

Cold-to-warm machine perfusion of the liver: a novel circuit for an uninterrupted combined perfusion protocol

C. Goumard¹, M.D., E. Savier¹, M.D., J. Danion¹, M.D., J. Pellissier², M.D., Cécile Legallais³, PhD., and O. Scatton¹ M.D, PhD.

¹ Sorbonne Université, INSERM Centre de Recherche Saint Antoine UMR S-938, Department of Hepatobiliary Surgery and Liver Transplantation, Hôpital Pitié-Salpêtrière, Assistance Publique-Hopitaux de Paris, France.

² Department of extracorporeal perfusion and vascular surgery, Hôpital Pitié-Salpêtrière, Assistance Publique- Hopitaux de Paris, France

³ Technological University of Compiègne (UTC), UMR CNRS 7338 Biomechanics and Bioengineering, Compiègne, France

Correspondence and reprint requests to:

Prof. Olivier Scatton, MD, PhD.

Department of Hepatobiliary surgery and liver transplantation, Sorbonne Université, UMRS-938, Hôpital Pitié-Salpêtrière, Assistance Publique- Hopitaux de Paris, Paris, France

47-83 Boulevard de l'Hôpital, 75013, Paris, France.

E-mail: olivier.scatton@aphp.fr

Article type: Short report

Keywords: liver transplantation, ex-vivo machine perfusion, organ perfusion, liver graft, cold-to-warm perfusion

Word count: 2052

Authorship statement

Claire Goumard drafted and wrote the manuscript, and made substantial contributions to data acquisition, analysis and interpretation.

Eric Savier and Jerome Danion actively contributed to the design and development of the circuit, and made substantial contributions to data acquisition, analysis and interpretation.

Jérôme Pelissier made substantial contributions to the design and technical improvement of the final circuit.

Cécile Legallais led the development of the circuit prototype.

Olivier Scatton designed and led the project, and revised it critically for important intellectual content, and gave final approval for publication.

Conflict of interest statement

The authors declare no conflicts of interest.

Financial disclosure statement

This study received partial funding by the IDEX SUPER Grant, *Institut Universitaire d'Ingénierie en Santé (Sorbonne University)*.

Abstract (word count: 197)

Background

Ex-vivo perfusion of liver grafts is associated with promising results for the preservation of marginal grafts. Recent studies highlight the need for a combination of perfusion conditions, such as hypothermic followed by normothermic perfusion. While comprehensive machines dedicated to liver perfusion have been developed, these systems remain costly and poorly adaptable to perfusion condition switch, which requires a complete interruption of the perfusion process.

Our team aimed at developing an adaptable and simple circuit for uninterrupted ex-vivo liver perfusion.

Methods

Together with specialized bioengineers, we developed a highly adaptable circuit that can fit on already pre-existing extracorporeal oxygenation machines routinely used in cardiovascular surgery. This circuit, owing to its reservoir, allows any type of perfusion conditions without interrupting the perfusion process.

Results

In a preliminary study, to assess the technical feasibility of liver perfusion using our circuit under different conditions, we performed 7 perfusions of discarded liver grafts. HOPE and DHOPE hypothermic perfusion could be performed, and a switch to normothermia was easily possible within seconds. From there, a dynamic perfusion sequence model was developed.

Conclusion

This circuit may represent a simpler alternative or a new refinement to existing perfusion systems allowing uninterrupted combined perfusion protocols.

Running head: cold-to-warm uninterrupted machine perfusion of the liver

The organ transplantation community has developed increasing interest over ex vivo machine perfusion as a tool to rescue marginal grafts and extend safely organ selection for transplantation. Recent prospective studies report successful testing, resuscitation and transplantation of human marginal liver grafts after ex-vivo machine perfusion, including one randomized controlled trial using normothermic machine perfusion¹⁻⁴. In the actual context of organ shortage and expansion of marginal grafts use, such rescue strategies appear as a promising advance for patients on waiting list.

In this context, different perfusion machines have been developed and marketed^{1,5,6}, and perfusion modalities are still under investigation to determine the best protocol. In brief, a liver graft can undergo three main types of ex-vivo machine perfusion: hypothermic perfusion, with an oxygenated cold preservation solution through portal vein alone (HOPE) or both arterial and portal vessels (DHOPE), which allows to restore ATP stocks and protects from reperfusion injury⁷; subnormothermic perfusion at 21°C, also called controlled oxygenated rewarming when used as a bridge from hypo to normothermia; and normothermic perfusion at 37°C, allowing to test graft viability in full metabolic conditions, as well as adding various pharmacological interventions^{8,9}.

While the debate between the pros and cons of hypothermic versus normothermic perfusion still persists to date, recent studies describe promising results for a sequential perfusion combining the three techniques in a dynamic fashion. Ideally, a few hours of hypothermic oxygenated perfusion (either HOPE or DHOPE), followed by a short controlled oxygenated rewarming and a variable normothermic perfusion phase, appears to be the most efficient and harmless method to concurrently assess and rescue a marginal organ^{5,10,10}. For example, in case of high grade steatosis, which is the most frequent cause of graft discard in France, this sequence would theoretically enable restoring ATP mitochondrial stocks before progressively returning to full metabolism and evaluating the graft function together with active resuscitation using various pharmacological agents⁹.

