Trial Title: Defatting of donor transplant livers during normothermic perfusion – a randomised clinical trial

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Funder:	NIHR Efficacy and Mechanism Evaluation Awards (NIHR131163)

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Conflicts of Interest

Peter Friend and Constantin Coussios are co-founders and shareholders in OrganOx (a University of Oxford spinout company). They receive consultancy payments as non-executive medical and technical directors of the company.

Simon Knight, David Nasralla and Carlo Ceresa have received consultancy income from OrganOx for assisting with the design and conduct of previous trials.

Peter Friend, Leanne Hodson and Simon Knight are advisors to OchreBio. Carlo Ceresa receives consultancy income from OchreBio for assisting with the design and conduct of pre-clinical studies.

Peter Friend, Constantin Coussios and Simon Knight will be not be involved in approaching, consenting, recruiting, or in the clinical management of patients in the proposed trial (the Oxford Transplant Centre is not a liver transplant unit).

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, HRA, host organisation and members of the Research Ethics Committee unless authorised to do so.

Protocol signatures continued

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The undersigned has read and understood the trial protocol detailed above and agrees to conduct the trial in compliance with the protocol.

Principal Investigator (Please print name)	**Signature**	**Site name or ID number**	**Date**

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1. KEY TRIAL CONTACTS

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2. LAY SUMMARY

Liver disease is the third leading cause of premature death in the UK. Liver transplantation is the only successful treatment for end-stage liver disease but is limited by a shortage of suitable donor organs. As a result, up to 20% of patients on the NHS liver transplant waiting list die before receiving a lifesaving transplant.

However, a third of donated livers cannot be used for transplants. A frequent reason for this is the presence of fat within the liver cells (known as non-alcoholic fatty liver disease). This affects a third of the UK population and is commonest with obesity. As the incidence of obesity in the general population increases, donated organs are more likely to be fatty.

Transplanting a fatty liver carries a much greater risk to the patient compared to a normal liver. This is because fatty livers do not tolerate being cooled down, and we currently store organs in an ice-box before the transplant. An alternative new technology (normothermic machine perfusion; NMP) stores the liver in very similar conditions to those in the body: it maintains the liver at body temperature and provides oxygen and nutrition. We know that this preserves it in better condition, with less damage to liver cells: it also allows the surgeon to test how well the organ is working before deciding whether to carry out the transplant. However, whilst beneficial, NMP technology does not completely resolve the problem of fatty livers, because fat remains in the liver cells.

We have been testing a new way to remove fat from the liver during NMP. We add a combination of (currently available) drugs to release fat from liver cells, and we remove the fat from the perfusion machine using a filter. This reduces the amount of fat in the liver and improves its function. None of the livers treated in this experimental study were actually transplanted: if used for patients, we believe that this might increase the number of livers that could be transplanted safely.

In the proposed trial, we will randomly assign 60 livers from donors with a high risk of fatty liver disease to either NMP alone or NMP with fat removal treatment. We will assess how many of these livers are safe to transplant and, in those that are transplanted, follow the outcomes after the operation. The main objective is to show whether this treatment is safe; it will also help us to design a future, larger study which will test the extent to which fat removal actually leads to additional transplants.

Patients and their families have contributed in the design of the study and will be members of the committee that run it. They believe that the study is addressing an important issue, particularly in the context of the global obesity crisis and its consequent implications for liver transplantation. They have concluded that this area of research is of great significance in order to reduce waiting list deaths.

We plan to present the results of this 3-year project at national and international conferences and publish this research in high-impact journals. This will ensure that transplant teams around the world become aware of this treatment.

3. SYNOPSIS

Trial Title	Defatting of donor transplant livers during normothermic perfusion – a randomised clinical trial
Internal ref. no. (or short title)	The DeFat Study
Trial registration	ISRCTN number: 14957538
Sponsor	University of Oxford
Funder	NIHR Efficacy and Mechanism Evaluation Awards (NIHR131163)
Clinical Phase	Phase II
Trial Design	Randomised pilot study (2-arm, 1:1 allocation), with randomisation at point of organ inspection by a surgeon from the implanting team
Trial Participants	Liver offers will be made to participating centres through NHSBT offering system (as per standard practice). Liver offers accepted by each participating transplant centre will be screened for a high likelihood of fatty liver disease at each point of the donor pathway based on: (i) waist circumference (>88 females and >102cm males) or BMI >30kg/m^2 or both at point of acceptance (ii) evidence of macroscopic moderate-severe steatosis identified by the retrieval surgeon (or biopsy result) at point of retrieval. In addition, any liver offer fast tracked due to moderate-severe steatosis (based on appearance or biopsy result) will also be considered for enrolment (regardless of WC and/or BMI). The final entry criterion will occur at the point of inspection upon arrival at the transplant hospital: A surgeon from the implanting team will assess the liver to confirm its suitability for inclusion into the trial (based on macroscopic characteristics: colour, texture, rounded edges, size, weight). The objective of this second entry criterion is to reduce the number of false positive (non-fatty) livers enrolled in the trial. Randomisation will be undertaken by the trial co-ordinator (clinical research fellow) after inspection of the liver by a surgeon from the implanting team. Where available, the results of clinical biopsies demonstrating moderate-severe steatosis (typically >30%) will also be taken into account to assess suitability for randomisation.
Sample Size	60 randomised livers (30 per arm).
Planned Trial Period	Total study: 01/04/2021 – 01/04/2024 (36 months) Participant follow-up: 6 months and one-year follow-up data will be obtained from the NHSBT registry

Planned Recruitment period	01/04/2022 – 01/10/2023 (18 months)		
	Objectives	**Outcome Measures**	**Timepoint(s)**
Primary	To confirm the safety and assess efficacy of the NMP-defatting protocol in steatotic livers intended for transplant	1. The proportion of fatty livers that achieve all functional criteria (based on adequate liver function during NMP) at 6 hours. Perfusion parameters: • Clearance of lactate to a level < 2.5mmol/L • Perfusate pH ≥ 7.20 • Evidence of glucose metabolism (spontaneous fall in perfusate glucose) • Minimum bile pH ≥ 7.5 (if bile produced) • Bile glucose concentration ≤3 mmol/L or ≥10 mmol less than perfusate glucose • Hepatic arterial flow ≥ 100ml/min; portal venous flow ≥ 500ml/min • Perfusate alanine aminotransferase (ALT) < 6000U/L at 6 hours	Baseline (pre-NMP) and during preservation (1,2,4 and 6 hours and end of perfusion) Lactate will also be measured at baseline (pre-NMP) and 5 minutes after start of NMP
Secondary (clinical, histological & imaging)	To test the feasibility of the (i) inclusion criteria (false positives & negatives); (ii) delivery of intervention; (iii) the study endpoints *Clinical*	Proportion of livers transplanted in the 2 arms	Day 1
		LiMAx (maximum liver function capacity) test, measured in [micro]g/kg/h	Performed after 1 hour of liver stabilisation during NMP, repeated at 5 hours and subsequently every 6 hours till end of perfusion where feasible
		Cell free DNA (cfDNA)	Baseline (pre-NMP) and during

			preservation (1, 2, 4, and 6 hours and end of perfusion) Peri-operative (before transplant) and post re-perfusion (in recipient) Post-transplant (days 1, 3, 7 & 14). If patient not admitted at day 14 (then sample at day of discharge) Follow-up (day 30, month 3 & 6).
		Biochemical liver function (ALT, GGT, INR, Bilirubin and Peak serum AST)	Days 1-7
		Model of Early Allograft Function (MEAF)	Days 1-3
		Primary non-function (PNF)	Days 1-10
		Post-reperfusion syndrome (PRS)	First 5 minutes following reperfusion
		Need for renal replacement therapy Length of ITU/HDU stay	Days 1-7, discharge from hospital
	Histological & biochemical	Graft and patient survival	Days 1-7, Day 30, Month 3 and Month 6
		Correlation of donor biopsy (histopathologist's steatosis report) with:	Retrospective analysis of baseline donor

		a. WC, BMI, fatty liver index (FLI) & hepatic steatosis index (HSI): where relevant data available b. Surgeon's assessment c. Non-invasive pocket-sized micro-spectrometer readings: where data available	biopsy taken prior to commencement of ex-situ perfusion Pre and post-perfusion (end-NMP) biopsy
		Evidence of ischaemia reperfusion injury: a. Histological including: neutrophil infiltration, leukocytosis, glycogen depletion and lipid peroxidation b. Biochemical including: cytokine profile	Pre-perfusion, post-perfusion and post re-perfusion (in recipient) Perioperative (before transplant) and post re-perfusion (in recipient)
	Imaging	Histological evidence of bile duct injury (BDI) such as: stroma necrosis, extramural peribiliary glands (loss/injury of cells) and presence of vascular lesions. Measurement of biliary viability including bile pH, bicarbonate, glucose and LDH.	Bile duct biopsies taken where feasible (if sufficient length of duct): Pre-perfusion, post-perfusion and re-perfusion (in recipient) During preservation (1,2,4 and 6 hours and end of perfusion)
		Biliary strictures (anastomotic and non-anastomotic, bile duct	6 +/- 1 month

		leaks) determined by MRI scan at 6 months (depending on site capacity) using MRCP+ (an advanced biliary visualisation software by Perspectum Diagnostics)	
		Graft hepatic steatosis determined using multiparametric liver MRI (proton density fat fraction, cT1 and T2* mapping) depending on site capacity and quantified using software such as Liver*MultiScan*™ developed by Perspectum Diagnostics	6 +/- 1 month
	To provide information on likely effect sizes in order to design a subsequent phase III study	The proportion of fatty livers that achieve functional criteria (based on adequate liver function during NMP) Proportion of livers transplanted in the 2 arms Graft and patient survival	During preservation Days 1-7, Day 30, Month 3 and Month 6

	To provide information on safety	• Organ discard rate
		• Positive perfusate cultures
		• Adverse events, graded according to the Clavien-Dindo classification:
		  • Recipient infection
		  • Biopsy proven acute rejection
		  • Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks)
		  • Vascular complications (bleeding, hepatic artery stenosis, hepatic artery
			Days 1-7, Day 30, Month 3 and Month 6

		thrombosis, portal vein thrombosis) • Reoperation rate • Technical complications/device failure	

	12-month clinical outcomes	• Graft and patient survival
		• Total number of days in hospital
		• Total number of re-admissions for:
		  • Recipient infection
		  • Acute rejection
		  • Chronic rejection
		  • Biliary complications
		  • Vascular complications
		  • Disease recurrence
		  • Other reasons
		• Transplant related renal dysfunction
		• Biochemistry (liver and renal function)
			One-year follow-up data (obtained from the NHSBT registry)

Mechanistic studies (to be analysed subsequent to main clinical outcomes)	To measure the effect of the intervention on the histological degree of steatosis	Histological quantification of MaS and severity of NAFLD activity score	Pre-perfusion, post-perfusion and re-perfusion (in recipient)
	To measure the effect of the intervention on markers on of hepatic lipid metabolism	Markers such as TG, ketone body, 3-hydroxybutrate, cytokines and FGF-21	Baseline (pre-NMP) and during preservation (1,2,4 and 6 hours and end of perfusion) Perioperative (before transplant) and post re-perfusion (in recipient)

	To understand the structural, cellular and metabolic effects of defatting on steatotic livers	Genomic analysis	Pre-perfusion, post-perfusion and re-perfusion (in recipient)
		Proteomic and glycomic analysis	Baseline (pre-NMP) and during preservation (1,2,4 and 6 hours and end of perfusion) Perioperative (before transplant) and post re-perfusion (in recipient)
Intervention(s)	Normothermic Machine Perfusion (NMP) with oxygenated blood using the OrganOx metra, prior to implantation, for a minimum of 6 hours and a maximum of 24 hours with the following adjuncts to the preservation system: • Lipoprotein apheresis filtration: This is licensed for patients with severe hyperlipidaemia refractory to maximal medical therapy. • L-carnitine: This is licensed for use in primary carnitine deficiency due to inborn errors of metabolism and prevention of L-carnitine deficiency in patients with kidney disease undergoing haemodialysis. It is important in ß-oxidation of fatty acids from the mitochondrial membrane. • Forskolin: This natural supplement, used in the treatment of obesity, is a glucagon mimetic cAMP activator which results in increased lipolysis of lipid droplets and fatty acid oxidation. • Insulin: This will be infused at a 50% lower concentration than in the OrganOx instructions for use. This reduces the stimulation of *de novo* lipogenesis (DNL), the only source of fatty acid production in the liver during isolated normothermic perfusion. • Glucose: The threshold to infuse nutrition will be reduced from 10 mmol/L (as per standard instructions for the OrganOx device) to 5 mmol/L. Glucose is a non-lipid precursor for DNL. This will reduce the liver's ability to synthesise fatty acids *de novo* during perfusion.		
Comparator	Normothermic Machine Perfusion (NMP) with oxygenated blood using the OrganOx metra, prior to implantation, for a minimum of 6 hours and a maximum of 24 hours, following the standard OrganOx instructions for use and normal centre protocols.		

4. ABBREVIATIONS

°C	Degrees Celsius
%	Percent
3-OHB	3-hydroxybuturate
AE	Adverse event
APHG	All-Party Parliamentary Hepatology Group
AR	Adverse reaction
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
BMI	Body Mass Index
CI	Chief Investigator
CIT	Cold ischaemia time
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CTA	Clinical Trials Authorisation
CTU	Clinical Trials Unit
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DLI	Donor Liver Index
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DNL	*De novo* lipogenesis
DSUR	Development Safety Update Report
EAD	Early Allograft Dysfunction
ECD	Extended Criteria Donor
FGF-21	Fibroblast growth factor-21
FLI	Fatty Liver Index
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GW7647	2-(4-(2-(1-Cyclohexanebutyl)-3-cyclohexylureido)ethyl)-phenyl-thio)-2-methyl-propionic acid
GW501516	2-(2-Methyl-4-(((4-methyl-2-(4-(trifluoromethyl)phenyl)-5-thiazolyl)methyl)thio)phenoxy)-acetic acid
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HD	Haemodialysis

HDF	Haemodiafiltration
HIV	Human immunodeficiency virus
HF	Haemofiltration
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
IFU	Instructions For Use
IHTG	Intrahepatocellular triglyceride
INR	International Normalised Ratio
IP	Intellectual Property
IRI	Ischaemia-reperfusion injury
IRB	Independent Review Board
ITU	Intensive Care Unit
IVC	Inferior Vena Cava
kg/m^2	Kilogram per square meter
LD	Lipid droplet
MAP	Mean Arterial Pressure
MaS	Macrovesicular steatosis
MEAF	Model of Early Allograft Function
MELD	Model for End-stage Liver Disease
MHRA	Medicines and Healthcare products Regulatory Agency
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NICE	National Institute for Health and Care Excellence
NHS	National Health Service
NHSBT	NHS Blood and Transplant
NMP	Normothermic Machine Preservation
RES	Research Ethics Service
RGEA	Research Governance, Ethics & Assurance, University of Oxford
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
PNF	Primary Non-Function
PPARα	Peroxisome proliferator-activated receptor alpha
PPARδ	Peroxisome proliferator-activated receptor delta

PPI	Patient and Public Involvement
PRISMA	Preferred Reporting Systematic Reviews and Meta-Analyses
PRS	Post reperfusion syndrome
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
RNA	Ribonucleic acid
RRT	Renal Replacement Therapy
RSI	Reference Safety Information
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SDV	Source Data Verification
SMPC	Summary of Medicinal Product Characteristics
SNOD	Specialist Nurses in Organ Donation
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reactions
TG	Triacylglycerol
TMF	Trial Master File
TMG	Trial Management Group
TPN	Total parenteral nutrition
TSC	Trial Steering Committee
UKCRC	UK Clinical Research Collaboration
UKTR	UK Transplant Registry
USADE	Unanticipated Serious Adverse Device Event
UW	University of Wisconsin
VLDL	Very low-density lipoprotein

5. BACKGROUND AND RATIONALE

5.1. Hepatic steatosis and liver transplantation

Liver disease kills almost 11,000 people annually, a 400% increase since 1970 (1). For many patients liver transplantation is a highly successful treatment, but its benefit is critically limited by a shortage of donor organs. As a result, up to 20% of patients die on the waiting list before receiving a transplant (2).

In order to increase the donor pool, more sub-optimal 'marginal' donor livers are being transplanted (3). These include livers with substantial intra-cellular fat accumulation (steatosis). Steatosis results from altered metabolism of fatty acids within hepatocytes and is characterised by cytoplasmic accumulation of triglyceride (TG) in the form of lipid droplets (LDs) (4). Large cytoplasmic LDs cause peripheral displacement of the cell nucleus resulting in macrovesicular steatosis (MaS). Livers with MaS are much more susceptible to post-transplant ischaemia-reperfusion injury (IRI), with the primary initiating event occurring during storage at ice temperature (static cold storage; SCS). The consequent organ injury, attributed to impaired microcirculation, reduced mitochondrial function, and excessive inflammatory response, is associated with poor post-transplant outcomes (5).

