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# Porcine Liver Normothermic Machine Perfusion: Methodological Framework and Potential Pitfalls

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**Abstract.** Porcine models of liver normothermic machine perfusion (NMP) are increasingly used in transplant research, although known to be challenging because of their complex methodology and their scarcely documented operational aspects. Here, we aimed to provide a methodological framework for researchers looking to adopt NMP technology in research setting by giving an in-detail account of the implementation of a previously validated porcine liver NMP model. We subjected groups of 3–5 porcine livers to 24 h NMP and, using a trial-and-error principle, introduced stepwise changes in the NMP setting with the objective to obtain stable preservation of liver function and histology for 24 h. Female porcine livers were procured, and packed red-blood-cell perfusate was prepared. Perfusate oxygenation, hemodynamics, markers of hepatic injury (aspartate transaminase [AST]), function (lactate, perfusate pH, bile production), and histology were analyzed. Intermediate analysis was performed within groups and a minimum of 3 (out of 5) failed experiments prompted methodological reevaluation. Overall, 13 liver NMP experiments were needed in 3 phases. In phase 1, loss of oxygenator performance occurred from 6 h onward in 3 consecutive experiments because of perfusate leakage. In phase 2, a plasma-tight hollow fiber oxygenator ensured adequate perfusate oxygenation in 5 experiments. However, portal vein resistance increased during all liver NMP, associated with high perfusate AST levels (range, 106–322 IU/L/100 g) and pan-lobular sinusoidal dilation and hemorrhage, suggesting liver outflow impairment. In phase 3, an improved inferior vena cava cannulation technique avoided liver outflow impairment, resulting in lower AST release (range, 29–101 IU/L/100 g), improved lactate clearance, preserved biliary excretion, and normal histology in 5 experiments. This study underscores the critical importance of auditing all equipment and operational components of NMP circuits to obtain successful and reproducible perfusion setup and advocates for in-detail reporting of methodological aspects and potential pitfalls.

(*Transplantation Direct* 2022;8: e1276; doi: 10.1097/TXD.0000000000001276).

## INTRODUCTION

Liver transplantation is the ultimate treatment for end-stage liver disease of many etiologies. The excellent post-transplant results achieved have created an ever-widening gap between transplant organ offer and demand, increasing mortality on a growing waitlist. The relaxation of

donor acceptance criteria, whereby high-risk donors are included, represents an imperfect solution. Livers procured from high-risk donors carrying risks such as advanced age, hepatic steatosis,<sup>1</sup> and donation after circulatory death<sup>2</sup> are more susceptible to Ischemia-Reperfusion Injury (IRI). Although total ischemia-free organ transplantation was recently described,<sup>3</sup> some degrees of IRI inevitably take

Received 20 May 2021. Revision received 14 September 2021.

Accepted 6 October 2021.

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The Lab of Abdominal Transplantation of KU Leuven has received Institute George Lopez 1 preservation solution by the Institute George Lopez (Lisseau, France) free of charge to be used in animal experiments.

I.J., J.P., and D.M. are recipients of a Centrale Afdeling Voor Fractionering chair for abdominal transplant surgery. J.P. is recipient of an Insitute George Lopez chair for abdominal transplant surgery. D.M. is a senior researcher of The Research Foundation—Flanders. The department of Abdominal Transplant Surgery has received unrestricted grants from Astellas and Roche. The other authors declare no conflicts of interest.

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ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001276

place during any liver transplantation, and its severity directly correlates to the risk of graft failure, posttransplant complications, and survival.<sup>1</sup> Therefore, understanding the effects of IRI on graft function and developing strategies to better preserve or protect liver grafts remain at the forefront of transplant research.

Animal models of liver transplantation have been pivotal, not only for the development of surgical techniques but also to understand the pathophysiology and immunology of liver preservation and transplantation. Whereas results from rodents experiments are difficult to translate to the clinical situation, the pig is considered a more appropriate model animal because the porcine liver is anatomically and physiologically close to humans, with comparable organ size and similar bile composition.<sup>4,5</sup> Nevertheless, large animal transplant models are labor-intensive, technically challenging, and carry higher financial costs.<sup>6</sup>