However, while switching from hypothermia to normothermia would be the best sequence for organ resuscitation, the actual machines do not enable an easy shift of perfusion conditions due the absence

of intermediate reservoir, excluding the possibility of a perfusate withdrawal during one perfusion sequence. To our view, replacing the hypothermic perfusion liquid, filled with degradation products and pro-inflammatory factors, by a fresh blood-derived perfusate for progressive switch to normothermia, is an important step in the dynamic perfusion sequence. The team of Boteon et al. recently evaluated an uninterrupted cold-to-warm perfusion protocol based on a haemoglobin-based carrier (HBOC) with encouraging results, highlighting the need for an uninterrupted combined perfusion¹¹. However, the use of HBOC is still unauthorized in E.U and the same perfusate was kept during the whole perfusion without any change possible. An open circuit with an easily accessible intermediate reservoir appears therefore a simple alternative to achieve a perfusate switch without interrupting a perfusion or having to re-prime the whole circuit.

Moreover, the machines that are available commercially remain extremely costly. Apart from the price of the machine in itself, each single liver perfusion would cost from 5000 to 30 000 euros, depending on the technology. Furthermore, despite these costs, they may not allow all types of perfusion conditions, some being only dedicated to normothermia. Therefore, some experts in the fields still remain reluctant to invest so much for complex perfusion technologies, which may be useful in only a small proportion of borderline grafts. Obviously, high costs may be justified by the expertise required for normothermic perfusion, which cannot suffer from technical problems; thus, in normothermic perfusion marketed devices, all parameters, such as flow, pressure or temperature, are highly regulated, leading to poor flexibility.

To overcome these barriers, our transplant surgical team, together with specialized engineers in innovation from *Université Technologique de Compiègne* (UTC), started to develop a simpler concept of perfusion circuit, directly based on extracorporeal perfusion devices that are used in cardiovascular surgery daily practice. Rather than developing a machine, we chose to design a novel and adaptable ex-vivo perfusion circuit, that fits on different types of pre-existing machines dedicated to human extracorporeal oxygenation, which are present in any transplantation or cardiovascular surgery center.

These machines routinely monitor pressures, flow, temperature or even basic biological markers, and have already demonstrated their safety in human extracorporeal oxygenation daily use. Since no specific feature or additional technology is required to assess organ viability, which is based on simple parameters such as bile output or lactate perfusate level, the use of such existing machines didn't require any adaptation for graft perfusion, which was handled by the circuit designed for adapting to extracorporeal oxygenation machines with standard universal connectors.

Our technical prerequisites for this circuit were: 1) to be highly adaptable, allowing HOPE or DHOPE perfusion and easy switch from hypothermia to normothermia, with possibility of quick perfusate change without having to stop the machine or changing pipes; 2) to enable an easy access to the graft, which would remain located on the open "back-table" floating in a basin (no organ compression) as for a usual graft preparation, in order to facilitate graft assessment and manipulation (i.e. sampling, biopsy, imaging, fluorescence...); 3) to allow easy addition of extra devices to the circuit, such as a second loop hemodiafiltration system that filtrates inflammatory molecules and helps assisting liver physiology during long term perfusions (more than 24 hours) or specific biological monitoring devices 5) to be cost effective and therefore affordable for any transplant team.

According to these terms, a specific perfusion kit was designed, in a preliminary study funded by the *Institut Universitaire d'Ingénierie en Santé (IDEX SUPER, Sorbonne University)*, and validated on a dedicated liver phantom designed using computer assist design software at UTC Innovation Center Fab Lab. The phantom mimicked the anatomy of the liver main vessels and the related pressure variations, which were set at 100-110 mmHg in the arterial system, and 5-10 mmHg in the portal venous system. As a result, we chose to design the perfusion circuit to be set on two independent roller pumps ensuring adequate flow rates in each vascular system (**Figure 1**). The flow levels were set to 0.75 ml/min/g in the portal vein and 0.25 ml/min/g in the artery, with pressure sensors and alarms. Owing to the reservoir, the content can be filled or changed without having to stop the running of the perfusion. Moreover, the circuit design prevents any bubble forming in the perfusate. The graft remains floating in a basin filled

with the chosen perfusate, connected to the circuit via the artery, the portal vein and a collector at the bottom of the basin. Thus, graft preparation can be performed simultaneously with hypothermic portal perfusion (HOPE), without any time loss spent in static cold storage for meticulous graft preparation, which is usually required prior to normothermic perfusion. The resulting circuit was selected by SATT Lutech to benefit from a “Déclaration d’Invention” and patent protection. The cost of one circuit for graft perfusion is less than 1000 euros, and it only needs to be fixed to an extracorporeal oxygenation machine equipped with roller pumps, which is quite common in transplant centers. We tested the proper adjustment of the circuit to Sorin-Livanova® systems, which are the main types of extracorporeal oxygenation machines used in cardiovascular surgery in our hospital.

