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Preliminary Single-Center Canadian Experience of Human Normothermic *Ex Vivo* Liver Perfusion: Results of a Clinical Trial

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After extensive experimentation, outcomes of a first clinical normothermic machine perfusion (NMP) liver trial in the United Kingdom demonstrated feasibility and clear safety, with improved liver function compared with standard static cold storage (SCS). We present a preliminary single-center North American experience using identical NMP technology. Ten donor liver grafts were procured, four (40%) from donation after circulatory death (DCD), of which nine were transplanted. One liver did not proceed because of a technical failure with portal cannulation and was discarded. Transplanted NMP grafts were matched 1:3 with transplanted SCS livers. Median NMP was 11.5 h (range 3.3–22.5 h) with one DCD liver perfused for 22.5 h. All transplanted livers functioned, and serum transaminases, bilirubin, international normalized ratio, and lactate levels corrected in NMP recipients similarly to controls. Graft survival at 30 days (primary outcome) was not statistically different between groups on an intent-to-treat basis ($p = 0.25$). Intensive care and hospital stays were significantly more prolonged in the NMP group. This preliminary experience demonstrates feasibility as well as potential technical risks of NMP in a North American setting and highlights a need for larger, randomized studies.

Abbreviations: AKI, acute kidney injury; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CIT, cold ischemia time; DBD, donation after brain death; DCD, donation after circulatory death; DRI, donor risk index; EAD, early allograft dysfunction; ECD, extended criteria donor; GDA, gastroduodenal artery; HA, hepatic artery; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HTK, histidine–tryptophan–ketoglutarate; ICU, intensive care unit; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; MRCP, Magnetic resonance cholangiopancreatography; NASH, nonalcoholic steatohepatitis; NMP, normothermic machine perfusion; OLTx, Orthotopic liver transplantation; PNF, primary nonfunction; POD, postoperative day; SCS, static cold storage; SD, standard deviation; SMA, superior mesenteric artery; T-Bilirubin, total bilirubin; UW, University of Wisconsin; WIT, warm ischemia time

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Introduction

With a critical shortage of donor liver organs across transplant centers, programs have increasingly resorted to using organs from extended criteria donors (ECDs) and donation after circulatory death (DCD) or from living liver donation to expand the limited donor pool. Standard static cold storage (SCS) fails to provide optimal preservation of DCD and ECD grafts, often resulting in early allograft dysfunction (EAD) and potential for long-term complications (1–3). It has long been recognized that prolonged cold storage of marginal and DCD livers further exacerbates cellular damage and markedly increases rates of ischemia–reperfusion, EAD, primary nonfunction (PNF), and ischemic cholangiopathy (4). As an alternative preservation strategy, *ex vivo* normothermic machine perfusion (NMP) proffers the potential of (i) extended-duration liver preservation; (ii) optimized liver function; (iii) ability to measure dynamic viability during the *ex vivo* phase, which is not possible with SCS; and (iv) the potential to resuscitate compromised livers through delivery of targeted additives (5,6).

Alternative approaches to machine perfusion have included hypothermic (7,8), subnormothermic (9–11), and normothermic perfusion (12–14). Initial clinical *ex vivo* liver perfusions involved cold oxygenated *ex vivo* perfusion of livers with positive outcomes (15,16), followed more

recently by warmer perfusate approaches (17–19). Large animal studies demonstrated consistently that *ex vivo* NMP technology can protect injured livers through elimination of prolonged SCS (14,20–22) with clinical translation of these studies following in rapid succession. Recently, novel portable normothermic *ex vivo* technology has been developed, and a first in-human pilot trial using portable NMP technology (OrganOx *metra*; OrganOx Ltd., Oxford, U.K.*)* was completed in the United Kingdom, demonstrating feasibility and safety (23). Ravikumar et al showed that NMP livers had lower median peak aspartate aminotransferase (AST) levels compared with matched SCS controls, suggesting enhanced hepatic protection.

In collaboration with the University of Oxford and OrganOx Ltd., we commenced our own investigator-initiated first-in–North America pilot clinical trial of liver NMP in Edmonton, Canada, and report on our preliminary experience with the first 10 cases.

Methods

Study design

From February to December 2015, a phase I nonrandomized pilot study was performed at the University of Alberta to evaluate outcomes of clinical livers perfused with NMP. The study was approved by the Health Research Ethics Board at the University of Alberta and by Health Canada (IRB 00043239, ID Pro00043239). All potential NMP recipients gave consent at the time of initial assessment, ahead of transplantation, and affirmed consent at the time of admission for transplantation. The primary objective was to assess safety of NMP in continuous liver preservation, with the primary outcome measure being graft survival at day 30 after transplant. Secondary outcomes included (i) patient survival at day 30; (ii) peak serum transaminase AST in the first 7 days; (iii) EAD incidence in the first 7 days (24); (iv) liver biochemistry in serum on days 1–7, 10, and 30; (v) major complications defined by a Clavien-Dindo score ≥3 (25); (vi) patient and graft survival at 6 mo; and (vii) biliary complications at 6 mo. Outcomes were compared 1:3 with matched control liver transplant recipients with conventional SCS grafts. Control participants were selected from a pool of 150 adult deceased donor liver transplants at the University of Alberta over the previous 36 mo based on closest matching for (i) recipient Model for End-Stage Liver Disease (MELD) score, (ii) donor risk index (DRI), (iii) donor age, (iv) recipient age, and (v) graft type (donation after brain death [DBD] vs. DCD).

