. After 24 hours of HMP, the grafts were transplanted into recipient pigs. After reperfusion, morphological integrity and AST levels did not differ between the two groups. However, TNF-α levels, an inflammation marker, were lower in the Aqix group, while hyaluronic acid clearance was better in the KPS group, suggesting better cellular function. The overall recipient survival at day 3 was only 33%. [230]

Speaking of KPS-1, another commercially available perfusion solution called Vasosol was compared to it during liver HMP. Vasosol, developed based on Belzer MPS, includes α-ketoglutarate, N-acetylcysteine, prostaglandin E1, nitroglycerin, and L-arginine. It was tested during liver HMP on porcine and discarded human livers, showing promising results, which led to its clinical application. To evaluate if the additional substances made a difference, it was compared to KPS-1. In a study on rat DCD livers, another group was included that was additionally supplemented with antioxidant α-tocopherol (vitamin E). Livers underwent 8 hours of HMP, followed by reperfusion with Krebs-Henseleit-Bicarbonate buffer. Both Vasosol groups demonstrated slightly decreased ALT levels during reperfusion. Additionally, a significant decrease in inflammatory markers such as IL-6, MCP-1, and TNF-α was observed in the Vasosol group, with further reductions in the vitamin E-supplemented Vasosol group. Apoptotic markers, including caspase 3/7 and to a lesser extent cytochrome C, were also reduced in the Vasosol groups, with the lowest levels in the vitamin E-Vasosol group. [278] At least two articles report successful clinical application of Vasosol, including HMP and transplantation of "orphaned" ECD livers. [303, 318] Following these positive results, Vasosol was used in a recent RCT of transplantation after liver HMP, where 63 livers were perfused using Vasosol. The trial demonstrated good results compared to the control SCS group. [279]

Another solution developed for MP is Polysol. It has a Na/K ratio of 120/20 mmol/L, originally includes HES as a colloid, and is enriched with more nutrients such as amino acids and vitamins compared to UW. To compare these solutions, rat livers underwent HMP for 24 hours with either Polysol or UW-Gluconate (UW-G, Na/K 125/25 mmol/L) and were reperfused for 1 hour afterward. Results showed similar pH, flow, lactate levels, and dry/wet ratios. Histopathological appearance also did not differ between the two groups. However, perfusion with Polysol resulted in slightly less liver enzyme release and higher bile production. [231] In a subsequent study focusing on Polysol, authors aimed to determine the optimal colloid for use in the solution. Rat livers underwent 24 hours of HMP followed by reperfusion with Polysol containing three different colloids: HES, dextran, and PEG. They were compared to a control group of livers perfused with UW-G. All Polysol groups demonstrated higher oxygen consumption, less hepatocellular damage, better flow, and increased bile production compared to the UW-G group. Regarding the different colloids, ALT levels, bile production, ammonia clearance, oxygen consumption, and dry/wet ratios were similar across all three Polysol groups, with slightly better results in the Polysol-PEG group and the poorest results in the Polysol-HES group. [232] In another study using porcine livers, the livers were divided into two groups: one

underwent 24 hours of HMP with Polysol, and the other underwent 24 hours of SCS with Celsior. AST and ALT levels, as well as vascular resistance at reperfusion, were significantly lower for the Polysol group, while ammonia and urea production were similar, and no bile was produced in either group. [233] It is important to note that the first report of clinical experience with Polysol during initial flush and SCS in 9 living kidney transplantations resulted in an increased rejection rate, leading to the premature termination of the study. [280]

Reviewing available reports on clinical trials of liver HMP, including RCTs, it is evident that Belzer-MPS has been and continues to be the preferred perfusion solution. Additionally, this solution and its equivalents, such as KPS-1 and PumpProtect, are essentially the only solutions documented as used in clinical trials of HMP in the literature. Specifically, Belzer-MPS is cited as the perfusion solution in six clinical trials and RCT reports published between 2010 and 2023. [71, 80, 81, 90, 301, 302] The only other solution mentioned in a clinical series and an RCT for liver HMP is Vasosol, as discussed earlier. [279, 303]

3.1.2 Literature on perfusion solutions for kidney HMP

Starting with older solutions, back in 2003 a comparison of Eurocollins and Belzer solution was conducted on porcine kidneys during 3 hours of HMP. Similarly to the aforementioned liver HMP study, UW proved to be superior to EC in kidneys as well. Here, perfusion with EC resulted in increased weight gain of the grafts and demonstrated worse histopathological outcomes. [234]

The Belzer solution and its variations have been widely used for kidney HMP. Besides comparing it to other popular or newly developed solutions, there are some experimental and clinical studies comparing different UW-based solutions. One of the earlier studies, for example, subjected canine kidneys to HMP with either Belzer-MPS developed for perfusion purposes or the cold storage UW solution (Viaspan). Prior to that, kidneys underwent either 60 minutes or 75 minutes of warm ischemia, followed by 72 hours or 24 hours of HMP, respectively. The experiment resulted in successful 72-hour preservation of kidneys after 60 minutes of warm ischemia with 100% 10-day post-transplant survival for either solution. On the other hand, authors observed better survival rates for kidneys after 75 minutes of warm ischemia perfused with the UW solution compared to the Belzer-MPS group with the same warm ischemia time (86% vs. 25%). [235]

A large retrospective analysis has been done on outcomes of kidney transplantation after HMP with different variations of Belzer solution. The study included 532 kidneys perfused with either Belzer-MPS, Belzer II albumin gluconate, or Belzer-MPS modified with nitroglycerin, prostaglandin E1, and polyethylene glycol-superoxide dismutase. The first two additives act mainly as vasodilators, while the last one is a free radical scavenger. This modification showed the best results of all three solutions, such as reduced DGF rate, better graft survival, and lower creatinine levels at 6 months. The results of the other two solutions did not differ significantly, although Belzer-MPS performed slightly better than Belzer II albumin gluconate. According to the authors, these findings suggest positive effects of the added substances, especially in the context of minimizing IRI. [236]

Another study compared HMP of DCD pig kidneys for 24 hours with either UW (Viaspan) or KPS-1 (Kidney Perfusion Solution), followed by autotransplantation. While both solutions are HES-based, some key differences are the sodium and potassium concentrations, with UW

containing more potassium and KPS containing more sodium, as well as UW containing lactobionate while KPS contains mannitol. At the start of perfusion, resistance was significantly lower and flow rate significantly higher for KPS compared to UW. Kidneys perfused with KPS also regained diuresis faster (on day 3 vs. day 4 for UW) and had lower serum creatinine levels. Authors also observed levels of urinary enzymes, such as alanine aminopeptidase and N-acetyl-β-D-glucosaminidase (β-NAG), discovering lower levels in the KPS group. The ratio of oxidized glutathione to total glutathione, indicating oxidative stress, was also lower in animals that received KPS-perfused kidneys. On top of that, KPS-perfused kidneys demonstrated lower immune response activation, better tissue histology, and lower fibrosis rates at 3 months post-transplantation. Although animal survival rates at 4 months did not differ significantly between the KPS and UW perfusion groups, other findings suggest the superiority of KPS-1 over UW (Viaspan). [237]

Furthermore, some studies compare UW-based solutions with HTK (Custodiol). The first study was performed on pig kidneys from non-heart-beating donors. The grafts underwent either 18 hours of HMP with Belzer-MPS or low-flow HMP with non-colloidal HTK. Viability of the grafts was followed up to 1 week after transplantation. In this study, no significant differences were found between HMP with non-colloidal HTK and Belzer-MPS. [238]

The next study was also conducted on pig kidneys, although this time the modern variation of HTK, Custodiol-N, supplemented with dextran 40, was compared to KPS-1 as the gold standard. Kidneys underwent 20 hours of oxygenated HMP, followed by 2 hours of reperfusion with diluted autologous blood at 37°C. The results demonstrated higher flow rates and oxygen consumption, as well as higher urine production for the supplemented Custodiol-N group. On top of that, creatinine clearance was 30% higher in Custodiol-N-perfused kidneys. Sodium excretion and LDH rates were only slightly better in the Custodiol-N group. This preclinical study suggests positive renoprotective effects of dextran 40-supplemented Custodiol-N during HMP. [239]

Acknowledging these results, a further study on porcine kidneys comparing these two solutions was conducted, this time with subsequent autologous transplantation. Here, grafts underwent 21 hours of HMP. One day after transplantation, authors detected higher free-radical-induced tissue damage and tubular cell injury in the KPS-1 group by analyzing specific biomarkers. Similarly to the previous study, creatinine clearance rates were higher and serum creatinine

levels lower in the Custodiol group. Similar differences were found concerning urea levels after transplantation. Nevertheless, creatinine and urea levels eventually normalized in both groups. Activation of TNF-alpha and some endothelial markers were higher in the KPS-1 group. During histological assessment, it was found that kidneys in both groups developed only slight changes, though the KPS-1-perfused kidneys had slightly higher epithelial vacuolization. In this study, as well as in the previous one, Custodiol-N supplemented with dextran seemed to optimally preserve porcine kidneys during HMP and protect them from IRI at implantation, which could result in lower DGF rates in clinical transplantation. [240]

The next well-known preservation solution, also tested for kidney HMP, is IGL-1, primarily designed and used for SCS. In a pig autotransplantation model, kidneys underwent 60 minutes of warm ischemia followed by 22 hours of SCS or HMP with either Belzer-MPS or IGL-1. Authors failed to find many significant differences in outcomes for these two solutions in the HMP groups. At 30 days post-transplant, kidneys perfused with MPS tended to demonstrate slightly less histological injury. However, at the 3-month follow-up, after observing levels of immune markers, the immunological response seemed to be weaker in the IGL group. Authors mentioned the effects of immunocamouflage of PEG as well as possible suppression of fibrosis pathways for IGL. [241]