The last decade has brought an increasing interest in normothermic machine perfusion (NMP) as a technique to enhance liver preservation.<sup>7,8</sup> At variance with conventional cold storage,<sup>9</sup> NMP preserves the liver graft *ex situ* at near-physiology conditions by recirculating a warm, oxygenated, and nutrient-enriched perfusate through the liver vasculature. Liver NMP can be used to mimic a liver transplantation *ex situ* for research purposes,<sup>7</sup> to preserve a liver *ex situ* before transplantation,<sup>10,11</sup> and/or to investigate its function before implantation.<sup>12</sup> A randomized clinical trial found NMP liver preservation to be associated with lower levels of injury than conventional cold storage (measured as posttransplant transaminase release).<sup>13</sup> In contrast, the use of a leukocyte-containing whole blood perfusate in NMP has been postulated to replicate the (early) events occurring during liver transplantation. Our recent systematic review of 11 different models of porcine liver NMP indicated that whole blood-based NMP can indeed replicate the IRI-related proinflammatory events during the first 24 h after liver transplantation.<sup>7</sup>

Because of its versatility, porcine liver NMP is used to address a growing number of research questions in the field of liver transplantation.<sup>7</sup> However, the adoption of these complex models is challenging in part because surgical and machine-related technical aspects are scarcely described, and potential pitfalls remain unpublished.<sup>7</sup> In our research laboratory, we used the original NMP setup described by Butler et al<sup>10</sup> to implement and validate a 24 h porcine liver NMP model that could be used to both preserve a liver graft for transplantation and to mimic a transplant in an *ex situ* experimental setup.<sup>7</sup> Here, we share our experience in implementing and validating this model and describe our adaptive stepwise approach to difficulties that arose with the objective to facilitate the widespread implementation of NMP.

## MATERIALS AND METHODS

### Experimental Design

The goal of this study was to implement a model of NMP allowing to preserve the function of porcine livers and prevent the occurrence of parenchymal damage (at the histological level) for up to 24 h. In preparation to this study, we first performed a systemic review of all published porcine models.<sup>7</sup> At the time of systematic search, the model of Butler et al<sup>10</sup> was the only one in which porcine livers were repeatedly and successfully transplanted after *ex situ* preservation for 24 h.

Therefore, this model was chosen for its capacity to preserve liver function and histology for 1 d. NMP was based on autologous concentrated red blood cells to avoid the interference of IRI-related events and prevent hemolysis secondary to blood group incompatibility. The 24 h timeframe was also chosen based on systematic review of the literature.<sup>7</sup>

We designed a pilot setup study with an adaptive and stepwise implementation of experimental methodology according to a trial-and-error principle. In a first step, experiments were conducted as described in the available literature until a minimum number of 3 to 5 experiments were performed before investigating the achievement of the predetermined goal. Intermediate results analysis guided hypothesis formulation and identification of methodological issues potentially affecting outcomes if the predetermined goal was not achieved. Changes in equipment, surgical, or technical aspects were made as deemed necessary and implemented in the following experimental step. The process was repeated until the predetermined goal was achieved. Single animals were the experimental unit, whereas the different phases constituted the experimental groups. Given the adaptive, stepwise optimization of the experimental methodology, animal allocation to the different phase was not randomized. The sample size was arbitrarily chosen to compensate for the biological variation between experimental units. If a methodological issue was unequivocally identified after 3 experiments, the corresponding phase was stopped. Outcomes measured to investigate whether the predetermined goal was achieved were perfusate oxygenation (Po<sub>2</sub> and oxygen consumption), hemodynamic stability (flows and intravascular resistances), graft injury (perfusate aspartate transaminase [AST] concentration), graft function (perfusate lactate concentration, perfusate pH, hourly bile production), and histology. Results are reported according to the Animal Research: Reporting of In Vivo Experiments guidelines 2.0.<sup>14</sup>

### Animals

Prepubescent female pigs (aged 3 mo; TOPIG TN70, Bilzen, Belgium) weighing approximately 30 kg (average liver weight, 720 g; range, 580–866) were housed in single pens with a 12 h light-dark cycle. Animals had access to food (MPig-H; ssniff, Soest, Germany) and tap water, and visual, olfactory, and auditory contact between them. Animals were allowed to get accustomed to their surroundings for at least 2 d and were fasted for 12 h before surgery while retaining access to water. At the end of the experiment, animals were euthanized by exsanguination under anesthesia. The KU Leuven Animal Care Committee approved the study (P027/2016) and the experiments were conducted in line with European guidelines.<sup>15</sup>