Deceased donors (≥40 kg in weight) were included in both groups. All DCD livers were procured with <30 min of warm ischemia. Living donors and livers intended for split transplant were excluded. Recipient participants with end-stage chronic nonfulminant liver disease were included. Patients undergoing retransplantation or transplantation of other organs were excluded. OrganOx Ltd. provided onsite training in Edmonton, and members of the surgical team also participated in clinical NMPs carried out in the United Kingdom as part of an ongoing randomized controlled trial (ISRCTN39731134). Allocation of livers to the NMP group was primarily determined by availability of the perfusion team coupled with prior recipient consent (see Figure S1).

Normothermic machine perfusion

Livers allocated to the intervention group were preserved using the NMP system (Organ Ox *metra*), as described recently (Figure S2) (23). Briefly, the OrganOx *metra* was calibrated and primed once a suitable liver graft was deemed acceptable for transplantation. All organ procurements were completed locally within Edmonton city hospitals, and the OrganOx *metra* was initiated at the donor center. Machine perfusate consisted of 500 mL of Gelofusine (B. Braun, Melsungen, Germany) and 3 U of type O packed red blood cells. All perfusate additives were otherwise identical to those described by Ravikumar et al (23). Sodium bicarbonate 30 mL 8.4% (Hospira, Montreal, Canada) was added as needed to maintain pH between 7.35 and 7.45. A full description of the OrganOx *metra* perfusate composition is listed under Appendix S1.

All livers were procured in standard fashion, flushed *in situ* with histidine–tryptophan–ketoglutarate solution (HTK; Custodiol HTK; Methapharm Inc., Brantford, Ontario, Canada), prepared, and cannulated on a "back table" at the donor hospital. Prior to perfusion, liver grafts 1–4 were primed only with HTK on the back table. Livers 5–9 were flushed additionally with 1 L Gelofusine and 500 mL 5% human albumin (Plasbumin-5; Grifols Therapeutics Inc., Mississauga, Ontario, Canada) prior to NMP, with the intention of washing out carryover of intrahepatic HTK to the perfusion circuit. A notable difference between the Canadian and U.K. studies was the use of HTK versus University of Wisconsin (UW) solution for donor vascular flush.

Metabolic parameters

Liver grafts were monitored during NMP with interval determination of blood gases (pH, lactate), alanine aminotransferase (ALT), AST, and total bilirubin at perfusion start and every 2 h thereafter. Blood glucose was manually entered every 4 h, and the Nutriflex (B. Braun) infusion was automatically adjusted by the device accordingly. Liver perfusion quality was documented by variation in perfusate pH, lactate concentration, and perfusion vascular stability and by hourly bile production. Once recipient hepatectomy was completed, the NMP was discontinued. To comply with accepted clinical practice, all liver grafts were further flushed with cold HTK solution immediately before being brought into the surgical field.

Intraoperative management

Surgical implantation techniques were identical between NMP and SCS groups. All livers were transplanted with standard caval replacement, without bypass or temporary portocaval shunt. The incidence of reperfusion syndrome, defined by a decrease in mean arterial pressure of >30% from baseline lasting >1 min during the first 5 min after reperfusion, was documented only in the NMP group (26).

Posttransplant care

Posttransplant care in both NMP and SCS groups was performed following standard protocols including tacrolimus-based immunosuppression, as appropriate, and sirolimus if preexisting renal dysfunction was present (27). Five of nine NMP liver grafts were deemed to have EAD, but only one case was due to a factor other than peak AST, specifically, elevated bilirubin on day 7. All participants who received DCD liver grafts underwent magnetic resonance cholangiopancreatography (MRCP) at 6 mo to rule out the presence of nonanastomotic biliary strictures secondary to ischemic cholangiopathy (28).