Another solution compared to Belzer-MPS in the context of kidney HMP is Celsior, a solution primarily designed for static cold preservation of heart grafts. In this comparative study, conducted on porcine kidneys, grafts underwent 6 hours of HMP with either Celsior, Belzer-MPS, or Celsior with the addition of 30 mg/L PEG 20,000 Da. The Celsior+PEG group demonstrated the best performance among the three groups, suggested by lower infrarenal resistance and better flow, lower histological injury scores, and less weight gain. Celsior without the addition of PEG, on the contrary, seemed to perform the worst when comparing the aforementioned parameters of the groups. [242] On the other hand, a report of clinical transplantation of 10 kidneys after 18.3 1.3 hours of hypothermic pulsatile perfusion with Celsior was published, presenting positive results of 100% patient and 90% graft survival at 1 year, as well as DGF in 10% and the need for post-transplant dialysis in 10% of the cases. Authors deemed such results comparable to historical UW perfusion outcomes and highlighted that Celsior can be used as a single solution during donor organ procurement and preservation,

without needing to be switched out in the context of further kidney preservation after the initial multi-organ flush. [243]

A less commonly used solution, Unisol (UHK), was also compared to Belzer-MPS during HMP of porcine kidneys. UHK is a solution containing Hepes as a buffer and dextran 40 as a colloid, with a Na/K ratio of 62/70 mM. It has high buffering capacity and was primarily developed for application at low temperatures (up to -15°C). In this experiment, porcine kidneys underwent 72 hours of HMP with either UHK or Belzer-MPS without much warm ischemia prior to cooling. During perfusion, authors observed similar renal flow and vascular resistance parameters, as well as minimal histological signs of injury for both groups, although tubular atrophy was more pronounced in the UHK group. The weight gain of grafts was likewise much higher in kidneys perfused with Unisol. On the other hand, authors detected much higher metabolic activity in the UHK group, as seen in changes in glucose, glutamate, and ammonium concentrations compared to baseline, which was not the case for Belzer-MPS. Concerning this, authors mentioned the possibility of better monitoring of the biochemical activity of grafts ex vivo with UHK, as it could correlate with post-transplant performance. [244] The same group of researchers also previously demonstrated in a similar setting a much better acid-base regulation with UHK. The use of this solution seemed to control pH at a stable level, compared to Belzer-MPS, where wide ranges of pH were recorded throughout the experiment. [245]

Adenosine and lidocaine solution (AL) is another medium that could potentially be used in HMP of the kidney, as shown in an experimental study comparing it to UW. AL is a solution primarily used in cardiac surgery to reanimate the heart, demonstrating many positive effects, including, most importantly, IRI reduction. In this study, a DCD porcine model was used, where kidney grafts underwent 30 minutes of warm ischemia time (WIT), followed by 4-5 hours of static cold storage (SCS) and 10 hours of HMP with either a modified AL solution with bovine serum albumin or the standard UW solution. Afterwards, kidneys were further evaluated during normothermic reperfusion. During HMP, higher systolic pressure and better flow were detected in the AL group, while resistance rates were similar. At reperfusion, these parameters were similar, with slightly better rates for the UW group. As for weight gain, it was much higher in AL-perfused kidneys after HMP, whereas, after reperfusion, weight gain was significantly lower in the AL group. Urine output was slightly higher in the UW group, while creatinine clearance, sodium excretion, and oxygen consumption were similar in both groups. On the other hand, better buffering capacity and lower lactate levels were observed in the AL group.

Histological findings were similar in both groups, with the exception of higher tubular dilation in the UW group. This study highlighted some advantages of an AL-based solution, potentially setting the stage for further developments. [246]

Another interesting study demonstrated the use of M101, an extracellular hemoglobin derived from a marine lugworm, in human kidney HMP as compared to simple Belzer-MPS. M101 has a high oxygen-binding capacity, releases oxygen gradually, and possesses antioxidant properties. The purpose of adding M101 to preservation solutions is to prevent the decline of dissolved oxygen levels. Its effectiveness in preventing IRI has been shown in preclinical studies involving the kidney, lung, and heart. In this study, a total of 15 human kidneys perfused with the addition of M101 were transplanted. Other grafts underwent SCS with simple UW or UW modified with M101. Results were compared to a control group consisting of contralateral kidneys perfused with Belzer-MPS or stored in UW without any addition. The mean cold ischemia time (CIT) for all groups was 740 minutes. Outcomes were assessed at a follow-up after 3 months. After transplantation, the delayed graft function (DGF) rate was significantly lower in the M101 groups. The rate of adverse events was comparable between groups, and graft and recipient loss was low in all groups, although somewhat better in those receiving M101. As a result, this study not only confirmed the safety of M101 in kidney preservation but also demonstrated improved function recovery due to M101 addition. [247]

Polysol, previously mentioned in the context of liver HMP, was also tested during kidney perfusion. In a study on porcine kidneys, 20 hours of HMP with Polysol was compared to SCS with Polysol or HTK, as well as control non-ischemic unilateral kidneys. The HMP group showed the best renal function among the three preservation groups, comparable to control kidneys. Although no comparison to another solution for HMP was made, the SCS group using Polysol also demonstrated better results than SCS with HTK. [281] As previously mentioned, in the pilot clinical study in 2013, Polysol seemed to increase rejection rates, and the study had to be terminated. Since then, not much literature on the solution can be found. [280]

Judging by descriptions of clinical trials and RCTs involving kidney HMP, Belzer-MPS and its equivalents, such as KPS-1, remain the predominant solutions used in the clinical setting of hypothermic kidney perfusion. These solutions were credited with being used in 14 clinical trials and RCTs of kidney HMP from 2001 through 2021. [85, 86, 88, 90, 304-313]

3.2 Subnormothermic perfusion solutions

3.2.1 Literature on liver SNMP solutions

In the context of SNMP perfusion solutions, an important consideration is the ability to provide a reliable oxygen supply. This is crucial because metabolic activity is higher and oxygen demand is greater due to the warmer temperatures compared to HMP. To address this challenge, two studies incorporated human-derived hemoglobin-containing vesicles (HbV) as non-cellular oxygen carriers into basic perfusion solutions.

In the first study, a pig DCD model was used, where livers were preserved for 240 minutes after procurement by either SCS, SNMP with UW, or SNMP with UW containing HbV. This was followed by reperfusion with autologous blood for 120 minutes. The study demonstrated significantly higher oxygen consumption during SNMP with HbV. Additionally, portal vein resistance was lower in the HbV group during perfusion, though at reperfusion, the rates were similar between the two SNMP groups. AST levels were also similar between the perfusion groups both during SNMP and after reperfusion. Histological evaluation after perfusion did not show differences in injury grade between the groups. [248]

In a further study under similar conditions, HbV showed more advantages compared to SNMP with simple UW solution. A HMP group with UW solution was also included for comparison. During perfusion, hepatic artery and portal vein pressure, as well as ASK and LDH concentrations, were lowest, and oxygen consumption was significantly higher in the HbV group compared to the other MP groups. After reperfusion, perfusate LDH and AST levels were again lowest in the HbV group. The dynamic change in perfusate pH towards acidosis was also smallest in livers preserved with HbV. Lactate levels were similar between both SNMP groups. Histological evaluation revealed less bile duct damage and disorder around the central veins in livers preserved with HbV. [249]

Another non-cellular oxygen carrier, HBOC-201 (Hemopure), has gained popularity in MP. This bovine hemoglobin-based oxygen-carrying solution was originally developed as a blood alternative for military use. In the context of liver SNMP, it was tested on porcine grafts, mixed with Belzer MPS and compared to SCS. The cold ischemia time (CIT) was 9 hours for all grafts, after which they underwent orthotopic transplantation and were followed up for 5 days. The

study demonstrated higher oxygen delivery and bile production, as well as generally better graft function and significantly higher survival in the SNMP group with Hemopure. [282]

The previously mentioned Celsior solution was also used in studies on liver SNMP. Although no comparison to a different solution was made, positive results were observed with Celsior. In one study, DCD porcine livers were preserved by either SCS or SNMP for 6 hours using Celsior, followed by reperfusion with autologous blood. SNMP with Celsior resulted in significantly reduced AST, LDH, and lactate levels, as well as better histopathologic findings. [283] In another study, Celsior was used during 6 hours of SNMP of rat livers with or without the addition of angiotensin IV. This was followed by reperfusion with Krebs-Henseleit solution. Livers perfused with the addition of angiotensin IV demonstrated lower AST and LDH levels, higher bile flow, decreased oxidative stress, and reduced apoptosis. [284]

3.2.2 Literature on kidney SNMP solutions

Acellular oxygen carriers have also been tested in kidney SNMP. For instance, a study compared HBOC-201 and autologous blood, both mixed 1:2 with an isotonic crystalloid solution, in a porcine kidney SNMP setting. The perfusion duration was 4 hours, and the kidneys were cold-stored in HTK for 4 hours beforehand. After SNMP, the kidneys were reperfused with autologous blood at normothermia. Oxygen saturation during perfusion was similar between groups, demonstrating HBOC as a reliable alternative oxygen carrier. Renal blood flow and urine output were higher during reperfusion in the HBOC group. Creatinine clearance and urine protein/creatinine ratios were similar between the two SNMP groups. Histological findings were also comparable between blood- and HBOC-perfused kidneys. Levels of damage markers (NGAL 1, KIM-1) and pro-inflammatory markers (IL-6) were slightly lower in the HBOC group. [250]