The next step was to test the circuit on human discarded liver grafts. After obtaining approval by the *Agence de la Biomedecine*, we conducted a pilot study on seven liver grafts from deceased donors deemed for transplantation due to heavy steatosis (up to 90%). The behavior of the discarded livers was assessed under various perfusion conditions, from hypothermia alone (HOPE and DHOPE) to normothermia after controlled rewarming (**Table 1**). A short movie describing the procedure is available online at the following link:

<https://www.youtube.com/watch?v=o6RfYtHoWJQ&feature=youtu.be>

One remaining pitfall for an easy access to liver perfusion was the need for an oxygen carrier for normothermic perfusion, bringing legal and safety constraints related to either human donor blood products or *de novo* synthetic molecules. We overcame this issue by harvesting the blood directly from the donor. During organ harvesting, the donor’s blood is usually replaced by a cold preservation solution after aortic clamping, and discarded. After acceptance by the *Agence de la Biomedecine* in the setting of a scientific protocol (PFS 12-005), we collected the discarded donor’s blood during organ procurement, and processed it for filtration with the Cell Saver® system, which is routinely used for major surgical interventions, at the transplant center after transportation with the graft in cold storage. This process allowed to obtain about one liter of concentrated autologous blood (hematocrit 50%),

which we diluted with Krebs medium and albumin to obtain a sufficient amount for normothermic perfusion. The hematocrit of the final perfusion solution was at minimum 15% and a systematic search for schizocytes was negative. A parallel study is currently being conducted by our team to assess the safety of donor's blood for organ perfusion use in terms of immunologic and inflammatory profile.

The characteristics of the seven organ perfusions are shown in **Table 1 and Figure 2**. The objective of the pilot study, which was to assess the technical feasibility of liver perfusion using our circuit under different conditions, was achieved. Both HOPE and DHOPE hypothermic perfusion could be performed, and a switch to subnormothermia and normothermia was easily possible within seconds, without any interruption of the perfusion or need for re-priming of the circuit. The gradual implementation first included two hypothermic-only perfusions (HOPE), and then five combined perfusions (**Table 1**). As these perfusions remain preliminary procedures, the function of the different livers we processed was highly variable across procedures and even within one perfusion sequence. Obviously, this is due to many factors such as perfusion conditions (hypothermia or normothermia), often long cold ischemia time prior to perfusion start, or inherent features of low-quality organs (amount of steatosis or fibrosis). The last perfusion case combined sequential DHOPE, followed by subnormothermic and normothermic perfusion with donor's blood, during 15h total. The lipid levels in both perfusate and bile of this latter graft perfusion are shown in Figure 2b. In this highly steatotic graft (90% macrosteatosis), the modification of the triglyceride, cholesterol and phospholipids levels in the perfusate and bile show that moving from hypo to normothermic conditions led to modify the composition of lipids without impairing the glucose production, albumin production and inflammatory profile along the perfusion. The results of this pilot study led to implement the "optimal" liver perfusion sequence, which is presented in **Figure 3**, and will serve as a template for future perfusions.

Herein, we showed that ex-vivo liver perfusion could be dramatically deescalated in terms of complexity by relying on pre-existing routine devices rather than expensive dedicated machines. By using an

innovative circuit designed to adapt to different types of extracorporeal oxygenation devices, which are already available in many hospitals, we were able to achieve liver perfusions in various conditions, without technical issues. Moreover, this highly flexible device allowed various manipulations of the liver during perfusion and therefore facilitated a comprehensive graft assessment.

While the cost of our circuit is relatively low due to the use of already marketed components and its adaptation on preexisting devices, the global cost-effectiveness of a perfusion with our circuit still warrants further evaluation, as well as a CE marking evaluation for its use in human clinical care. This aspect is currently being handled by the company manufacturing our circuit, which is one of the leading companies in routine tubing for human extracorporeal perfusion machines (Livanova). Indeed, even though many transplant centers already use extracorporeal circulation machines and Cell Saver systems, the cost of such devices must certainly be taken into account in a global cost-evaluation comparison with the available comprehensive perfusion systems. Nevertheless, our primary goal was to provide a cheaper and flexible solution for scientific purpose and was not a commercial project.

Obviously, this preliminary experience requires further investigations but demonstrated the feasibility of the concept. This adaptable perfusion circuit may help opening the restricted world of organ machine perfusion to a broader level, with the perspective to democratize perfusion to any transplant center. One may also consider using this device for in vivo liver perfusion, or even for other transplanted organs such as heart or kidney.

Acknowledgements: To Carine Asensio from SAT LUTECH for supporting us at the very beginning and launching the patent. To Livanova company (Sandra Spiritelli and John Migliazza) for supporting us and kindly providing the first-generation machine (S3 Sorin device) to start our program. Note that Livanova did not support financially this program. To Dr Fouzi Mestari, from the biology department, for kindly performing all perfusate biochemical measurements.

