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Peritransplant energy changes and their correlation to outcome after human liver transplantation

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Abstract

Background—The ongoing shortage of donor livers for transplantation and the increased use of marginal livers necessitate the development of accurate pretransplant tests of viability. Considering the importance energy status during transplantation, we aimed to correlate peritransplant energy cofactors to posttransplant outcome and subsequently model this in an ex vivo setting.

Methods—Sequential biopsies were taken from 19 donor livers postpreservation, as well as 30 min after portal venous (PVR) and hepatic arterial reperfusion (HAR) and analyzed by LC-MS for energetic cofactors (ATP/ADP/AMP, NADH/NAD⁺, NADPH/NADP⁺, FAD⁺, GSSG/GSH). Energy status was correlated to posttransplant outcome. In addition, 4 discarded human DCD livers were subjected to ex vivo reperfusion, modeling reperfusion injury and were similarly analyzed for energetic cofactors.

Results—A rapid shift towards higher energy adenine nucleotides was observed following clinical reperfusion, with a 2.45-, 3.17- and 2.12-fold increase in ATP:ADP, ATP:AMP and energy charge (EC) after PVR, respectively. Seven of the 19 grafts developed early allograft dysfunction (EAD). Correlation with peritransplant cofactors revealed a significant difference in EC between

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Author contributions

B.G.B., J.A., J.F.M., K.U. and H.Y. designed the study. M.L.J., K.C., B.A., H.Y. participated in clinical indications and enrolment. B.G.B., J.A., G.V.S., P.W. conducted the laboratory experiments. B.G.B., J.A., G.V.S., R.J.P., K.U., H.Y. participated in data analysis and interpretation. The manuscript was written and reviewed by all authors.

Disclosures

Dr. Uygün is inventor on pending patents relevant to this study (WO/2011/002926; WO/2011/35223). Dr. Uygün and Dr. Bruinsma have a provision patent application relevant to this study (MGH 22743). Dr. Uygün has a financial interest in Organ Solutions, a company focused on developing organ preservation technology. Dr. Uygün's interests are managed by the MGH and Partners HealthCare in accordance with their conflict of interest policies.

EAD and normal functioning grafts (0.09 vs. 0.31, $P < 0.05$). In the simulated reperfusion model, a similar trend in adenine nucleotide changes was observed.

Conclusion—A preserved energy status appears critical in the peritransplant period. Levels of adenine nucleotides change rapidly following reperfusion and ratios of ATP/ADP/AMP following reperfusion are significantly correlated to graft function. Using these markers as a viability test in combination with ex vivo reperfusion may provide a useful predictor of outcome that incorporates donor, preservation and reperfusion factors.

INTRODUCTION

Annually over 10 000 patients are added to the liver transplant wait list in the United States, while only 6000 donor livers are deemed transplantable¹. These donors are selected based on criteria that result in a sufficiently high chance of good transplant outcome. Currently, transplantation of livers that meet these criteria results in 1-years survival rates of nearly 90%². However, in an attempt to satisfy the increasing demand for donor livers the use of more marginal grafts is being pushed. These grafts, including livers from cardiac death donors (DCD) pose an increased risk of poor posttransplant function. This risk further accentuates the need for reliable determinants of posttransplantation function^{3,4}.

Numerous clinical cohort studies have identified risk factors for poor graft function and graft failure in liver transplantation⁵⁻⁷. Moreover, long-term clinical data has been used to create the donor risk index (DRI) to predict graft failure, using exclusively donor characteristics⁸. However, posttransplant function is not just the result of donor liver quality, but is also highly dependent on the quality of liver preservation and the degree of reperfusion injury. Consequently, transplant outcome remains difficult to predict based on current clinical classifications of liver viability⁷. Although many preservation and reperfusion factors that influence liver function have been thoroughly studied, useful predictors of outcome that incorporate these factors are still lacking.