Statistical analysis

Data are represented as medians and ranges and as means plus or minus standard deviations as necessary. The Mann–Whitney *U* test, and two-way analysis of variance with Bonferroni multiple comparisons were used to analyze differences between continuous variables. The Fisher exact test was used to compare proportions between groups for categorical outcomes. Overall comparisons between NMP and SCS groups was performed with a 95% confidence interval. A p-value <0.05 was considered significant, and all analyses were performed using Stata (StataCorp, College Station, TX) and GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

Results

Donor characteristics

In total, 10 liver grafts were procured, nine of which were successfully perfused using NMP and transplanted. Four grafts (40%) were from DCD donors (Maastricht category III), and six (60%) were from DBD donors. One liver from a 60-year-old DCD donor was procured and cannulated for NMP but was promptly discarded because of an occult portal venous twist that retracted into the liver hilum, preventing perfusion (Video S1). The median donor age was 56 years (range 14–71 years) in the NMP group versus 52 years (range 20–77 years) in SCS controls ($p = 0.91$) (Tables 1 and 2). Back-table reconstruction of aberrant hepatic arterial vasculature was completed if present (three of 10, 30%) before perfusion to ensure that all liver segments received adequate arterial inflow once warmed. Temporary iliac arterial extension grafts were added in the last three cases to minimize arterial desiccation surrounding the cannula tip (Table 1, Figure S3). The median DRI was 1.6 (range 0.92–2.66) in the NMP group versus 1.6 (range 0.95–2.71) in SCS controls ($p = 0.82$). A summary comparison of donor characteristics between NMP and SCS control grafts is provided in Table 2 and demonstrates no significant differences between variables.

Normothermic machine perfusion

The OrganOx *metra* was primed concomitantly with donor procurement surgery; NMP was established in all but one case. Mean hemoglobin concentration of the perfusate was 104 ± 18 g/L, and mean hematocrit was 32 ± 5%. For the nine livers perfused successfully, there were no further technical complications during NMP. Median NMP time was 11.5 h (range 3.3–22.5 h). NMP duration was determined by the recipient surgeon based on logistics surrounding the transplant (Table 1). Graft 9, procured from a DCD donor, underwent NMP for 22.5 h prior to implantation and recovered normal function after a 1-mo period of gradually improving cholestasis.

Hepatic transaminases (AST and ALT) in the recirculating NMP *ex vivo* perfusate circuit rose progressively in all cases. Perfusate AST was notably higher for DCD compared with DBD livers ($p < 0.001$). During the longest perfusion (22.5 h), AST levels on circuit peaked at 15 009 IU (Figure 1A), but the terminal perfusion lactate was 1.0 mmol/L; this graft functioned appropriately in the recipient. Most perfusions required occasional supplemental sodium bicarbonate to maintain physiological pH, but all grafts cleared lactate rapidly while on circuit (Figure 1B) ($p = 0.20$). During NMP, total bilirubin levels increased in all cases (Figure 1C), with higher levels in DCD livers ($p = 0.002$). Total bile output for all perfused livers occurred at a median rate of 6.2 mL/h (range 1.9–32.2 mL/h). Bile output was lower for NMP DCD livers, with a median of 4.0 mL/h (range 1.9–16.7 mL/h) versus NMP DBD livers with a median of 10.0 mL/h (range 1.9–32.2 mL/h) ($p < 0.001$) (Figure 1D). All NMP grafts demonstrated stable portal vein and hepatic artery flow rates. Histological samples were not obtained routinely and would have been helpful.

Recipients

In total, nine participants underwent transplantation with livers perfused by NMP. Transplant indications and preoperative MELD are shown in Table 1. Median recipient MELD was 13 (range 9–32) in the NMP group versus 19 (range 7–34) in the SCS group ($p = 0.37$). Median recipient age was 53 years (range 28–67 years) in the NMP group versus 59 years (range 43–69 years) in the SCS group ($p = 0.28$) (Table 3). There were no significant differences between recipient characteristics in the NMP versus control SCS groups (Table 3).

On an intent-to-treat basis, the primary outcome measure of 30-day graft survival was not significantly different between groups (nine of 10 NMP vs. 30 of 30 SCS, $p = 0.25$). The 30-day transplanted patient survival was equivalent (100%) in both groups (Table 3). Six-month graft survival, a secondary outcome measure, was also not significantly different (eight of 10 NMP vs. 30 of 30 SCS, $p = 0.06$) (Table 3). No PNF was observed in either NMP or SCS groups (29). Incidence of EAD in NMP livers was five of nine (55.5%) compared with eight of 27 (29.6%) for SCS controls ($p = 0.23$). EAD was caused principally by elevated transaminases in the initial 24 h and resolved promptly without other markers of graft dysfunction (Figure 2). There were no cases of postreperfusion syndrome in NMP grafts. NMP graft recipients had substantially longer median intensive care unit (ICU) stay: 16 days (range 2–65 days) versus 4 days (range 1–29 days) in SCS controls ($p = 0.004$) (Table 3). Median hospital stay in the NMP group was also much longer: 45 days (range 13–114 days) versus 25 days (range 9–89 days) in SCS controls ($p = 0.01$) (Table 3).