### Anesthesia and Surgery

Pigs were premedicated with xylazine 2 mg/kg (VMD, Arendonk, Belgium) and tiletamine 8 mg/kg (Virbac, Barneveld, The Netherlands) allowing for orotracheal intubation and mechanical ventilation. Anesthesia was induced and maintained with isoflurane 1% (Dechra Veterinary, Belgium). Analgesia was achieved with fentanyl 8 µg/kg/h (Janssen-Cilag, Beerse, Belgium). The left external jugular vein and left carotid artery were isolated to insert a triple lumen 12F venous catheter and an arterial line for invasive monitoring of systemic blood pressure, respectively. After a midline laparotomy, the

infra-hepatic inferior vena cava (IVC) and abdominal aorta were dissected free and prepared for cannulation. The bile duct, portal vein (PV), and hepatic artery (HA) were dissected free. Cannulas were inserted in the IVC and abdominal aorta following the systemic administration of 500 U/Kg heparin (Leo Pharma, Ballerup, Denmark). The liver was flushed with 2000 mL of cold (4–6 °C) Institute George Lopez 1 preservation solution (Institute George Lopez, Lissieu, France) via the aorta and 2000 mL via the PV. Blood was collected during the flush-out and washed with a cell-saver (Electa; Sorin Group, Mirandola, Italy) to obtain concentrated red blood cells. The liver was then completely mobilized and procured by sectioning the supra- and infra-hepatic IVC, the PV, and HA with a patch of aorta. After hepatectomy, the liver was kept on melting ice and prepared for perfusion. The diaphragm was removed and the phrenic veins ligated. The supra-hepatic IVC was closed. The IVC, PV, and HA were cannulated with a 20F, an 18F, and a 10F cannula (Medtronic, Dublin, Ireland), respectively. The cystic duct was ligated, and the bile duct was cannulated for bile collection during NMP. After flushing of the liver and de-airing of the cannulas with 500 mL of gelofusine (Braun, Melsungen, Germany), the liver was ready for NMP.