There is evidence that moderate to severe steatosis (more than 30% MaS) is associated with primary non-function and a 71% increase in risk of graft loss, and such high-risk organs are frequently declined for transplantation (6). Around one thousand steatotic livers are retrieved but discarded for this reason each year in the USA (7); in the UK, 39% of liver discards are primarily due to steatosis (8).

The prevalence of hepatic steatosis, which is commonly associated with obesity, is increasing and currently affects 33% of the UK population (9,10). Increasing obesity in the population is reflected in the donor pool; 29% of deceased donors in the UK have a BMI >30kg/m^2 (11). Steatosis in donor livers is increasing and methods to render these organs suitable for transplantation are urgently needed.

NHS Blood and Transplant (NHSBT) has been highly successful in increasing organ donation rates in order to meet waiting list demands, but the quality and utilisation of donated organs is now a key concern. We hypothesise that through the reduction of IHTG content, steatotic livers can be optimised for safe transplantation. This will benefit liver transplant patients by reducing waiting list mortality and post-operative complications. It will reduce the economic burden of chronic liver disease on the NHS and society and maximise the benefit of every donor's generous gift.

5.2. Normothermic machine perfusion (NMP) in liver transplantation

Conventional storage of donor organs between retrieval from the donor and implantation in the recipient involves cooling on ice to 4°C (to reduce metabolic activity) and the use of specialist solutions (to reduce cellular swelling). Recently, the benefits of normothermic perfusion have been shown (12–14); normal physiological functions are maintained during preservation by using a blood-based perfusate at body temperature (37°C) and providing oxygen and nutrients.

This has several benefits: (i) recovery from acute injury (hypoxia) sustained prior to or during retrieval (12); (ii) objective assessment of organ function prior to transplantation: a number of studies have shown that this enables identification of organs in the 'high-risk' category that can safely be transplanted (14–17); (iii)

extended preservation times (up to 24 hours) (14). Crucially, it also provides the opportunity for therapeutic intervention to a functioning organ before it is transplanted.

Attempts to improve post-transplant outcomes in steatotic livers have included treatments to attenuate the IRI to which these grafts are particularly susceptible. However, in experimental models, levels of injury remained higher in treated steatotic than in lean livers (18–20). Rather than identifying methods to reduce IRI, targeting the primary cause, accumulation of intra-hepatocellular triglyceride (IHTG), may yield improved transplantation outcomes. By eliminating the root of the problem, the associated complications may be avoided. Several groups have explored this approach, particularly using NMP as a method to enhance the quality of steatotic grafts by actively removing IHTG during preservation.

Our collaborators in Birmingham recently published a systematic review of ex-situ machine preservation of steatotic donor livers, covering both non-pharmacological and pharmacological strategies (21) in their literature search and included studies up to March 2018. 15 studies were identified, covering all aspects of machine perfusion (including hypothermic machine perfusion, HMP) relevant to hepatic steatosis and defatting strategies in both animal studies and studies involving discarded human livers.

Out of the 15 original articles, only 4 were relevant to defatting steatotic livers during NMP (22–25). We undertook a further systematic literature search to identify further experimental and clinical studies relating to defatting interventions during NMP of the liver published since this review and identified two more recent studies (26,27) (see Table 1).

Table 1. Summary of defatting interventions and effect on MaS

Ref.	Defatting interventions	Model	Total ex-situ perfusion time (h)	Percentage (%) reduction in macrovesicular steatosis (MaS)
Jamieson et al, 2011	NMP alone	Porcine (*N* = 8)	48	13
Nagarth et al, 2009	GW501516, GW7647, forskolin, hypericin, visfatin and scorparone	Zucker rats (*N* = 12)	3	50
Raigani et al, 2019	GW501516, GW7647, forskolin, hypericin, visfatin, scorparone and L-carnitine	Zucker rats (*N* = 12)	6	33
Banan et al, 2016	L-carnitine and exendin-4	Discarded human livers (*N* = 2)	8	10

Liu et al, 2018	NMP alone	Discarded human livers (*N* = 10)	24	-
Boteon et al, 2019	GW501516, GW7647, forskolin, hypericin, visfatin, scorparone and L-carnitine	Discarded human livers (*N* = 10)	6	40
			12	50

5.3. NMP, hepatic steatosis and pre-clinical animal studies

Steatotic livers constitute the largest individual cohort of organs which might be salvaged through active intervention during NMP (22,23,25,27,28). Pre-clinical models demonstrate that ex-situ liver function can be enhanced and IHTG content reduced using NMP(22,23).

Jamieson et al. (23) investigated the effect of NMP alone on steatotic porcine livers during 48 hour perfusions. Steatotic porcine livers maintained perfusate base excess, factor V and bile production during NMP and demonstrated comparable haemodynamics and markers of liver injury to lean controls. MaS was reduced from 28% to 15% with reduction in lipid droplet size by the end of preservation (23). This study demonstrated mobilisation of fat from the liver into the perfusate. Indeed, one limitation of the study was the recirculation of secreted TGs in the circuit, thereby making the perfusate extremely lipaemic. It was thought that this might be a factor that limited the amount of fat that could be extracted by perfusion alone.

Nagrath et al. (22) used an experimental oxygenated normothermic model to investigate the effect of a 'defatting cocktail' on steatotic livers from Zucker rats over 3-hour perfusions. The 'defatting cocktail' combined 6 pharmacological agents (see Table 2). IHTG content was reduced by 65% with increased hepatic lipid metabolism. Notably, a 30% reduction in IHTG content was seen in the control group with no defatting agents.

Table 2. Defatting agents used in Nagrath et al (14)

Defatting agent	Function
PPARδ ligand GW501516	Increase fatty acid β-oxidation
Peroxisome proliferator-activated receptor (PPAR) α ligand GW7647	Increase mitochondrial fatty acid oxidation
Cyclic adenosine monophosphate (cAMP) activator forskolin	A glucagon mimetic cAMP activator, increases lipolysis and fatty acid oxidation
Pregnane X receptor ligand hypericin	Increase β-oxidation (very long chain fatty acids)
Visfatin	An insulin-memetic adipokine, role not fully understood
Scorparone	An androstane receptor ligand, upregulates PPAR

Raigani et al. (26) demonstrated similar results in a Zucker rat model using the addition of L-carnitine (to increase fatty acid β-oxidation) to the 'defatting cocktail' described by Nagrath et al (22). MaS was reduced from 41.5% to 8.5% during defatting perfusion over 6 hours. There was an increase in perfusate ketone content (a marker of fatty acid β-oxidation), bile bicarbonate content and lactate clearance in treated livers.

These pre-clinical animal studies show the potential of NMP as a platform to deliver targeted intervention(s) to treat donor hepatic steatosis, with evidence that both IHTG and MaS can be reduced during ex-situ NMP. However, these studies involved artificially-induced steatosis and it is not clear how well this replicates the clinical situation, and whether a clinically-relevant effect is achievable in steatotic human livers.

5.4. NMP, hepatic steatosis and discarded human livers

Liu et al. (24) perfused 10 discarded livers with variable degrees of baseline steatosis for 24 hours and demonstrated a significant increase in perfusate TG levels over the duration of the perfusion, suggesting mobilisation of IHTG. However, no histological reduction in IHTG content was observed. Banan et al. reported results from two human livers which were preserved normothermically with 2 defatting agents (L-carnitine and exendin-4); one of these showed a 10% reduction in the degree of MaS after 8 hours NMP (25).

When our group explored the effect of NMP (alone) on transplanted steatotic human livers (as part of a larger trial), we observed clear differences in TG metabolism during preservation compared to lean livers. As with previous groups (24,25,27) we observed significant increases in perfusate TG and 3-hydroxybutyrate (3-OHB) (a marker of hepatic fatty acid oxidation) during perfusion (Figure 1) (28). This suggests that a steatotic liver upregulates pathways to dispose of intrahepatic fatty acids, including mobilising and secreting more very low-density lipoprotein (VLDL)-TG and increasing ketone body production (Figure 2). Despite these changes in TG metabolism, the amount of IHTG did not change when assessed histologically. Furthermore, although steatotic NMP livers demonstrated significantly superior post-transplant biochemical function compared to steatotic cold-stored livers (implying less preservation injury), there was still evidence of greater injury than in lean counterparts, with a significantly higher post-operative peak serum AST level ($p = 0.02$) (28).

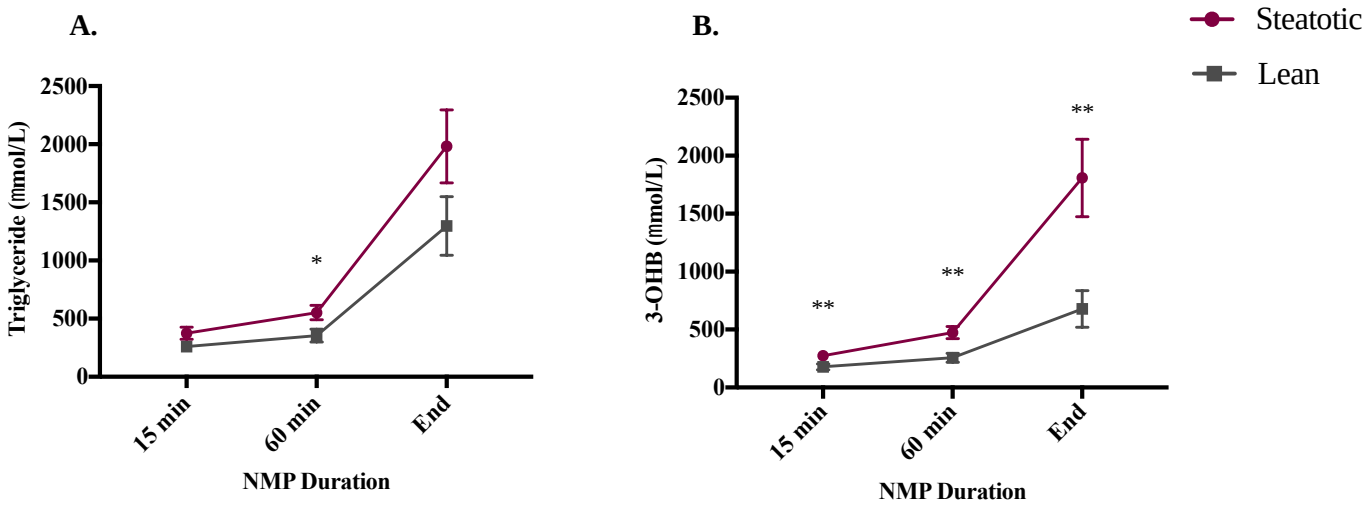

Figure 1A-B. Comparison of circulating TG **(A)** and 3-OHB **(B)** in the perfusate during NMP between steatotic (n = 18) and lean (n = 15) livers. Data presented as mean ± SD. * $p < 0.05$, ** $p < 0.01$

Further evidence supporting the need for targeted intervention for steatotic livers beyond NMP alone comes from our collaborators in Birmingham. Previously-declined livers were perfused and those meeting pre-defined functional criteria were transplanted: 22 of 31 perfused organs were transplanted, all with immediate function (17). Notably, of the livers that did not meet 'viability criteria' and were therefore not transplanted, 71% had histological evidence of moderate to severe steatosis. These data suggest that steatotic livers require more active intervention beyond simply replacing static cold storage with NMP.

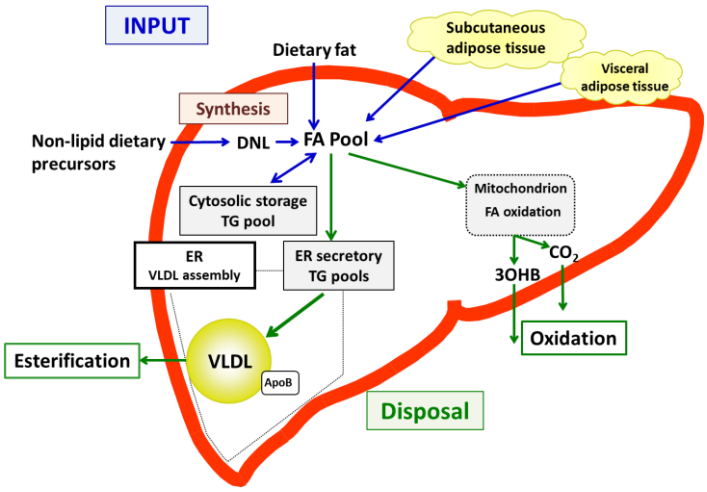

Figure 2. Overview of hepatic lipid metabolism

FAs derived from diet or the lipolysis of adipose tissue TG, enter the liver. Here, these mix with FA already stored in the liver (from the cytosolic TG pool) and from those synthesised via DNL. From the FA pool, FAs are partitioned into: i) esterification pathways where the TG produced can be secreted in VLDL particles or be stored in cystolic lipid droplets; or ii) mitochondrial oxidation pathways where these enter either the Krebs' Cycle to produce ATP and CO_2 or the ketogenic pathway, producing 3-OHB. Abbreviations: ApoB, Apolipoprotein; DNL, de novo lipogenesis; ER, endoplasmic reticulum; FA, fatty acid; TG, triglyceride; VLDL, very low density lipoprotein; 3-OHB, 3-hydroxybutyrate.

Using the same 'defatting cocktail' as that of Nagrath et al (22) with the addition of L-carnitine, Boteon et al. (27) treated organs retrieved for clinical transplantation but discarded due to steatosis. Using groups of 5 livers (only 4 in each group had evidence of MaS), pharmacological intervention was associated with improved metabolic function, reduced vascular resistance, lower levels of liver injury and increased bile production. There was evidence of reduction in markers of oxidative injury, immune cell activation, release of inflammatory cytokines and tissue TG. There was 40% reduction of MaS at 6 hours of perfusion. All 5 livers that received defatting therapies achieved viability criteria for transplantation compared to 2/5 in the control group (P = 0.04). However, not all treated livers that met the viability criteria achieved the clinical threshold of MaS of <30% (27). This calls into question the correlation between histology and function It is possible that activation of cytoprotective and vaso-protective pathways are the key elements that render such organs suitable for transplantation (29). NMP and defatting may have synergistic effects in achieving viability criteria for transplantation.

Although Boteon et al. demonstrated favourable outcomes, careful evaluation of the safety profile of this proposed 'defatting cocktail' is required prior to clinical use. Currently, many of the agents included in the 'defatting cocktail' lack important safety data (although there is some cytotoxicity tested reported in vitro) (30). Hypericin is a component of St John's Wort that is involved in up-regulation of the cytochrome P450 3A4 enzyme (31). This enzyme is involved in the metabolism of medications including cyclosporine and tacrolimus. In addition the peroxisome proliferator-activated receptor agonists GW501516 and GW7647 have not been tested in human trials and concern has been raised regarding carcinogenesis in preliminary animal studies (32).

The recent work from our own group has been directed to treating steatotic livers with the intention that these should function as well as lean counterparts, by active intervention to remove fat during preservation (33). Whilst designing this research, we considered subsequent clinical translation and avoided use of unlicensed chemical compounds which would require extensive testing and optimisation prior to use in a clinical trial. We also considered the conclusions of our earlier porcine perfusions (15) and the potential benefit of removing mobilised fat from the perfusate.

Our preliminary results in organs retrieved for clinical transplantation but discarded due to steatosis have demonstrated the potential of a novel defatting strategy (33). Using the commercially-available OrganOx *metra* device, 18 livers were perfused: 6 using a standard NMP protocol (Group 1); 6 using a circuit including a lipoprotein apheresis filter to remove circulating lipids (Group 2), and 6 using the lipoprotein apheresis filter and pharmacological interventions (Group 3). All livers were perfused over 48 hours.

Figure 3. Lipoprotein apheresis filter in NMP circuit

The first intervention was aimed at reducing the amount of VLDL-TG circulating in the perfusate; these are thought to be pro-inflammatory and might contribute to on-going IHTG accumulation (these can be recycled through the liver). To remove VLDL, a lipoprotein apheresis (DALI® 500) filter (Fresenius Medical Care (UK) Ltd, Huthwaite, UK) was incorporated into the circuit (Figure 3). In clinical practice, this haemofiltration system is used for patients with severe hyperlipidaemia, refractory to medical therapy (34). The filter consists of a matrix of polyacrylate beads, effective for the adsorption of cholesterol, lipoprotein (a) and triglycerides (34). Following this, we further modified the perfusate to include the following

1. **L-carnitine**: is comprised of amino acids including lysine and methionine. It is naturally present in meat, fish dairy products and plants (35). Humans can synthesise carnitine, therefore its availability is not limited to dietary intake (36). L-carnitine can increase the rate of fatty acid transport to mitochondria and is important in β-oxidation of fatty acids from the mitochondrial membrane (36). For this reason, it has been proposed as a weight loss supplement. It is licensed for use in primary carnitine deficiency due to inborn errors of metabolism and prevention of L-carnitine deficiency in patients with kidney disease undergoing haemodialysis.