The importance of preserving and restoring energy metabolism throughout the transplant process has been duly acknowledged⁹. Consequently, current methods of organ preservation aim to best preserve the viability of the liver by reducing the rate of this energy depletion¹⁰. Practically, it is conjectured that once energy levels have fallen beyond a critical point, the resulting hepatocellular injury is irreversible¹¹. Furthermore, we have previously emphasized the importance of preserving the cellular machinery to subsequently recover energy levels of reperfusion¹². A number of studies have demonstrated the importance of the peritransplant period, indicating a correlation between early graft ATP levels and outcome^{13,14}. Despite the importance of adenine nucleotide levels in liver viability, these or surrogate parameters are currently not used in the consideration of liver transplantability. Clinical applicability has been limited in part by time-consuming methods of ATP analysis, which are now quickly being replaced by new, rapid mass-spectrometry techniques. Moreover, despite an abundance of experimental data, recent studies correlating peritransplant ATP levels to transplant outcome in the clinical setting are lacking

To address the increasing uncertainty and facilitate expanded use of more marginal grafts, attempts are made to more accurately predict outcome, using either preoperative biomarkers^{15,16}, or by means of functional testing in an ex vivo setting¹².

Various ex vivo machine perfusion modalities have been explored for the preservation and viability testing of human livers^{17–19}. However, an ex vivo model for reperfusion of the human liver does not yet exist. Such a model would provide a platform for both physiologically relevant pretransplant viability testing as well as for use as an experimental outcome. Development of an accurate reperfusion model is met with various challenges including timing of donor liver procurement and the availability of whole blood for accurate simulation of reperfusion injury.

Here we present a preliminary clinical study that demonstrates a significant correlation between energy cofactor recovery in transplanted livers and early allograft dysfunction. Moreover, we further explore this reperfusion energy dynamics in a novel simulated human liver transplant model with ex vivo whole blood reperfusion, and demonstrate the clinical relevance of this model for early posttransplant metabolism.

MATERIALS AND METHODS

Patient and clinical data collection

Adult candidate recipients (aged 18–75 years) for deceased donor liver transplantation were included in this study following informed consent. Potential study candidates were approached during an inpatient admission or during 2 outpatient clinical appointments where the study could be sequentially introduced and consented. Moreover, cognitively compromised patients (sedated, or severe encephalopathic) could not be included. Biopsies were obtained from 19 recipients, transplanted between October 2013 and March 2015 (Table 1). Three recipients were transplanted in this period where no biopsies could be obtained for logistic reasons. HIV-1 seropositivity was an exclusion criterion. Patients received standard of care as directed by their surgical, anesthesia, and critical care teams. Posttransplant liver function tests, INR, ICU length of stay (LOS), pressor use, HCV status and recipient WIT were determined by retrospective chart review. Donor age, gender, donor type (DCD vs. DBD) and CIT were determined by retrospective review of the recipient medical chart, which had information transferred from the donor packet accompanying the donor organ. The Massachusetts General Hospital Investigational Review Board (IRB) approved this clinical study (protocol #2013P000872).

Surgical and biopsy procedure

All donor livers for transplantation were obtained following standard procurement procedure for brain death or cardiac death donation. Livers were cold-flushed *in situ* with University of Wisconsin (UW) solution or histidine-tryptophan-ketoglutarate (HTK) solution and preserved on ice until transplantation. Livers were flushed through the portal vein with lactated ringers (LR)-albumin solution either immediately prior to implantation or during the infra-hepatic caval anastomosis, depending on whether the piggyback or bicaval technique was used for implantation. Arterial anastomoses and reperfusion were performed separately

and after portal reperfusion. A maximum of 3 needle biopsies (2×25 mm) were taken at sequential time points: 1) immediately prior to implantation (out of ice, OOI) 2) 30 minutes after portal vein reperfusion (PVR) and 3) 30 minutes after hepatic artery reperfusion (HAR). Samples were immediately (<10 seconds) flash-frozen in the operating room using liquid nitrogen and stored at −80 °C until further analysis.

Discarded human livers

Human DCD livers were used for simulated ex vivo reperfusion (n=4). The grafts were turned down for transplantation due to excessive warm ischemia time (>30 minutes), or donor age in combination with DCD status. The livers were procured in the New England Organ Bank (NEOB) region, with consent for research from the donor family. Procurement of the DCD liver was performed following standard procurement protocol, as described in detail elsewhere¹⁷. WIT was defined as the time from donor extubation to the time of *in situ* cold flush with preservation solution. Livers were then transported to our center on ice.