Lifor is a relatively new preservation solution designed to protect grafts from IRI. It contains nutrients, growth factors, amino acids, salts, and buffers. It has been tested for kidney perfusion in a porcine DCD model. In this study, three groups were compared: perfusion with Lifor at room temperature, Belzer-MPS at room temperature, and hypothermic perfusion with Belzer-MPS. All kidneys were perfused for 24 hours following an initial 2 hours of cold storage. The flow rates were lowest in the HMP group, while the room temperature Belzer-MPS group demonstrated the highest flow rates during the first 15 hours, which declined thereafter. The Lifor group maintained stable high flow rates throughout the 24 hours. Resistance rates were similar, with the Lifor group showing consistently low levels of resistance. Pro-inflammatory cytokine levels (IL-8, TNF-α, IL-1β, and IFN-γ) were significantly higher in the room temperature Belzer-MPS group, especially after the first 5 hours of perfusion. At 24 hours, the levels of these substances were lowest in the HMP group, with the Lifor group showing somewhat higher levels, though still much lower than those in the room temperature Belzer-MPS group. These results favor SNMP with the Lifor solution. [251]

A recent study reported the successful preservation of declined human kidney grafts for 4 days using SNMP on a novel platform with a newly developed perfusate. This medium is acellular and based on Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12, which includes glutamine, human serum albumin, and HEPES. It is additionally supplemented with components required by kidneys during perfusion, such as insulin-transferrin sodium selenite,

citric acid, and acetic acid. The study demonstrated maintained metabolic activity and functional integrity of the grafts for up to 4 days of SNMP, setting the stage for further research. [285]

3.3 Normothermic perfusion solutions

3.3.1 Literature on liver NMP solutions

In the context of NMP, reliable and sufficient oxygen delivery is of utmost importance. Traditionally, this is addressed by using whole or diluted blood, or by adding packed red blood cells (RBCs) to the perfusate. Some studies, however, have examined liver NMP without the addition of blood or RBCs.

In a 2000 study on rat livers, outcomes of NMP with an RBC-free buffer were compared to those with an RBC-supplemented buffer. The study observed increased H2O2 production, as well as injury and apoptosis in the sinusoidal endothelial cells, followed by H2O2 occurrence in hepatocytes. These effects were less pronounced with the addition of RBCs, catalase (an H2O2 radical scavenger), and a specific xanthine oxidase inhibitor. [252]

A similar study was conducted on rabbit livers, which underwent 180 minutes of oxygenated NMP with either a modified Krebs-Henseleit solution (containing electrolytes, HEPES, and glucose) or the same solution with washed rabbit RBCs. Mitochondrial cytochrome oxidase levels, used as a marker for oxygenation in hepatocytes, initially dropped in both groups, but more drastically in the RBC-free group. Livers perfused with the addition of RBCs showed increased levels of mitochondrial cytochrome oxidase, which were significantly higher by the end of the perfusion compared to the RBC-free group. However, bile output was the same in both groups. [253]

Another study on a rat model of NMP for 150 minutes achieved similar positive results when human RBCs were added to Dulbecco's Modified Eagle Medium, which is used for cell cultivation and contains a broad spectrum of amino acids and vitamins. Livers perfused with the addition of RBCs exhibited higher levels of oxygen delivery and consumption, as well as increased lactate clearance, glucose and potassium uptake, and ATP levels. Portal vein pressure and resistance were higher in the RBC group, while bile production was increased. AST and LDH levels were similar between the two groups. Histological analysis showed normal histology in both groups, with less edema in the RBC group. No damage related to RBC use was detected. [254]

An important consideration in NMP is determining the optimal hematocrit for liver perfusion. A study using a DCD pig model compared 3 hours of NMP with either collected whole blood or blood diluted with pig serum and Leibovitz s Medium, which is primarily used for cell cultivation. The diluted medium contained only 10% of the collected whole blood. The groups were compared to a control group with heterotopic transplantation without NMP. During perfusion, flow levels were similar between groups. pH levels remained stable in the whole blood group but became acidic in the dilution group. The whole blood NMP group demonstrated the lowest rates of guanosine (a marker of IRI) and lower levels of lactate. Additionally, choline metabolism in the whole blood group was more similar to the control group, which can be used as a marker of liver function. [255] In contrast, a clinical study reported liver transplantation outcomes after NMP using an FFP-based solution, which included RBCs. Compared to a control SCS cohort, outcomes after NMP with the described perfusate were comparable or better. The authors described this perfusate option as the most physiological, despite not containing leukocytes and thrombocytes, which play a significant role in IRI. [289]

An alternative strategy in NMP is to avoid blood and RBCs altogether by using cell-free oxygen carriers. Several studies have explored this approach. In 1991, purified human hemoglobin was tested as an alternative to RBCs during 120 minutes of NMP. The study included four groups with different media: the first contained Krebs-Ringer-bicarbonate solution with 20% washed human RBCs and bovine serum albumin; the second contained Krebs-Ringer-bicarbonate with purified human hemoglobin; the third contained Krebs-Ringer-bicarbonate with bovine serum albumin; and the fourth contained perfluorochemical emulsion FC 43, commercially available at that time as "artificial blood." The first three groups were also supplemented with amino acids, glucose, and lactic acid. The RBC and hemoglobin groups showed less weight gain in perfused livers, lower pH levels, and stabilized glucose and lactate levels. Potassium outflow was lowest in the RBC group, which also had the highest levels of glycogen and ATP. Methemoglobin levels were 2.2% in the hemoglobin group and less than 1% in the RBC group, which might explain the slightly decreased oxygen utilization in the hemoglobin group. No major alterations in liver morphology were detected after NMP with RBCs or hemoglobin, in contrast to the other two groups. The authors concluded that hemoglobin-containing solutions could serve as a blood substitute during liver perfusion, maintaining sufficient oxygenation and organ integrity. [256]

Another artificial blood" solution containing hemoglobin is Neo Red Cell, a Japanese medium also tested during liver NMP. Combined with L-15 medium and bovine serum albumin, Neo Red Cell was used to perfuse porcine livers for 10 hours. The study observed sufficient oxygenation, decreasing lactate levels, and almost unchanged liver histology by the end of the experiment. [290]

A popular RBC substitute today is Hemopure (HBOC-201), a bovine hemoglobin-based acellular oxygen carrier, previously mentioned in the context of SNMP. This promising erythrocyte substitute has been successfully tested on discarded human livers. One study compared a Hemopure-based solution to an RBC-based solution during 6 hours of NMP. Both oxygen carriers were mixed with a custom medium containing human albumin, heparin, and antibiotics. The perfusion parameters were similar between the two groups. Lactate and ATP levels, as well as the concentration of 8-hydroxy-2 -deoxyguanosine (a marker of oxidative stress), remained similar in both groups. A significantly higher oxygen extraction was observed in the HBOC group. No differences were found during histological assessment, and no Hemopure-related damage or thrombosis was observed in the HBOC group, further confirming its viability. [257]

Another study compared Hemopure to RBCs during 6 hours of liver NMP, exploring which medium is more suitable for mixing with HBOC-201—fresh frozen plasma (FFP) or Gelofusine. Gelofusine is a commercial colloid solution containing bovine-origin gelatin and is considered a viable plasma substitute. The groups in this study included RBCs with FFP (control historical cohort), Hemopure with FFP, and Hemopure with Gelofusine. Portal and arterial flow rates were higher in both Hemopure groups, while resistance rates were similar across all three groups. ATP content was much higher in the HBOC groups compared to the RBC group, with Hemopure+Gelofusine demonstrating the highest levels. As with the previous study, the Hemopure groups showed increased oxygen extraction. Bile production was also much higher in the Hemopure groups, and lactate and glucose levels decreased slightly faster in these groups. Both HBOC groups demonstrated a lower increase in ALT levels. The buffering capacity was similar across all groups, and methemoglobin levels were under 1% in all study groups. Histological evaluation showed no significant differences between the groups.