The perfusate was supplemented with 1 g of L-carnitine hydrochloride, in 20 ml of 0.9% sodium chloride. This dose was based on *in vivo* human studies investigating the effect L-carnitine in treatment of hyper-lipoproteinemia, chronic myocardial ischaemia and deficiency in paediatric patients on peritoneal dialysis. The intravenous pharmacokinetics were determined from an *in vivo* study involving healthy subjects on a low-carnitine diet (37–40).L-carnitine has a half-life of around 15 hours (41), therefore a further 1 g was administered at 24 hours of perfusion.

Efficacy: L-carnitine has been investigated in cardiovascular disease and type 2 diabetes studies, in which plasma lipid levels and weight loss were secondary outcomes. In a trial of 258 patients with uncontrolled type 2 diabetes 2g/day of L-carnitine with orlistat (360mg/day) for 1 year significantly increased weight loss compared to orlistat alone (42). A recent metanalysis of 911 patients showed an average 1.33 kg excess weight loss compared to placebo. The doses administered ranged from 1.8-4g/day (36).

Safety: L-carnitine supplements are generally well tolerated at doses of up to 4g/day. Some side effects reported during *in vivo* human studies include nausea, vomiting and increased frequency of bowel movement. Rarer side effects reported include muscle weakness in patients with uraemia and seizures (in patients with underlying seizure disorders) (43,44).

2. **Forskolin**: is a dietary supplement originating from the roots of *Coleus forskohlii*, a plant prevalent in India and Thailand. It has reported to facilitate weight loss through lipolysis and appetite suppression (45,46). It is a glucagon mimetic cAMP activator which results in increased lipolysis and fatty acid oxidation (47).

The perfusate was supplemented with 1 mg NKH477 hydrochloride (a water-soluble version of forskolin), a dose based on data from previous studies in patients with cardiomyopathy (48) and schizophrenia (49) at doses of 0.1-0.5mg/kg.

Efficacy: Forskolin has demonstrated suppression of appetite in pre-clinical animal studies. A randomised double-blind trial showed significant (4%) reduction in body fat in 30 overweight men compared to placebo (46).

Safety: During *in vivo* human studies, forskolin has been reported to increase frequency of bowel movement. Doses of 500mg/day have not been associated with any serious or adverse events (50).

3. **Insulin reduction**: *De novo* lipogenesis (DNL) is the process through which the liver synthesises fatty acid, namely the 16-carbon saturated fatty acid palmitate, from non-lipid precursors. This is stimulated by insulin (51). Enhanced DNL may have significant effects on cellular metabolism as the primary fatty acid product is saturated (palmitoyl-CoA) (52,53) which may interfere with cellular function (54). In the absence of peripheral fat stores or dietary fat in this model, the only source of fatty acid production in the liver is via the DNL pathway. In order to lower DNL, we reduced the amount of insulin delivered during the perfusion by 50%. The perfusate was infused with 100 units of Actrapid, dissolved in 30 ml 0.9% NaCl, at 1 ml/hour.

4. **Glucose reduction:** Glucose acts as a non-lipid precursor for DNL (55). In order to reduce the liver's ability to *de novo* synthesise fatty acids, the glucose threshold to commence infusion of parenteral nutrition (TPN) infusion was reduced from 10 mmol/L to 5 mmol/L to reduce perfusate glucose concentration.

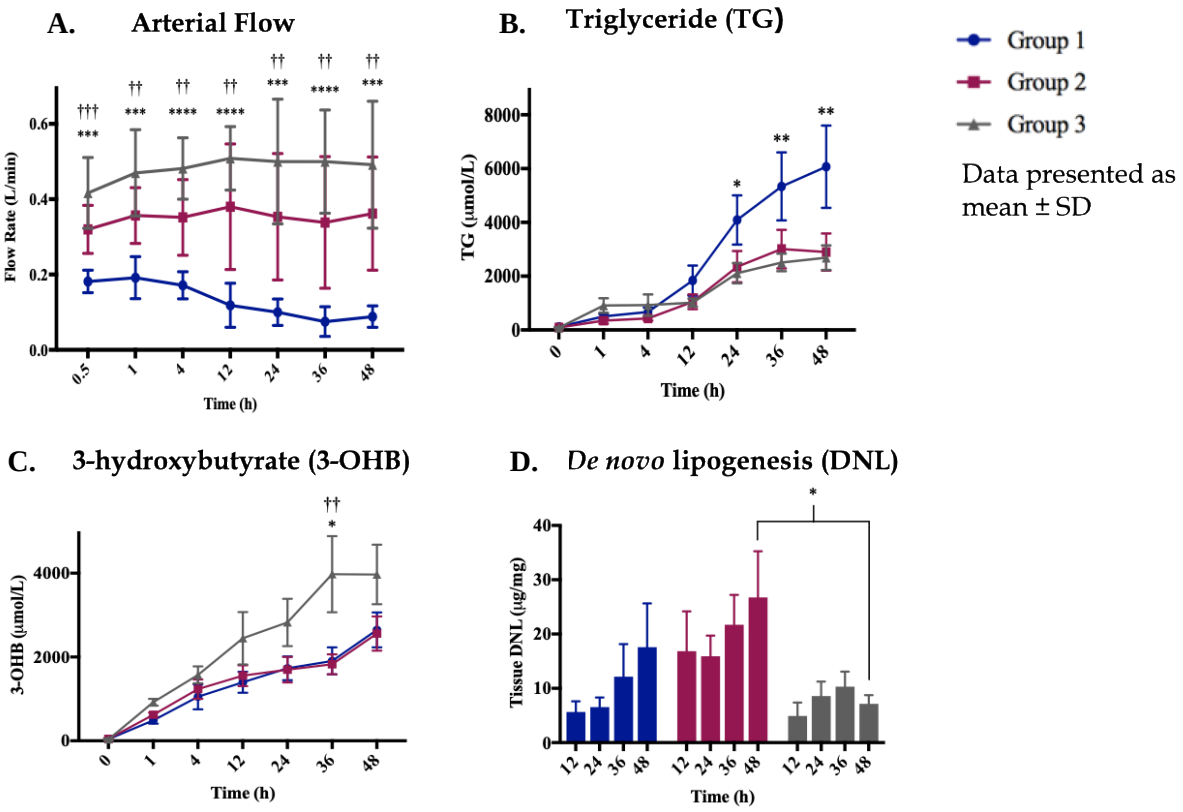

Figure 4A-D. Group 1 – NMP alone, Group 2 – filter, Group 3 – filter plus defatting agents

Over the 48 hour perfusion, a significantly increased arterial flow was seen in both intervention groups (Groups 2 and 3) (Figure 4A). The lipoprotein apheresis filter (Groups 2 and 3) significantly reduced circulating TG concentrations (Figure 4B). 3-OHB measurements showed a significant increase in fatty acid β-oxidation in Group 3, where L-carnitine and forskolin had been added (Figure 4C). Significant reduction in intrahepatic DNL (as measured in liver tissue using stable-isotope methodology) was seen in Group 3 (Figure 4D). Other functional benefits observed in both Groups 2 and 3 were increased hepatic glycogen production, less rise in perfusate transaminase, and a reduction in haemolysis (which we previously identified as a marker of ex-situ liver function (14)).

The combination of lipoprotein apheresis filtration and defatting interventions significantly reduced the amount of fat within the liver by 45% at 48 hours. However, the functional improvements were seen much earlier, by 6 hours. From this experimental study, we concluded that a combination of lipoprotein apheresis filtration and perfusate modification reduces hepatic steatosis and improves ex-situ liver function.

5.5. Summary

The pre-clinical studies of ex-situ defatting in discarded human livers, from both Oxford and Birmingham, have demonstrated a 'proof of concept' (27,33): if this translates into clinical practice, it will significantly increase the number of safely-transplantable organs by increasing utilisation of 'marginal' organs. The interventional agents proposed are safe, well-tolerated and available for clinical use (in contrast to previous studies) (22,27). We do not anticipate any systemic side effects following transplantation; first, because the doses are much lower than those used in human studies and, second, because, these agents will be administered ex-situ to livers that are then thoroughly flushed prior to transplant (as per standard practice following NMP) removing the agents from the liver prior to transplantation.

In our discarded liver study, structural and functional differences were evident after 6 hours of perfusion. These were associated with improved perfusion and biochemical metrics that would have rendered these organs transplantable on current functional criteria. This suggests that 6 hours of perfusion should be the minimum required prior to considering implantation of the organ into the recipient (with a maximum of 24 hours as per OrganOx *metra* instructions for use).

In the proposed trial we intend to enrol livers that have been retrieved for the purpose of transplantation, and that have been identified as high-risk of steatosis. We know that such livers are likely to be discarded after retrieval either because of appearance, histology or unfavourable perfusion metrics on NMP. We will test the targeted defatting protocol described above, using objective measures of function to assess outcomes.

6. OBJECTIVES AND OUTCOME MEASURES

6.1. Primary Objective and Outcome Measure

The experimental data outlined above suggest that the combination of NMP and defatting may be effective in reducing the fat content of livers and improving perfusion parameters to meet functional criteria for transplantation. In this first clinical study we intend to test the safety and feasibility of the intervention, and to obtain initial data regarding efficacy and effect size.

Primary Objective

To confirm the safety and assess efficacy of the NMP-defatting protocol in steatotic donor livers intended for transplant

Primary outcome measure

The primary endpoint is the proportion of livers that achieve all of the following functional criteria at 6 hours of perfusion (15,17), as defined by:

- Clearance of lactate to a level < 2.5mmol/L
- Perfusate pH ≥ 7.20
- Evidence of glucose metabolism (spontaneous fall in perfusate glucose)
- Minimum bile pH ≥ 7.5 (if bile produced)
- Bile glucose concentration ≤3 mmol/L or ≥10 mmol less than perfusate glucose
- Hepatic arterial flow ≥ 100ml/min; portal venous flow ≥ 500ml/min
- Perfusate alanine aminotransferase (ALT) < 6000U/L at 6 hours

These objective criteria, reflecting hepatic metabolism and injury, have been derived by a process of consensus amongst current NMP users. These parameters are increasingly recognised as a way to discriminate livers with favourable post-transplant outcomes and will be measured at baseline (pre-NMP) and throughout perfusion (1,2,4 and 6 hours and end of perfusion) (15,17). Lactate measurements will also be taken at baseline (pre-NMP) and 5 minutes after start of NMP.

These functional criteria are not intended as an instruction to the implanting surgeon, but rather as a consistent endpoint for the trial. The decision as to whether a liver is actually transplanted will remain with the implanting surgeon, who will base this on a number of criteria, including some that are recipient-related rather than donor organ-related (e.g. the urgency with which the patient needs a transplant may determine the decision).

6.2. Clinical Secondary Objectives and Outcome Measures

Objective	Outcome Measures
To test the feasibility of the (i) inclusion criteria (false positives & negatives); (ii) delivery of intervention; (iii) the study endpoints	*Clinical* 1. Proportion of livers transplanted in the 2 arms 2. LiMAx (maximum liver function capacity) test performed after 1 hour of liver stabilisation during NMP, repeated at 5 hours and subsequently every 6 hours till end of perfusion where feasible. If the decision to transplant has been made by 6 hours of NMP, the test will not be repeated. The LiMAx test will allow real time monitoring of CYP1A2 (prominent in functional livers cells and less prominent in damaged cells) and is based on the metabolism of ^{13}C-methacetin. This will enable measurement of liver capacity and functional reserve during perfusion (56). 3. Cell free DNA (cfDNA) measured at baseline (pre-NMP) and during preservation (1, 2, 4 and 6 hours and end of perfusion). Further measurements taken from the recipient peri-operatively (before transplant) and re-perfusion (following liver transplantation). Post-operative samples collected on days 1, 3, 7 and 14 (if the patient is discharged prior to day 14 – a sample will be collected on the day of discharge instead). Outpatient sample collection will align with clinic visits on day 30, month 3 & 6. cfDNA has been correlated with allograft injury, rejection and formation of *de novo* donor specific antibodies (57). 4. Biochemical liver function in the first 7 days post-transplant: ALT, GGT, INR, Bilirubin and peak serum AST (where AST measurements available) in the first 7 days post-transplant. Peak serum AST is a validated surrogate marker, predictive of PNF as well as graft and patient survival (58). It is also associated with histological evidence of moderate to severe reperfusion injury (59). 5. Model of Early Allograft Function (MEAF) (60): A score (between 0-10) based on bilirubin, INR and ALT within the first 3 post-operative days. 6. Primary non-function (PNF): irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes. 7. Post-reperfusion syndrome (PRS) (61): a decrease in mean arterial pressure (MAP) of more than 30% for more than one minute during the first five minutes after reperfusion (61). 8. Need for renal replacement therapy (haemodialysis, haemofiltration, peritoneal dialysis) during the first 7 days post-operatively. 9. Duration of ITU/HDU and hospital stay. 10. Graft survival (defined as a functioning transplant in the absence of death and re-transplantation) at day 7, day 30, month 3, and month 6. 11. Patient survival at day 7, day 30, month 3 and month 6. *Histological & Biochemical* 1. Correlation of pre-perfusion donor biopsy (histopathologist's steatosis report) with: a. WC, BMI and clinical risk scores such as the fatty liver index (FLI) and hepatic steatosis index (HSI) (62,63) where relevant data available

b. Surgeon's assessment (64)
c. Non-invasive pocket-sized micro-spectrometer reading. The device has been developed by SCIO - Consumer Physics (http://www.consumer-physics.com) and is CE marked. It utilises spectroscopy (absorption of near infrared light, 700-1,100nm). The commercially available device is able to quantify composition of foods, estimate body fat levels and identify analgesic agents (65). Readings will be taken sequentially over perfusion (where feasible): at baseline (pre-NMP) and during preservation (1, 6 hours and end of perfusion) to facilitate correlation with post-perfusion (end-NMP) biopsy in addition to the pre-perfusion biopsy.

2. Histological and biochemical evidence of ischaemia -reperfusion injury (IRI):

   a. Histology (formalin fixed paraffin embedded) (33):
      (i) Neutrophil infiltration & leucocytosis determined using Haematoxylin & Eosin (H&E) stain
      (ii) Glycogen depletion determined using periodic acid-Schiff (PAS) stain
      (iii) Lipid peroxidation determined using 4-HNE (4-hydroxynonenal) stain

   Biopsy samples collected pre-perfusion, post-perfusion and re-perfusion in the recipient (following liver transplantation).

   b. Cytokine profile implicated in liver transplantation including (66): CXCl8/IL-8, IL-10, IL-2, TNF-a, IFN-γ, IL-13, IL-4, IL-1β, IL-17A and IL-6.

   Blood samples collected peri-operatively (before transplant) and re-perfusion in the recipient (following liver transplantation).

3. Histological and biochemical evidence of bile duct injury (BDI) and biliary viability (67) such as:
   a. Histology (formalin fixed paraffin embedded) biopsy samples pre-perfusion, post-perfusion and following re-perfusion in the recipient (following liver transplantation). For example, evidence of stroma necrosis, loss/injury to peribiliary glands and vascular lesions. Bile duct biopsies will only be taken if sufficient length on the bile duct and feasible to do so.
   b. Bile composition measurements (if produced and measured at 1, 2, 4 and 6 hours and end of perfusion) for example: low pH and bicarbonate with high glucose and lactate dehydrogenase (LDH) as indicators of poor biliary viability.

Imaging

1. Biliary strictures (anastomotic and non-anastomotic) determined by MRI scan at month 6 (+/- 1 month) depending on site capacity using MRCP^{+} (an advanced biliary visualisation software by Perspectum Diagnostics) which is CE marked (68).
2. Graft hepatic steatosis determined using multiparametric liver MRI (proton density fat fraction, cT1 and T2* mapping) at 6 (+/- 1 month) depending on site capacity. MRI proton density fat fraction (MRI-PDFF) has demonstrated high diagnostic accuracy in both the detection and grading of hepatic steatosis with histology as a reference standard (69,70). Software such as

	Liver*MultiScan*™ which is CE marked and developed by Perspectum Diagnostics will aide in the quantification of steatosis.
To provide information of likely effect sizes in order to design a subsequent phase III study	1. The proportion of fatty livers that achieve functional criteria (based on adequate liver function during NMP) measured at 6 hours of perfusion (primary outcome). 2. Proportion of livers transplanted in the 2 arms (secondary outcome). 3. Graft and patient survival at day 7, day 30, month 3 and month 6.
To provide information on safety	1. Organ discard rate 2. Perfusate culture. At the end of preservation a sample will be taken for microbiological culture. 3. Adverse event rates and severity, graded according to the Clavien-Dindo classification (71) during the first 7 days, day 30, month 3 and month 6: a. Recipient infection b. Biopsy proven acute rejection c. Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks) d. Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis) e. Reoperation rate 4. Technical complications/device failures
12-month clinical outcomes (obtained from NHSBT registry)	1. Graft and patient survival 2. Total number of days in hospital in the last year (excluding transplant admission) 3. Total number of re-admissions for: a. Recipient infection b. Acute rejection c. Chronic rejection d. Biliary complications e. Vascular complications f. Disease recurrence g. Other reasons 4. Transplant related renal dysfunction 5. Biochemistry (liver and renal function)

6.3. Mechanistic Secondary Objectives and Outcome Measures

The mechanistic studies will be analysed subsequent to the main clinical outcomes. These will be carried out for two broad reasons:

- To identify more sensitive and specific markers of transplantability
- To understand the process of defatting that leads to a steatotic organ being reconditioned

This is of particular importance in the context of high-risk livers. Identification of such markers could augment current practice by predicting the outcome of each liver with objectivity. The mechanistic studies proposed will test hypotheses based on previous published studies that have investigated markers in the field of NMP and will inform development of functional criteria and optimisation of future defatting protocols.