Posttransplant liver function

There was no statistical difference between peak AST levels within the first 7 days in NMP versus SCS preserved grafts ($p = 0.52$) (Table 3). Overall, there was no statistical difference between posttransplant AST trends in both groups ($p = 0.24$) (Figure 2A). There was no difference in bilirubin levels between groups on day 7 ($p = 0.35$) (Table 3) and globally ($p = 0.17$) (Figure 2B). Posttransplant alkaline phosphatase levels were similar in recipients of NMP and SCS grafts ($p = 0.82$) (Figure 2C). Comparison of coagulation parameters demonstrated stable, normal, uncorrected international normalized ratio (INR) synthetic function in both groups ($p = 0.63$) (Figure 2D). Arterial lactate was not significantly different between groups and normalized within 1 day ($p = 0.07$) (Figure 2E).

Table 1: Donor and recipient characteristics for NMP liver grafts

Participant no.	Donor age, y	Donor cause of death	Type	WIT, DCD (min)	CIT (min)	Notes/anatomical variations	NMP time (h:min)	Recipient age, y	Indication for transplant	MELD	Posttransplant complications
1	14	Asphyxiation	DCD	20	208	Replaced right HA; right HA onto GDA	12:15	69	HBV + HCC	9	EAD, laparotomy POD 2 for intra-abdominal bleed
2	19	Head trauma	DBD	–	192	–	6:51	43	Autoimmune	13	–
3	38	Head trauma	DBD	–	95	–	11:31	50	Autoimmune	29	EAD
4	35	Asphyxiation	DCD	16	108	–	3:16	62	HCV + alcohol	16	EAD
5	60	Brain stem stroke	DCD	26	130	Liver discarded due to portal vein torsion on *metra* plus age/warm ischemia/ operating room team availability	–	–	–	–	–
6	56	Intracranial hemorrhage	DBD	–	150	Start of back-table albumin flush	15:52	50	NASH	12	–
7	71	Intracranial hemorrhage	DBD	–	184	–	8:14	54	NASH	11	–
8	61	Subdural hematoma	DBD	–	293	Left accessory HA, right replaced HA; right HA onto GDA; temporary iliac extension	7:41	53	HCV + HCC	11	EAD, AKI requiring hemodialysis, death secondary to untreated HCV
9	56	Pulmonary fibrosis	DCD	23	284	Complete occlusion of celiac origin with replaced right HA; donor left HA onto right gastric origin, single SMA inflow; temporary iliac extension	22:27	62	HCV	26	EAD
10	59	Intracranial hemorrhage	DBD	–	129	Temporary iliac extension	12:49	46	NASH	32	–

DCD donors denoted in gray shading. AKI, acute kidney injury; CIT, cold ischemia time; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; GDA, gastroduodenal artery; HA, hepatic artery; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; NMP, normothermic machine perfusion; POD, postoperative day; SMA, superior mesenteric artery; WIT, warm ischemia time.

Table 2: Summary of donor and graft characteristics

	NMP (n = 10)	SCS (n = 30)	p-value
Donor age, y, median (range)	56 (14–71)	52 (20–77)	0.91
Donor risk index, median (range)	1.6 (0.92–2.66)	1.6 (0.95–2.71)	0.82
Donor BMI, median (range)	27.5 (19.6–36.7)	25.6 (18.8–34.1)	0.34
Cold ischemia time, min, median (range)	167 (95–293)	233 (64–890)	0.09
DCD donor proportion	4/10 (40%)	8/30 (27%)	0.45

DCD, donation after circulatory death; NMP, normothermic machine perfusion; SCS, static cold storage.

Complications

After transplant, there was no hepatic arterial or portal vein thrombosis in either group, as determined by serial ultrasound Doppler interrogation. Comparing Clavien-Dindo scores ≥3, there was no significant difference in major complication rate in the initial 30 days between groups (two of nine (22%) NMP vs. 10 of 27 (37%) SCS controls, p = 0.69) (Table 3). In the NMP group, complications included one reoperation for intra-abdominal bleeding and one patient with renal insufficiency requiring transient hemodialysis. One NMP graft (participant 8) developed early aggressive cholestatic hepatitis secondary to uncontrolled recurrent hepatitis C infection; the recipient was unable to access preemptive sofosbuvir and ledipasvir and died at 3 mo after transplant. Six-month patient survival for transplanted grafts was not statistically different between groups (eight of nine NMP vs. 27 of 27 SCS, p = 0.25) (Table 3).

Biliary reconstructions in NMP grafts were duct to duct in five of nine (56%) cases and Roux-en-Y hepaticojejunostomy in the remainder. All eight surviving participants reached 6 mo of follow-up with no biliary complications in NMP livers compared with four of 27 (14.8%) in SCS controls (p = 0.25), as assessed by liver function, ultrasound, and MRCP (Table 3). There was no ischemic cholangiopathy or anastomotic structuring by MRCP in any recipients in the NMP group.