Diluted whole-blood collection and preparation

Autologous blood was collected from the liver donor in conjunction with procurement of the donor liver. Two 5-L suction canisters were primed with 10⁶ U of sodium heparin and were connected to surgical suction devices just prior to transection of the vena cava. Roughly 6 L of blood, combined with UW solution, was collected and transported on ice along with the organ. On arrival, blood was processed to isolate a cell concentrate and remove any accidental debris, or small blood clots. First, the blood was filtered through a 90-μm mesh and centrifuged at 2400 rpm. The acellular top layer was removed and the cell suspension was refiltered and washed twice in 10% dextrose in 0.9% NaCl solution. The final concentrate was corrected to a hematocrit of 21% using type-matched fresh frozen plasma. A final blood volume of 1.5 L was supplemented with 100 U of insulin (Humulin), an additional 200 units of sodiumheparin, ceftriaxone 1 g/L, and the pH was corrected to 7.30–7.50 using sodiumbicarbonate 8.4%. A complete blood count (CBD) was performed on samples at random, confirming leukocyte presence (not shown).

Simulated ex vivo reperfusion

Ex vivo reperfusion was performed on a device designed for machine perfusion preservation of the liver (Liver Assist, Organ Assist, Groningen, The Netherlands). The system was primed with the diluted whole-blood and set to warm at 37 °C. Simultaneously, the liver was prepared for perfusion by cannulation of the portal vein, arterial system and bile duct as described previously^{17,20}. The liver was then flushed with ice-cold Lactated Ringer's solution supplemented with 100 mL/L albumin 25%. The portal vein was flushed with 2 L, followed by a 1 L flush through the hepatic artery. The liver was connected to the machine perfusion device and pressure was set to 6 mmHg on the portal vein, and 60 mmHg on the artery. Oxygenators on both the portal and arterial side were connected to 95% O₂ and 5% CO₂. Throughout perfusion, liver tissue and blood samples were taken, blood gas measurements were performed and pressure and flow dynamics were recorded. Three livers were perfused for 3 hours and 1 liver for 2 hours.

Biochemical analyses

Alanine transaminase—Levels of alanine transaminase (ALT) were measured in the blood during ex vivo reperfusion using a point-of-care blood chemistry analyzer (Piccolo, Abaxis, Union City, CA).

C-reactive protein and cytokines—Levels of C-reactive protein (CRP), TNF- α , IL-1 β , and IL-12 were determined using a multiplex bead array (Eve Technologies, Calgary, Canada).

Cofactor analysis

Frozen tissue biopsies were pulverized (averaging ~25 mg) and analyzed for energetic cofactors using a targeted MRM (multiple reaction monitoring) analysis on a 3200 triple quadrupole liquid chromatography-mass spectrometry (QTRAP LC/MS-MS) system (AB Sciex, Foster City, CA). First, metabolites were extracted using 250 μ L of a 2/1 (v/v) mixture of methanol/chloroform and following 3 freeze-thaw cycles, which entailed 30 seconds of rapid freezing in liquid nitrogen, thawing at room temperature, and a 10 second vortex. Ice-cold water (200 μ L) was then added to each extract and after a 1-min centrifugation at 15 000 $\times$ g, the top phase of the resulting biphasic mixture was transferred to an autosampler vial for LC/MS analysis. The chromatographic separation conditions and the analyte-specific MS parameter optimization routines were the same as that reported in Quinn et al, for which NAD⁺, NADH, and FAD were quantified ²¹. In this study, MRM transitions for ATP, ADP, AMP, NADPH, NADP⁺, GSH, and GSSG were also quantified in addition to NAD⁺, NADH, and FAD. The precursor/product ion transitions for all the compounds are presented in Table S1. The peak area of each MRM transition was correlated to extract concentration based on serial dilutions of pure chemical standards. Pertinent redox ratios were then computed based on the relative concentrations of cofactors calculated to be in the tissue extract.

Statistical analysis

Differences in donor characteristics between groups were tested using a Mann Whitney U or chi squared test where applicable. A logistical regression was performed to test differences in cofactor ratios and DRI between groups. Analysis was performed using Prism 5.0a for Mac OSX (GraphPad Software, La Jolla, CA) and MATLAB (MathWorks, Natick, MA). Data is presented as median (Q1–Q3) unless otherwise noted.