Objective	Outcome Measures
To measure the effect of the intervention on the histological degree of steatosis	1. Histological quantification of MaS measured pre-perfusion, post-perfusion, and during re-perfusion in the recipient. We hypothesise that the intervention of defatting will reduce the degree of MaS and severity of NAFLD activity score (72).
To measure the effect of the intervention on markers on of hepatic lipid metabolism	1. Perfusate TG, insulin, ketone bodies and cytokines associated with IRI will be measured at baseline (pre-NMP) and during preservation (1,2,4 and 6 hours and end of perfusion). Further measurements taken from the recipient peri-operatively (before transplant) and re-perfusion (following liver transplantation). This will provide insight into changes in intrahepatic lipid handling and inflammation. We hypothesise that the intervention of defatting will 'repartition' intrahepatic fatty acids away from esterification into oxidation pathways leading to a decrease in IHTG and a decrease in IRI-associated cytokine production (66). 2. Perfusate FGF-21 will be measured at baseline (pre-NMP) and during preservation (1,2,4 and 6 hours and end of perfusion). Further measurements taken from the recipient peri-operatively (before transplant) and re-perfusion (following liver transplantation). FGF-21 is a hormone produced in the liver involved in energy homeostasis; its secretion is attributed to metabolic stress. There is evidence that serum FGF-21 is a useful marker for steatosis and correlates with increasing steatosis grade (73). We hypothesise that the defatting intervention will reduce FGF-21.
To understand the structural, cellular and metabolic effects of defatting on steatotic livers	1. Genomic analysis of samples taken pre-perfusion, post-perfusion and following re-perfusion in the recipient: a. Transcriptomics: A complex signalling cascade regulates metabolic processes within the liver. To understand the effect of NMP and the defatting intervention, genomic analysis of liver tissue will be undertaken. We hypothesise that the defatting intervention will lead to downregulation in pathways related to fat synthesis and inflammation and an upregulation in pathways related to fat disposal. Samples from livers will undergo RNA sequencing of the liver and this will be correlated with clinical outcomes. Changes in gene expression will be mapped with changes occurring in biological pathways, inferring biological changes during NMP. 2. Proteomic and glycomic analysis of perfusate samples taken at baseline (pre-NMP) and during preservation. Further measurements taken from the recipient peri-operatively (before transplant) and re-perfusion (following liver transplantation): a. Proteomics: A recent study investigating the use of NMP to increase utilisation of high-risk donor livers, identified protein clusters that were able to discriminate between transplantable and non-transplantable livers (22 out of 31) as well as markers predictive of post-transplant complications (74). We aim to determine the effect of the intervention on protein expression associated with hepatic steatosis, inflammation and IRI.

	b. Glycomics: The liver perfusate glycome profile may form part of future functional criteria (75). A recent study found that the abundance of a single glycan, agalacto core-alpha-1,6-fucosylated biantennary glycan (NGA2F) was significantly higher in the perfusate of livers that developed PNF. We will test this hypothesis in sequential perfusate samples.

7. TRIAL DESIGN

This is a prospective, blinded randomised pilot study, which will test the effect of normothermic defatting of steatotic donor livers. Donor organs meeting enrolment criteria will be randomised, using a 1:1 allocation ration, using permuted blocks of varying undisclosed size and will be stratified by donor organ type (DCD/DCD). Livers will be perfused using the OrganOx *metra* NMP device and assigned to either NMP alone (n=30) or NMP with defatting interventions (n=30). An interim safety review will be undertaken after perfusion of the first 10 livers.

All recruiting centres have extensive experience in the clinical use of NMP and are current users of the OrganOx *metra* device.

Perfusions will be supervised by a member of the central trial team. Randomisation will be carried out by the trial co-ordinator (clinical research fellow) after inspection of the organ with the transplanting surgeon. Following randomisation, setting up the NMP device will follow standard practice, with addition of the apheresis filter and pharmacological protocol (see below). The presence or absence of the lipoprotein apheresis filter will be blinded through use of a 'dummy' filter covered by a drape. This will prevent the local transplant team (and therefore the patient) from knowing the study allocation. An interim safety review will be undertaken after perfusion of the first 10 livers.

Study visits will align with routine outpatient clinics to avoid extra hospital visits where possible. These will be at post-operative days 1-7, day 30 and, months 3 and 6. At each study visit, details of adverse events, biochemical liver function tests and graft and patient survival will be documented.

The collaboration with the NHSBT Clinical Trials Unit (CTU) will facilitate longer-term (12 month) follow-up of basic parameters (where data is available) beyond the end of the trial and we will request consent to do so. This data will be collected from the UK Transplant Registry (UKTR) held by NHSBT.

Data will be collected into a secure central online electronic database (MACRO) using electronic case report forms.

The study will close after the final patient has completed 12 months of follow-up.

Anticipated flow of liver offers through the trial is depicted in *Appendix 1*.

8. PARTICIPANT IDENTIFICATION

8.1. Trial Participants

The randomised entity in this study is a donor liver, rather than a transplant recipient. Donor livers accepted by each participating transplant centre will be screened for a high likelihood of fatty liver disease at each point of the donor pathway based on: (i) waist circumference (>88 females and >102cm males) or BMI >30kg/m^2 or both at point of acceptance (76) (ii) evidence of macroscopic moderate-severe steatosis identified by the retrieval surgeon (or biopsy result) at point of retrieval.

In addition, any liver offer fast tracked due to moderate-severe steatosis (based on appearance or biopsy result) will also be considered for enrolment (regardless of WC and/or BMI).

The final entry criterion will occur at the point of inspection upon arrival at the transplant hospital: A surgeon from the implanting team will assess the liver to confirm its suitability for inclusion into the trial (based on macroscopic characteristics: colour, texture, rounded edges, size, weight) (64). The objective of this second entry criterion is to reduce the number of false positive (non-fatty) livers enrolled in the trial. Where available, the results of clinical biopsies demonstrating moderate-severe steatosis (typically >30%) will also be taken into account to assess suitability for randomisation.

Outcomes of livers transplanted during the study will be assessed. Liver transplant recipients will be those on the waiting list in participating centres to whom the liver offers are offered, and recipients will be consented for use of their data. This study does not alter the normal UK offering process in any way.

8.2. Inclusion Criteria

Donor Livers:

- Donors aged 18 years or over
- Offered through the national offering scheme and accepted by participating liver transplant centre
- Moderate-severe steatosis: macroscopic characteristics based on colour, texture, rounded edges, size and weight at point of inspection at the transplant hospital to confirm suitability for randomisation. Where available, the results of clinical biopsies demonstrating moderate-severe steatosis (typically >30%) will also be taken into account to assess suitability for randomisation.

Liver transplant recipients:

- Recipients 18 years of age or above
- Elective waiting list at a participating centre
- Willing to consent for inclusion into the study and collection and use of their data

8.3. Exclusion Criteria

Donor Livers:

- Donors from outside of the UK
- Donor is HIV, hepatitis B or C positive
- Cold ischaemia time (CIT) expected to exceed > 10 hours

- Macroscopic evidence of fibrosis
- Livers undergoing normothermic regional perfusion (NRP)
- Livers undergoing any other form of ex-situ machine preservation
- Participating centre cannot offer NMP due to device, logistical or staffing reasons

Liver transplant recipients:

- Receipt of a liver that has not undergone randomisation
- Receipt of super urgent transplant for acute liver failure
- Receipt of a split liver transplant
- Receipt of a multi-organ transplant
- Transplanted outside of the participating centres
- Contra-indication to MRI e.g. pacemaker

9. TRIAL PROCEDURES

All trial procedures are summarised in *Appendix 2 – Schedule of procedures.*

9.1. Recruitment

All UK liver offers meeting the inclusion criteria will be eligible for consideration. Offers are managed by NHS Blood and Transplant Hub Operations using the electronic offering system (EOS). Following NHSBT standard practice potential donors are identified by the donor hospital ITU staff and referred to the specialist nurse for organ donation (SNOD). The SNOD will obtain donor family consent for donation, and/or research samples, arrange any necessary investigations and register the donor with Hub Operations as per standard practice.

Liver offering will follow standard NHSBT policy, and offering will not be altered in any way by participation in the study (77).

9.2. Screening and Eligibility Assessment

Donor liver offers accepted by each participating transplant centre will be screened for a high likelihood of fatty liver disease at each point of the donor pathway based on: (i) waist circumference (>88 females and >102cm males) or BMI >30kg/m^2 or both at point of acceptance (ii) evidence of macroscopic moderate-severe steatosis identified by the retrieval surgeon (or biopsy result) at point of retrieval. In addition, any liver offer fast tracked due to moderate-severe steatosis (based on appearance or biopsy result) will also be considered for enrolment (regardless of WC and/or BMI).

Randomisation will be undertaken by the trial co-ordinator (after inspection of the liver by a surgeon from the implanting team). Where available, the results of clinical biopsies demonstrating moderate-severe steatosis (typically >30%) will also be taken into account to assess suitability for randomisation.

At each point of the donor pathway, the recipient co-ordinator from the participating site will communicate relevant information to the trial co-ordinator (clinical research fellow) in order to identify

suitable and eligible liver offers. This information will influence the trial co-ordinator's decision to mobilise to the recruiting liver transplant centre. The distance of the donor liver and the central trial (Oxford) team from the recipient liver transplant centre will also influence this decision. The trial co-ordinator will exercise his judgement to avoid prolonged cold ischaemia times (CIT) and any undue delay.

For offers to a named recipient, the recipient co-ordinator will determine whether the recipient has indicated intention to consent or has already consented to the trial. If so, the recipient co-ordinator will contact the central trial team to mobilise to the site. Where an offer is made without a named recipient, the recipient co-ordinator will wait until a recipient is identified in-centre. The central trial team will only be informed if an eligible liver has been allocated to a consented patient (or a patient who has indicated intention to consent on admission for transplant). On arrival to the recipient hospital, the central trial team will only randomise and carry out perfusion providing that there is evidence of signed informed consent. In the absence of consent, the liver will be excluded from the study. Screening logs of all offers will be maintained at each site and will record if donor family consent for research samples has been provided.

Allocation of organs will not be affected in any way by this study. NHSBT matching runs and in-centre allocation of organs will follow usual practice, irrespective of eligibility for the study.

9.3. Randomisation

If the liver is eligible for the study (at the point of organ inspection by a surgeon from the implanting team at the liver transplant centre) or with results of a clinical biopsy, randomisation will be conducted by the trial co-ordinator (clinical research fellow) who will deliver and be unblinded to the intervention.

Once eligibility is confirmed, the central trial team will use on-line randomisation (sealedenvelope.com) to allocate the liver to NMP or NMP with defatting interventions. The allocation sequence will be produced by Sealed Envelope and quality checked by the trial statistician. Donor organs meeting enrolment criteria will be randomised, using a 1:1 allocation ratio, using permuted blocks of varying undisclosed size and will be stratified by donor organ type (DCD/DBD). The randomisation list will only be accessible to the trial statisticians and Sealed Envelope. Randomised livers that are not perfused due to unforeseen reasons will not be replaced. It is anticipated that non-perfusion of a randomised liver will be a very uncommon event.

9.4. Informed Consent

9.4.1. Consent procedures

Consent for organ donation and/or research samples from the donor family will be obtained and recorded by the SNOD as per NHSBT standard practice.

All participating liver transplant centres have an OrganOx device available for routine clinical use. Patients in all centres are informed of different methods of preservation at the time of listing and/or explicitly consented for the use of preservation technologies.

Eligible patients on the transplant waiting list in each centre will be approached during a routine outpatient visit or by phone. Written information and a copy of the Informed Consent Form (ICF) will be provided in person or via post. In order to allow sufficient time for considering participation in the study, this initial

approach will be followed up by a further phone call or clinic visit at which point a consent discussion will take place and consent will be requested. If followed-up by phone call, the patient will be asked to confirm consent by signing the ICF and posting or emailing the signed ICF back to the site team. In cases where email is used, the patient will be requested to scan/photograph and send the signed ICF back by replying to an email sent by the local site team/consenter. Similarly, if the patient is followed-up in clinic, they will be asked to sign the ICF in-person during their clinic visit.

If a patient on the waiting list has indicated intention to consent and has not returned the signed consent form prior to admission for transplant, they will be asked to sign and confirm consent on admission for transplant.

In the event of a fast-track liver offer, where the liver may arrive at the site before the patient, there is a chance that randomisation and perfusion of the liver may commence prior to the arrival of the patient. This will only be permitted if a signed ICF has been received from the intended recipient in advance, either by post, email or in-person. In this scenario, the patient will be contacted by telephone prior to randomisation to reaffirm consent.

If the patient chooses to withdraw from the study at any point between consent and randomisation, the liver will not be randomised, it will be excluded from the study and offered as per standard care.

Written and verbal versions of the Participant Information Sheet and Informed Consent Form will be presented to the participants detailing no less than: (i) the exact nature of the trial; (ii) what it will involve for the participant; (iii) the implications and constraints of the protocol; (iv) the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as possible to consider the information, and the opportunity to question the Investigator or other independent parties to decide whether they will participate in the trial. Written Informed Consent will then be obtained by means of dated signatures of the participant and the person who presented and obtained the Informed Consent. The participant must personally sign and date the Informed Consent form before any trial related procedures are performed. The person who obtained the consent must be suitably qualified and experienced and have been authorised to do so by the Chief or Local Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed Informed Consent Form will be retained at the trial site.

If a recipient refuses consent, the liver will be preserved and transplanted according to usual centre practice and will not be randomised into the trial. In addition, patients who are unable to consent for themselves at baseline will not be recruited to this study.

Whether or not a patient consents does not affect organ offering or the chances of receiving a transplant in any way. Once a liver has been offered to the recipient, this offer will be maintained unless the recipient surgeon feels that the liver is not suitable for transplant or the recipient is not medically fit to undergo the procedure. Similarly, if the liver is not deemed eligible for the study by the trial co-ordinator (clinical research fellow), it will be excluded from the trial and offered as per standard care.

Post-operatively, there is a small risk that participants in this study may lose capacity to consent to continued involvement in this study. Sometimes, it is necessary for patients to be cared for on intensive care or a high dependency unit for a short period of time following a liver transplant. They would occasionally remain sedated following their surgery as part of this higher level care. The risk of a permanent loss in capacity (for example, due to a peri-operative stroke) following a liver transplantation is very low. In the event of a prolonged loss of capacity to consent to continued involvement in the trial, we would provide their designated consultee with information about the study (as the Patient Information Sheet) and seek advice from them as consultee about continuing to collect samples and data from the participant whilst their capacity is impaired.

9.4.2. Patients who lack understanding of verbal or written English

Patients and parents/carers with an insufficient understanding of the English language should not be approached to discuss trial participation unless there are adequate arrangements at the site for translation or interpretation of the trial documents. The Sponsor is unable to cover the cost of translation due to financial constraints. However, most participating sites will make use of translation services for communication and procedure consent and use of these services is permissible if feasible.

9.5. Blinding and code-breaking

This is a single blinded randomised clinical trial. Perfusions will be performed by a member of the central trial team. The trial co-ordinator (clinical research fellow) and/or member of the central trial team will be responsible for randomisation and will be unblinded to the intervention. After randomisation, setting up the NMP device will follow standard practice, with addition of the apheresis filter and pharmacological protocol. The presence or absence of the lipoprotein apheresis filter will be concealed through the use of a 'dummy' filter covered by a drape. This will prevent the local transplant and research team (and therefore the patient) from knowing the study allocation. In the case of a medical emergency or safety concern ascribed to the perfusion, rapid identification of the trial treatment and randomisation code will be permitted and documented.

In the few cases when urgent unblinding is considered necessary during the liver perfusion, the trial co-ordinator (clinical research fellow) or a member of the central trial team who will be co-ordinating the perfusion and therefore aware of the liver randomisation will disclose allocation to the clinical team. In other circumstances, the PI (or assigned deputy) will request and be given access to the unblinding facility for the individual randomisation through the web-based service (www.sealedenvelope.com). Unblinding of randomisations will be documented along with the reasons triggering them. Automatic notification of the unblinding will inform the investigators of the site that the randomisation originates from as well as the PIs. Details of the randomisation process and emergency code breaking will be located in the Site File. The person performing the unblinding will be sent an e-mail detailing a recipient's treatment allocation. The trial co-ordinator (clinical research fellow) will not be involved in any clinical decision making or any of the study assessments.