According to established criteria, 9 out of 10 livers in the Hemopure groups could be considered viable for transplantation. [258]

Oxyglobin, another medium containing polymerized bovine hemoglobin, was developed by the same company as Hemopure but differs in molecule size. A recent study on rat livers compared Oxyglobin to solutions containing either human or rat RBCs during 4 hours of NMP. William s E medium, primarily used for liver cell cultivation, was the base for all three perfusates. No differences were observed in lactate clearance and bile production between the groups. Levels of liver injury markers also did not differ, though AST levels were higher in the human RBC group. Histopathological analysis revealed similar changes across all groups. Importantly, the study demonstrated that Oxyglobin caused the least pronounced activation of liver-resident immune cells, which are mediators of rejection. [259]

Steen is an acellular buffered solution designed for warm perfusion, containing extracellular sodium and potassium concentrations, as well as dextran and albumin. Some studies have attempted to perform acellular NMP with Steen, hypothesizing that the solution has good oxygenation abilities. There was also a comparison study conducted using DCD pig livers, where a control SCS group and three NMP groups were formed: using only the Steen solution as perfusate, Steen solution with RBCs, or whole blood. After 10 hours of preservation, livers were flushed and reperfused with whole blood for 24 hours to simulate transplantation. The hemodynamic parameters were similar between all groups during NMP. At the end of perfusion and during reperfusion, the Steen group demonstrated increased AST, ALT, and LDH levels, while other perfused livers showed lower rates, comparable between the two groups. As for bile production and bilirubin levels, the whole blood group demonstrated the best results, while the worst rates were observed in the Steen group. Furthermore, the Steen group demonstrated lower VO2 and higher lactate rates during perfusion. Histological findings were similar after the preservation period, but some changes were seen after the reperfusion phase. Hemorrhage and necrosis were found in the centrilobular zone of livers perfused with only Steen. The group perfused with Steen and RBCs showed milder changes, which were almost absent in the whole blood group. Authors concluded that the best outcomes seem to be achieved with a medium that includes an oxygen carrier, additionally highlighting that whole blood as a perfusate performed the best among the three groups. [262]

Steen and Gelofusine were further explored as two potential base mediums for NMP with RBCs as oxygen carriers, compared with whole blood. In this study, DCD pig livers underwent 5 hours of NMP with either RBCs added to Steen, RBCs added to Gelofusine, or whole blood, followed by subsequent transplantation. During perfusion, hepatic artery flow rates were higher and resistance rates slightly lower in the Steen group compared to the other two. AST levels were higher in the whole blood group. Hyaluronic acid clearance, measured as a marker of endothelial function, was highest in the Steen group. Lactate clearance and oxygen consumption were similar between all three groups. Livers perfused with whole blood produced much more bile, and its pH was less acidic in comparison to the other two groups. Weight gain was lower in the Steen and whole blood groups compared to Gelofusine. After transplantation, livers were observed for 3 days, where the Steen group demonstrated lower AST, AP, and bilirubin levels. Endothelial integrity also seemed to be best maintained in the Steen group. Furthermore, the Steen group demonstrated the lowest INR rates and lactate levels after transplantation, which correlated with 100% animal survival. In contrast to the previously described study, this study demonstrated good performance of the Steen solution combined with RBCs, with some rates being superior to the whole blood group. [263]

Staying on the topic of Steen solution, it was investigated during multiple other liver NMP studies, although without a direct comparison to other perfusion solutions. For example, it was tested during 8 hours of acellular NMP of DCD porcine livers or in a porcine DCD model of orthotopic transplantation after 4 hours of NMP, both experimental groups compared to cold storage groups. In comparison to these, Steen-perfused livers demonstrated lower enzyme levels, higher oxygenation parameters, and less necrosis. [286] Furthermore, there is a clinical trial report of successful use of the solution during liver NMP. Here, a total of 10 out of 12 perfused human livers were transplanted after 340-580 minutes of NMP with Steen, supplemented with RBCs. This resulted in positive outcomes, including good graft function, no graft or patient loss, and a major complication occurrence in only one out of ten cases. [287] A study focusing on establishing a rat liver NMP model also reported improved results after the addition of the Steen solution to Dulbecco's modified Eagle's medium, also supplemented with plasma and erythrocytes. In livers perfused with the addition of Steen, authors observed a decrease in enzyme levels and oxidative stress markers, as well as better-preserved tissue integrity and anti-inflammatory effects of the solution. [288]

A Chinese group presented a novel artificial oxygen-carrying perfusate based on perfluoronaphthalene-albumin nanoparticles, comparing it to whole blood in a pig DCD model undergoing 24 hours of NMP. The nanoparticle group demonstrated lower resistance rates, while lactate clearance, AST, ALT, and bile production were similar between the two groups. There were also no significant differences found during the histological examination of livers from the two groups. The authors concluded that the perfusate was able to meet oxygen demand and, considering the possible positive effects and advantages of this medium as an artificial oxygen carrier, it should be investigated further. [260]

Regarding available literature on clinical trials and RCTs concerning liver NMP, the procedure involves the addition of packed RBCs to the perfusate in all five articles that specify the perfusate composition. In three of these, Gelofusine is used as the medium to which RBCs are added, while the other two articles describe using a center-specific solution composition or packed human plasma as the base perfusate. [149, 153, 156, 189, 314]

3.3.2 Literature on kidney NMP solutions

Sufficient oxygenation plays a crucial role in kidney NMP, prompting extensive research to identify the best oxygen carrier and/or medium. While the standard approach in kidney NMP involves the use of RBCs or whole blood, alternative options are being actively explored. Whole blood is known to potentially increase immunological response due to the presence of donor immune cells and substances. A recent study comparing RBC-based solutions with whole blood during kidney NMP investigated the immunological effects of both mediums. The study tested both perfusates during 4 hours of NMP of pig kidneys, either directly after explantation or in kidneys that had previously undergone 22 hours of cold storage (SCS) or 60 minutes of warm ischemia time (WIT). Creatinine clearance, oxygen consumption, and AST levels did not differ significantly between the RBC and whole blood groups. Additionally, no significant differences were observed in pro- and anti-inflammatory cytokine levels between the groups using RBCs or whole blood as perfusate. [264]

The choice of base medium for a perfusion solution containing RBCs as an oxygen carrier is another important consideration. A study on porcine kidneys compared four different base solutions combined with RBCs over 7 hours of NMP. The solutions used with autologous pig RBCs were: William s E medium with 10g bovine albumin (group 1), 200 ml Albuman with saline (group 2), Ringer's lactate with saline, adenine, glucose, and mannitol (SAG-M, group 3), and 100 ml Albuman with saline (group 4). Group 3 used a colloid-free solution commonly used in the UK. After NMP, kidneys in group 2 gained the least weight, while group 1 had the highest weight gain. Flow parameters in group 3 were low but stable, while the first two groups showed high flow rates initially, which decreased over time. Group 3 also had higher diuresis compared to the other groups, with group 2 having the lowest urine output. Sodium and potassium levels fluctuated and varied among the groups, with the least stable parameters in group 3. The pH levels of the perfusate in group 3 started higher but became acidic, whereas other groups started with lower pH rates that stabilized around 7.4. Creatinine clearance was significantly higher in group 3, with the lowest rates in group 2. AST and LDH levels were higher in groups 3 and 4 compared to groups 1 and 2, with the highest levels in group 4. Histological analysis showed less glomerular dilation in group 2, with similar findings across the other groups. Increased Bax-gene (pro-apoptotic) activation was observed in group 1, while N-acetyl-β-D glucosaminidase levels, an injury marker, were considerably lower in group 2.

Although this study did not identify the best medium, it highlighted how perfusate composition affects various parameters during perfusion. [265]

A few studies have also explored potential negative effects of RBCs during kidney perfusion. Given that RBCs may be damaged during perfusion and release free heme into the perfusate, a study measured heme concentrations in kidneys undergoing 1 hour of NMP within a randomized controlled trial (RCT). The trial used packed RBCs added to a medium based on Ringer s solution and mannitol. The authors hypothesized that free heme could activate inflammatory pathways, which was investigated in declined human kidneys. The study confirmed the release of free heme correlated with the increasing age of packed RBCs but did not find a correlation between free heme concentrations or RBC age and inflammatory markers. [295] Another study focused on hemolysis during NMP with RBCs. Discarded human kidneys underwent 6 hours of NMP with a custom perfusate, including albumin, mannitol, and calcium gluconate, with added washed RBCs. The study confirmed the occurrence of hemolysis, correlating with the duration of NMP and interaction between RBCs and the graft, observing erythrostasis in the glomeruli and peri-glomerular blood vessels. Free hemoglobin and heme increased oxidative stress and had generally toxic effects, especially at higher concentrations. The authors concluded that a more optimal oxygen carrier should be used during kidney NMP. [296]

Similarly to research in the field of liver NMP, many studies in kidney NMP question the use of RBCs altogether and explore alternative oxygen carriers or acellular media. For instance, a study on porcine kidneys undergoing 6 hours of NMP compared three different strategies: perfusion with an acellular medium at a supra-physiological pO2 level of 500 mmHg; the same medium and pO2 with the addition of autologous RBCs; and the same medium with RBCs and a lower pO2 of 200 mmHg. The base solution used was Aqix RS-I, a universal tissue storage solution, supplemented with bovine albumin, sodium bicarbonate, and dexamethasone. The solutions in the two RBC groups were also optimized with heparin and verapamil. During perfusion, the flow rates of the RBC groups were slightly lower than in the acellular group. Oxygen consumption and saturation were stable across all three groups. Creatinine clearance was similar in all groups at 4 hours of perfusion and remained stable at 6 hours in the acellular group, compared to a slight decline in the RBC groups. Urine production and albuminuria were similar in all groups. Due to the high oxygen pressure, which could increase free radical content,

lipid peroxidation was measured and did not differ significantly between groups. Erythropoietin induction, a marker for hypoxic changes, was also similar across all groups. Evaluation of biomarkers HMGB1 and tenascin C showed worse results in the RBC groups and in the lower pO2 group, respectively. Nevertheless, no histological differences were found between the three groups at the end of perfusion. The study concluded that hyper-oxygenated acellular NMP could be a safe alternative to RBC-enriched perfusion. [266]