RESULTS

Cofactor changes during clinical reperfusion

Nineteen human livers were included and biopsied in the peritransplant period. Average cold ischemia time was 375 (298–420) min (Table 1). Two transplanted grafts were donated after cardiac death with a donor WIT of 22 and 28 minutes. Average recipient WIT was 30 (27–42) min. PVR and HAR biopsies were successfully collected after 31.0 (29.3–37.0) and 30.5 (30–33.5) min, respectively. For technical reasons no biopsy could be taken after PVR in 3 recipients and after HAR in a single recipient. Overall a rapid shift towards higher energy adenine nucleotides was observed in the early phase of reperfusion. The ratio of ATP:ADP

as well as ATP:AMP increased significantly after PVR (2.45- and 3.17-fold, respectively, $P < 0.01$; Fig. 1A/B). No additional shift in adenine nucleotides was observed after 30 minutes of HAR. Energy charge ($EC = [2ATP + ADP]/[ATP + ADP + AMP]$) showed a similar shift, with a 2.12-fold increase after PVR ($P < 0.005$; Fig. 1C). NADH:NAD⁺ dropped 2-fold in the first 30 minutes ($P = 0.02$), followed by a slight increase that was not statistically significant (Fig. 1D). Concurrently, FAD levels increased after PVR ($P = 0.02$; Fig. 1E). The ratio of oxidized to reduced glutathione remained unchanged throughout the measured reperfusion period (Fig. 1F), while a slight decrease in NADPH:NADP⁺ was observed after HAR (1.39-fold, $P = 0.02$; Fig. 1G).

Correlation of adenine nucleotides to clinical outcome

To test the suitability of adenine nucleotides as a predictor of posttransplant outcome these cofactors were correlated to early allograft dysfunction (EAD), defined as ALT or AST > 2000 U/L in the first 7 days after transplantation or INR > 1.6 on postop day 7 or bilirubin > 10 mg/dL on postop day 7 as validated by Olthoff et al.⁷. Of the 19 transplanted livers, 7 developed EAD and 12 functioned normally (NF). There was no significant difference in CIT, recipient WIT, or other donor characteristics between the 2 groups (Table 2). ATP:ADP, ATP:AMP ratios as well as the total energy charge were compared between EAD and NF groups by logistical regression, which revealed a significant difference in energy charge at the final time point (0.09 (0.05–0.26) vs. 0.31 (0.25–0.33), $P < 0.05$) (Table 3). Surprisingly, a drop is seen in EC between PVR and HAR in livers with EAD (Fig. 2). Notably, DRI correlated poorly with EAD (1.43 (1.18–1.70) vs. 1.40 (1.09–1.76), $P = 0.92$).

Cofactor changes during simulated reperfusion

Four discarded human livers were studied in a novel model of ex vivo human liver reperfusion (Table 4). The ratios of ATP:ADP and ATP:AMP increased by 9.6-fold ($P = 0.03$) and 58.1-fold ($P = 0.03$), respectively, at the end of the reperfusion period (Fig. 3A/B). The energy charge was 0.41 (0.40–0.42) preperfusion and ended at 0.50 (0.46–0.53) ($P = 0.06$; Fig. 3C). There was no significant change in levels of NADH:NAD⁺ ($P = 0.34$; Fig. 3D). During 3 hours of simulated reperfusion, increases in ATP:ADP, ATP:AMP were substantially higher than during the early stages of clinical reperfusion, while this difference was less pronounced for EC change.

Perfusion parameters and injury during simulated reperfusion

The discarded human livers were visually well-perfused within 10 minutes of reperfusion and maintained a homogeneously perfused appearance throughout the simulated ex vivo reperfusion (Fig. 4A). ALT increased sharply in the first 30 minutes to 2111 (2012–2209) U/L as the expected washout effect, and continued to increase marginally thereafter to a final ALT level of 2872 (2646–3098) U/L (Fig. 4A). Lactate levels peaked after 60 minutes (10.9 (10.1–13.3) mmol/L), which was followed by a gradual decrease to 1.37 (1.00–2.10) mmol/L after 180 minutes of reperfusion (Fig. 4B). The inflow pH was acidotic immediately after reperfusion (7.10 (7.00–7.15), but also normalized after 30 minutes (Fig. 4C).

As a marker of inflammatory response to reperfusion CRP was measured and increased from 23.4 (17.6–68.2) $\mu\text{g/mL}$ to 90.1 (66.3–202.6) $\mu\text{g/mL}$ (Fig. 5A). Moreover, inflammatory

cytokines TNF- α , IL-1 β , and IL-12 were determined hourly, and increased to 1887 (1312–2210) pg/mL, 252 (127–698) pg/mL, and 0.58 (0.3–23.58) pg/mL, respectively (Fig. 5B).