9.6. Baseline Assessments

9.6.1 Donor Demographics

Donor demographics to be recorded will include, but not limited to the following:

- Age
- Sex
- Ethnic origin
- Co-morbidities (e.g. ischaemic heart disease, hypertension, diabetes, malignancy)
- Cause of death (CVA, hypoxia, trauma, other)
- Type of donor (DBD, DCD)
- Donor height
- Donor weight
- Donor body mass index (BMI)
- Donor waist circumference (WC)
- Donor smoking history
- Donor alcohol consumption
- Last and peak serum aspartate transaminase (AST)
- Last and peak serum alanine transaminase (ALT)
- Last and peak serum gamma-glutamyl transferase (GGT)
- Last and peak serum alkaline phosphatase (ALP)
- Last and peak serum bilirubin
- Last and peak serum sodium
- Last and fasting triglyceride (TG), if available
- Patient on TPN or enteral feed, if information available
- Length of ITU stay
-

9.6.2 Recipient Demographics

Recipient demographics to be recorded will include, but not limited to the following:

- Age
- Sex
- Ethnic origin
- Co-morbidities (e.g. ischaemic heart disease, hypertension, diabetes, malignancy)
- Recipient requiring renal support at the time of transplant (minimum 24 hours of continuous renal replacement therapy) or 2 haemodialysis sessions in the previous week
- Aetiology of liver disease
- Indication for transplant
- Height
- Weight
- BMI
- Waist circumference (WC)
- Recipient smoking history

- Recipient alcohol consumption
- Pre-transplant INR
- Pre-transplant creatinine
- Pre-transplant bilirubin
- Pre-transplant sodium

9.7 Trial Interventions

9.7.1. NMP (Control Group)

All livers included in the study will undergo NMP using the OrganOx *metra*, a CE-marked device already in use in liver transplant units in both clinical trials and routine practice. NMP technology gained National Institute for Health and Care Excellence (NICE) approval in January 2019 (14,78).

Livers will be transported on ice to the transplant centre, and those meeting the inclusion criteria will undergo NMP (minimum 6 hours, maximum 24 hours), in accordance with the manufacturer's instructions for use and current local protocols. The procedure for preparing the device for use and placing the organ on the device is described in detail in the instructions for use (IFU) document (L300-0437ReV1.0 RoW Version 25/09/2017). All livers will be perfused with 3 units of donor-type (or O-negative) red blood cells and cross-match will be arranged by the recipient surgical team at the recruiting liver transplant centre.

The procedure for removing the liver from the device is also described in the IFU. Implantation and reperfusion of the liver proceed as per the usual practice of the implanting centre.

9.7.2. NMP with defatting interventions (Study Group)

In addition to NMP, livers randomised to the study group will undergo the defatting protocol developed in our previous experimental study (33). All components of this protocol are licensed for clinical use, and comprise:

- Lipoprotein apheresis filtration DALI ® 500 (Fresenius Medical Care (UK) Ltd, Huthwaite, UK) : This is licensed for patients with severe hyperlipidaemia refractory to maximal medical therapy (34). The filter consists of a matrix of polyacrylate beads, effective for the adsorption of cholesterol, lipoprotein (a) and triglycerides. The adsorption of lipoproteins occurs by polyacrylate ligands covalently binding to the polyacrylamide surface. Polyacrylate, consists of polyanions, with negatively charged carboxylate groups. The polyanions interact selectively with the cationic groups in the lipoproteins, and due to this electrochemical interaction, the lipoproteins are immobilized on the beads. Prior to filtration, the filter will be primed with 2 L of Gelaspan (B Braun, Sheffield, UK). Besides lipoproteins, the filter adsorbs the positively charged ions calcium and magnesium. Priming therefore saturates the adsorber with these cations, preventing

hypocalcaemia and hypomagnesaemia. In this context, priming also prevented excessive volume loss from the circuit upon commencement of filtration.

- L-carnitine: This is licensed for use in primary carnitine deficiency due to inborn errors of metabolism and prevention of L-carnitine deficiency in patients with kidney disease undergoing haemodialysis. It has been shown to increase β-oxidation of fatty acids from the mitochondrial membrane (37). The perfusate will be supplemented with of L-carnitine 1g/5ml aqueous solution. This dose is based on *in vivo* human studies investigating the effect L-carnitine in treatment of hyper-lipoproteinemia, chronic myocardial ischaemia and deficiency in paediatric patients on peritoneal dialysis. The intravenous pharmacokinetics have been determined from an *in vivo* study involving healthy subjects on a low-carnitine diet (37–40).
- Forskolin: This natural supplement, used in the treatment of obesity, is a glucagon mimetic cAMP activator which results in increased lipolysis of lipid droplets and fatty acid oxidation (47). The perfusate will be supplemented with 1 mg of NKH477 in 2 ml of 0.9% sodium chloride (from a stock solution of 5mg of NKH477 in 10 ml of 0.9% sodium chloride). NKH477 hydrochloride is a water-soluble version of forskolin and the dose is based on data from previous studies in patients with cardiomyopathy (48) and schizophrenia (49) at doses of 0.1-0.5mg/kg.
- Insulin: This will be infused at a 50% lower concentration than in the OrganOx instructions for use. This reduces the stimulation of *de novo* lipogenesis (DNL), the only source of fatty acid production in the liver during NMP (51). The perfusate will be infused with 100 units of Actrapid, dissolved in 30 ml 0.9% NaCl, at 1 ml/hour.
- Glucose: The threshold to infuse parenteral nutrition (TPN) will be reduced from 10 mmol/L to 5 mmol/L. As glucose is a non-lipid precursor for DNL, this will reduce the liver's ability to synthesise fatty acids *de novo* during perfusion (55).

Normothermic defatting will treat the liver in the ex-situ setting. Following treatment, prior to transplantation, the liver will be flushed with 2L of preservation solution, as per standard NMP practice. The investigational agents will therefore be effectively removed from the liver prior to implantation.

9.7.3. Recording of operative and perfusion parameters

The following data will be recorded on MACRO:

Donor timings

These are all routinely collected at the time of retrieval and will be obtained from the NHSBT database.

The parameters to be recorded include:

- Timings:
    - Withdrawal of support (DCD donors only)
    - Onset of functional warm ischaemia (DCD donors only)
    - Cessation of donor circulation (cross clamp or asystole in DCD donors)
    - Start of cold perfusion
    - Liver removal and placement on ice
- Perfusion solution used for aortic perfusion
- Perfusion solution used for storage and transport

- Degree of steatosis (graded mild, moderate, severe) – surgeon's assessment
- Quality of *in-situ* perfusion (graded poor, moderate, good)

Preservation parameters

In addition to timings, a number of other preservation parameters will be recorded. These will include:

- Time of initiation of normothermic machine preservation
- Time of cessation of normothermic machine preservation (end flush)
- Flush solution (UW, HTK, other)
- Perfusion parameters (for NMP livers; logged automatically by the device):
    - Arterial, and caval pressures (in mmHg)
    - Arterial, portal and caval flow rates (in L/min)
    - pO_2, pCO_2 and pH
    - Blood temperature (^oC), Glucose (mmol/L) and bile production (ml/h)
- Perfusate biochemistry
    - Perfusate lactate at baseline (pre-NMP), 5 minutes after start of NMP and during preservation (1, 2, 4 and 6 hours and the end of NMP)
    - Perfusate pH at baseline (pre-NMP) and during preservation (1, 2, 4 and 6 hours and end of NMP)
    - Perfusate ALT at baseline (pre-NMP) and during preservation (1, 2, 4 and 6 hours and end of NMP)
    - Glucose levels at baseline (pre-NMP) and during preservation (1, 2, 4 and 6 hours and at the end of NMP)
    - Bile pH, glucose and bicarbonate (if bile produced) at 1, 2, 4 and 6 hours and at the end of NMP
    - Bicarbonate use (time and dose of each bolus)
- Sampling:
    - Perfusate at baseline (pre-NMP) and during preservation (1, 2, 4 and 6 hours and end of NMP)
    - Liver core biopsies taken pre-perfusion and post-perfusion
    - Bile duct biopsies taken pre-perfusion and post-perfusion where feasible i.e. if sufficient bile duct length

At the end of preservation a sample of perfusate/storage solution will be taken for microbiological culture as per standard practice.

Operative parameters

These will include:

- Total operative time: defined as time from knife-to-skin to skin closure.
- Time of flush at end of NMP
- Time of liver in body (start of anastomosis)
- Time of reperfusion (portal or arterial, whichever occurs first)
- Portal reperfusion time
- Arterial reperfusion time
- Intraoperative transfusion of blood products measured in units.

- The use of veno-venous bypass or porto-caval shunts
- Type of caval anastomosis (standard end-end, piggyback (end-side or side-side))

Intra-operative outcome assessment

Recipient blood samples (before and after transplant) and post-reperfusion liver biopsy (as well as bile duct biopsy where feasible) will be taken:

  - Peri-operative blood taken (before transplant) and re-perfusion (following liver transplantation)
  - Liver core biopsy taken post-reperfusion
  - Bile duct biopsy taken post-reperfusion where feasible i.e. if sufficient bile duct length

These samples will be taken to determine the severity of ischaemia-reperfusion injury and changes in mean arterial pressure will be recorded to assess for post-reperfusion syndrome:

- Histological and biochemical evidence of ischaemia-reperfusion injury (IRI) in recipient (66):
  - Cytokine profile implicated in liver transplantation including (66): CXCl8/IL-8, IL-10, IL-2, TNF-a, IFN-γ, IL-13, IL-4, IL-1β, IL-17A and IL-6.
  - Liver histology (formalin fixed paraffin embedded biopsy) (33):
    - Neutrophil infiltration & leucocytosis determined using Haematoxylin & Eosin (H&E) stain
    - Glycogen depletion determined using periodic acid-Schiff (PAS) stain
    - Lipid peroxidation determined using 4-HNE (4-hydroxynonenal) stain

- Bile duct histology (formalin fixed paraffin embedded biopsy) (67):
  - Evidence of stroma necrosis, extramural peribiliary glands (loss/injury to cells) and presence of vascular lesions

- Post-reperfusion syndrome (PRS) (61), a decrease in mean arterial pressure (MAP) of more than 30% for more than one minute during the first five minutes after reperfusion (61).

Declines and discards

If a decision is made to decline an organ at any point after retrieval (but before randomisation), any donor and preservation data recorded will be kept, and the reason for decline clearly documented in the eCRF. If deemed appropriate by the OTDT Hub, the organ may be offered to other centres on the matching run with liver allocation as per standard of care. If the liver is already on the OrganOx *metra* device, every attempt should be made to keep it on the device (as per national agreement). If all centres subsequently decline an organ, the organ will be documented as a discard and it will be offered for research or disposed of as per standard procedures.

Data will also be collected for all discards (including reason for discard) from point of randomisation.

9.7.4. Concomitant care

Recipient management including the implantation procedure, postoperative care, immunosuppression and other medications, and post-transplant monitoring will follow local protocols.

9.8. Subsequent Visits

9.8.1. Inpatient stay

Patients will be assessed daily by their clinical team and managed according to standard care protocols at the site with clinical information obtained from medical records.

Post-operative outcome assessment

The investigations performed form part of routine clinical care.

The following biochemical outcomes will be recorded:

- Daily serum samples for the first 7 days post-transplant, to include:
    - Serum bilirubin (measured in µmol/l)
    - Serum gamma-glutamyl transferase (GGT; measured in IU/L)
    - Serum aspartate transaminase (AST; measured in IU/L) or serum alanine transaminase (ALT; measured in IU/L) depending on liver transplant centre
    - International normalised ratio (INR)
    - Serum alkaline phosphatase (ALP; measured in IU/L)
    - Blood urea (mmol/L)
    - Serum creatinine (µmol/l)
- Daily serum lactate (measured in mmol/L) whilst on ITU/HDU.

The first measurements should be taken at 12 to 24 (±6 hours) hours post-transplant. For subsequent measurements, in the event that more than one measurement is taken in a 24 hour period, the measurement taken closest to the specified follow-up time-point should be used.

- cfDNA measurements perioperatively in the recipient (before and after transplant) and on days 1, 3, 7 and 14 (if the patient is discharged prior to day 14 – a sample will be collected on the day of discharge instead). cfDNA has been correlated with allograft injury, rejection and formation of *de novo* donor specific antibodies (57). These research samples will be taken alongside routine clinical samples in order to minimise any additional tests.
- Model of Early Allograft Function (MEAF) (60): A score (between 0-10) based on bilirubin, INR and ALT within the first 3 post-operative days.

Other outcomes to be recorded include:

- Length of stay in Level 2/ Level 3 care (ITU/HDU) (days)
- Total length of hospital stay (days)
- Requirement for renal replacement therapy during transplant admission (haemodialysis (HD), haemodiafiltration (HDF), haemofiltration (HF), peritoneal dialysis (PFD))
- Graft and patient survival at day 7 post-transplant

- Primary non-function: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes.

Safety outcomes

- Recipient infection (defined as both clinically diagnosed treated infection and infection with a positive microbiological culture result)
- Clinically suspected treated rejection
- Biopsy-proven acute rejection episodes
- Biliary complications diagnosed radiologically e.g. a non-protocol MRI or CT scan in clinically symptomatic patient:
    - Biliary strictures - anastomotic and non-anastomotic. Defined as those requiring surgical or radiological intervention
    - Bile duct leaks. Defined as those requiring drainage, refashioning of anastomosis or stenting.
- Vascular complications
    - Bleeding. Defined as bleeding requiring transfusion and/or radiological/surgical intervention.
    - Hepatic artery stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
    - Hepatic artery thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
    - IVC/hepatic vein occlusion. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
- Reoperation rate
- Technical complications and device failures
- Any other reported adverse event

Severity will be graded according to the Clavien-Dindo classification (71) – *Appendix 3*.

Immunosuppression

Details of induction immunosuppression and maintenance immunosuppression (including doses) at day 7 post-transplant will be recorded.

9.8.2. Study visit 2 - Day 30 (± 2 weeks)

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate.

Outcome assessment

The following biochemical outcomes will be recorded at day 30 post-transplant:

- Serum bilirubin (measured in µmol/l)
- Serum gamma-glutamyl transferase (GGT; measured in IU/L)
- Serum aspartate transaminase (AST; measured in IU/L) or serum alanine transaminase (ALT; measured in IU/L) depending on liver transplant centre
- International normalised ratio (INR)
- Serum alkaline phosphatase (ALP; measured in IU/L)
- Blood urea (mmol/L)
- Serum creatinine (µmol/l)
- cfDNA measurements. These research samples will be taken alongside routine clinical samples in order to minimise any additional tests.

Other outcomes to be recorded include:

- Graft and patient survival at day 30 post-transplant
- Requirement for renal replacement therapy (HD, HF, HDF, PD) at any time

Safety outcomes

- Recipient infection (defined as both clinically diagnosed treated infection and infection with a positive microbiological culture result)
- Biopsy-proven acute rejection episodes
- Biliary complications diagnosed radiologically e.g. a non-protocol MRI or CT scan in clinically symptomatic patient:
    - Biliary strictures - anastomotic and non-anastomotic. Defined as those requiring surgical or radiological intervention
    - Bile duct leaks. Defined as those requiring drainage, refashioning of anastomosis or stenting.
- Vascular complications
    - Bleeding. Defined as bleeding requiring transfusion and/or radiological/surgical intervention.
    - Hepatic artery stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
    - Hepatic artery thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
    - Venous outflow obstruction. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
- Reoperation rate
- Any other reported adverse event

Severity will be graded according to the Clavien-Dindo classification – *Appendix 3*.

Immunosuppression

Details of maintenance immunosuppression (including doses) at day 7 and day 30 post-transplant will be recorded.

9.8.3. Study visit 3 – Month 3 (± 1 month)

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate.

Outcome assessment

The following biochemical outcomes will be recorded at month 3 post-transplant:

- Serum bilirubin (measured in µmol/l)
- Serum gamma-glutamyl transferase (GGT; measured in IU/L)
- Serum aspartate transaminase (AST; measured in IU/L) or serum alanine transaminase (ALT; measured in IU/L) depending on liver transplant centre
- International normalised ratio (INR)
- Serum alkaline phosphatase (ALP; measured in IU/L)
- Blood urea (mmol/L)
- Serum creatinine (µmol/l)
- cfDNA measurements. These research samples will be taken alongside routine clinical samples in order to minimise any additional tests.

Other outcomes to be recorded include:

- Graft and patient survival at month 3 post-transplant
- Requirement for renal replacement therapy (HD, HF, HDF, PD) at any time

Safety outcomes

- Recipient infection (CMV infection, fungal infection, post-operative sepsis)
- Biopsy-proven acute rejection episodes
- Biliary complications diagnosed radiologically e.g. a non-protocol MRI or CT scan in clinically symptomatic patient:
    - Biliary strictures - anastomotic and non-anastomotic. Defined as those requiring surgical or radiological intervention
    - Bile duct leaks. Defined as those requiring drainage, refashioning of anastomosis or stenting.
- Vascular complications
    - Bleeding. Defined as bleeding requiring transfusion and/or radiological/surgical intervention.
    - Hepatic artery stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
    - Hepatic artery thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
    - Portal vein stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
    - Venous outflow obstruction. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
- Reoperation rate

- Any other reported adverse event

Severity will be graded according to the Clavien-Dindo classification – *Appendix 3*.