Aqix was further investigated in another porcine kidney NMP study, used either with or without RBCs and with either bovine serum albumin or dextran 40. The study also included a control group of kidneys perfused with leukocyte-depleted diluted blood. All kidneys underwent 3 hours of hypothermic machine perfusion (HMP) with Belzer MPS, followed by 4 hours of NMP using the 5 described solutions. During NMP, oxygen consumption was higher in all RBC-containing groups, with the highest rates in the blood group. Similarly, fractional sodium excretion rates were lower and sodium reabsorption rates were higher in the blood group, particularly compared to the acellular groups. The Aqix group containing RBCs and bovine albumin was closest to the blood group in terms of sodium excretion and demonstrated high sodium reabsorption during the first hours of NMP. ATP content was highest in the blood group and low in both acellular groups at 30 minutes and at the end of NMP. The Aqix group with RBCs and albumin had almost as high ATP rates initially as the blood group but had low ATP content by the end, whereas the Aqix groups with RBCs and dextran showed comparable ATP content initially, which did not drop as drastically by the end of NMP. Lactate levels increased more in all Aqix groups compared to the blood group. Flow rates were stable across all groups, with higher rates in the blood group and both Aqix-albumin groups compared to both Aqix-dextran groups. Creatinine clearance rates were highest in the control group during hours 2-4 and lowest in the Aqix-RBC-dextran group, with second lowest results in the Aqix-dextran group. The Aqix-RBC-albumin group showed the highest creatinine clearance rates during the first two hours of NMP, followed by a significant drop, while the Aqix-albumin group had stable rates higher than both Aqix-dextran groups. Kidneys perfused with Aqix and albumin without RBCs gained the most weight and produced the most urine among all groups. The control group had the least weight gain and produced a good amount of urine. The urine production of the Aqix-RBC-albumin group was comparable to the control, while the weight gain was higher. Both dextran groups had low urine output and high weight gain. The Aqix-albumin group had much higher AST and LDH rates, which did not correlate with injury (uNAG) and oxidative stress (TBARS) markers. Among all five groups, uNAG levels were

similar, while TBARS levels were higher in the RBC groups compared to the control and acellular groups. The percentage of damage found during histological examination was comparable between the control and RBC groups and higher in the acellular groups. The authors concluded that although Aqix-RBC-albumin showed good results concerning metabolism and renal function, blood perfusion remains superior. [267]

William's E medium was also tested without addition of RBCs as an acellular medium in a NMP study. Here, human kidneys, declined for transplantation, underwent 6 h of NMP with either sole William's E medium or with addition of packed RBCs to the William's E medium. In the acellular group, flow tended to be higher and resistance lower, compared to the RBC group. Urine output tended to be higher in the acellular group as well. Creatinine clearance was similar between groups, while a more pronounced increase of lactate rates was observed in the acellular group. Oxygen consumption was the same between groups, at the same time renal oxygen extraction was higher in the acellular group. Here, authors mentioned, that oxygen extraction rates in both groups signalized active metabolism during the whole time of NMP. ATP parameters tended to increase and be generally higher in the acellular group. During histological evaluation no differences were found between the two groups. Thus, authors concluded, that NMP with acellular William's E medium could be a convenient alternative to RBC-enriched perfusion. [268]

Another medium tested as acellular perfusion solution for kidney NMP is Ecosol. It is a newly developed preservation solution, primarily used for SCS of kidney grafts. It contains, among other things, PEG 35, HEPES, taurine as antioxidant and nutritious components. In a study on porcine kidneys, perfusion with Ecosol was compared to perfusion with RBCs mixed with Ringer's solution containing mannitol. Both groups underwent NMP for 6 hours. During perfusion, pH levels of the RBC group increased, while the rates in Ecosol group remained more stable and tended to be acidotic. Arterial flow and resistance rates did not differ significantly between groups. Arterial pO2 levels were high and comparable between both groups, while concentrations of HIF1-α, as a marker of oxidative stress, were slightly higher in the Ecosol group. AST and lactate levels did not differ significantly between groups. LDH and urine protein rates were somewhat higher in the RBC group. IL-6 levels did not differ at all between the two groups. Antioxidant capacity was higher in the Ecosol group, but was also increasing during perfusion in the RBC group, on the contrary oxidation-reduction potential was higher in the RBC group, but also increased in the Ecosol group. Creatinine clearance was

similar between the two groups at the beginning and at the end of the perfusion, although it did increase in the RBC after 30 min of perfusion and was higher than in the other group for a couple of hours. The weight gain was higher in the Ecosol group. Caspase 3 levels were higher in the Ecosol group. Histopathological examination revealed more prominent tubular injury in the Ecosol group, and higher tubular vacuolization in the RBC group. Additionally, a formation of a gel-like mass was observed around the hilus in the Ecosol group, according to authors possibly caused by the wrong PEG molecule size used. Authors also suggested, that increased edema and tubular injury in the Ecosol group are probably connected to the suboptimal PEG used and concluded, that considering otherwise good results of perfusion with Ecosol, it could be a feasible perfusion solution for NMP. [272]

The previously mentioned popular alternative acellular oxygen carrier HBOC-201 (Hemopure) was also studied in context of kidney NMP. In this study, human kidneys, that were declined for transplantation, underwent 6 h of NMP with William's E medium as base solution, combined with either Hemopure or packed RBCs as oxygen carriers. During perfusion, both groups demonstrated similar renal artery flow and resistance rates. Such parameters as pH, creatinine clearance and urine output as well as FENa, a renal tubule function marker, did not differ between groups. Lactate levels were higher in the RBC group, compared to the Hemopure group. Some of the kidneys in the RBC group gained weight, while all grafts perfused with Hemopure ended up losing weight. Oxygen delivery and extraction as well as ATP parameters did not differ between the two groups. During histological assessment, increased loss of tubular integrity, epithelial vacuolization and coagulation necrosis were observed in the RBC group, compared to the other group. These results show not only feasibility of Hemopure as oxygen carrier during NMP, but also demonstrate its possible superiority over RBCs. [269] On the other hand, the results of HBOC-301 (Oxyglobin) in another study on kidney NMP were not as positive. Here, porcine kidneys were perfused with addition of either Oxyglobin or autologous RBCs for 6 hours. The base medium included mannitol, sodium bicarbonate, glucose and creatinine added to Ringer solution and was supplemented with nutrients. Additionally, ascorbic acid was added as bolus and infusion to the perfusion of half of the kidneys in attempt to reduce the increased production of methaemoglobin, previously observed after use of HBOC-201, creating two additional groups. During perfusion, an increase in infrarenal resistance as well as a decrease in flow rates was observed in Oxyglobin groups, while the parameters in RBC groups remained stable. The Oxyglobin groups demonstrated significantly lower urine production and creatinine clearance. Salt excretion also tended to be lower in the RBC groups.

The pH levels were similar between groups, although RBC groups demonstrated more stable rates, while HBOC-groups tended to get more acidotic at the end of perfusion. Lactate levels were also significantly higher in HBOC groups after 2 hours of perfusion. During perfusion, sodium levels rose in RBC groups and potassium — in HBOC groups. LDH levels were significantly lower in RBC groups, and the HBOC group with added vitamin C did not show lower LDH levels, compared to the other HBOC group. There were no significant differences detected in weight gain of all groups at the end of perfusion. Methaemoglobin levels increased significantly in HBOC groups, ending up around 23% in the non-vitamin C group, and around 19% in the group with vitamin C addition. The RBC groups both showed physiological levels of methaemoglobin. Urine expression of Hb was much higher in HBOC groups, with the highest levels observed in the HBOC group without vitamin C. Nevertheless, no significant differences between all groups were observed during histological assessment. In this study clear inferiority of Oxyglobin in comparison to RBCs was demonstrated. Vitamin C supplementation failed to improve the results. [270]

A newly developed artificial oxygen carrier, that has also been tested during kidney NMP, is albumin-derived and perfluorocarbon-based (A-AOC) and can dissolve big amounts of oxygen, depending on its partial pressure. In a study on rat kidneys, a base solution containing bovine serum albumin and Ringer solution was either used alone as perfusate, or enriched with described emulsified artificial oxygen carrier in different dosages — 2%, 4% and 8%. Kidneys underwent 2 hours of NMP. As expected, the higher the A-AOC content, the higher was the oxygen capacity of the perfusate. HIF-1α and HIF-2α were observed as markers of hypoxia, with lower levels being similarly detected in correlation with A-AOC concentration. Out of all A-AOC groups, only the 4% one demonstrated higher glomerular filtration rates, compared to the control group, while the other two groups showed rates, that were lower than in the control group. The perfusate AST rates were slightly lower and the urine AST activity significantly lower in the 4% group, compared to other groups. Malondialdehyde was used as a marker of oxidative stress, its levels being similar to control in the 4% A-AOC group, and higher in the other two groups. Cell death was investigated using TUNEL staining, where yet again, the 4% A-AOC group showed the least apoptosis. Interestingly, the most cell death was detected in the 8% A-AOC group. [271]

The only available report of a RCT on kidney NMP, conducted recently, describes using a RBC-based perfusate during perfusion. Authors emphasized, that it contained no leukocytes,

thrombocytes or complement. Other than RBCs, the solution included Ringer's lactate with addition of mannitol, sodium bicarbonate, nutrients, etc. [158]

3.4 Perfusion solutions during rewarming

3.4.1 Literature on solutions for rewarming of liver

Previously described solutions, Belzer MPS and Steen solution, were also investigated and compared during rewarming. In a study on rat livers, which had been cold stored in HTK for 18 hours, these two solutions were used as perfusates during 60 minutes of rewarming from 8°C to 35°C, followed by 60 minutes of warm perfusion. After this, the organs were additionally reperfused with oxygenated William s E medium supplemented with bovine serum albumin at 37°C for 60 minutes. During perfusion, transhepatic flow rates and lactate levels were similar between groups. However, ALT levels were significantly lower in the Belzer MPS group. During reperfusion, resistance rates were similar between the two groups. At the end of reperfusion, ALT levels were only slightly higher in the Steen group. Bile production was similar between the two groups during reperfusion. Levels of caspase-cleaved keratin 18, a marker of apoptosis, were significantly lower in the Belzer MPS group. Additionally, levels of the inflammatory marker TNFα were lower in the Belzer MPS group. On the other hand, lipid peroxidation rates were similar between the two groups. Histological analysis did not reveal significant differences between the groups. To conclude, rewarming with Belzer MPS showed more optimal parameters compared to Steen. [273]

UW-gluconate solution was also used in an experimental study on DCD pig livers undergoing rewarming to 25°C. Compared to HMP, grafts demonstrated lower ALT and lactate levels, and overall better histological findings were observed in the rewarming group. [293]

Another solution described in the literature regarding liver rewarming is Custodiol-N. Initially, it was tested on a porcine liver perfusion model, where, after 18 hours of cold storage, grafts underwent 90 minutes of either HMP, COR to 20°C, or SNMP. Good results were observed, especially in the rewarming group. [292] Custodiol-N was later applied in a clinical setting, where the perfusion temperature was gradually increased from 10°C to 20°C over 90 minutes. Compared to a historical SCS cohort, this approach resulted in decreased peak AST levels and improved graft and patient survival rates (both 100% after 6 months). [291]

Considering the need for adequate oxygenation as temperature and metabolism rise during rewarming, alternatives to blood products have also been explored in the rewarming procedure.