Discussion

Friend and colleagues recently partnered with a spin-off company associated with the University of Oxford, OrganOx Ltd., to fully automate, miniaturize, and make portable an NMP liver system called the OrganOx *metra*. Their clinical experience in 20 initial livers undergoing NMP on the OrganOx *metra* and subsequently transplanted at King's College Hospital London and University Hospital Birmingham demonstrated NMP technology to be safe and feasible (23). When outcomes were compared with those of recipients of control SCS grafts, AST in recipients of NMP livers was significantly lower (23). In a recent, similar study using identical technology, Selzner et al from the University of Toronto perfused 12 liver grafts on NMP using Steen solution in place of Gelofusine to prime, with transplanted graft outcomes comparable to SCS controls. No differences in postoperative graft function, duration of ICU stay, or posttransplant hospital stay were observed compared with SCS. NMP grafts had lower AST and ALT levels on days 1–3, without reaching statistical significance (30).

Using the OrganOx *metra* technology, we were the first in North America to successfully perfuse and clinically transplant a liver with NMP on February 19, 2015. Our findings are notable, as one liver intended for NMP perfusion was discarded because of unrecognized operator error. Furthermore, in contrast to Ravikumar et al and Selzner et al, we did not observe significant differences in early recipient transaminases between NMP and SCS preserved grafts (23,30). Our median early peak AST in NMP grafts was higher (1252 IU, range 383 to >2600 IU) than that observed in the Oxford/U.K. pilot study (417 IU, range 84–4681 IU), but our proportion of NMP DCD donors was also higher (44% Edmonton vs. 20% U.K. vs. 20% Toronto) (23,30). ICU and total hospital stay was also more prolonged for recipients of NMP livers in our study.

In addition to our increased proportion of DCD donors, we frequently prolonged the NMP period for logistical convenience (Figure S4). Furthermore, our cold ischemic period before initiation of NMP (Tables 1 and 2) was prolonged, mostly due to a higher proportion of complex back-table vascular reconstructions, potentially limiting the potential benefit of NMP (31). The median cold ischemia time of our control livers was notably shorter (3.9 h, range 1.07–14.8 h) than that reported in the study by Ravikumar et al (8.9 h, range 4.2–11.4 h) (23). We cannot discount the effect of the use of different initial cold vascular flush preservation solution (HTK in Canada vs. UW solution in the United Kingdom). Although, theoretically, this should not contribute to graft injury, the potential for carryover of HTK to the warm NMP perfusate may have been additive to injury, which we attempted to correct in our more recent cases, without obvious detectable effect. Nonetheless, all transplanted livers demonstrated equivalent survival at 30 days compared with SCS controls.

Markers of cholestasis were nonsignificantly elevated within the initial 14 days after transplant in recipients of NMP grafts. Despite this, no NMP grafts had late biliary complications detected by MRCP (zero of eight at 6 mo). In the Oxford/U.K. series, biliary strictures occurred in four of