Immunosuppression

Details of maintenance immunosuppression (including doses) at 3 months post-transplant will be recorded.

9.8.4. Study visit 4 – Month 6 (± 1 month)

This visit will, where possible, coincide with a routine outpatient appointment. If the recipient is an inpatient, assessment will be made in hospital where appropriate.

Outcome assessment

The following biochemical outcomes will be recorded at month 6 post-transplant:

- Serum bilirubin (measured in µmol/l)
- Serum gamma-glutamyl transferase (GGT; measured in IU/L)
- Serum aspartate transaminase (AST; measured in IU/L)
- Serum alanine transaminase (ALT; measured in IU/L)
- International normalised ratio (INR)
- Serum alkaline phosphatase (ALP; measured in IU/L)
- Blood urea (mmol/L)
- Serum creatinine (µmol/l)
- cfDNA measurements. These research samples will be taken alongside routine clinical samples in order to minimise any additional tests.

Other outcomes to be recorded include:

- Graft and patient survival at month 6 post-transplant
- Protocol MRI Scan (depending on site capacity) at 6 +/- 1 month to assess: (i) Biliary strictures (anastomotic and non-anastomotic) determined using MRCP^{+} (an advanced biliary visualisation software by Perspectum Diagnostics) (68) and; (ii) Donor graft hepatic steatosis using software such as Liver*MultiScan*^{TM} (MRI proton density fat fraction, MRI-PDFF protocol by Perspectum Diagnostics) (69,70).
- Requirement for renal replacement therapy (HD, HF, HDF, PD) at any time

Safety outcomes

- Recipient infection (CMV infection, fungal infection, post-operative sepsis)
- Biopsy-proven acute rejection episodes
- Biliary complications diagnosed radiologically e.g. a non-protocol MRI or CT scan in clinically symptomatic patient:
    - Biliary strictures - anastomotic and non-anastomotic. Defined as those requiring surgical or radiological intervention
- Vascular complications

  - Bleeding. Defined as bleeding requiring transfusion and/or radiological/surgical intervention.
  - Hepatic artery stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
  - Hepatic artery thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
  - Portal vein thrombosis. Defined as formation of new clot resulting in graft dysfunction or loss, or requiring pharmacological, radiological or surgical intervention.
  - Portal vein stenosis. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
  - Venous outflow obstruction. Defined as causing graft dysfunction requiring radiological or surgical intervention or resulting in graft loss.
- Reoperation rate
- Any other reported adverse event

Severity will be graded according to the Clavien-Dindo classification.

Immunosuppression

Details of maintenance immunosuppression (including doses) at 6 months post-transplant will be recorded.

9.8.5. Later outcomes

Whilst the end-point for trial participation will be 6 months, patients will also be consented for ongoing follow-up (12 months) by linkage to outcomes recorded by in the NHSBT transplant registry. This will allow the ongoing assessment of resource use (hospital stay and reasons for re-admission), biochemistry results (liver and renal function), transplant related renal dysfunction and longer-term patient/graft survival.

9.9. Sample Handling

9.9.1. Sample handling for trial purposes

The trial co-ordinator (clinical research fellow) and/or member of the central trial team will be responsible for collection of perfusate and peri-operative samples.

Perfusate samples will be collected at baseline (pre-NMP) and during preservation (1, 2, 4 and 6 hours and at the end of perfusion). Blood samples will also be collected peri-operatively before transplant and post-reperfusion. 3 samples will be taken at each timepoint:

- 1x EDTA separator tube (or universal tube if EDTA not available)
- 1x Serum separator tube
- 1x Streck tube for measurement of cfDNA

To ensure minimal sample degradation and pre analytical variability, perfusate and peri-operative samples should be kept at room temperature prior to separation of plasma from cellular parts. Separation of cells

from plasma and serum should be achieved by centrifugation at 1500g for 10 min at room temperature as close as possible to blood collection. After centrifugation plasma and serum samples should be kept at 4°C. The perfusate samples will be transferred into 1.0-2.0mL aliquots and subsequently transported to Oxford University Hospitals NHS Foundation Trust and stored frozen at -80 °C.

In addition to perfusate and peri-operative measurements, cfDNA will also be measured post-operatively. Samples will be collected from recipients in Streck tubes on days 1, 3, 7 and 14 (if discharged before day 14, a sample will be collected on date of discharge). These post-operative samples will be taken by the clinical team at each liver transplant centre and collection will align with routine clinical samples. Follow-up measurements will align with clinic visits on day 30, months 3 and 6. The Streck tubes are stable at room temperature for up to 7 days and will be shipped to an accredited laboratory in the United Kingdom (UK) or abroad.

Bile (if produced) will be collected at 1, 2, 4 and 6 hours and at the end of perfusion and transferred into 1.0-2.0mL aliquots for subsequent transport to Oxford University Hospitals NHS Foundation Trust and stored frozen at -80 °C.

Liver and bile duct biopsies will be taken before perfusion, at the end of perfusion and following reperfusion in the recipient (prior to skin closure). A total of 5 core liver biopsies will be taken: 2 biopsies before perfusion, 2 at the end of perfusion and 1 following reperfusion. Each liver biopsy will be divided into two segments. Per liver, one segment will be stored in formalin and the remaining segments will be frozen. A single bile duct biopsy will be taken at each timepoint where feasible i.e. if sufficient length on the bile duct. The bile duct biopsies will not be divided into two and only stored in formalin. The formalin samples will be stored at Oxford University Hospitals NHS Foundation Trust. Frozen samples will also be stored at this location at -80 °C .

Pre-implantation research biopsy samples will only be taken where donor family consent to research is in place. Sample collection will follow national regulations and standard operating procedures. Following collection, storage and transportation will be in accordance with the Human Tissue Authority guidelines and Trust policies. The trial team will have access to the samples and will ensure storage at Oxford University Hospitals NHS Foundation Trust. All research samples will be stored for future research and the mechanistic studies described in the study protocol.

Overall, the trial ID will be used as an identifier for all stored samples. Only personnel authorised by the Chief Investigator will be responsible for the storage, access and release of these samples for analysis.

9.9.2. Sample handling for standard of care

Routine blood samples taken for this study (donor and recipients) are part of standard clinical care, and will be processed in local laboratories for clinical purposes as per normal protocols. For study purposes, the results of these investigations will be documented.

9.10. Early Discontinuation/Withdrawal of Participants

All patients completing the 6-month follow-up assessment will be regarded as having completed the primary study. All patients will be encouraged to complete study follow-up, and all reasonable efforts will be made to ensure completeness of follow-up. Measures include ensuring that sample collection and

assessments are made, where possible, at routine hospital visits rather than additional appointments, and that patients do not incur extra financial costs (e.g. travelling costs) as a result of study participation.

It is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. The investigators may also withdraw a recipient from the study in order to protect their safety and/or if they are unwilling or unable to comply with the required study procedures. We will keep all data accrued to the point of withdrawal, as is stipulated in the trial consent form.

Possible reasons for investigator-led withdrawal of a participant from the trial include:

- Major protocol deviation
- Withdrawal of consent
- Loss to follow-up
- SAE/SUSAR
- Early termination of study

In the event of a patient withdrawing from the trial, the reason for withdrawal must be documented on the eCRF. Such patients will be asked whether they consent to the use of ongoing data collected as standard in the national transplant registry for the purposes of this study.

9.11. Definition of End of Trial

Data will be collected from participants for 6 months post-transplant. Once all data from all participants has been collated, entered and cleaned, then the database will be locked and the trial will end.

The procedures for the early termination/suspension of the study at one or more clinical sites in light of safety or compliance concerns are detailed in section 11.6.

10. THE ORGANOX METRA DEVICE

10.1. Device description

10.1.1 OrganOx Limited

OrganOx Limited is a late-stage medical device development company that was founded in April 2008 as a spin-out from the University of Oxford.

10.1.2. The OrganOx metra

The OrganOx *metra* is a normothermic preservation device for use in human liver transplantation. It perfuses the donor liver with blood, oxygen and nutrients, as well as a number of medications, at normal body temperature to replicate physiological conditions and preserve the organ for up to 24 hours. The device provides information as to the haemodynamic, synthetic and metabolic function of the liver during perfusion, which may assist the clinician in assessing the organ's suitability for transplantation. The device is available at all recruiting liver transplant centres.

10.1.3. The OrganOx metra Base Unit

The OrganOx *metra* normothermic perfusion device incorporates a centrifugal pump, an oxygenator, oxygen concentrator, heat exchanger, reservoir, flow probes, pressure sensors, infusions and blood gas analyser together with tubing and connector components. The device is comprised of three main components:

- a reusable base unit which contains software and hardware
- a disposable plastic circuit
- a set of perfusion solutions suitable for 24 hours perfusion

10.1.4. Disposable Set

The disposable set used with the core base unit of the OrganOx *metra* contains all the disposables used with each organ recovery on the *metra* and comprises:

1. A disposable tubing set, including a blood reservoir, perfusion lines, a blood oxygenator and centrifugal pump-head together with flow and pressure sensors.
2. An organ storage bowl which is pre-connected to the tubing set to contain the organ while on the device.
3. Cannulae for the coeliac artery, portal vein and inferior vena cava with easy connection attachment to the perfusion circuit.
4. A cannula and connection point for bile collection
5. Blood gas sensors for monitoring pO_2, pCO_2 and pH by means of on-line blood gas analysis.

10.1.5. Perfusion Solutions

For the present study all the additives necessary to perfuse and maintain the organ during the storage process, with the exception of sodium taurocholate, are not included and will be sourced locally (OrganOx will provide a list of recommended suppliers in the Instructions for Use (IFU) document). These solutions include bolus injections (given at the start of perfusion) and the maintenance infusions (given throughout perfusion).

The primary perfusion fluid for the liver comprises packed red blood cells, supplemented by colloid solution to normalise the haematocrit and osmolarity– these two components are not included and will be sourced locally.

Before connection of the liver the blood-based perfusate is supplemented with:

- Antibiotic and antifungal agents as per current local protocols. Heparin (anticoagulant) to prevent thrombosis in the circuit. In clinical use, a half-life of ~90 minutes is assumed; on this basis heparin is also given as a maintenance infusion.
- Sodium bicarbonate (buffer) for adjusting the pH of the perfusate.
- Calcium gluconate/calcium chloride to correct the binding of citrate to calcium.

During the perfusion the following are infused at a constant rate:

- Parenteral nutrition solution - a source of amino acids and glucose for liver maintenance.
- Insulin to control the perfusate glucose level
- Heparin to maintain anticoagulation.

- A 2% solution of sodium taurocholate in isotonic saline to compensate for loss of bile salts.
- Prostacyclin to optimise micro-perfusion.

The primary fluid for perfusing the organ is packed red cells supplied from blood transfusion centres and supplemented by a commercially-available colloid solution (human albumin solution or gelofusin as per local protocol) to normalise the haematocrit and osmolarity. Further additions are made to the perfusate to support the liver. All solutions required will be attached to the circuit during set-up and before the liver is attached. The recipient centre will provide the solutions necessary for perfusion with the *metra* including the packed red blood cells. All solutions are prepared immediately before the organ is attached to the device and contain sufficient solution for 24 hours operation, the intended maximum perfusion time for a liver on the device.

10.2. Device Safety

In designing the *metra*, OrganOx has made every attempt to maintain the current practices of organ retrieval and transplant teams, in order to minimise the risk of complications or errors that would prevent a successful retrieval. From a regulatory standpoint, it is important to note that the *metra* is an organ preservation system and its use does not involve direct connection to either the donor or recipient at any time.

The device has been designed according to ISO 13485, the standard that stipulates the requirements for a comprehensive management system for the design and manufacture of medical devices. In addition ISO 14971 specifies a process for a manufacturer to identify the hazards associated with medical devices to estimate and evaluate the associated risks, to control these risks, and to monitor the effectiveness of the controls. As part of the development of the device an extensive risk analysis has been undertaken and the risks identified and minimised in accordance with this standard.

The OrganOx perfusion system is based on the principle that all the perfusion solutions, additives and packed red cells must be removed from the organ prior to transplant. Therefore following the completion of the perfusion, the perfusion solution is flushed out of the organ with UW or HTK solution. OrganOx has deliberately designed the operation of the device such that it will require minimal changes to current transplant clinical practice.

10.3. Regulatory Aspects

The OrganOx metra has been used in over 1100 clinical liver perfusions worldwide. It has been tested in a multicentre Phase III clinical trial demonstrating both safety and efficacy.

The device carries a CE mark in Europe. FDA approval is currently being sought in the US, where a 266-patient randomised controlled trial has completed recruitment.

10.4. Device Accountability

All participating centres have access to an OrganOx metra device that is also available for general clinical use. Disposable sets will be provided for the study by OrganOx and should only be used for the preservation

of livers randomised into this study. Any livers being perfused outside of the current study should use the hospitals own supply of disposable sets.

Device accountability will be undertaken at each local site throughout the study for the reusable unit(s) and disposable sets (sterilisation/assembly batch number and disposable set number). The manufacturer and lot number for each perfusion solution will also be recorded on the case report forms (CRFs). The site will maintain a log of usage of both the retained unit, disposable set and perfusion solutions used throughout the study recording the lot number used against each subject (on the CRF).

At the end of each procedure the OrganOx *metra*, and any unused disposable and perfusion solutions will be disposed of on site. Details of total numbers of disposable sets provided for trial use will be recorded.

10.5. Device Maintenance

Device cleaning and routine maintenance will be the responsibility of the local transplant centre storing the device. Full details for cleaning and routine maintenance required will be provided in the instructions for use (IFU), and appropriate training will be provided as part of the device training described in section 14.3.

If a device develops a fault during the study, it will be removed from service and a replacement loan device provided as soon as practically possible to allow continuation of recruitment.

10.6. Ex-Situ Liver Defatting

The intervention including dosage, treatment duration and administration of liver defatting interventions to the perfusate is described in detail in section 5.4. and 9.7.

Briefly, the normothermic machine used in the study, the OrganOx *metra*, is CE-marked has been shown to be safe and effective in a previous phase III clinical trial. The device perfuses the liver ex-situ prior to transplantation and at no point is it connected to the patient. Following ex-situ perfusion, the liver is thoroughly flushed prior to transplantation.

There is also increasing clinical evidence that normothermic machine perfusion (NMP) is effective in reducing the immediate liver injury associated with transplantation of fatty (steatotic) organs (which suffer exacerbated ischaemia-reperfusion injury). The study arm of the proposed trial will combine the use of a lipid filter to the normothermic circuit (for a minimum of 6 hours and a maximum of 24 hours), with targeted pharmacological strategies during ex-situ perfusion (described in detail in section 9.7.2). Briefly, these include:

- Lipoprotein apheresis filtration: This is licensed for patients with severe hyperlipidaemia refractory to maximal medical therapy (34).
- L-carnitine: This is licensed for use in primary carnitine deficiency due to inborn errors of metabolism and prevention of L-carnitine deficiency in patients with kidney disease undergoing haemodialysis. It is important in ß-oxidation of fatty acids from the mitochondrial membrane (37–40).

- Forskolin: This natural supplement, used in the treatment of obesity, is a glucagon mimetic cAMP activator which results in increased lipolysis of lipid droplets and fatty acid oxidation (47).
- Insulin: This will be infused at a 50% lower concentration than in the OrganOx instructions for use. This reduces the stimulation of *de novo* lipogenesis (DNL), the only source of fatty acid production in the liver during isolated normothermic perfusion (51).
- Glucose: The threshold to infuse nutrition will be reduced from 10 mmol/L (as per standard instructions for the OrganOx device) to 5 mmol/L. Glucose is a non-lipid precursor for DNL. This will reduce the liver's ability to synthesise fatty acids *de novo* during perfusion (55).

These interventions have been tested in discarded human livers and proven effective in reducing fat content without compromising perfusion. The pharmacological agents are all widely available (Forskolin) and/or licensed for human use (L-carnitine). At the end of perfusion, the liver will be flushed with 2 litres of preservation solution prior to transplant, meaning that the investigational agents will be effectively removed from the liver prior to implantation.

The MHRA has been consulted regarding the proposed clinical trial interventions. The MHRA has reviewed our application and advised that our proposal is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC and no submission to the Clinical Trials Unit at the MHRA is required. This outcome is in line with that of a previous comparable negotiation between OrganOx Ltd. and the MHRA, over the use of sodium taurocholate. This is a choleretic agent, a bile salt of bovine origin, that is used to optimise biliary function in perfused livers and infused continuously throughout perfusion. The MHRA determined that the flushing of the liver at the end of perfusion (with 2 litres of preservation solution) removed such a large majority of the infused bile salt that any small amount carried over to the patient would be at a level highly unlikely to have a pharmacological effect.