For example, a recent study compared the results of the Hemopure clinical trial with the application of RBCs during a similar rewarming procedure, followed by transplantation of viable grafts. During preservation, human DCD liver grafts underwent 1 hour of HOPE, 1 hour of oxygenated rewarming, and 150 minutes of NMP with Gelofusine-based albumin-supplemented solutions, which included either Hemopure or RBCs as oxygen carriers. The difference in procedure between the two groups was the need to use UW-MPS for HOPE, then rinse the grafts and replace the medium with the RBC-supplemented solution at 20°C in the RBC group, which was not necessary in the Hemopure group. During NMP, the two groups demonstrated similar lactate and glucose utilization and stable pH levels. Sufficient lactate clearance, pH stabilization, and bile production as viability criteria were observed in 53 out of 54 livers. Cholangiocellular viability was achieved by 63% of the livers, making them eligible for transplantation. Out of 18 livers in the HBOC group, 12 were transplanted; similarly, 22 of 36 livers perfused with RBCs underwent transplantation. Graft survival at 3, 6, and 12 months was high and comparable between groups, with each group having one case of re-transplantation. Recipient survival at 12 months was 100% in both groups. ALT, AST, GGT, AP, INR, bilirubin levels, and the number of complications were comparable between the two groups. Thus, Hemopure could be considered a feasible alternative to RBC-supplemented rewarming, offering logistical advantages. [261] Hemopure has also been used during a clinical trial of a perfusion strategy where HOPE was followed by COR and NMP. In this setting, the addition of RBCs to the perfusate would be limited, as it cannot be used cold. Thus, Hemopure is an ideal oxygen carrier, as it can be used throughout the entire perfusion procedure. During the clinical trial, discarded grafts underwent 60 minutes of DHOPE, followed by 60 minutes of rewarming from 10°C to 37°C, and finally, NMP, where viability assessment was conducted during the first 2.5 hours. The viability criteria included bile production, lactate concentration, and pH levels of the perfusate and bile. Out of 16 perfused livers, 11 were deemed viable and successfully transplanted. Patient and graft survival were 100% at 3 and 6 months. The authors concluded that this strategy increased the number of liver transplantations from deceased donors by 20%. [294]

3.4.2 Literature on solutions for rewarming of kidney

Previously described novel modification of HTK, Custodiol-MP, was compared to Belzer-MPS in a rewarming procedure of porcine kidneys. Here, grafts underwent 20 hours of SCS in HTK, followed by perfusion with either Custodiol-MP or Belzer-MPS, starting with rewarming from 8°C to 20°C for 60 minutes and continuing with an additional 30 minutes of perfusion at subnormothermia. Kidneys were evaluated during reperfusion at 37°C with Krebs-Henseleit buffer supplemented with bovine serum albumin and amino acids for 90 minutes. Flow rates and oxygen consumption during reperfusion did not differ between the two groups. However, slightly higher and tendentially increasing rates of creatinine clearance were observed in the Custodiol-MP group. Glomerular protein leakage and fractional sodium excretion did not differ between the two groups. Similarly, levels of molecular markers of renal injury, including AST, were not significantly different between groups. Tissue damage evaluated during histological analysis was also similar between the two groups. The authors stated that Custodiol-MP is feasible for kidney rewarming and noted that the flexibility in colloid choices for Custodiol-MP could be advantageous. [274]

The addition of HBOC-201 for improved oxygenation was also evaluated in kidney rewarming. In this study, rat kidneys were preserved for 2 hours in UW solution, followed by 60 minutes of gradual rewarming from 10°C to 37°C, and then 30 minutes of warm perfusion. The perfusate consisted of William s E medium with or without the addition of 25% HBOC-201. All kidneys were then reperfused at 37°C with HBOC-enriched perfusate for 120 minutes. During initial perfusion, no differences in resistance and pH rates were found between groups. Oxygen consumption during the rewarming phase could only be measured in the HBOC groups and showed a gradual increase. During reperfusion, no significant differences were observed in resistance, lactate, LDH, and pH rates between the two groups. Weight increase and oxygen consumption were also similar between groups. Ultrafiltrate production, GFR, and sodium reabsorption rates during reperfusion were all superior in the HBOC group. Histological analysis did not show significant differences between groups. Methaemoglobin levels gradually increased up to 7% in the HBOC group during the 90 minutes of perfusion. During reperfusion with the Hemopure solution, both groups demonstrated a similar increase in methaemoglobin up to 20%. Nevertheless, the authors concluded that perfusion with Hemopure is more beneficial than perfusion without an oxygen carrier. [275]

There is also a case report of a human kidney from an ECD that underwent rewarming from 8°C to 35°C using diluted acellular Steen solution, oxygenated with 100% oxygen. The rewarming was conducted over 90 minutes, after which the graft was further perfused at normothermia for 30 minutes. Following successful transplantation, the kidney exhibited immediate function and showed a good decrease in creatinine concentration and improved creatinine clearance rates during the first 16 days, as described in the report. [297]

3.5 Perfusion solutions used for machine perfusion of pancreas

As for literature directly comparing different perfusion solutions for pancreas perfusion, only one such article could be found. In this study, on a porcine model, oxygenated HMP of the pancreas was performed with either UW or IGL-2 for 6 hours. The perfused organs were then assessed during 1 hour of normothermic reperfusion with autologous leukocyte-depleted blood. During HMP, flow rates were lower in the IGL-2 group. Both groups demonstrated mean tissue weight loss that did not differ significantly. After glucose delivery during reperfusion, a rise in glucose levels and induced insulin secretion was observed only in the UW group. During initial perfusion, amylase, lipase, and lactate levels were higher in the UW group, while LDH rates remained similar. All these biochemical parameters were similar between groups during reperfusion, so the authors could not conclude which perfusion solution is more beneficial for pancreas HMP. [299]

Continuing on the topic of hypothermic perfusion of the pancreas, IGL-2 was reported to be used in an experimental study that resulted in allotransplantation on a porcine diabetic model. Either 2 or 6 hours of HMP using IGL-2 were performed without complications, including no graft thrombosis. [298]

As for pancreas NMP, the procedure was tested on porcine pancreata by a group of researchers who also developed a step-by-step protocol for it. The perfusion solution used in the successful experiments of this group was based on Steen, Ringer s lactate, and washed RBCs. [300] Steen solution combined with RBCs, although without the addition of Ringer s lactate, was also the solution of choice in an NMP study on discarded human pancreata, demonstrating promising results. [163]

4. Discussion

This review examines the existing literature comparing various perfusion solutions across different perfusion modalities in liver, kidney, and pancreas transplantation. Additionally, it includes studies without direct comparisons to provide a comprehensive overview of the experimental and clinical applications of specific perfusion solutions and their outcomes in various scenarios.

Beginning with the older solution, Eurocollins, it is safe to say that, in an MP setting, it is inferior to its successors, as demonstrated in studies on both liver and kidney HMP [223, 234].

On the other hand, UW and Belzer solutions, which were first developed decades ago, continue to show superior performance. In the context of HMP, Belzer-MPS remains the most frequently used solution. Its variations have been explored in multiple studies. For example, supplementation of Belzer-MPS with substances such as antioxidants and vasodilators has shown promising results in experimental studies. Conversely, Belzer II albumin gluconate did not result in better outcomes in a human HMP study compared to Belzer-MPS [236].
Comparing Belzer-MPS (KPS-1) to UW solution (Viaspan) for cold storage has produced conflicting results, with UW showing superiority in canine kidney HMP studies and Belzer-MPS being more effective in porcine kidney studies [235, 237].
In a study on human kidney transplantation, the addition of M101, an oxygen carrier derived from a marine lungworm, to Belzer-MPS resulted in lower rates of delayed graft function (DGF) after preservation [247]. These studies highlight the potential for improving Belzer-MPS.
However, Belzer-MPS has its drawbacks. For example, it contains hydroxyethyl starch (HES), which has been associated with erythrocyte hyperaggregation [317]. Additionally, it is not intended for use during cold storage or warm perfusion, requiring a switch to another solution if a combination of preservation strategies is employed. Therefore, it is reasonable to explore other perfusion solutions that, according to reports, are practical and demonstrate equal or superior results.