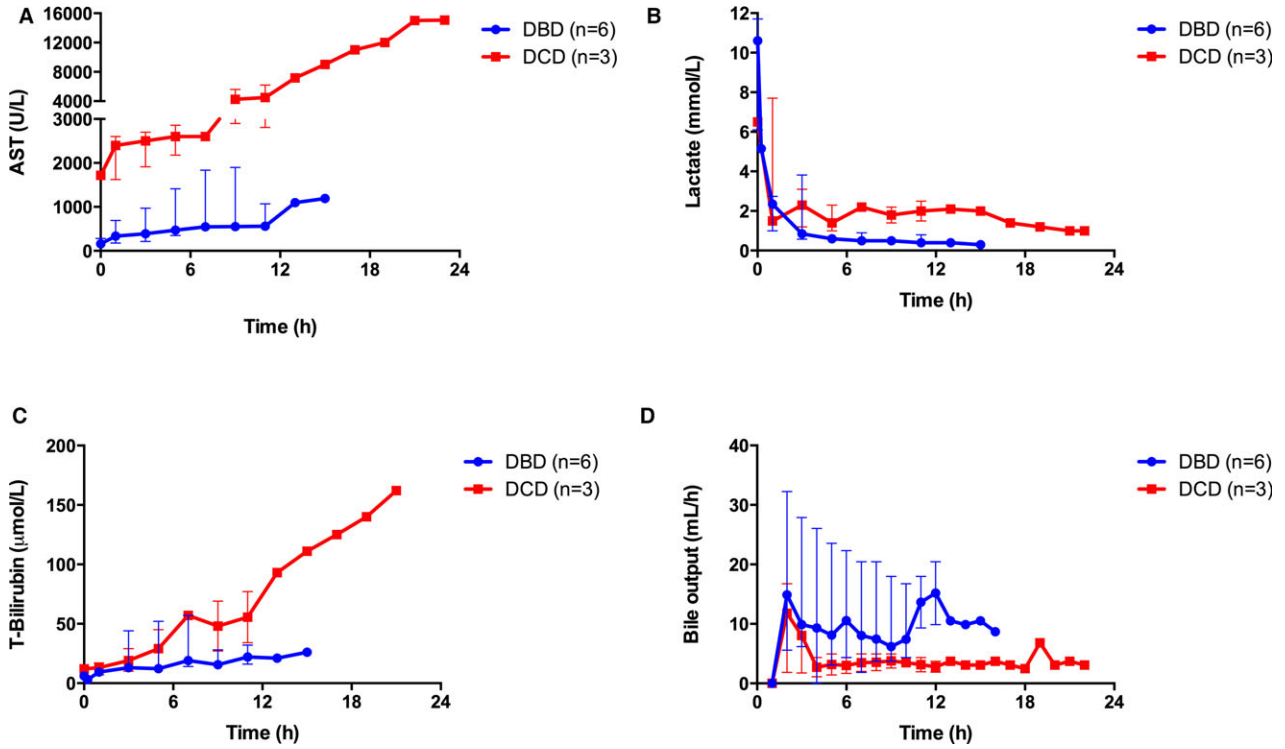

Figure 1: *Ex vivo* circulating perfusate liver biochemistry over time during NMP. (A) Circulating AST perfusate levels during NMP, separated by donor type (DBD vs. DCD) ($p < 0.001$; 95% CI 1760–10 636). (B) Circulating perfusate lactate levels during NMP, separated by donor type (DBD vs. DCD; $p = 0.20$; 95% CI −0.91 to 1.6). (C) *Ex vivo* circulating perfusate total bilirubin levels of liver grafts during NMP, separated by donor type (DBD vs. DCD; $p = 0.002$; 95% CI 10–114). (D) Bile output for all livers during NMP, separated by donor type (DBD vs. DCD; $p < 0.001$; CI −8.3 to −6.0). Data points show medians and ranges, 95% CI. AST, aspartate aminotransferase; CI, confidence interval; DBD, donation after brain death; DCD, donation after circulatory death; NMP, normothermic machine perfusion; T-Bilirubin, total bilirubin.

20 cases (20%) by 6 mo in NMP grafts, possibly reflecting a lower predilection for Roux-en-Y hepaticojejunostomy in that series. We used Roux biliary reconstructions in 44% of cases, largely because of donor–recipient duct size mismatch, which may have mitigated this risk.

ICU and total hospital stays were substantially prolonged in the NMP group compared with the SCS controls in the present study, and this is concerning. Although we largely attribute this to patient and concurrent disease-related factors, we can neither exclude nor confirm any association with use of NMP. Cost savings in terms of both ICU and total hospital stays will be an important measure in future adoption and reimbursement of NMP technology and requires a randomized controlled trial to further clarify the costs and benefits.

The loss of a potentially transplantable liver graft occurred in our fifth perfusion. A DCD liver from a donor aged 60 years was discarded as a result of inadvertent operator error. There was an unrecognized twist in the donor portal vein that was hidden above tissues of the hilar plate (Video S1). Despite confirming that the cannula tip was sited within the mid–portal vein and checking that the portal tubing was not kinked, and despite completely disconnecting and reconnecting the tubing circuits, we still failed to recognize this error. In retrospect, the problem was detected and the operator notified immediately by means of the low portal flow alarm on the device; however, the operator did not identify and resolve the precise problem until after ≈10 min because the twist was obscured by surrounding tissue within the hilum of the liver. Although the compromise to the liver due to interruption of portal flow was considered modest, the implanting surgeon felt that, on aggregate, the liver should be discarded because the donor organ was marginal. Recurrence of this problem should be prevented by specific training, as has been implemented by OrganOx.

The current study provides an analysis of perfusate biochemistry of human livers during NMP, including perfusate transaminases, lactate, total bilirubin, and bile production. We found that circulating transaminase leak increased progressively over time, as did circulating bilirubin, with markedly elevated levels in NMP perfusates from DCD grafts. Similarly, bile output of DCD livers was less than that of

Table 3: Outcome comparisons between NMP and SCS control liver transplant recipients