11. SAFETY REPORTING

The below sections describe the required reporting for adverse events within the clinical trial. This is in addition to the standard incident reporting to the device manufacturer and to Clinical Governance at NHSBT. It is a statutory condition of a licence for procurement or transplantation activity to rapidly report to NHSBT (acting on behalf of the HTA), relevant and necessary information concerning adverse events which may influence the quality and safety of organs. All study sites will therefore follow their usual procedures for highlighting concerns – by completing an NHSBT incident submission form:

https://safe.nhsbt.nhs.uk/IncidentSubmission/Pages/IncidentSubmissionForm.aspx

These reports will be reviewed periodically by the Data Monitoring Committee (DMC). A safety review will be conducted by DMC after the first 10 liver perfusions. All available data will be reviewed with a focus on adverse events, graft and patient survival, as well as organ utilisation.

Untoward incidents related to the process of organ retrieval and transplantation is routinely collected by NHSBT. Further detail may be found here:

http://www.odt.nhs.uk/odt/governance-and-quality/incident-reporting/.

11.1. Adverse Event Definitions

Adverse Event (AE)	Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) whether or not related to the study intervention.
Serious Adverse Event (SAE)	An adverse event that: • Led to death • Resulted in serious deterioration in the health of the subject that: o resulted in a life-threatening illness or injury o resulted in a permanent impairment of a body structure or a body function o required in-patient care or prolongation of hospitalisation o resulted in persistent or significant disability or incapacity o resulted in congenital anomaly or birth defect o resulted in medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function. This includes device deficiencies that might have led to a serious adverse event if: a) suitable action had not been taken or b) intervention had not been made or c) circumstances had been less fortunate.

Severity definitions

The following definitions will be used to determine the severity rating for all adverse events:

Mild: awareness of signs or symptoms, that does not interfere with the subject's usual activity or is transient that resolved without treatment and with no sequelae.

Moderate: a sign or symptom, which interferes with the subject's usual activity.

Severe: incapacity with inability to do work or perform usual activities.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

11.2. Anticipated Adverse Events

As liver transplant recipients, all recruits to the DeFat trial are at high risk of experiencing AEs due to the complexity of their condition. Many of these events are anticipated as a result of the patient's medical condition and standard treatment received in hospital. We will only document adverse events if in the opinion of the investigator they are likely to be associated with the trial intervention.

All adverse events meeting the definition of serious adverse event in section 11.1 will be recorded.

11.3. Assessment of Causality

The relationship of each adverse event to the trial procedures, conduct or intervention must be determined by a medically qualified individual according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from the trial procedures, conduct or intervention. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

11.4. Procedures for Reporting Adverse Events

It is the responsibility of the local investigator to ensure that all adverse events considered related to the intervention and occurring during the course of the study are recorded. This will include but not be limited to:

- A description of the event
- The dates of the onset and resolution
- Action taken
- Outcome
- Assessment of relatedness to the trial procedures, conduct or intervention
- Whether the AE is serious or not

Whether the AE arises from errors in OrganOx *Metra* device functioning or use, adverse events that occur during the course of the study should be treated by established standards of care that will protect the life and health of the study subjects.

Adverse events considered related to the intervention should be recorded on the eCRF via the MACRO database provided. If the eCRF is unavailable for any reason, a paper version of the form should be completed.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

Non-serious AEs, considered related to the trial procedures, conduct or intervention as judged by a medically qualified investigator or the Sponsor, will be followed up until resolution.

11.5. Reporting Procedures for Serious Adverse Events

It is the responsibility of the local investigator to ensure that all adverse events which fall in to the category of Serious Adverse Events (SAEs) meeting criteria defined in 11.1 are reported to NHSBT Clinical Trials

Unit, chief investigator, central investigators and, if required, to their local R&D department as soon as possible after becoming aware of the event but no later than 24 hours. This will include but not be limited to:

- A description of the event
- The dates of the onset and resolution
- Action taken
- Outcome
- Assessment of relatedness to the trial procedures, conduct or intervention

Serious adverse events will be collected from transplant until 6 months following the transplant, via a purposely designed MACRO database (access via www.ctu.nhsbt.nhs.uk/macro). SAEs will be automatically notified to NHSBT CTU. If the eCRF is unavailable for any reason, a paper version of the form should be completed, scanned and emailed to serious_adverse_events@nhsbt.nhs.uk. Within the following 5 working days, the local investigator may be required to provide additional information on the SAE in the form of a written narrative. This should include a copy of the completed SAE form, and any other diagnostic or relevant information that will assist the understanding of the event.

Additional and further requested information (follow-up or corrections to the original case) should also be added to eCRF using a new SAE Report Form. NHSBT CTU will ensure that all SAEs are reported to the Sponsor.

The clinical reviewers will review the SAEs and, if they agree that the SAEs are unexpected and related, or pose an immediate risk to patient health or safety, then they will report them to the DMC immediately and to the device manufacturer and the REC within 15 calendar days of the Chief Investigator becoming aware of the event. The DMC will review the accumulating data at regular intervals.

11.6. Study Suspension or Early Termination

The DMC or sponsor may recommend suspension or termination of the study either at an individual investigation site or the entire study for significant and documented reasons. An investigator, ethics committee may suspend or prematurely terminate participation in the study at the investigation sites for which they are responsible. If suspicion of an unacceptable risk to subjects arises during the study, or when so instructed by the ethics committee, the sponsor shall suspend the study while the risk is assessed. The sponsor shall terminate the study if an unacceptable risk is confirmed.

The sponsor shall consider terminating or suspending the participation of a particular study site or investigator in the study if monitoring or auditing identifies serious or repeated deviations on the part of an investigator.

If suspension or premature termination occurs, the terminating party shall justify its decision in writing and promptly inform the other parties with whom they are in direct communication. The chief investigator and sponsor shall keep each other informed of any communication received from either the ethics committee.

If, for any reason, the sponsor suspends or prematurely terminates the study at an individual investigation site, the sponsor shall inform the Ethics Committee, either through the chief investigator or the sponsor.

If the suspension or premature termination was in the interest of safety, the sponsor shall inform all other investigators.

If suspension or premature termination occurs,

a) the sponsor shall remain responsible for providing resources to fulfil the obligations from the study protocol and existing agreements for following up the subjects enrolled in the study, and
b) the chief investigator or authorized designee shall promptly inform the enrolled subjects at his/her study site, if appropriate.

12. STATISTICS

12.1. Statistical Analysis Plan (SAP)

The statistical aspects of the study are summarised here with details fully described in a separate statistical analysis plan. The SAP will be finalised before any analysis takes place.

12.2. Description of Statistical Methods

Primary endpoint data will be presented for each arm separately, and the primary analysis will be a logistic regression model, with adjustment for donor organ type (DCD/DBD) to assess whether there is a statistically significant difference between treatment arms. An additional analysis where the model is adjusted for transplant centre will also be considered given sufficient counts within centre. The additional model will employ Firth's penalised maximum likelihood to mitigate for small sample bias and overfitting. This analysis will include all livers randomised in an intention-to-treat (ITT) analysis. The proportion of livers actually transplanted will be presented and analysed in a similar way. A modified intention to treat analysis (mITT) will be considered for livers that were randomised but did not subsequently perfused. Reasons for not undergoing perfusion will be documented and an independent adjudication panel will consider inclusions to a mITT analyses after consideration of these reasons, on a case-by-case basis. There maybe indirect logistical reasons rendering the inclusion of non-perfused livers to an ITT inappropriate i.e. these reasons are not completely unrelated to the allocated intervention.

Many of the secondary endpoints are only relevant for livers that are actually transplanted, and so these analyses will be conducted on a modified intention-to-treat (mITT) population of all livers randomised and transplanted, analysed according to randomised treatment. Outcomes will be presented as counts and proportions, means and standard deviations, or medians and interquartile ranges as appropriate, and analysed using linear regression for continuous outcomes; logistic regression for binary outcomes, Cox regression analysis for time to event outcomes. In addition to the peak ALT/AST in the first 7 days post-transplant, the area under the curve will be used summarise the post-operative biochemical markers (ALT, AST, GGT, INR and Bilirubin) levels over time. There will be very limited statistical testing of secondary endpoints in this small pilot trial, and the focus will be on presenting the effect size of the defatting + NMP intervention relative to standard NMP with 95% confidence interval, to help inform the design of a future definitive trial.

For the mechanistic work measures will be compared before and after perfusion to assess for a change during machine perfusion. A paired t-test will be used to compare the means of these levels pre and post treatment to determine whether any change is statistically significant. For all analyses with statistical testing, a p-value of < 0.05 will be used to determine statistical significance.

12.3. Sample Size Determination

Our preliminary data described above showed that 40% more livers met functional criteria for transplantation where NMP was combined with defatting versus NMP alone (100% vs.60%) (33). However, this is based on a small sample size and the interventions were tested on a very high-risk group of livers that had all been previously discarded. Using the proposed inclusion criteria, a smaller effect size is anticipated. Whilst the present pilot study is not primarily intended to demonstrate efficacy, a sample size of 60 livers (30 per group) will provide greater than 80% power to detect a difference of 30% (from 65% in the control NMP arm) in those meeting criteria for transplantation (at 5% significance): this is a clinically significant outcome. This sample size should provide sufficient information for the design of a larger, phase III study to formally test the efficacy of the intervention.

The annual NHSBT report (2018-19) shows that of 735 adult elective liver transplants, 618 (84%) were performed at the participating liver transplant centres (11). Data from within Eurotransplant show that 23% of livers have moderate to severe steatosis (>30%) on histology (79). This predicts that 142 livers (annually) and 213 livers (over 18 months) with moderate to severe steatosis would be available at the centres participating in this study. Allowing for a 50% recruitment rate, the recruitment of 60 livers in 18 months is feasible (allowing for small proportion non-steatotic livers to be randomised).

12.4. Analysis Populations

An ITT analysis will be performed for the primary outcome and secondary (donor liver related) outcomes. Secondary (recipient) outcomes will be analysed using a mITT. This analysis will exclude livers perfused but not transplanted for any reason.

12.5. Decision Points

Data will be reviewed by the Data Safety Monitoring Board (DSMB) after first 10 liver perfusions. If there are no safety concerns recruitment will continue as per the study protocol.

12.6. Stopping Rules

There will be no formal stopping rules.

12.7. The Level of Statistical Significance

The level of statistical significance will be set at 5% (p=0.05).

12.8. Procedure for Accounting for Missing, Unused, and Spurious Data.

Withdrawals from the trial after implantation will be documented, and a narrative analysis of withdrawals will be performed. Recipients withdrawing from the trial after implantation will be included in analysis using all available data. Consideration will be given to model-based and multiple imputation methods and detailed in the SAP. The rational for this is briefly described below.

The primary outcome will be available for all livers randomised and perfused. Missing data will be described and reported, although it is anticipated very few patients will be lost to follow-up. The reason for missingness for variables implicated in the primary analyses will be explored through regression of the missing variable indicator on other observables and detailed in the SAP. All analyses will include all data available.

The small sample size poses significant limitations for building robust multiple imputation models for handling missing data. Consideration of multiple imputation model will be given only for the analyses of selected/primary outcomes when it is valid to do so:

- The proportion of missing data is less than 5% and the impact of missing is negligible
- When no additional information can be obtained (no auxiliary variables to use for imputation can be identified)
- When missing data can be assumed to be missing completely at random from the outset
- When missingness can be assumed missing at random conditional on other observable data

In the case where the primary outcomes are not missing at random then a “worst-best-case” scenario sensitivity analyses will be undertaken to show the range of uncertainty due to missing. Briefly, in such analyses a “worse-best-case” scenario dataset will be generated where it is assumed that all participants missing the primary outcome in one group had a harmful outcome and all missing the outcome in the other group had a beneficial outcome.

12.9. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

These will be described, reported and justified in the final data analysis report.

13. DATA MANAGEMENT

A detailed Data Management Plan will be developed to outline the data management processing, data cleaning and QC procedures for the trial. The data management aspects of the study are summarised here with details fully described in the Data Management Plan.

13.1. Source Data

Source documents are where data are first recorded, and from which participants’ CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the patient trial ID, not by name.

13.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor and the host institution to permit trial-related monitoring, audits and inspections.

13.3. Data Recording and Record Keeping

Randomised liver and participant data will be entered onto the trial database designed and administered by the NHSBT CTU data management team using MACRO™, a commercially available FDA 21 Code of Federal Regulations (CFR) Part 11 compliant clinical trial database system produced by InferMed. Following completion of analysis, the trial database will be archived in accordance with NHSBT's policies.

The study team must keep the signed Informed Consent forms, all trial documentation and source documents collected during the trial in a secure location (e.g. locked filing cabinets in a room with restricted access). All data must be accessible to the competent authorities and the Sponsor with suitable notice for inspection.

The participants will be identified by a unique patient trial ID in any database. Participant identifiers (e.g. NHS number) will only be stored where required for linkage to external data sources (e.g. NHSBT). Individual participants will not be identified in the resulting publications and presentations from the trial. This trial will comply with the UK Data Protection Act (2018) and the General Data Protection Regulation

All trial documentation must be retained for at least 5 years after trial completion or termination. In addition, the Investigator must not discard or destroy any trial specific materials unless otherwise instructed by NHSBT.

13.4. Use of registry data

The UK Transplant Registry will be the primary source of data about resource use (hospital stay and reasons for re-admission), biochemistry results (liver and renal function), transplant related renal dysfunction and graft/patient survival at 12 months. Where available, the primary source of recipient outcome data will be that collected from the electronic case report forms. Where primary or secondary outcome data are missing, we will attempt to link to the NHS Blood and Transplant registry to obtain missing data where recorded. The primary source for 12- months outcome data will be the UK Transplant Registry. Linkage between trial and registry data will only be undertaken by statisticians working on the trial and registry identifiers will be removed from datasets after linkage has been undertaken. NHS Blood and Transplant Information Governance have conducted a Data Protection Impact Assessment, are satisfied that

confidentiality and data protection measures are in place and approved the use of UK Transplant Registry Data for this study.

14. QUALITY ASSURANCE PROCEDURES

14.1. Risk assessment

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

14.2. Monitoring

Regular monitoring will be performed according to the trial specific Monitoring Plan. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents as these are defined in the trial specific Monitoring Plan. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

14.3. Local Investigator and Site Personnel Training

All key site personnel must undergo relevant training in advance of the site initiation in accordance with Good Clinical Practice (GCP) guidelines. Such training will be documented.

In addition, training for site staff will be provided by OrganOx Ltd in advance of recruitment of the first patient. A record of all device training will be maintained. All personnel involved in randomisation and data entry will also be trained in the use of the online randomisation and data collection tool by members of the clinical trials unit, and records of such training will be maintained.

14.4. Study Documentation

It is the responsibility of the local investigator to maintain complete, accurate and current study records. Each investigator will be provided with an investigator site file, online access to the case reporting system and other associated study specific documentation by the co-ordinating centre. Such records will be maintained during the course of the study and for up to 5 years following the date on which the study is terminated or completed, in accordance with local regulatory requirements.

14.5. Trial committees

There are a number of committees involved with the oversight of the trial. These committees are detailed below.

14.5.1. Trial Management group (TMG)

A TMG comprising the CI, other lead investigators, local principal investigators and members of the CTU. The TMG will be responsible for the day to day running and management of the trial. It will meet at least twice a year, more often during set up and close down phases of the trial. At least one face to face meeting will be held each year.

14.5.2. Trial Steering Committee (TSC)

The role of the TSC is to:

- provide expert oversight of the trial
- maintain confidentiality of all trial information not already in the public domain
- make decisions as to the continuation of the trial
- monitor recruitment rates and advise the TMG on recruitment issues
- review and approve V1.0 of the protocol, and any substantial amendments
- review regular progress reports of the trial from the Trial team
- receive feedback from the DMC and consider their recommendations, including any ethical implications arising from their advice
- assess the impact and relevance of any accumulating external evidence
- monitor completion of Case Report Forms (CRFs) and comment on strategies from TMG to deal with problems
- monitor protocol deviations and advise the TMG on remedial action
- monitor any quality issues e.g. serious breaches and advise TMG on remedial action approve additional sub-studies
- oversee the timely reporting of trial results
- approve the statistical analysis plan
- approve the publication policy
- approve the main trial manuscript
- approve abstracts and presentations of results during the trial and on completion
- approve any requests for release of data or samples including clinical data and stored biological samples

The ultimate decision on continuation of the trial lies with the TSC.

14.5.3. Safety Monitoring Committee

The trial has a data monitoring committee (DMC) which consists of at least three independent members, including clinicians with relevant expertise and a statistical expert, independent from the Investigators and the funding source. The DMC will periodically review accruing data to safeguard the interests of the trial participants, potential participants and future patients and assess the safety of the interventions. As a result of the reviews the DMC may make recommendations to the TSC, including premature termination of the trial, should they feel it is indicated.

A separate DMC charter will contain full details of the committee and its roles and reporting structure.

15. PROTOCOL DEVIATIONS

15.1. Definitions

The investigators shall conduct this study in accordance with this protocol and any conditions of approval/notification imposed by the Research Ethics Committee and Competent Authority. Failure to comply with and/or inability to meet these regulations may jeopardize further participation of the investigator or investigative site in this and future clinical studies.

A "protocol deviation" is a failure to adhere to the requirements specified in this study protocol without adequate justification. Examples may include the enrolment of a liver or recipient not meeting all of the inclusion/exclusion criteria specified in section 8 or missed study procedures without documentation. Livers excluded after randomisation due to factors not known at the time of randomisation (see section 11.4) will not be deemed protocol deviations.

