Poster Round I, Session 1, 2, 3: Machine Perfusion

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Viability testing and transplantation of marginal donor livers (VITTAL) Trial: Metabolomics of normothermically perfused livers discloses molecular signatures predictive of graft viability and postoperative outcomes

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Background and Aim: Donor organ shortage has led to increased reliance on high-risk livers for transplantation. Furthermore, emergence of machine perfusion has led to a paradigm shift in organ preservation from functional suppression to full ex vivo metabolic support. The recently completed VITTAL trial demonstrated that normothermic machine perfusion (NMP-L) provides an opportunity to objectively assess graft quality and functional integrity to safely transplant high-risk livers rejected by all transplant centres. This study investigated the potential of metabolic profiling to elucidate molecular signatures during NMP-L predictive of graft viability, post-reperfusion syndrome (PRS) and early allograft dysfunction (EAD) in these discarded livers.
Methodology: All livers were deemed marginal according to established high-risk criteria, and rejected by all transplant centres. 31 livers underwent NMP-L for a minimum of four hours, at which point a decision was made to transplant based on established viability criteria. Perfusate aliquots from graft perfusion commencement to 4-hour time point were collected. Samples were centrifuged and the supernatant subjected to an untargeted metabolomics analysis using Ultra High Performance Liquid Chromatography-Mass Spectrometry.
Results: 22 livers were transplanted after achieving viability criteria. Univariate statistical analyses revealed more than 20 metabolites ($q < 0.05$) differentiating liver metabolic profiles according to viability criteria fulfilment following 4 hours of perfusion. Key changes were detected in metabolites relating to lipid, phospholipid and sphingolipid metabolism, notably upregulated in the non-viable group. In transplanted cohort; after 4 hours of perfusion 52 metabolites distinguished EAD(n=7) from non-EAD livers(n=15) and PRS group(n=10) revealed changes in 36 metabolites compared to non-PRS group(n=12).
Conclusion: This study reveals a metabolic signature of high-risk livers that correlates with VITTAL criteria for graft viability for transplantation during NMP-L. It also demonstrates the potential of NMP-L metabolic profiling as a clinical tool to objectively assess the quality and predict functional integrity of these livers pre-implantation.

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Initial report of siRNA uptake during normothermic and hypothermic liver machine perfusion: a promising new therapy

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Background: RNA interference (RNAi) is a natural gene modulation process that was recently approved for clinical use and has great potential for treatment of many pathological conditions. The surface receptor FAS and tumor suppressor p53 are implicated in ischemic liver damage. Both may be silenced via RNAi with limited off-target effects. We determined the efficiency of FAS siRNA delivery with lipid nanoparticles during normothermic (37°C) and hypothermic (4°C) ex vivo machine perfusion. We also show silencing p53 via siRNA reduces inflammation and caspase expression during liver ischemia.
Methods: Rat portal veins were cannulated (Fig. 1A) and perfused at 10mmHg on a closed-loop pump circuit regulated by circulating water bath (Fig. 1B). Controls were perfused with Williams E+10U insulin and lipid nanoparticles (Fig. 1C). Normothermic livers were perfused with medium plus nanoparticle/FAS-siRNA complex with 3'-AlexaFluor-555 modification at 37°C. Hypothermic livers were perfused similarly at 4°C. Biopsies were collected at 4 hours, formalin fixed, stained, then imaged with confocal microscopy. For p53 studies, siRNA was delivered 24 hours before hilum clamping. Tissue/blood was collected 3 days after siRNA injection.
Results: Compared to empty vector controls (Fig. 2A-D), normothermic (Fig. 2E-H) and hypothermic livers (Fig. 2I-L) perfused with FAS siRNA demonstrate diffuse uptake in sinusoids and surrounding central veins. SiRNA is observed together in sinusoid membranes and in hepatocyte cytoplasm. Livers pretreated with p53 siRNA showed reduced cellular infiltration, vacuolization, and fewer caspase-3 positive cells.

Conclusions: By incorporating RNAi into preservation solutions or delivering before procurement, we may reliably improve the quality of livers during the ischemic period. It will be important to determine the ideal perfusion temperature. Further studies will quantify uptake with peptide-nucleic acid hybridization at both perfusion temperatures and the degree of FAS or p53 silencing in a transplant model.

Figure 1:

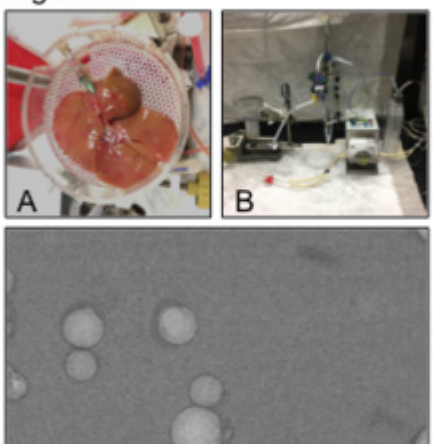
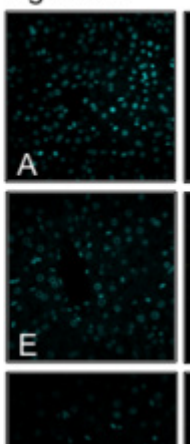


Figure 2:



[FAS Data Figure]