DISCUSSION

Herein, we report the changes in cofactors during early reperfusion in liver transplantation and assess the correlation between adenine nucleotide cofactors and posttransplant hepatic function. Moreover, we present for the first time a model of ex vivo reperfusion of the human liver and present changes in energy metabolism during 3 hours of simulated ex vivo reperfusion as a comparison for the clinical data. Our most important findings are 1) a rapid change in, most notably, adenine nucleotide ratios during clinical reperfusion; 2) a correlation between adenine nucleotide ratios and EAD after reperfusion of the arterial system; 3) human liver reperfusion system can be simulated ex vivo, mimicking ischemia/reperfusion injury as well as accurate cofactor shifts that are similar to the in vivo changes.

The importance of maintaining sufficient levels of high-energy adenine nucleotides such as ATP has been thoroughly emphasized. ATP is therefore often used as a viability marker in preservation research^{17,22} but despite seemingly good correlation with outcome, energy status is yet to be used routinely for clinical testing. Previous clinical studies have reported a correlation between peritransplant adenine nucleotide levels and posttransplant outcome. Lanir et al, and Gonzalez et al, found a correlation between both prereperfusion energy charge and ATP levels and transplant outcome^{13,23}. Kamiike et al, found that not the pre, but the postreperfusion ATP levels were predictive of transplant success¹⁴. Looking at primary nonfunction as an outcome measure Hamamoto et al,²⁴ also found ATP levels to be predictive when measured at ~1.5 hours after reperfusion, in line with our findings.

In terms of the role of energy levels, these studies suggest that transplant success is explained not just by remaining stores, but also the ability to recover energy levels on reperfusion, although these measures are likely to be related. In this study we show that while energy charge does not differ significantly at the end of preservation, the reconstitution of ATP during reperfusion is inferior in graft that develop EAD. Experimental studies in an ex vivo setting have corroborated this fact. For instance, in our own work we confirmed the finding that ATP levels gradually decline during ischemia²⁵, and demonstrate that successful transplantation is contingent on a significant recovery of ATP levels during reoxygenation²⁶. Metabolomic analysis in discarded human livers has suggested different mechanisms for poor recovery of ATP in marginal grafts. For instance, highly steatotic livers seem to predominantly suffer for poor perfusion, while excessively warm ischemic DCD livers are more susceptible to mitochondrial injury¹². Alternatively, poor ATP recovery in dysfunctioning livers may be the result of regional micro-perfusion defects during early reperfusion²⁷.

The finding here that energy charge after reperfusion appears most relevant poses a practical issue if we are to use this for viability testing. To make this applicable as a pretransplant test, ex vivo perfusion may offer an elegant outcome. Machine perfusion modalities are gaining significant interest as an improved method of liver preservation. Besides being used as a means to recover the liver before transplantation, or reduce cold ischemic injury, ex vivo

machine perfusion enables viability testing by offering a dynamic environment and, in warm perfusion, facilitating organ function. Various parameters have been proposed as predictive biomarkers, ranging from ALT levels in perfusate and bile production¹⁸, to more complex indices of metabolism shown to correlate to transplant outcome^{28,29}. In various experimental machine perfusion models, ATP levels have been shown to correlate to transplant^{26,30,31} or reperfusion³² outcome parameters. Since ATP analysis is arduous, surrogates have also been found and include bile production on reperfusion³³, and ALT levels during ex vivo perfusion¹⁷. We recently showed that biomarkers can also be measured in the posttransplant flush solution and can also be correlated to ATP levels¹⁵. In this study we present a new model of reperfusion, simulating early reperfusion in an ex vivo setting, so as to facilitate the use of energy status as a biomarker in a first in field approach. This model distinguishes itself from other normothermic blood models by the inclusion of leukocytes in the whole blood composition of the perfusion solution¹⁹, thereby more accurately early representing reperfusion. Early liver injury has been shown to result from neutrophil recruitment through an IL-12 dependent pathway³⁴. In our ex vivo model we show the IL-12 response and increase in TNF α and IFN- γ required for accurate modeling of ischemia/reperfusion injury.

Besides showing relevant injury as is expected during whole-blood reperfusion, we demonstrate clinically relevant shifts in adenine nucleotide ratios. Application of a pretransplant simulated reperfusion would facilitate pretransplant viability testing. Moreover, we suggest that this platform provides an experimental means to study early reperfusion, specifically aimed at identifying predictive markers.