Compared to Belzer-MPS and UW, HTK and its modifications have demonstrated at least equal or better results. Specifically, HTK has shown superior outcomes over UW and UW-gluconate in two porcine liver HMP studies and comparable results to Belzer-MPS in a rat liver HMP

study [225, 226, 277]. In porcine kidney HMP, HTK produced similar results to Belzer-MPS [238]. However, Custodiol-N exhibited better performance in two studies compared to KPS-1 [239, 240]. Furthermore, in a study on porcine kidney rewarming followed by SNMP, Custodiol-MP demonstrated slightly better results than Belzer-MPS [274]. These preclinical studies suggest that Custodiol might offer advantages over Belzer-MPS in both liver and kidney HMP, although further clinical testing is necessary to draw definitive conclusions about its superiority. Regarding HTK itself, its newer modification, Custodiol-N, appears to be superior to the original formulation, although this has only been investigated in a rat liver HMP study [224].

Regarding the IGL-2 solution, there are fewer comparative studies available. Two articles that examined IGL-2 during HOPE, compared to Belzer-MPS, reported better outcomes for rat livers and equivalent results in a porcine liver model [227, 228]. In kidney HMP, IGL-1 was compared to Belzer-MPS in a porcine model with 60 minutes of warm ischemia time (WIT). Notably, at 3 months post-transplantation, recipients of IGL-1 perfused kidneys exhibited a weaker immune response [241]. This observation may reflect the previously noted immunocamouflaging" properties of PEG. Additionally, in a perfused rat kidney model, PEG has been reported to have beneficial effects in minimizing ischemia-reperfusion injury (IRI), compared to perfusion with EC and UW [317]. Given its positive attributes, PEG-containing solutions are also suggested to potentially protect and preserve fatty liver grafts optimally, although further research is needed to confirm these benefits [181]. Another advantage of IGL-2 is its versatility; the solution can be used for both static storage and machine perfusion, offering logistical benefits.

The next solution evaluated for machine perfusion (MP) of abdominal organs is Celsior. Although the number of studies is limited, the existing literature suggests promising outcomes. Notably, Celsior with the addition of PEG has shown better results compared to Belzer-MPS in a porcine kidney HMP study [242]. Additionally, the results of clinical kidney transplantation following HMP with Celsior were very promising and comparable to those from a historical cohort using UW solution. According to the authors, Celsior can also serve as a singular solution for organ procurement, static storage, and perfusion, which provides a logistical advantage over both UW and Belzer-MPS [243]. Regarding liver perfusion, Celsior has been

successfully utilized in two SNMP studies involving rat and porcine livers, although these studies did not include a direct comparison to other perfusion solutions [283, 284].

A solution based on Belzer-MPS, known as Vasosol, has been tested in the context of liver HMP in rats, demonstrating superior results compared to KPS-1 [278]. Vasosol was also used in the first clinical series of liver transplantation after HMP in 2010 [303], and has shown success with declined ECD livers, resulting in successful transplantation and favorable outcomes [319]. More recently, Vasosol was employed in a randomized controlled trial (RCT) of liver transplantation after HMP, making it one of the few solutions used in clinical HMP trials, alongside the standard Belzer-MPS [279]. Although direct comparison studies between Vasosol and KPS-1 are limited, it appears to be a promising option for liver HMP.

Another solution to note is Polysol, which has been tested in HMP studies for both liver and kidney [231-233, 281]. Despite showing good experimental results, its clinical application in kidney transplantation led to increased rejection rates, which resulted in the termination of the study and the subsequent decline in use of this solution [280].

It is important to note that many comparative studies on MP solutions evaluate the perfusate in question primarily against Belzer-MPS or KPS-1. Consequently, there is a lack of direct comparisons between other seemingly viable solutions. For instance, there are no existing studies that compare Custodiol and IGL-2 in the context of MP. This gap represents a valuable opportunity for future research to explore and clarify the relative efficacy of these alternatives.

Regarding SNMP, there is a limited number of comparative studies available. However, evidence suggests that incorporating an oxygen-carrying substance into the perfusion solution provides benefits over using a simple perfusate. Experimental studies have shown that adding human-derived hemoglobin-containing vesicles or Hemopure to the perfusate during liver and kidney SNMP results in superior outcomes compared to using unmodified solutions. [248, 250, 282] For liver SNMP, Celsior has also been successfully applied in two experimental studies, though these studies did not include comparisons with other solutions. [283, 284] Additionally, Lifor solution has been compared to Belzer-MPS in the context of kidney SNMP, demonstrating superior results, such as improved perfusion parameters and a reduced immune response. [251]

Regarding NMP, the primary concern is meeting the graft's oxygen demands during perfusion. Research consistently shows that the inclusion of an oxygen-carrying substance, such as RBCs or other oxygen carriers, improves outcomes compared to using solutions without such carriers. While whole blood, being the most physiological, often yields the best results in experimental NMP studies, its use can be limited by the presence of leukocytes and thrombocytes, which may provoke an undesirable immune response. Consequently, the clinical preference for NMP of liver and kidney often involves using packed RBCs in conjunction with a base medium.

However, the logistical challenges associated with packed RBCs—such as procurement, limited shelf life, and blood group compatibility testing—highlight the need for effective alternative oxygen carriers. One extensively researched acellular oxygen carrier is Hemopure (HBOC-201). Multiple experimental studies and clinical reports indicate that Hemopure can match or even surpass RBC-based perfusion in terms of oxygen extraction and graft viability, both in liver and kidney NMP. [257, 258, 269] For instance, Hemopure has been successfully used in liver and kidney NMP, demonstrating results comparable to or better than those achieved with RBCs, leading to viable grafts suitable for transplantation. [261, 275]

In contrast, Oxyglobin has shown mixed results. It performed better than RBCs in rat liver studies but yielded poorer results in porcine kidney studies. [259, 270] An important consideration with HBOCs like Hemopure is the formation of methemoglobin, which varies widely across studies. Methemoglobin levels can remain within physiological ranges or exceed 20%, even with the addition of vitamin C to the perfusate. Some researchers suggest that methemoglobin formation is not a significant concern as long as the solution is adequately washed out before reperfusion and does not impact the quality of the MP.

Future research should also explore other, less-studied oxygen carriers, such as human-derived hemoglobin vesicles, perfluoronaphthalene-albumin nanoparticles, or albumin-derived perfluorocarbon-based artificial oxygen carriers. These emerging alternatives show promise in the contexts of NMP and SNMP and warrant further investigation.

Continuing with the topic of NMP, it is essential to determine the optimal solution to use as a medium, either combined with RBCs or alternative oxygen carriers. Gelofusine is frequently cited as a base medium in experimental and clinical NMP strategies, including randomized controlled trials (RCTs), though comparative data with other solutions is limited. One study on

porcine liver NMP found that RBC-based perfusion using Gelofusine yielded inferior results compared to Steen solution, notably showing higher weight gain. [263] Conversely, in human liver NMP with Hemopure, Gelofusine demonstrated favorable outcomes, including higher ATP content, compared to using FFP in conjunction with Hemopure. [258]

Another solution examined for NMP and rewarming, either with RBCs or in its acellular form, is Steen solution. The results from experimental and clinical studies are mixed. For instance, two studies on NMP of DCD porcine kidneys reported better outcomes with Steen when RBCs were added compared to using Steen alone. When compared to whole blood perfusion, Steen performed inferiorly in only one of the studies. [262, 263] Additionally, during a rewarming procedure followed by NMP of rat livers, acellular Steen showed worse results compared to Belzer-MPS. [273] Nonetheless, Steen solution has been successfully used in other experimental and clinical settings. For example, human liver transplantations have been successfully performed following RBC-supplemented NMP with Steen, and a case report describes successful human kidney transplantation after rewarming and NMP using Steen without an oxygen carrier. [287, 297]

In summary, while some studies suggest that Steen might not be the optimal medium, it remains a popular choice in NMP protocols. The addition of RBCs to Steen appears to enhance performance compared to using the solution alone, particularly in experimental liver NMP. Further research could clarify the comparative advantages of Steen and other solutions, including Gelofusine, in different NMP scenarios.

William s E medium is another solution frequently used in experimental NMP when combined with an oxygen carrier. However, in a study on porcine kidney NMP, the William s E + bovine albumin medium group exhibited the highest weight gain and increased activity of a pro-apoptotic gene, raising concerns about its clinical applicability. [265]

Aqix is also commonly used as a base medium for RBC-based NMP in animal models and discarded human grafts. Although comparative data are limited, a study on porcine kidney NMP showed that Aqix with RBCs and bovine albumin yielded superior results compared to acellular Aqix perfusion and groups where dextran was added to Aqix. However, perfusion with diluted

blood produced the best outcomes in that study. [267] Additionally, Aqix demonstrated good performance compared to KPS-1 in a liver HMP study. [230]

Another potential NMP strategy, though less frequently described, involves using a combination of packed FFP and RBCs. This approach could offer a more physiological perfusate while being both leukocyte- and thrombocyte-depleted. However, the logistical and economic implications of this method remain to be fully evaluated, given the limited availability of both components.

As highlighted in the results, there is limited data on pancreas MP and perfusion solutions. IGL-2 has been investigated as a perfusion solution for experimental HMP of the pancreas, showing promising results. For NMP, RBC-based perfusates, including those with Steen solution, are the preferred choice in the few available studies involving porcine and discarded human pancreata.

It is important to note that most of the literature on different perfusion solutions is based on experimental MP using animal models. Currently, there are only a few randomized controlled trials (RCTs) in clinical settings, although some new trials are underway. In most available clinical trials and RCTs, Belzer-MPS remains the standard perfusion solution for HMP of liver and kidney. Similarly, RBC-based perfusates are commonly used for NMP in clinical practice. The scarcity of reliable clinical reports makes it difficult to definitively assess the validity of other perfusion mediums, despite often promising experimental data.