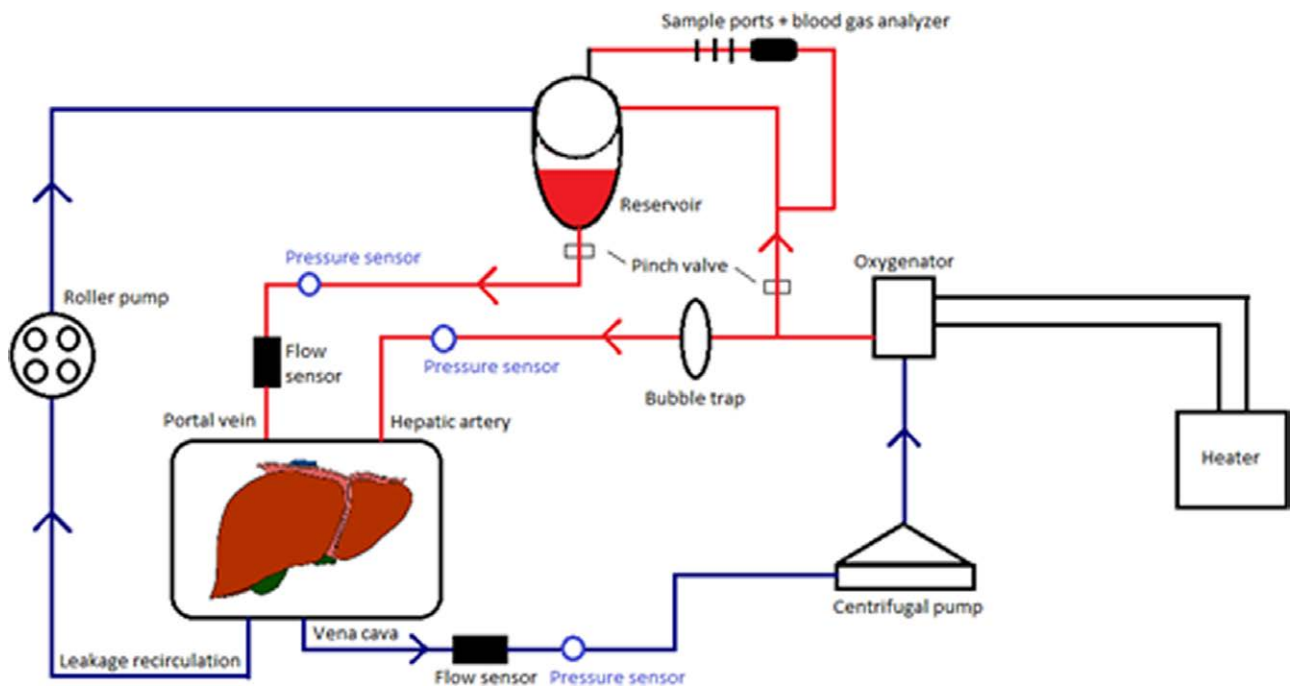
### Normothermic Machine Perfusion

The Terumo FX05 pediatric kit for extracorporeal membrane oxygenation (Terumo Europe, Leuven, Belgium) was modified to reproduce the circuit depicted in Figure 1. The liver was placed in a receptacle allowing for the collection and recirculation of ascites and perfusate leaking from surgical surfaces via a roller pump (Medtronic, Dublin, Ireland). A centrifugal pump (Medtronic, Dublin, Ireland) delivering a continuous flow pumped the packed red blood cells perfusate to the hollow fiber oxygenator included in the Terumo FX05 pediatric kit coupled to a heat exchanger (Stockert; Sorin Group, Munich, Germany). After reaching 38 °C (physiological core temperature in pigs<sup>7</sup>) and a  $P_{O_2}$  of 70–100 mmHg

(1–2 L/min of 21% oxygen), the perfusate flow was split into 2 lines, one connected directly to the cannula inserted in the HA and one connected to a hard-shell reservoir placed at a fixed position. The reservoir was connected to the PV cannula, thus supplying the portal circulation by gravity. Pinch valves positioned before and after the PV reservoir allowed for a pressure-controlled recirculation of the perfusate. The outflow from the liver is collected via the IVC cannula and recirculated via the centrifugal pump, closing the circuit. Flow and pressure probes were placed along the PV, HA, and IVC lines. A pressure servo regulator (BM 9505-2 pressure servo regulator, Medtronic) prevented the formation of excessively negative pressure in the IVC line. The total flow (1 mL/min/g liver) was divided 75% via the PV and 25% via the HA to respect normal physiology of porcine hepatic circulation.<sup>7</sup> The bile duct was cannulated for bile collection during NMP.

The circuit was primed with 500 mL of gelofusine. The volume of washed red blood cells to be added to reach a hematocrit of 30% was calculated with the formula:  $([\text{weight of the liver} + \text{priming volume}] \times \text{desired hematocrit}) / \text{hematocrit}$  after washing. The perfusate was then supplemented with 10 000 U heparin (Leo Pharma, Ballerup, Denmark), 1000 mg cefotaxime (Sanofi-Aventis Belgium, Diegem, Belgium), 10 mL calcium gluconate 10% (Braun, Melsungen, Germany), and 5–10 mL of bicarbonate 8.4% (Braun, Melsungen, Germany).