Outcomes	NMP	SCS	p-value
Intent-to-treat graft survival, n	10	30	
30-day graft survival (primary outcome), intent to treat	9/10 (90%)	(30/30) (100%)	0.25
6-mo graft survival, intent to treat	8/10 (80%)	(30/30) (100%)	0.06
Recipient outcomes for transplanted grafts per protocol, n	9	27	
Total graft preservation time, min, median (range)	786 (304–1631)	235 (64–890)	<0.001
Recipient age, y, median (range)	53 (28–67)	59 (43–69)	0.28
Recipient MELD, median (range)	13 (9–32)	19 (7–34)	0.37
Peak AST, days 1–7, U/L, median (range)	1252 (383 to >2600)	839 (153 to >2600)	0.52
Bilirubin day 7, median (range)	79 (17–344)	53 (8–340)	0.35
INR day 7, median (range)	1.1 (1.1–1.6)	1.1 (0.9–1.5)	0.44
PNF	0/9 (0%)	0/27 (0%)	–
EAD	5/9 (55.5%)	8/27 (29.6%)	0.23
DBD	2/6 (33%)	3/20 (15%)	0.56
DCD	3/3 (100%)	5/7 (71%)	0.30
ICU stay, days, median (range)	16 (2–65)	4 (1–29)	0.004
Hospital stay, days, median (range)	45 (13–114)	25 (9–89)	0.01
Major complications (Clavien-Dindo ≥3)	2/9 (22%)	10/27 (37%)	0.69
6-mo biliary complications	0/8 (0%)	4/27 (14.8%)	0.55
30-day patient survival per protocol	9/9 (100%)	27/27 (100%)	–
6-mo patient survival per protocol	8/9 (89%)	27/27 (100%)	0.25

AST, aspartate aminotransferase; DBD, donation after brain death; DCD, donation after circulatory death; EAD, early allograft dysfunction; ICU, intensive care unit; INR, international normalized ratio; MELD, Model for End-stage Liver Disease; NMP, normothermic machine perfusion; PNF, primary nonfunction; SCS, static cold storage.

DBD grafts. All NMP grafts demonstrated stable portal vein and hepatic artery flow rates. All of these livers were transplanted successfully and functioned adequately in recipients, as shown by postoperative normalizing lactate and INR. These data extend but differ from previous observations from large animal studies (14,22) and from perfusion studies of discarded human livers (32,33), in which both "on circuit" transaminases and bile output were deemed to be surrogate markers of graft viability. Our study was not designed or powered to shed light on this issue, which remains an unmet critical determinant for future assessment of graft viability in marginal livers.

Limitations of the present study included a nonrandomized design, a small cohort size, limited matching between NMP and SCS groups, and relatively short (6-mo) follow-up. We acknowledge an imbalance in selection and quality of liver donor organs between NMP and SCS controls, and although imperfect, the controls provided valuable comparative data for context and perspective. We further acknowledged potential for inclusion bias in the NMP group, with selection of participants with considerable comorbidities and other cofounders that could have detracted from the robustness of the study. The cold ischemic times taken to complete back-table preparation, cannulation, and complex arterial reconstructions were considerable (median 2 h 47 min) and likely offset the potential benefit of NMP technology in the present study. We anticipate that with greater experience, these times will become markedly curtailed.

A large prospective U.K./Europe phase III randomized controlled trial comparing NMP on OrganOx *metra* with SCS is completed but not yet published (ISRCTN14355416). This trial will provide a more complete and robust comparison of outcomes. It further remains to be established whether NMP technology will expand the limited liver donor pool in a safe manner or salvage livers that are routinely discarded presently. Development of reliable *ex vivo* predictive potency scores remains a priority to guide surgical teams when more extreme marginal grafts are preserved with NMP.

This study provides important groundwork for future trials that will explore the feasibility of this technology in expanding the currently limited donor pool.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. All clinical data were acquired as part of a regulated phase I clinical study, and none of the clinicians responsible for organ selection, organ perfusion, transplantation, and patient care (M.B., B.G., S.L., J.E., A.M., L.J.W., D.L.B., N.M.K., A.M.J.S.) have any conflicts of interest to disclose. Collaborative members of the OrganOx Ltd. team (L.R., C.C., P.F.) hold relevant patents, are involved with device manufacture, or receive consultancy support from OrganOx Ltd.