Table 1. Summary of the characteristics and perfusion conditions of the seven discarded liver grafts that underwent ex-vivo perfusion with the Pitié-Salpêtrière perfusion circuit.

| N. perfusion | Temperature conditions, °C | Static cold storage duration, min | Hypothermic perfusion duration, min | Hypothermic perfusion type | Subnormothermic perfusion duration, min | Normothermic perfusion duration, min | Total duration of the perfusion | Donor age, years | Donor's BMI (kg/m ²) | Reason for encephalic death | Lactate perfusate level, mmol/l (end of perfusion) | Liver Weight, g | Liver macrovesicular steatosis, % |
|--------------|----------------------------|-----------------------------------|-------------------------------------|----------------------------|---|--------------------------------------|---------------------------------|------------------|----------------------------------|-----------------------------|--|-----------------|-----------------------------------|
| Liver 1 | 4° | 270 | 630 | HOPE | - | - | 630 | 53 | 33.3 | Anoxia | 4.98 | 1300 | 60 |
| Liver 2 | 4° | 747 | 755 | HOPE | - | - | 755 | 82 | 29.4 | Stroke | nr | 1500 | 60 |
| Liver 3 | 4° to 21° to 37° | 535 | 50 | DHOPE | 40 | 580 | 670 | 39 | 25.9 | Pulmonary embolism | 3.11 | 1500 | 80 necrosis |
| Liver 4 | 4° to 21° to 37° | 1016 | 71 | DHOPE | 52 | 293 | 416 | 66 | 26.1 | Stroke | 11.6 | 1640 | 70 |
| Liver 5 | 4° to 21° to 37° | 718 | 84 | DHOPE | 79 | 717 | 880 | 45 | 24.2 | Trauma | 10.8 | 1265 | 60 |
| Liver 6 | 4° to 21° to 37° | 174 | 98 | DHOPE | 47 | 575 | 720 | 33 | 41 | Stroke | 10.1 | 1550 | 70 |
| Liver 7 | 4° to 21° to 37° | 630 | 240 | DHOPE | 60 | 570 | 900 | 63 | 32.4 | Stroke | 3.4 | 2090 | 90 |

Figure legends

Figure 1a. The « adaptable » liver perfusion circuit designed by Pitie-Salpêtrière team and UTC

Figure 1b. Pictures of the circuit adapted on the extracorporeal perfusion machine (upper left), during a perfusion in normothermic conditions (lower left and right) and in hypothermic conditions (upper right).

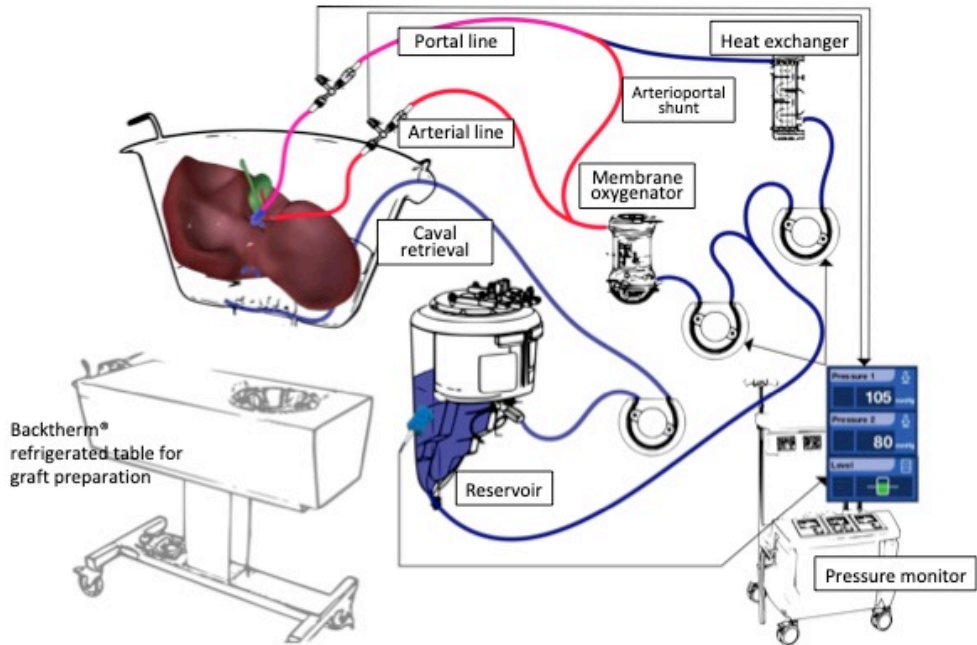
Figure 2a. Main biological values measured throughout the 7 consecutive liver perfusions with the Pitié-Salpêtrière perfusion circuit.