After a period of cold storage of circa 2 h (average time needed for cold flush, hepatectomy, and back-table preparation), the liver was connected to the circuit and NMP started. Epoprostenol 0.5 mg (GlaxoSmithKline Belgium, Wavre, Belgium), insulin 200 U (Novo Nordisk, Bagsværd, Denmark), heparin 25 000 u, sodium taurocholate 5.6 g (kindly provided by OrganOx Ltd., Oxford, United Kingdom), and fat-free parental nutrition (Baxter, Lessines, Belgium) were continuously infused during NMP, as previously described.<sup>10,16</sup> The NMP setup was manually operated by the same operator in all experiments. The target pressure in the HA (70 mmHg)



**FIGURE 1.** Schematic overview of the circuit utilized for normothermic machine perfusion of porcine livers.

was reached by adjusting the pinch valve proximal to the PV reservoir and the speed of the pump, whereas PV target pressure (10 mmHg) was maintained by the fixed position of the reservoir. In contrast, PV flow was approximated to 75% of the total flow by adjusting the pinch valve between the reservoir and the PV cannula. These settings were not changed after NMP started.

### Sample Collection and Analyses

The perfusate concentration of AST was measured as marker of hepatocellular injury during NMP, whereas the perfusate concentration of lactate, perfusate pH, and bile volume were measured as markers of hepatocellular function during NMP. Gas exchange was also monitored throughout perfusion, measuring both arterial and venous perfusate pH, bicarbonate concentration,  $PO_2$ , and  $PCO_2$ . Oxygen consumption was calculated as the difference between arterial and venous  $PO_2$  and saturation, corrected for total blood flow and hemoglobin concentration.<sup>17</sup> Perfusate samples were collected at 15, 30, and 45 min, and 1, 2, 3, 6, 12, and 24 h of NMP. Pressure and flow in the IVC, PV, and HA were monitored continuously and pointwise data were recorded at the same time points as for perfusate sampling. The resistance in the PV and HA circulation was calculated as the ratio between pressure and flow and expressed per 100 g of liver. A wedge biopsy was taken at 24 h of NMP.