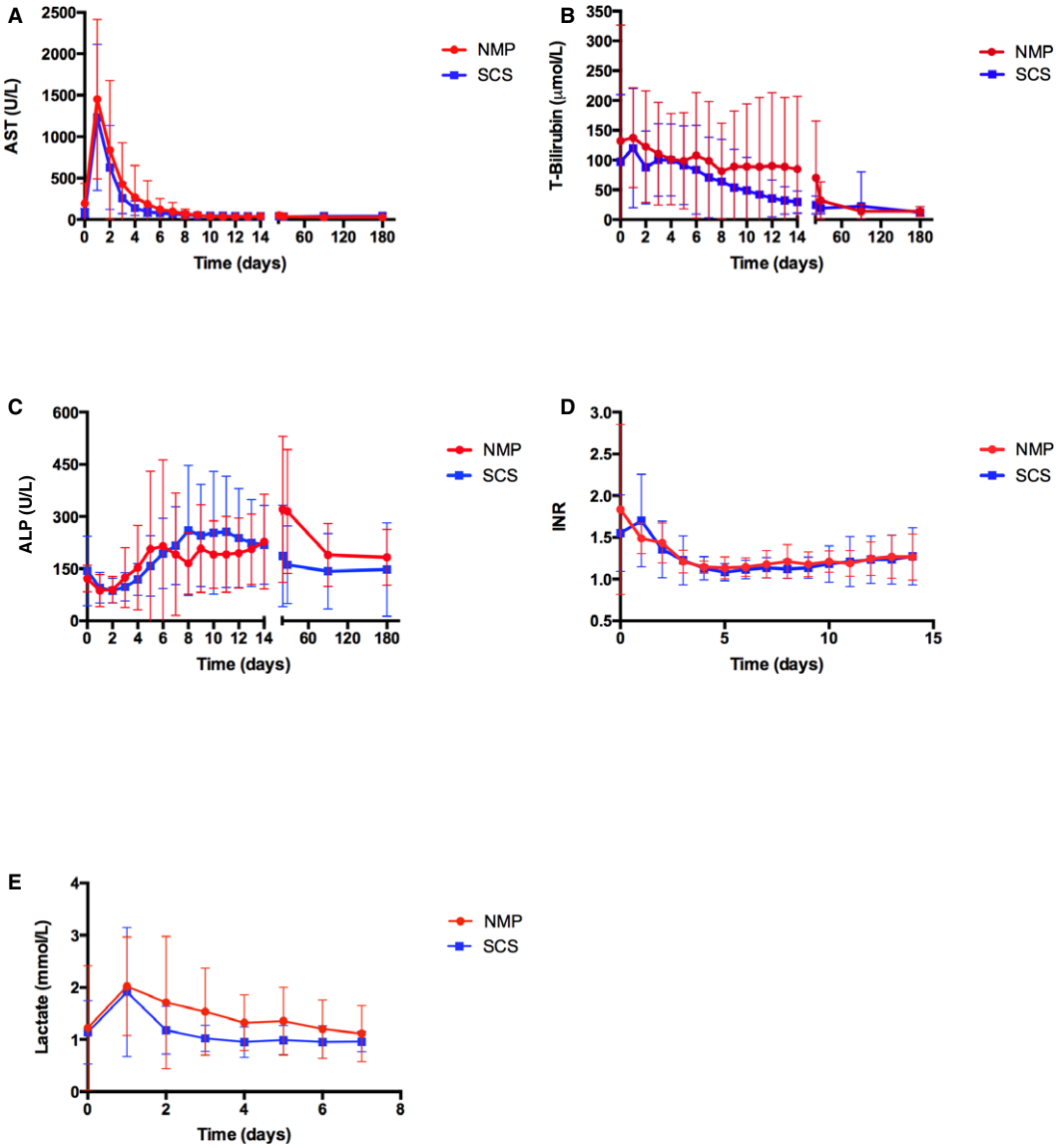

Figure 2: Posttransplant AST levels, markers of cholestasis, and posttransplant serum metabolic profiles. (A) Postoperative AST levels of graft recipients who received NMP and SCS livers. There was no statistical significance between recipients of NMP versus SCS control livers ($p = 0.24$). Data points show mean, SD, and 95% CI. (B) Postoperative total bilirubin levels of graft recipients who received NMP and SCS livers ($p = 0.17$). (C) ALP levels in graft recipients who received NMP and SCS livers ($p = 0.82$). Data points show mean, SD, and 95% CI. (D) Postoperative uncorrected INR levels of graft recipients who received NMP and SCS livers ($p = 0.63$). (E) Postoperative lactate levels of graft recipients who received NMP and SCS livers ($p = 0.07$). Data points show mean, SD, and 95% CI. ALP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval; INR, international normalized ratio; NMP, normothermic machine perfusion; SCS, static cold storage; SD, standard deviation; T-Bilirubin, total bilirubin.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Consort diagram demonstrating how livers were allocated to normothermic machine perfusion versus standard cold storage.

Figure S2: Schematic diagram of the OrganOx *metra* circuit.

Figure S3: Schematic diagram of iliac artery extension graft anastomosed to the donor graft celiac artery to prevent desiccation of hepatic artery during extended normothermic machine perfusion.

Figure S4: Timing logistics of normothermic machine perfusion (NMP) grafts. Purple bar, DCD withdrawal of life-support; green bar, donation after brain death (DBD) surgical retrieval period; blue bar, cold ischemia time (CIT) for liver preparation, complex microvascular reconstruction (denoted *Complex Recon*); red bar, duration of NMP; yellow bar, timing of liver transplantation (OLTx); white box, competing transplant surgical activity by the recipient team.

Appendix S1: Summarized protocol for priming of the OrganOx *metra*.

Video S1: Video of donor 5 demonstrating occult torsion of the proximal portal vein at the level of the hepatic bifurcation. This had retracted beneath tissues at the level of the hilar plate. The twist was not recognized despite a series of troubleshooting measures, and this liver (from a deceased circulatory death donor aged 60 years) was discarded because it otherwise fell outside of our local acceptance criteria at the time (upper age limit 55 years).