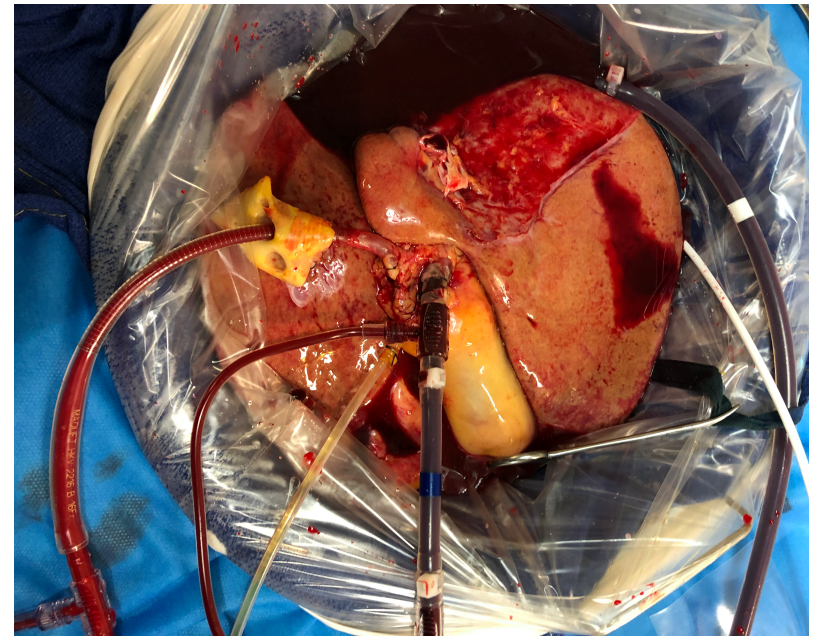
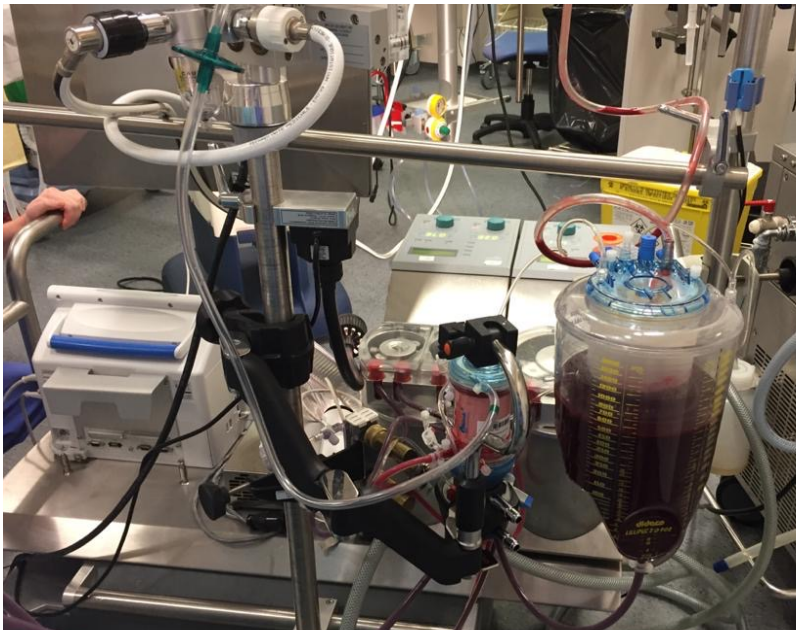
Figure 2b: Biological profile of graft n°7 showing lipids profile, metabolism and inflammation in both perfusate and bile throughout the perfusion.