15.2. Reporting of protocol deviations

All protocol deviations must be recorded and reported to the data monitoring committee. The DMC will review all deviations and assess their impact on patient safety. Serious breaches must be reported as per section 15.3.

15.3. Reporting of serious breaches

A "serious breach" is defined as a breach of GCP or the trial protocol which is likely to affect to a significant degree:

a) The safety or physical or mental integrity of the subjects of the trial; or
b) The scientific value of the trial

In the event that a serious breach is suspected the NHSBT CTU must be contacted within 1 working day. In collaboration with the chief investigator and the DMC, the serious breach will be reviewed by the NHSBT CTU. If appropriate, NHSBT CTU, in conjunction with the sponsor will report it to the REC committee and the host institution within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki (2008).

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in in compliance with the approved protocol, Good Clinical Practice(GCP), the General Data Protection Regulation and the UK Policy Framework for health and social care research..

16.3. Approvals

Following Sponsor approval, the protocol, informed consent form, participant information sheet will be submitted to an appropriate Research Ethics Committee (REC), HRA (where required), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Other Ethical Considerations

It is possible that, through participation in this trial, incidental findings may be made that are unrelated to a participant's liver disease, transplantation or involvement in the trial, but are of relevance to their health or wellbeing. If this does happen, the patient will be informed of the findings and, with their consent, so too will their GP and other relevant members of their local health team.

Participation in this trial will not affect a patient's position on the liver transplant waiting list or their likelihood or receiving a liver transplant. Similarly, withdrawal of a participant from the trial at any point and for any reason will not affect their position on the liver transplant waiting list or their likelihood or receiving a liver transplant.

16.5. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation, funder (where required) and Sponsor. In addition, an End of Trial notification and final report will be submitted to the REC, host organisation and Sponsor.

16.6. Donor and Recipient Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of both donors and recipients will be minimised by making use of unique liver and patient trial IDs only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

16.7. Expenses and Benefits

Where possible, study visits and investigations will be conducted during routine hospital attendances. Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, in accordance with the requirements of the Declaration of Helsinki 2008.

17. FINANCE AND INSURANCE

17.1. Funding

This study is funded by an NIHR Efficacy and Mechanism Evaluation Award (NIHR131163). Funding will be managed through the Nuffield Department of Surgical Sciences (NDS) finance office.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. DISSEMINATION POLICY

18.1. Data analysis and release of results

By conducting the study, the local investigators agree that all information provided by the sponsor and co-ordinating centre will be maintained by the local investigators and the site personnel in strict confidence. It is understood that the confidential information provided to local investigators will not be disclosed to others without authorization from the sponsor and/or co-ordinating centre.

The scientific integrity of the study requires that all data must be analysed study-wide and reported as such.

No data from the study will be presented in oral or written form without permission of the TSC. Approval to submit papers for publication will include all authors of the paper.

18.2. Primary outcome publications

All publications, abstracts and other outputs will be reviewed by the Trial Steering Committee (TSC) prior to publication. Publications will reflect the input of all participating centres in authorship, which will be agreed by the TSC.

Reports relating to primary outcomes will be published in peer-reviewed journals of appropriate relevance. Individual centres will undertake not to report any trial data independently. A final report on the primary outcomes of the study will be compiled by the chief investigator and NHSBT CTU and approved and signed off by each local investigator.

18.3. Other study papers, abstracts and presentations

Study investigators wishing to publish secondary data analyses will submit a proposal to the TSC for approval. If the committee accepts the proposal, then the author of the proposal may decide on the lead in each publication resulting from such a proposal.

18.4. Identification

The ISCRTN trial identifier will be included on all presentations and publications.

18.5. Timing

No data may be made public before publication and never without agreement from the CI.

18.6. Acknowledgements

For the main report of this study submitted for publication, together with associated methodology and health economic papers or posters/presentations, we will use the International Committee of Medical Journal Editors definitions of Authorship and Contributorship (http://www.icmje.org/ethical_1author.html). The members of the TSC and DMC should be listed with their affiliations in the Acknowledgements/Appendix of the main publication and the support of the NHSBT CTU, and funder acknowledged in all publications/presentations. The NIHR must be acknowledged in all research publications and carry a disclaimer:

"The research is funded by the National Institute for Health Research (NIHR). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care"

19. MANAGEMENT OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The protection and exploitation of any new IP is managed by the University's technology transfer office, Oxford University Innovations.

20. ARCHIVING

Archiving will be authorised by the Sponsor following submission of the end of study report. The TMF including all essential documents will be retained for at least 5 years after the completion of the study.

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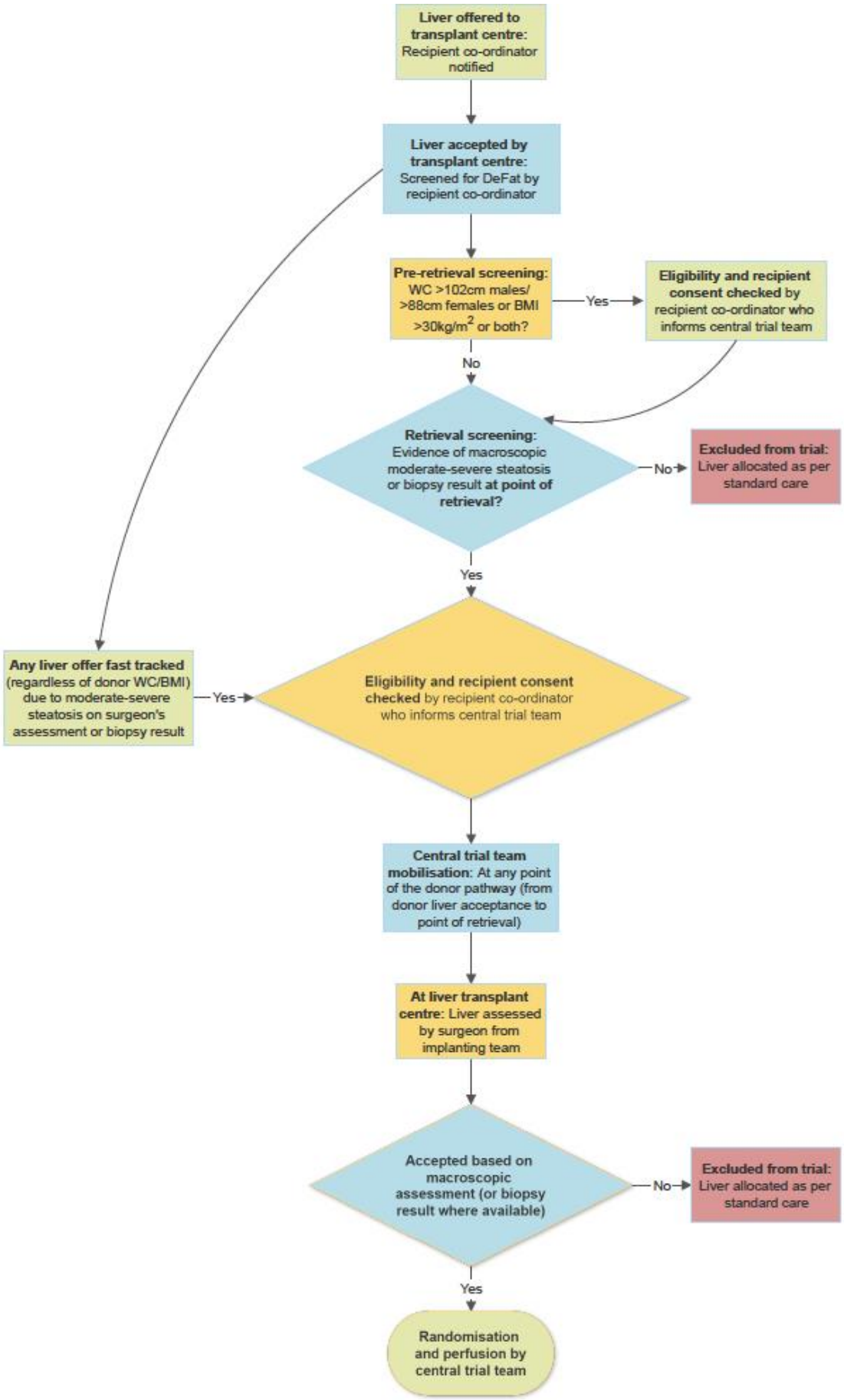
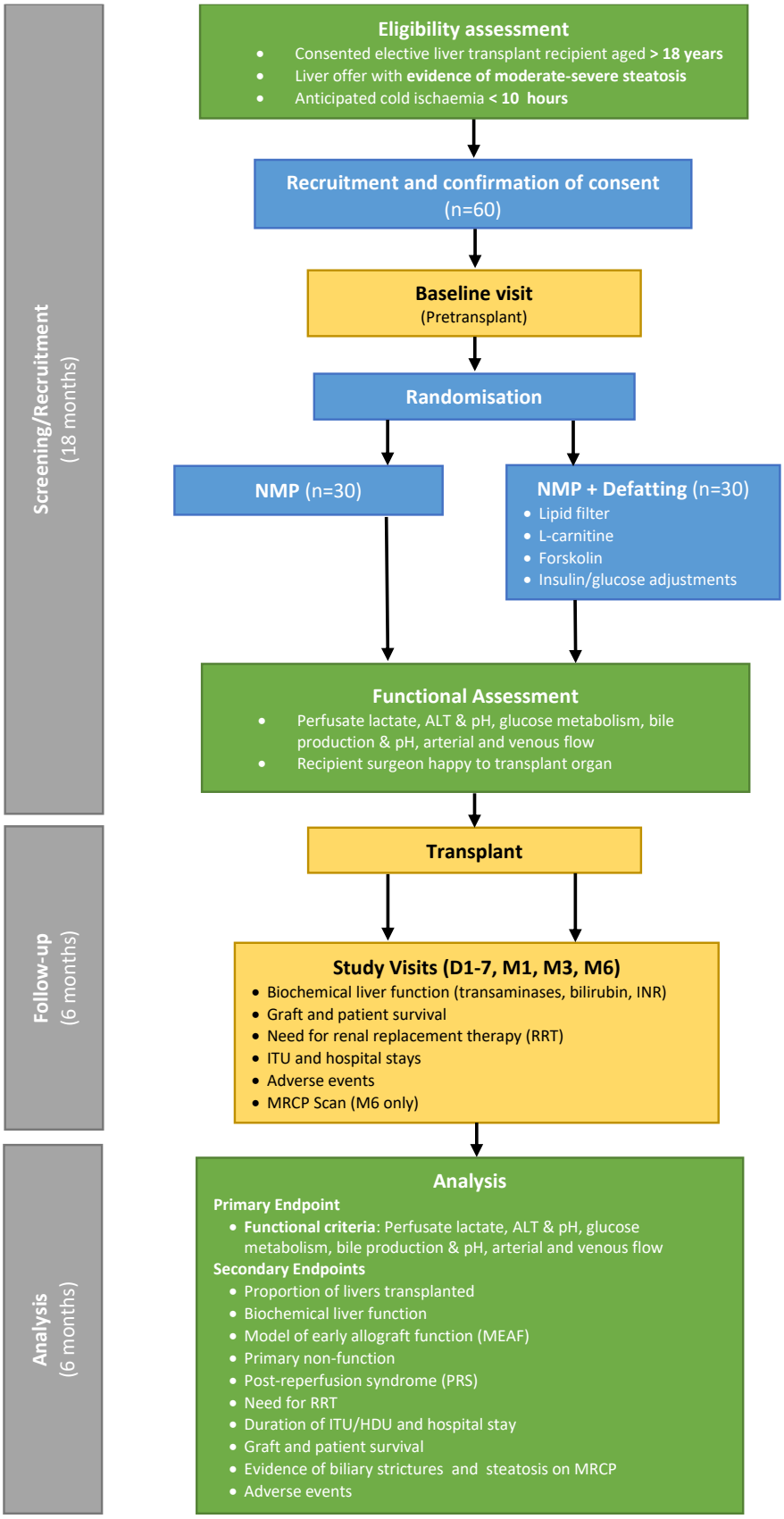
22. APPENDIX 1: FLOW OF LIVERS AND PARTICIPANTS THROUGH THE STUDY





Flow of participants through the study. NMP – Normothermic Machine Preservation; MRCP – Magnetic Resonance CholangioPancreatography, ALT – Alanine Transaminase

23. APPENDIX 2: SCHEDULE OF PROCEDURES

Activity	Pre-study Screening	Pre-study Baseline	Pre-perfusion	During & end of perfusion	Pre-transplant	Post-reperfusion	Postoperative									Follow-up			
							D1	D2	D3	D4	D5	D6	D7	D10	D14	D30	M3	M6	M12
Informed consent	X																		
Meets inclusion/ exclusion criteria	X																		
Randomisation		X																	
Donor & recipient demographics		X																	
Perfusion parameters/samples			X	X	X	X													
cfDNA samples			X	X	X	X	X		X				X		X	X	X	X	
Surgical variables						X													
Serum ALT & AST							X	X	X	X	X	X	X			X	X	X	X
Serum Bilirubin							X	X	X	X	X	X	X			X	X	X	X
Serum GGT							X	X	X	X	X	X	X			X	X	X	X
INR							X	X	X	X	X	X	X			X	X	X	X
Serum lactate*							X	X	X	X	X	X	X						
Primary non-function														X					
Graft survival							X	X	X	X	X	X	X	X		X	X	X	X
Patient survival							X	X	X	X	X	X	X	X		X	X	X	X
Resource use							X	X	X	X	X	X	X	X		X	X	X	X
Safety outcomes						X	X	X	X	X	X	X	X	X		X	X	X	X
MRI (depending on site capacity)																		X	

24. APPENDIX 3: CLAVIEN-DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS

Grade	Definition
I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions.
II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.
III	Requiring surgical, endoscopic or radiological intervention.
IIIa	Intervention not under general anaesthesia.
IIIb	Intervention under general anaesthesia.
IV	Life-threatening complications (including CNS complications) requiring HDU/ITU management.
IVa	Single organ dysfunction (including dialysis).
IVb	Multi-organ dysfunction.
V	Death of a patient.
Suffix 'd'	If the patient suffers from a complication at the time of discharge, the suffix 'd' (for disability) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication.

25. APPENDIX 4: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	v1.1	22/06/2022	Hussain Abbas Simon Knight Peter Friend	Updates: PI in Birmingham (Thamara Perera), PI in Leeds (Abdul Hakeem), Co-investigator (Rachel Johnson), Trial Steering committee (Helen Thomas). Use of 12 month NHSBT registry data itemised.
2	V1.2	20/09/2022	Hussain Abbas Simon Knight Kerrie Brusby Fotini Kaloyirou Peter Friend	To make clearer the following points: • It may not be possible to perform the LiMAx test in all perfusions – therefore, the LiMAx test will only be performed where feasible. • Preparation of defatting agents (L-carnitine and Forskolin) for perfusate supplementation. • Livers recruited to this study will have evidence of at least moderate steatosis i.e. this includes those with moderate-severe steatosis. • Pre-implantation research biopsy samples will only be taken where donor family consent to research is in place. • All research samples will be stored for future research and the mechanistic studies described in the study protocol. This update is to match description already provided in the participant information sheet and consent form. • Research blood samples will be sent to an accredited laboratory in the UK or abroad
3	V1.3	26/10/2022	Hussain Abbas Fotini Kaloyirou Simon Knight Peter Friend	Update: • PI in Cambridge updated to Mr Rohit Gaurav

4	V2.0	09/03/2023	Hussain Abbas Kerrie Brusby Simon Knight Peter Friend	Update: • Addition of ISRCTN registration number • Removal of confidential watermark from footer • Update of trial statistician from Daphne Kounali to Helen Thomas • Removal of trial steering committee, data monitoring committee and trial management committee lists from protocol. These lists are subject to change over the course of the study and this information can be accessed from the respective charters of each committee • To make clearer that the study MRI will be performed depending on site capacity • To make clearer reporting of SAEs i.e. both unexpected and related rather than unexpected alone • To provide a cover letter for the participant information sheet
5	V3.0	26/06/2023	Hussain Abbas Kerrie Brusby Fotini Kaloyirou Simon Knight Peter Friend	Update: • To make clearer when the central trial team can mobilise to the trial site in order to avoid delays and ensure timely arrival. • To make clearer the process for obtaining informed consent for eligible patients on the transplant waiting list i.e. giving the option to sign consent in-person (in clinic) or post/email the signed consent form back to the trial site. This is also updated in the PIS. • To make clearer that in the event of a fast-track liver offer arriving in hospital before the patient, if the participant has signed the informed consent whilst on the waiting list, randomisation and perfusion

				may commence before the participant is on-site. This is also updated in the PIS. • To update the consent form documenting time of consent • To update the cover letter stating that along with the PIS sheet, a copy of the consent form is also provided (for future use) • To add an additional timepoint for the LiMAx test during perfusion at 5 hours

List details of all protocol amendments here whenever a new version of the protocol is produced.

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC committee, HRA (where required) or MHRA.