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Normothermic ex situ liver perfusion of donation after cardiac death grafts improves mitochondrial function

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Background: The Normothermic ex vivo liver perfusion (NEsLP) technique reduces reperfusion injury of donation after circulatory death (DCD) grafts and improves post-transplant outcomes. We aimed to understand the mechanistic basis of this improvement using high-throughput metabolomics.
Methods: Pig livers from 8 DCD donors with 30 minutes of warm ischemic time were preserved for 8 hours at cold static storage (CS, n=4) or perfused for 5 hours on NEsLP machine (n=4). Grafts were then transplanted into recipients, and animals sacrificed at day 7 post-transplantation. Liver tissues were collected at: 8 hours following preservation (T0) and 2 hours following liver transplantation (T1). Snap-frozen tissue was processed and analyzed by Sciex 6600 Q-TOF high resolution mass spectrometer. Three platforms of metabolites were compared atT1 vs T0 for both Cold storage and NEsLP: Fatty Acids, Amino Acids and Pentose Phosphate/TCA/Glycolysis Pathway. Data analysis was performed using MetaboAnalyst 4.0, with ANOVA-Simultaneous Component Analysis and Multivariate Empirical Bayes Analysis.
Results: A total of 50 statistical relevant metabolites were identified in the time course analysis obtained in NEsLP compared to COLD grafts **(Table 1)**. Lactate and acyl fatty acid derivative esters of carnitine were most significantly decreased following transplantation with NEsLP versus COLD organs. Tryptophan and alpha-ketoglutarate were significantly higher in NEsLP versus COLD group over time **(Figure 1)**.
Discussion: Compared to cold stored preservation, NEsLP-preserved DCD grafts have lower lactate and Acyl Carnitine-related metabolite concentrations following transplantation, indicating better mitochondrial function and β-oxidation. Additionally, tryptophan and alpha-ketoglutarate production were significantly increased in NEsLP organs, reflecting improved amino acid metabolism leading to protein synthesis. This high-throughput metabolomics study clearly provides a mechanistic rationale for NEsLP in improving graft function in DCD grafts as compared to cold storage alone.

Figure 1: Changes in Abundance of Metabolites Over Time

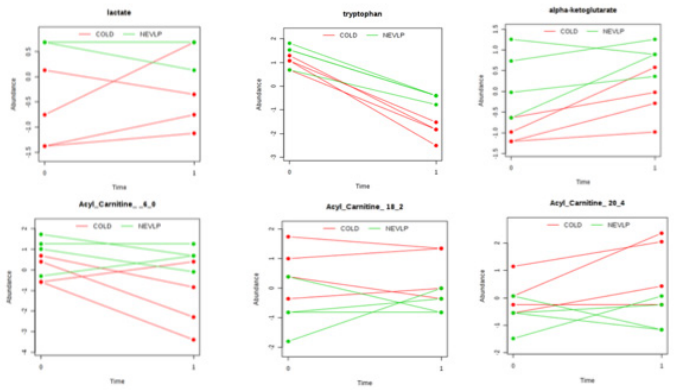

Table 1: Top 50 features identified by MEBA

	Compounds	Hotelling-T2
1	lactate	15.7192
2	tryptophan	15.34322
3	alpha-ketoglutarate	12.06549
4	Acyl_Carnitine_ _6_0	10.86261
5	Acyl_Carnitine_ 18_2	9.03194
6	Acyl_Carnitine_ 20_4	8.74487
7	phenylalanine	6.5822
8	Acyl_Carnitine_ 22_5	6.03624
9	4-aminobutyrate	5.97618
10	lysine	5.93937
11	tyrosine	5.75745
12	arginine	5.68636
13	dihydroxyacetonephosphate	5.53434
14	Acyl_Carnitine_ C4-OH	5.50636
15	Valeryl _Carnitine	5.32356
16	ornithine	5.22639
17	Propionyl _Carnitine	5.08382
18	leucine	4.97065
19	Acyl_Carnitine_ C5DC	4.84827
20	Acyl_Carnitine_5_1	4.81208
21	malate	4.33057
22	Acyl_Carnitine_ 18-OH	3.81352
23	glutamate	3.52683
24	Acyl_Carnitine_ 18_3	3.31857
25	Hydroxy-Palmitoyl _Carnitine	3.22837
26	proline	3.04153
27	phosphoenolpyruvate	3.00638
28	3-phosphoglycerate	2.86685
29	isoleucine	2.86198
30	2-Hydroxygluterate	2.80692
31	citrulline	2.72672
32	Acyl_Carnitine_ 16_1	2.63376
33	ribose-5-phosphate	2.53454
34	methionine	2.45862
35	fructose-6-phosphate	2.22689
36	alanine	2.07741
37	valine	1.91406
38	Acyl_Carnitine_ 10_0	1.89921
39	Acyl_Carnitine_ 18_1	1.57521
40	serine	1.19755
41	_Carnitine	1.05521
42	Hypoxanthine	1.05377
43	Acetyl_Carnitine	0.89599
44	Palmitoyl _Carnitine	0.87803
45	threonine	0.7078
46	Pipecolic Acid	0.69802
47	Butyryl _Carnitine	0.66416
48	Acyl_Carnitine_ 20_0	0.62729
49	Stearoyl _Carnitine	0.33799
50	succinate	0.28704

[figure 1]