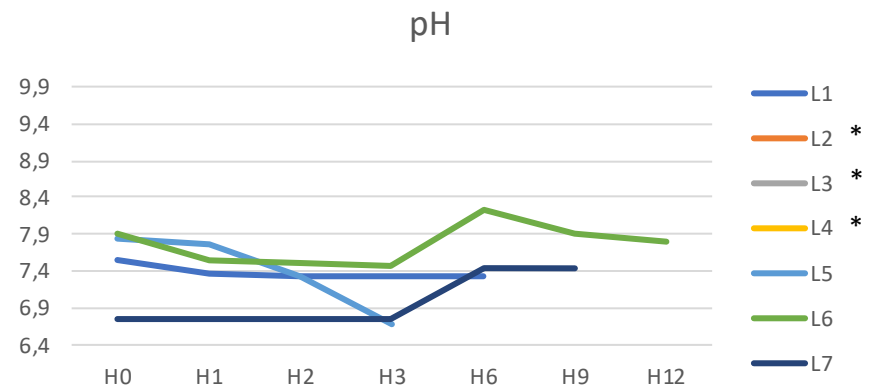
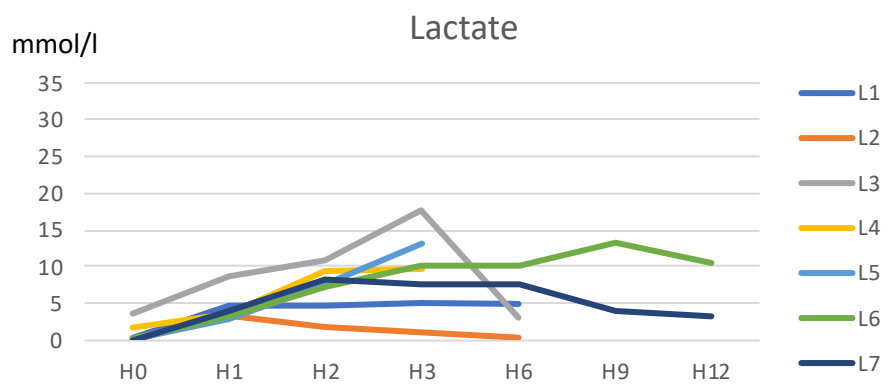
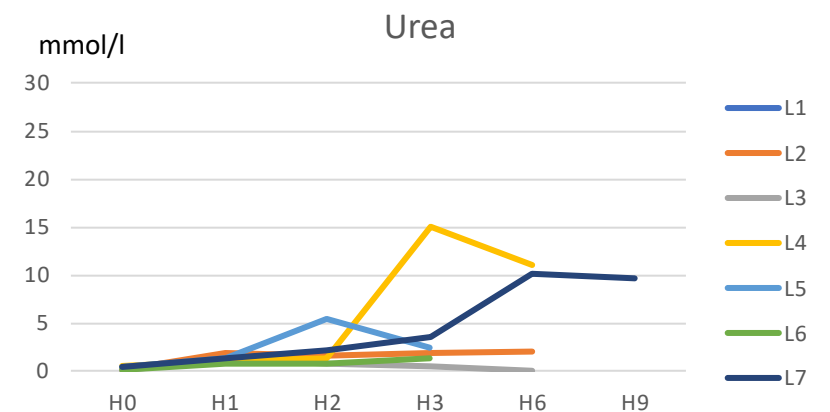
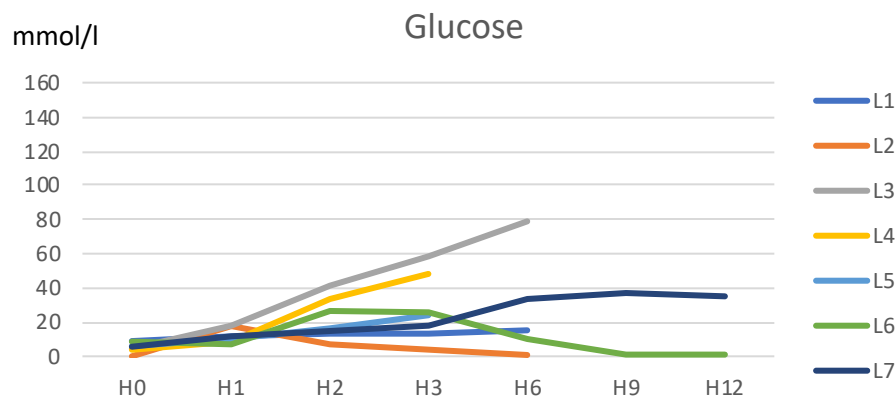
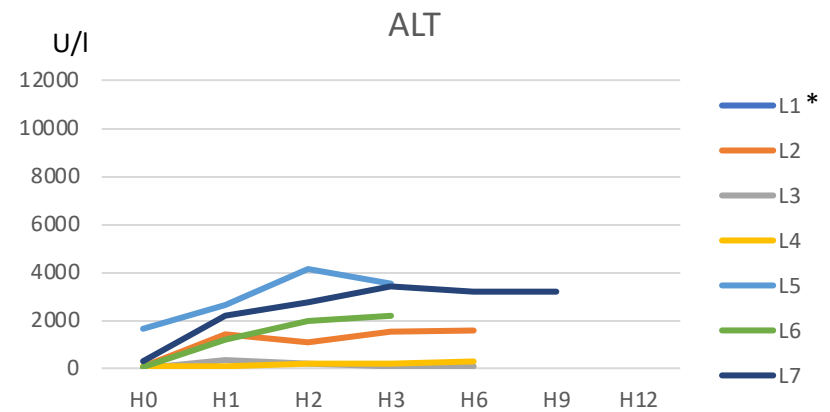
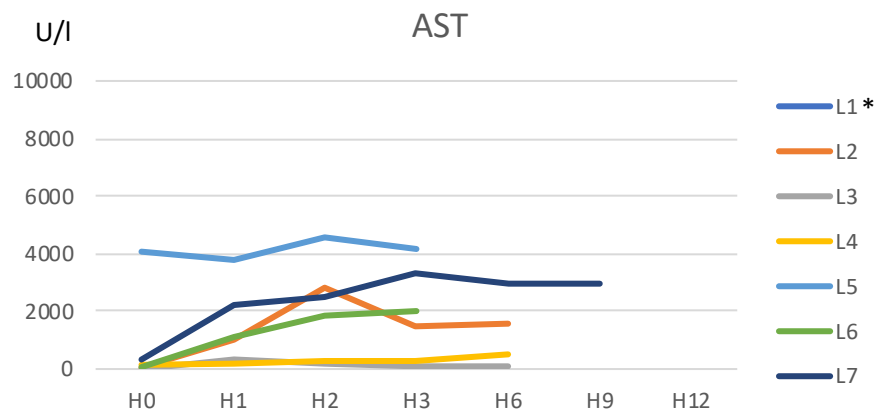
Figure 3. Perfusion protocol for marginal grafts using the Pitié-Salpêtrière perfusion circuit