Perfusate samples were collected in EDTA tubes, centrifuged at 1000 g at 4 °C, aliquoted in 1 mL microtubes, snap-frozen, and stored at -80 °C until further analysis. AST was measured according to the International Federation of Clinical Chemistry method (detection limit 4 U/L; Hitachi/Roche Modular P Chemistry Analyzer; Roche Belgium, Vilvoorde, Belgium). The perfusate concentration of AST was expressed per 100 g of liver to account for differences in liver weight. Perfusate pH,  $PO_2$ ,  $PCO_2$ , bicarbonate, lactate, and hemoglobin concentration were measured with a point-of-care instrument (ABL 800-flex; Radiometer, Zoetermeer, The Netherlands). Tissue samples were stored in 6% formalin, paraffin-embedded, and stained with hematoxylin and eosin.

## RESULTS

Livers from a total of 13 animals were used in 3 different methodological phases before the study goal was achieved in 5 consecutive NMP experiments. In the first phase, 3 consecutive experiments were prematurely stopped at 15, 18, and 18 h. From 12 h onward, the arterial  $PO_2$  was below 50 mmHg and continued to decrease considerably below this value despite an increase in supplied oxygen concentration/flow. In addition, macroscopically, all 3 livers appeared ischemic. The prolonged anoxia, unresponsive to the administration of higher concentrations of oxygen, was attributed to the oxygenator, which lost performance after being used for more than 6 h. Perfusate samples from this first experimental phase were not processed for further analysis since changing the type of oxygenator was deemed unavoidable.

In phase 2, the oxygenator included in the Terumo FX05 pediatric kit was replaced by an oxygenator equipped with plasma-tight hollow fiber membranes (Hilite 2400 LT; Medos, Stolberg, Germany). Five experiments were conducted, each of them lasting 24 h. Intermediate analysis confirmed adequate perfusate oxygenation ( $PO_2$  stably in the

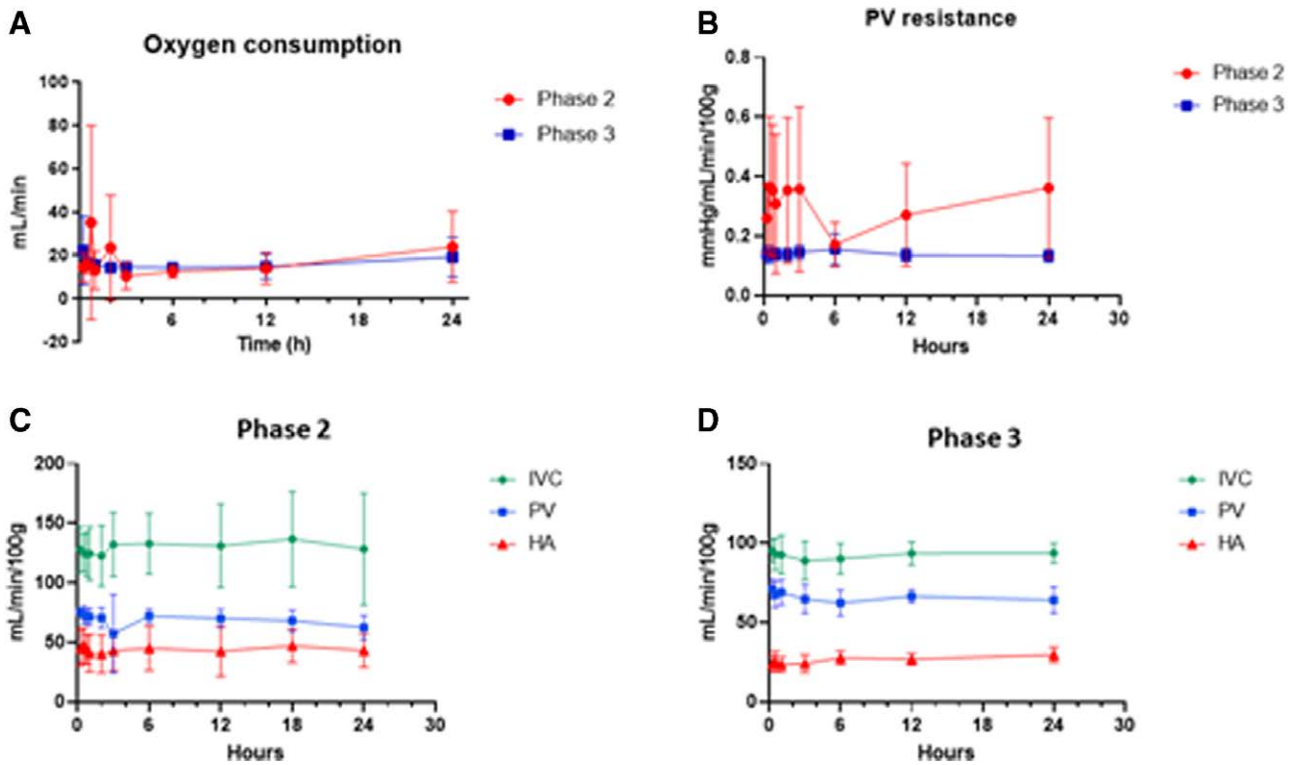
predetermined range of 70–100 mmHg) and stable oxygen consumption starting from 3 h of NMP onward (Figure 2A). Nevertheless, hemodynamic alterations were observed in all experiments. After an initial decrease, PV resistance increased from 6 h onward until the end of NMP, although considerable variation was observed between single experimental units (Figure 2B). Significant alterations of HA resistance were not observed (data not shown). As a result, PV flow tended to decrease and the total blood flow was divided 50% between the PV and HA circulation (Figure 2C). In parallel, the perfusate concentration of AST increased from 12 h in all experiments, reaching a peak at the end of perfusion with measured concentrations ranging between 106 and 322 IU/L/100 g (Figure 3A). The perfusate lactate concentration remained at <1 mmol/L during NMP in all experiments, and the perfusate pH remained stable with an average value of 7.21 (Figure 3B and C). Bile production peaked at 3 h of NMP and decreased thereafter, to stop completely at the end of NMP (Figure 3D). Histological analysis revealed pan-lobular sinusoidal dilatation, erythrocyte congestion, hemorrhagic dissociation, and necrosis of hepatocellular trabeculae (Figure 3E), observed in all experiments. We suspected liver outflow obstruction to be the underlying cause.