Another significant challenge is the variability in experimental settings. Different research groups use various equipment for MP, and animal models differ in species, donor conditions, and pre-perfusion cold and warm ischemia times. Additionally, the duration of perfusion can range widely from 1 to 24 hours. Some studies involve MP following static cold storage (SCS) of varying durations, while others are performed immediately after organ removal. These factors complicate the evaluation and comparison of results across different studies on perfusion solutions.

Additionally, there is no clear consensus on which parameters are most critical when evaluating different solutions for experimental MP. Various studies report different positive outcomes for each solution, for example, one solution would result in less weight gain, at the same time the

other one - in lower enzyme levels, making it challenging to determine which parameters should be prioritized. Therefore, it is essential to identify and standardize the key parameters that most accurately predict outcomes after perfusion and transplantation of specific organs. This standardization would provide clearer guidance in selecting the optimal solution for organ preservation and improve the reliability of comparative studies.

To address these issues, there is a pressing need for a standardized approach to perfusion strategies. Such standardization could help in identifying the most optimal perfusion solutions and resolving other unresolved questions regarding MP. Standardized parameters and methodologies would enhance the reliability and comparability of experimental data, ultimately improving clinical outcomes and advancing the field of organ preservation and transplantation.

Table 4. Summary of perfusion solutions used for different MP modalities in preservation of abdominal organs, as mentioned above

	Composition	MP modalities	Abdominal organs	Modifications tested (MP)	Clinical use (MP)	Comment
Eurocollins	Intracellular sodium/potassium, high glucose, phosphate buffer	HMP	Liver, kidney	-	Clinically used for SCS in the past	Inferior to UW
UW-solution (Viaspan)	Intracellular sodium/potassium, lactobionate and raffinose instead of glucose, HES, allopurinol and glutathione, adenosine, magnesium sulfate, phosphate buffer	HMP, SNMP, rewarming to 25°C	Liver, kidney, pancreas	UW-gluconate, supplementation with antioxidants, calcium chloride, thromboxane A2 inhibitor, etc	Widely clinically used for SCS	Developed and used primarily for SCS
Belzer-MPS (KPS-1, PumpProtect)	Intracellular sodium/potassium, calcium chloride, HES, HEPES, adenine, glutathione, gluconate, mannitol, phosphate, ribose	HMP, SNMP, rewarming	Liver, kidney	Belzer II albumin gluconate, Belzer-MPS modified with nitroglycerin, prostaglandin E1, and polyethylene glycol-superoxide dismutase	Widely clinically used for HMP of liver and kidney	Not suited for SCS
HTK (Custodiol)	Intracellular sodium, low potassium,no colloid, mannitol, α-	HMP	Liver, kidney	-	In context of MP of abdominal organs	Clinically used for SCS of liver and kidney

	ketoglutarate, histidine, tryptophan				only experimental works available	
Custodiol-N	HTK + glycine, alanine, iron chelators (deferoxamine and LK-614), sucrose, without mannitol	HMP, SNMP, rewarming to 20°C	Liver, kidney	Addition of dextran 40	In context of MP of abdominal organs only experimental works available	According to some studies superior to HTK
IGL-1	Extracellular solium-potassium, lactobionate and raffinose, phosphate buffer, glutathione, allopurinol, adenosine	HMP	Kidney	-	In context of MP of abdominal organs only experimental works available	Mostly suited for SCS
IGL-2	Increased PEG35 and glutathione, compared to IGL-1	HMP	Liver, pancreas	-	In context of MP of abdominal organs only experimental works available	Developed for MP
Celsior	Colloid-free, extracellular sodium-potassium balance, mannitol, lactobionate, reduced glutathione, histidine, and glutamate, slight acidosis and high magnesium	HMP, SNMP	Liver, kidney	PEG addition ; angiotensin IV addition	Clinical report of kidney HMP	Primarily used as cardioplegia solution
Vasosol	Based on Belzer MPS, α-ketoglutarate, N-acetylcysteine,	HMP	Liver	Vitamin E supplementaiton	Reports of clinical use available, including RCT of liver HMP	Not much data available

	prostaglandin E1, nitroglycerin, L-arginine					
Polysol	Na/K 120/20 mmol/L, HES, enriched with more nutrients such as amino acids and vitamins compared to UW	HMP	Liver, kidney	Addition of different colloids	Clinical use in kidney HMP resulted in increased rejection rate	Not used anymore
Unisol	HEPES, dextran 40, has high buffering capacity	HMP	Kidney	-	In context of MP of abdominal organs only experimental works available	Primarily developed for application at low temperatures (up to -15°C).
Aqix RS-1	Created as a universal solution for transportation and/or storage of any tissue or organ at any temperature, composition similar to serum,sodium bicarbonate and BES as buffers	HMP (liver), NMP (kidney)	Liver, kidney	Supplemented with bovine albumin, sodium bicarbonate, and dexamethasone; dextran 40	In context of MP of abdominal organs only experimental works available	As perfusion solution or a base solution in NMP
M101	Extracellular hemoglobin derived from a marine lungworm	HMP	Kidney	-	Report of clinical kidney transplantation	As addition to a base solution to increase oxygenation

Human-derived hemoglobin	Human-derived hemoglobin, sometimes used in form of vesicles	SNMP, NMP	Liver	-	In context of MP of abdominal organs only experimental works available	Addition to a base solution as an oxygen carrier
HBOC-201 (Hemopure)	Bovine hemoglobin-based oxygen-carrier	SNMP, NMP, rewarming	Liver, kidney	-	Clinical trial of a rewarming procedure of liver	Addition to a base solution as an oxygen carrier
HBOC-301 (Oxyglobin)	Bovine hemoglobin-based oxygen-carrier, different molecule size from Hemoglobin	NMP	Liver, kidney	-	In context of MP of abdominal organs only experimental works available	Addition to a base solution as an oxygen carrier, most likely inferior to Hemopure
Lifor	Nutrients, growth factors, amino acids, salts, buffers, non-protein oxygen carrier	SNMP	Kidney	-	In context of MP of abdominal organs only experimental works available	New preservation solution designed to minimize IRI
Whole blood	Autologous whole blood	SNMP (kidney), NMP	Liver, kidney	Diluted whole blood	Animal studies	Possible immune response increase, hemolysis
pRBCs	Cross-matched packed RBCs from blood banks	NMP	Liver, kidney, pancreas	-	The most clinically used oxygen carrier	Addition to a base solution as an oxygen carrier; can cause free heme release and/or hemolysis, limited availability

Steen	Buffered solution with extracellular sodium and potassium concentrations, as well as dextran and albumin	NMP, rewarming	Liver, kidney, pancreas	-	Case report of human kidney rewarming with subsequent transplantation	As perfusion solution or as base solution, designed for warm perfusion
Ecosol	Contains PEG 35, HEPES, taurine and nutritious components	NMP	Kidney	-	In context of MP of abdominal organs only experimental works available	Primarily used for SCS of kidney grafts
Modified Krebs-Henselheit solution	With electrolytes, HEPES, and glucose	NMP	Liver	Albumin	In context of MP of abdominal organs only experimental works available	As base solution, oft used at reperfusion for evaluation in experimental studies
Dulbecco's Modified Eagle Medium	Amino acids and vitamins, glutamine, human serum albumin, and HEPES	NMP	Liver	-	In context of MP of abdominal organs only experimental works available	As base solution, otherwise used for cell cultivation
Gelofusine	Commercial colloid solution containing bovine-origin gelatin and is considered a viable plasma substitute	NMP, rewarming	Liver	Albumin	As a base medium in liver NMP RCTs	As base solution

William's E medium	Unique composition for liver cell cultivation	NMP	Liver, kidney	Albumin	In context of MP of abdominal organs only experimental works available	As perfusion solution or as base solution; primarily used for liver cell cultivation
FFP	Human plasma from blood banks	NMP	Liver	-	As a base medium in a liver NMP RCT	As base solution, in combination with RBCs contains no leukocytes or thrombocytes, advantage in comparison to whole blood; limited availability

Abbreviations

ALT - alanine transaminase

AST - aspartate transaminase

ATP - adenosine triphosphate

CIT - cold ischemia time

COR - controlled oxygenated rewarming

DBD - donation after brain death

DCD - donation after circulatory death

DGF - delayed graft function

EC - Eurocollins solution

ECD - extended criteria donors

EV - extracellular vesicles

FFP - fresh frozen plasma

GGT - gamma-glutamyltransferase

HCC - hepatocellular carcinoma

HES - hydroxyethyl starch

HMP - hypothermic machine perfusion

HOC - hyperosmolar citrate solution

HOPE - hypothermic oxygenated perfusion

HTK - histidine-tryptophan-ketoglutarate solution

ICU - intensive care unit

IGL - Institute-George-Lopez solution

IL - interleukin

IRI - ischemia reperfusion injury

LDH - lactate dehydrogenase

MP - machine perfusion

NMP - normothermin machine

NRP - normothermic regional perfusion

PEG - polyethylene glycol

RBCs - red blood cells

RCT - randomized controlled trial

ROS - reactive oxygen species

SCOT - Solution de Conservation des Organes et des Tissus

SCS - static cold storage

SNMP - subnormothermic machine perfusion

TNF - tumor necrosis factor

UW - University of Wisconsin solution

WIT - warm ischemia time

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