References

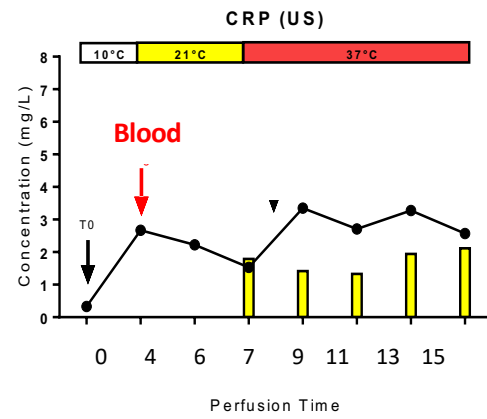
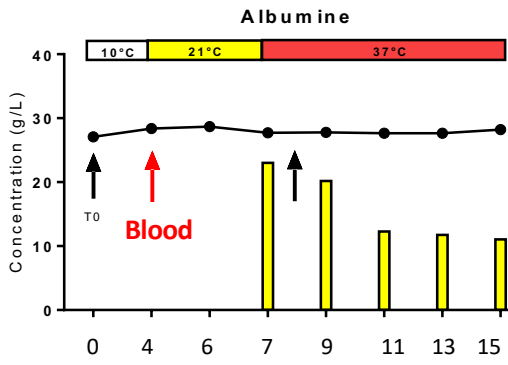
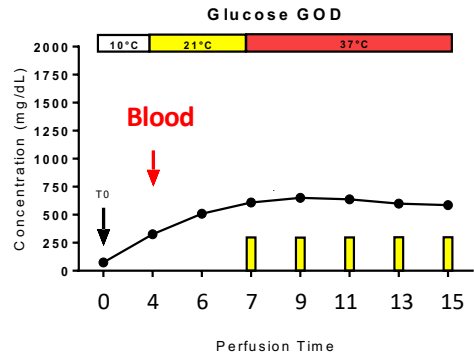
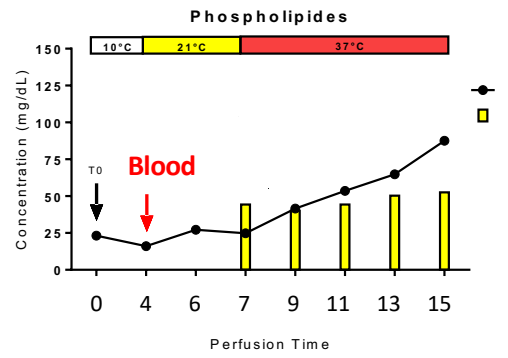
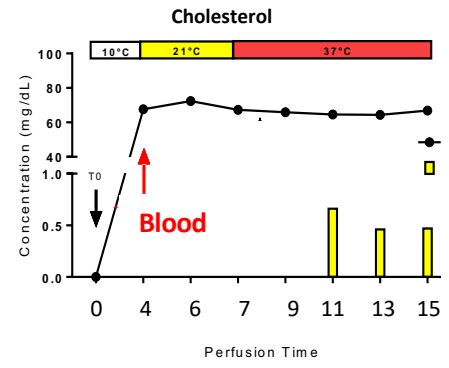
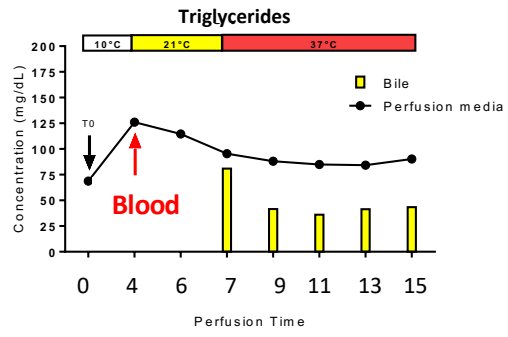
1. Nasralla, D. *et al.* A randomized trial of normothermic preservation in liver transplantation. *Nature* **557**, 50–56 (2018).
2. Boteon, Y. L., Afford, S. C. & Mergental, H. Pushing the Limits: Machine Preservation of the Liver as a Tool to Recondition High-Risk Grafts. *Curr. Transplant. Rep.* **5**, 113–120 (2018).
3. Czigany, Z. *et al.* Machine perfusion for liver transplantation in the era of marginal organs-New kids on the block. *Liver Int. Off. J. Int. Assoc. Study Liver* **39**, 228–249 (2019).
4. de Meijer, V. E., Fujiyoshi, M. & Porte, R. J. Ex situ machine perfusion strategies in liver transplantation. *J. Hepatol.* **70**, 203–205 (2019).
5. van Leeuwen, O. B. *et al.* Transplantation of High-risk Donor Livers After Ex Situ Resuscitation and Assessment Using Combined Hypo- and Normothermic Machine Perfusion: A Prospective Clinical Trial. *Ann. Surg.* (2019) doi:10.1097/SLA.0000000000003540.
6. de Vries, Y. *et al.* Transplantation of high-risk donor livers after resuscitation and viability assessment using a combined protocol of oxygenated hypothermic, rewarming and normothermic machine perfusion: study protocol for a prospective, single-arm study (DHOPE-COR-NMP trial). *BMJ Open* **9**, e028596 (2019).
7. Schlegel, A., Muller, X. & Dutkowski, P. Hypothermic Machine Preservation of the Liver: State of the Art. *Curr. Transplant. Rep.* **5**, 93–102 (2018).
8. Allard, M.-A. *et al.* Suitability of livers for transplantation when treated by normothermic machine perfusion. *Clin. Transplant.* **32**, e13256 (2018).
9. Boteon, Y. L. *et al.* Manipulation of Lipid Metabolism During Normothermic Machine Perfusion: Effect of Defatting Therapies on Donor Liver Functional Recovery: Liver Transplantation. *Liver Transpl.* **25**, 1007–1022 (2019).
10. Boteon, Y. L. *et al.* Combined Hypothermic and Normothermic Machine Perfusion Improves Functional Recovery of Extended Criteria Donor Livers. *Liver Transplant. Off. Publ. Am. Assoc. Study Liver Dis. Int. Liver Transplant. Soc.* (2018) doi:10.1002/lt.25315.
11. Boteon, Y. L. *et al.* The impact on the bioenergetic status and oxidative-mediated tissue injury of a combined protocol of hypothermic and normothermic machine perfusion using an acellular haemoglobin-based oxygen carrier: The cold-to-warm machine perfusion of the liver. *PLoS One* **14**, e0224066 (2019).