In phase 3, the IVC cannulation technique was modified to include direct visualization of the hepatic veins, ensuring that the tip of the multiperforated cannula was placed at this level without obstruction. Five experiments were conducted in phase 3, all of them lasting the full 24 h. Intermediate analysis confirmed stable oxygen consumption throughout perfusion (Figure 2A). In contrast to the previous experimental phase, PV resistance remained stable during the entire duration of NMP and variation between experimental units was less pronounced (Figure 2B). The total blood flow to the liver remained stable and approached the physiological repartition of 75% via the PV and 25% via the HA circulation (Figure 2D). The perfusate concentrations of AST observed in phase 3 were in a lower range (29–101 IU/L/100 g) than that observed during phase 2 (39–323 IU/L/100 g; Figure 3A). Lactate concentration and perfusate pH remained unaltered, whereas bile production was maintained until the end of perfusion in experiments of phase 3, with a mean rate of 4 mL/h at the end of NMP (Figure 3B–D). Histological findings showed hepatic lobules with normally preserved architecture and only minimal signs of hepatic injury (Figure 3E).

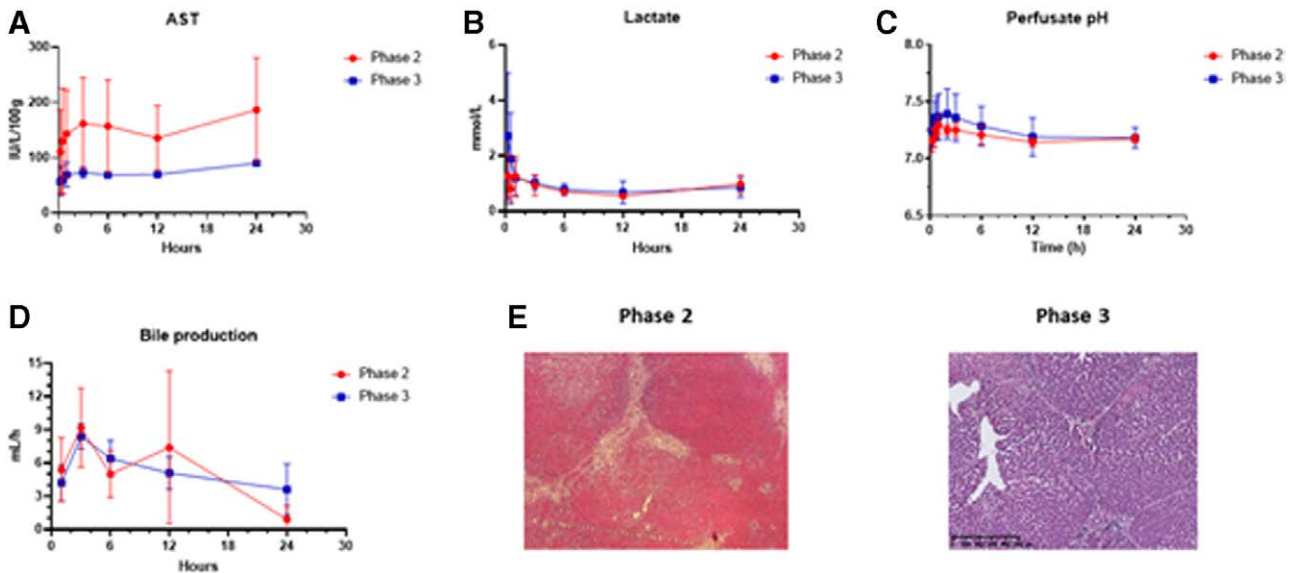
## DISCUSSION

This study aimed to provide guidance to transplant researchers wanting to adopt or refine a porcine NMP model, known to be labor-intensive and technically challenging. In addition to giving a comprehensive description of the surgical and perfusion circuit aspects of a validated model, this report shows how equipment characteristics and vascular cannulation methods critically influence the successful perfusion of the liver graft and illustrate how outcome parameters can be used as a problem-solving guide.

In the model chosen, the cold ischemia time was limited to circa 2 h. This allowed to minimize interference by IRI-related biological processes and to identify the essential methodological characteristics for successful liver function preservation for 24 h. Given the complexity of both technical and surgical aspects of this model, we proceeded with an adaptive stepwise



**FIGURE 2.** Overview of results of experiments of phase 2 and phase 3 (phase 1 experiments were terminated early) of the adaptive, stepwise, methodological optimization during implementation of a porcine model of liver NMP. Results concerning oxygen consumption and hemodynamic are shown. In both phases, stable oxygen delivery and oxygen consumption were observed during NMP (A). A progressive increase of PV resistance was observed during NMP in phase 2 (B), accompanied by a 50% repartition of the total blood flow between portal and arterial circulation (C). After changing the technique for IVC cannulation in phase 3, hemodynamic stability was achieved, approaching the physiological repartition of total hepatic flow in 75% via the PV and 25% via the HA circulation (D). HA, hepatic artery; IVC, inferior vena cava; NMP, normothermic machine perfusion; PV, portal vein.



**FIGURE 3.** Overview of results of experiments of phase 2 and phase 3 (phase 1 experiments were terminated early) of the adaptive, stepwise, methodological optimization during implementation of a porcine model of liver NMP. Results concerning markers of graft injury and function, and histology are shown. The perfusate concentration of AST tended to be higher in experiments of phase 2 (A), whereas the perfusate concentration of lactate (B) and perfusate pH (C) appeared to be similar. Bile production was the highest in both phases during early time point but was maintained until end of perfusion only in experiments of phase 3 (D). Histology from samples taken after 24 h of NMP (E) showed pan-lobular sinusoidal dilatation and erythrocytes congestion associated with intrahepatic hemorrhage in all experiments of phase 2, whereas in phase 3, the architecture of the hepatic lobules was preserved in all experiments (representative micrographs). AST, aspartate transaminase; NMP, normothermic machine perfusion.

approach and a trial-and-error principle, guided by the predefined outcome of achieving preserved liver function and histological architecture for up to 24 h. In a set of 13 experiments, issues with both oxygenation and hemodynamics prompted adaptations of both equipment and surgical technique.

Oxygenation is a key factor to adequately preserve and support metabolism during NMP. In our experience, the choice of the oxygenator built into the perfusion circuit was of paramount importance. Experience with extracorporeal circulation for cardiac surgery has taught that plasma may leak in the hollow fibers of the built-in oxygenator, which may drive protein deposition and erythrocyte aggregation. Erythrocyte aggregation increases the distance between the oxygen supply and perfusate flow, compromising gas exchange and oxygenator performance in prolonged perfusion. Since we used a perfusate based on concentrated red blood cells, we expected a negligible residue of plasma in the perfusate and stratified the risk of impaired oxygenation during 24 h NMP as low. Consequently, we initially used an oxygenator not equipped with plasma-tight hollow fibers. Nevertheless, in phase 1, we repeatedly observed reduced performance of the oxygenator from 12 h NMP onward. The introduction in phase 2 of an oxygenator that was equipped with plasma-tight hollow fiber membranes certified for extended use resulted in adequate oxygenation during the full 24-h NMP.