An interesting observation is that the shifts in adenine nucleotide levels occur rapidly and additional oxygenation offered by oxygen-rich arterial blood does not significantly improve adenine nucleotide ratios. It would appear that an oxygen supply immediately fuels oxidative phosphorylation, concurrent with the rapid oxidation of NADH seen in this study and elsewhere¹¹. We also observe a more pronounced increase in ATP:ADP and ATP:AMP levels during simulated reperfusion, when compared to clinical reperfusion. This may be explained by more depleted energy levels at baseline as a result of DCD status and a longer reperfusion period in the former. Secondly, it can be hypothesized that reperfusion in a stressed human body is less conducive to effective repletion of energy stores through an unknown mechanism. In grafts exhibiting EAD we even observe a slight drop in EC between PVR and HAR. This drop causes the discriminatory difference that distinguishes these inferior grafts. Although this study does not offer an explanation for this drop, we can speculate that I/R manifests as an inability to maintain ATP levels after the hyperacute phase of reperfusion, either by dysfunction at cellular level or through perfusion-vascularization defects.

Although this study was broadly inclusive in participation criteria, logistical factors reduced the number of patients included from the number transplanted at our center. There was selection of in house patients, as well as those with multiple outpatient clinic appointments where the study could be introduced and consented. We do not anticipate a significant effect from this, as this does not affect those receiving a transplant. Cognitively impaired patients

could not be included due to inability to consent, as a small bias towards less sick patients is possible.

In conclusion, cofactor metabolites change rapidly in the early peritransplant period. Moreover, postreperfusion adenine nucleotides ratios are correlated to posttransplant outcome. This finding supports the consensus that energy status is essential in successful liver transplantation and could be used to improve pretransplant viability testing, particularly in combination with the expansion of marginal liver transplantation. The presented model of simulated reperfusion is a feasible preclinical model to evaluate the success of alternative preservation techniques directly with human grafts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ADP	adenosine diphosphate
ALT	alanine transaminase
AMP	adenosine monophosphate
ATP	adenosine triphosphate
HTK	histidine-tryptophan-ketoglutarate
LR	lactated ringers
NEOB	New England Organ Bank
DCD	donation after circulatory death
DBD	donation after brain death
DRI	donor risk index
EAD	early allograft dysfunction
EC	energy charge
FAD	flavin adenine dinucleotide
GSSG	glutathione disulfide
GSH	glutathione

HAR	hepatic arterial reperfusion
NADH	nicotinamide adenine dinucleotide
NADP(H)	nicotinamide adenine dinucleotide phosphate
NF	normal function
OOI	out of ice
PVR	portal venous reperfusion
UW	University of Wisconsin

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Fig. 1. Cofactor changes during clinical reperfusion

ATP:ADP (A), ATP:AMP (B), Energy charge (C), NADH:NAD⁺ (D), FAD (E), GSSG:GSH (F) and NADPH:NADP (G) ratios before and after clinical reperfusion of the human liver.

OOI, out of ice; PVR; portal vein reperfusion; HAR, hepatic artery reperfusion.

Fig. 2. Peritransplant energy charge changes

Difference in the dynamics of energy charge during clinical reperfusion of the human liver in normally functioning (NF) livers and early allograft dysfunction (EAD) livers. OOI, out of ice; PVR; portal vein reperfusion; HAR, hepatic artery reperfusion. Thick, uninterrupted lines present the mean for the 2 groups, while the interrupted lines present individual grafts.

Fig. 3. Cofactor changes during simulated reperfusion

ATP:ADP (A), ATP:AMP (B), Energy charge (C), NADH:NAD⁺ (D) ratio changes during simulated ex vivo reperfusion of the human liver. EC, energy charge.

Fig. 4. Simulated ex vivo reperfusion

Human liver at various stages of simulated ex vivo reperfusion (representative images), showing portal vein, hepatic artery and bile duct cannulation (**A**). ALT levels, pH (**B**) and lactate levels (**C**) during during reperfusion. Error bars depict s.e.m.

Fig. 5. Inflammatory injury markers during simulated ex vivo reperfusion

Levels of c-reactive protein (A) and TNF- α , IL-1 β , and IL-12 during reperfusion. Error bars depict s.e.m.