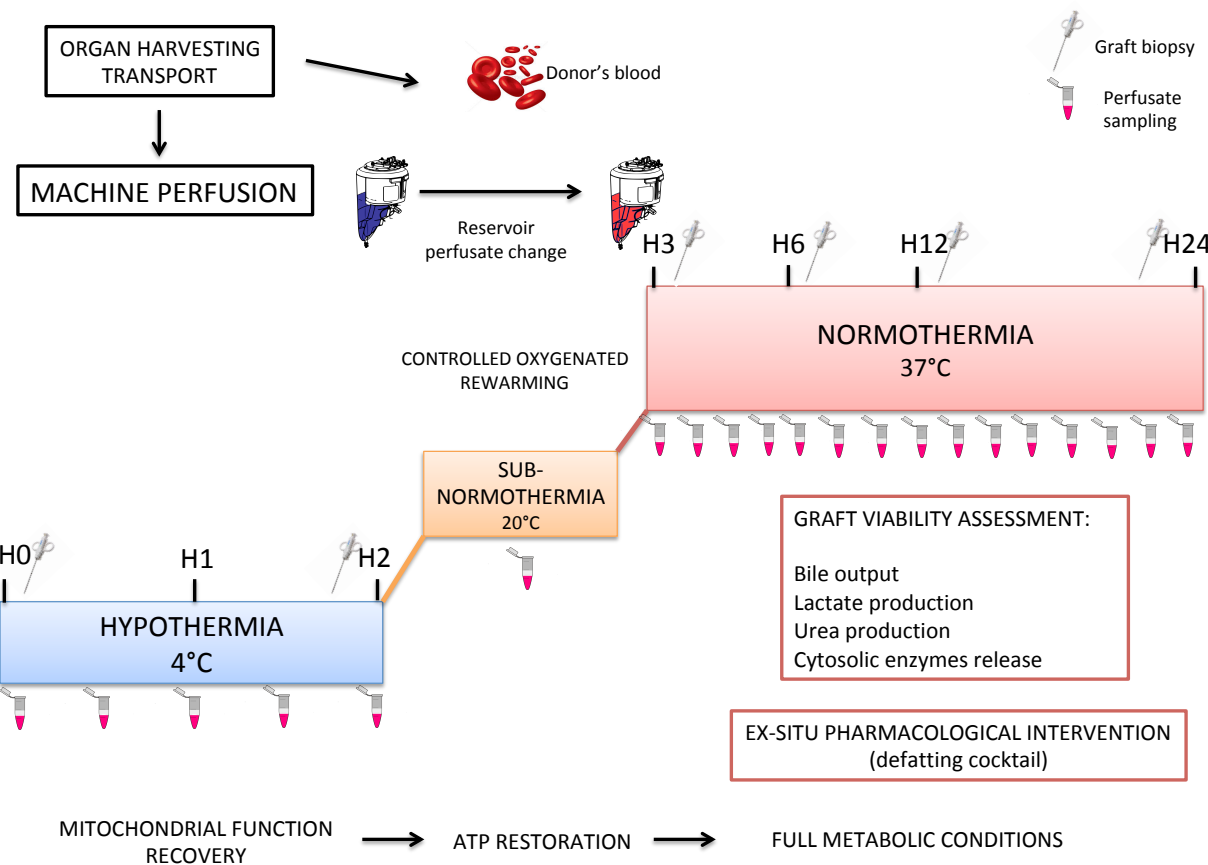






* Missing values for technical reasons





ORGAN HARVESTING TRANSPORT

MACHINE PERFUSSION

Donor's blood

Reservoir perfusate change

Graft biopsy
Perfusate sampling

H3

H6

H12

H24

NORMOTHERMIA
37°C

CONTROLLED OXYGENATED REWARMING

HYPOTHERMIA
4°C

H0

H1

H2

SUB-NORMOTHERMIA
20°C

GRAFT VIABILITY ASSESSMENT:

- Bile output
- Lactate production
- Urea production
- Cytosolic enzymes release

EX-SITU PHARMACOLOGICAL INTERVENTION (defatting cocktail)

MITOCHONDRIAL FUNCTION RECOVERY

ATP RESTORATION

FULL METABOLIC CONDITIONS