Hemodynamic stability during perfusion is equally paramount to preserve liver integrity and function. With a closed-circuit setup,<sup>7</sup> cannulation of the IVC is pivotal to prevent hepatic vein obstruction and collapse of the vessel walls on the tip of the cannula. Although we used a venous cannula with a multiperforated tip and a pressure servo regulator, we still observed some degrees of liver outflow impairment during perfusion, as shown by both hemodynamic alterations and histology findings in phase 2 experiments. Impairment of liver outflow determines sinusoidal stasis, increases PV resistance, and reduces portal flow. The liver self-regulates and keeps blood flow constant via the HA buffer response, which leads to a compensatory increase of arterial flow as observed in phase 2. Indeed, flow competition between the portal and arterial circulation has been observed as well during liver machine perfusion<sup>18</sup> and, in controlled experiments, obstructing the IVC led to hemodynamic alterations similar to that observed in phase 2 experiments.<sup>18</sup> Additionally, histological findings revealed sinusoidal dilatation, erythrocytes clogging, and panlobular intrahepatic hemorrhage, which are all consistent with hepatic outflow impairment. Consequently, we assumed the positioning of the venous cannula in the IVC to be the cause, and we revisited the positioning technique. So far, a technique was used similarly to that reported for human liver NMP: the supra-hepatic IVC was closed during back-table preparation and the venous cannula was slid via the sectioned infra-hepatic IVC until above the superior margin of the liver. Its tip was positioned neither too deep in the parenchyma, neither too close to the inner walls of the supra-hepatic IVC by digital palpation. In pigs, the complete intrahepatic anatomical course of the IVC results in a sharper angle between the hepatic veins and the supra-hepatic IVC. To avoid any obstruction of the ostia of the hepatic veins, we adapted the IVC cannulation technique. The supra-hepatic IVC was left open during back-table preparation, the IVC cannula was inserted via the infra-hepatic IVC and slid cranially under direct visualization of its tip, which was placed at the level of the septa delimiting the

ostia of the hepatic veins. After implementing these changes, hemodynamic disturbances were no longer encountered during 24 h perfusion. Importantly, this also resulted in lower range release of AST, suggesting that additional hepatocellular damage was also reduced. Consistent with this, we observed preservation of normal architecture of hepatic lobules in all livers perfused during phase 3.

Previous preclinical and clinical studies have concluded that perfusate lactate clearance reflects graft function during NMP and may be used to predict posttransplant outcomes.<sup>7,19</sup> As lactate clearance was observed to occur quickly, even during NMP of grafts with pan-lobular intrahepatic hemorrhage and necrosis, our findings question this assumption. Given the abundant capacity of hepatocytes to metabolize the lactate, few surviving hepatocytes may be sufficient to clear the lactate in the small volume of perfusate (circa 1.5 L). Therefore, in this model, lactate clearance may not accurately reflect the overall viability of hepatocytes. This observation underscores our incomplete understanding of liver functioning during NMP and the lack of reliable functional biomarkers. Further investigating how liver cells biology changes in response to and during NMP will be essential not only to deepen our knowledge of its mechanisms of action but also to allow for the discovery of new biomarkers.

The fact that the liver NMP device was manually operated may lead to operator-dependent outcomes, thereby limiting the reproducibility of the results. Nevertheless, results of phase 3 experiments were in line to that previously reported by Butler et al,<sup>10</sup> indicating that this is not the case. Another potential disadvantage is that the perfusion system is not reusable. However, costs associated to porcine liver NMP were rather contained (500 euros per perfusion run in average). Additionally, this system has some degrees of flexibility as it can be used also to mimic a liver transplant on the perfusion device. This way, costs associated to the surgical procedure and postoperative care of a recipient animal can be avoided.

In conclusion, this in-detail real-life account of the implementation of a validated porcine NMP model of liver preservation wants to provide a methodological framework for researchers looking to adopt and/or refine NMP technology into their research. This study underscores the critical importance of auditing all equipment and operational components to obtain a successful perfusion setup, specifically the oxygenator and vascular access, and advocates for in-detail reporting of methodological aspects to allow for models to be reliably and successfully reproduced.

## ACKNOWLEDGMENTS

We would like to thank all the students of our study team who helped with the porcine experiments: Lander Dewitte, Kristof Swinnen, Laurence Verstraeten, Adriaan Van Assche, Jolien Segers, Barbara Donck, Léonie Verbeke, Boudewijn Heggen, Diederik Koenders, Bob van Houten, Maurits Sikking, Julie Lelièvre, Gaëtan Devos. The authors thank An Billiau for the medical writing support and Anne Kaiser for the preparation or the article.

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