Table 1

Donor and recipient characteristics

	Age	Sex	type	CIT	donor WIT	recipient WIT	HCV	donor COD	DRI	EAD
1	63	M	DBD	384	0	29	–	anoxia	1.57	–
2	58	M	DBD	362	0	39	–	anoxia	1.36	–
3	48	M	DBD	427	0	30	–	anoxia	1.16	+
4	65	F	DBD	413	0	50	–	CVA	2.15	–
5	48	F	DCD	401	22	38	–	trauma	1.81	+
6	62	F	DBD	482	0	29	–	anoxia	1.74	–
7	61	F	DBD	401	0	22	–	CVA	1.87	–
8	43	M	DBD	375	0	45	–	anoxia	1.19	+
9	38	F	DBD	253	0	51	+	anoxia	1.10	–
10	48	F	DBD	340	0	37	–	anoxia	1.43	–
11	59	F	DBD	289	0	28	–	anoxia	1.84	–
12	29	F	DBD	244	0	16	–	anoxia	1.08	–
13	53	M	DBD	434	0	46	–	CVA	1.58	+
14	17	M	DBD	249	0	31	–	trauma	1.21	+
15	14	M	DCD	366	28	21	–	trauma	1.87	+
16	54	M	DBD	180	0	23	–	trauma	1.17	+
17	21	M	DBD	533	0	27	–	anoxia	1.21	–
18	23	M	DBD	306	0	27	–	trauma	1.04	–
19	19	M	DBD	443	0	45	–	trauma	0.93	–

CIT, cold ischemia time; WIT, warm ischemia time; HCV, hepatitis C virus; COD, cause of death; DRI, donor risk index; EAD, early allograft dysfunction

Table 2
Differences in donor and posttransplant outcomes between EAD and NF groups

	EAD (n=7)	(Q1–Q3)	NF (n=12)	(Q1–Q3)	p-value
% male (donor)	86%		42%		0.06
DCD/DBD	2/5		0/12		0.09
Donor age	39.6	(14–54)	53.00	(19–65)	0.25
CIT	375	(180–434)	373	(244–533)	0.8
Recipient WIT	31	(21–46)	29.0	(16–51)	0.9
peak INR	2.2	(1.5–4.0)	1.8	(1.5–2.6)	0.27
peak bili	8.2	(2.1–35.1)	9.2	(3.6–36.1)	0.7
peak AST	7058	(1760–9910)	1062	(303–1492)	0.0004
peak ALT	1972	(757–4511)	474	(157–650)	0.0004
ICU LOS (days)	4	(0–8)	3.5	(2–8)	0.83
Pressor use	5/7		8/12		0.93
HCV	0/12		1/12		0.45

EAD, early allograft dysfunction; NF, normal function; CIT, cold ischemia time; WIT, warm ischemia time; ICU LOS, intensive care unit length of stay; HCV, hepatitis C virus

Table 3
Peritransplant adenine nucleotide level differences between EAD and NF groups

	EAD (n=7)	(Q1-Q3)	NF (n=12)	(Q1-Q3)	p-value
ATP:ADP					
	OOI	0.068 (0.04-0.08)	0.192 (0.12-0.34)	0.45	
	PVR	0.498 (0.44-0.55)	0.713 (0.56-1.02)	0.33	
	HAR	0.178 (0.11-0.73)	0.779 (0.64-1.08)	0.18	
ATP:AMP					
	OOI	0.003 (0.002-0.006)	0.069 (0.01-0.09)	0.69	
	PVR	0.155 (0.09-0.16)	0.208 (0.15-0.41)	0.14	
	HAR	0.023 (0.01-0.25)	0.339 (0.20-0.41)	0.11	
Energy Charge					
	OOI	0.027 (0.02-0.04)	0.093 (0.06-0.15)	0.41	
	PVR	0.217 (0.14-0.21)	0.235 (0.19-0.34)	0.13	
	HAR	0.090 (0.05-0.26)	0.313 (0.25-0.33)	<0.05	
DRI					
		1.21 (1.18-1.70)	1.4 (1.09-1.76)	0.92	

EAD, early allograft dysfunction; NF, normal function; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; DRI, donor risk index

Table 4

Discarded donor liver characteristics

	Age	Sex	type	CIT	donor WIT	HCV	donor COD
1	52	M	DCD	436	38	-	anoxia
2	64	M	DCD	424	21	-	anoxia
3	59	M	DCD	432	27	-	resp. failure
4	51	M	DCD	427	35	-	anoxia

CIT, cold ischemia time; WIT, warm ischemia time; HCV, hepatitis C virus; COD cause of death