Assessment of mitochondrial function during prolonged *ex vivo* liver normothermic machine perfusion

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INTRODUCTION

Extracorporeal regeneration of diseased livers may help to overcome organ shortage. Thus, in-depth assessment of cellular mechanisms occurring during prolonged normothermic machine perfusion (NMP) is required.

Mitochondria play a central role in tissue bioenergetics [1,2]. Analysis of mitochondrial respiration using **high-resolution respirometry** (HRR) [3] may provide direct information on **hepatic function** during prolonged *ex vivo* NMP.

OBJECTIVE

We aimed for an in-depth assessment of mitochondrial respiration during prolonged liver NMP.

METHODS

Discarded human liver allografts (n = 14) were machine perfused for up to 5 days.

Liver function was monitored by analyzing perfusate lactate levels every 6 h.

Mitochondrial respiration in tissue homogenates was assessed before perfusion start and every 24 h by HRR for the succinate-linked pathway [3]. The coupling control states oxidative phosphorylation (OXPHOS), resting respiration (LEAK), and electron transfer (ET) capacity were determined [4].

Cell viability and tissue integrity were analyzed by real-time confocal microscopy (RTCM) [5].

RESULTS

Liver NMP was performed with a mean duration of **92 ± 33 h** (mean ± SD), whereas 3 livers were preserved for more than **120 h.**

Close to **physiological lactate** levels could be maintained for at least **72 h** (Fig. 1).

Calculation of ***P-L* control efficacy** revealed that efficacy of the oxidative phosphorylation was not deteriorated for **96 h** of NMP (Fig. 2a).

Mass-specific OXPHOS capacity halved during the prolonged NMP (Fig. 2b). This could be confirmed by a significant increase of the RTCM score (Fig. 2c+d), demonstrating loss of viable hepatocytes.

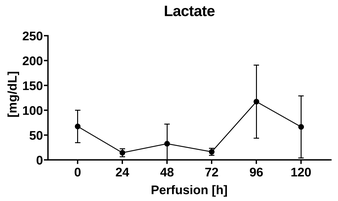

Fig 1: *Perfusate lactate levels during prolonged NMP (shown as mean* ± SD).

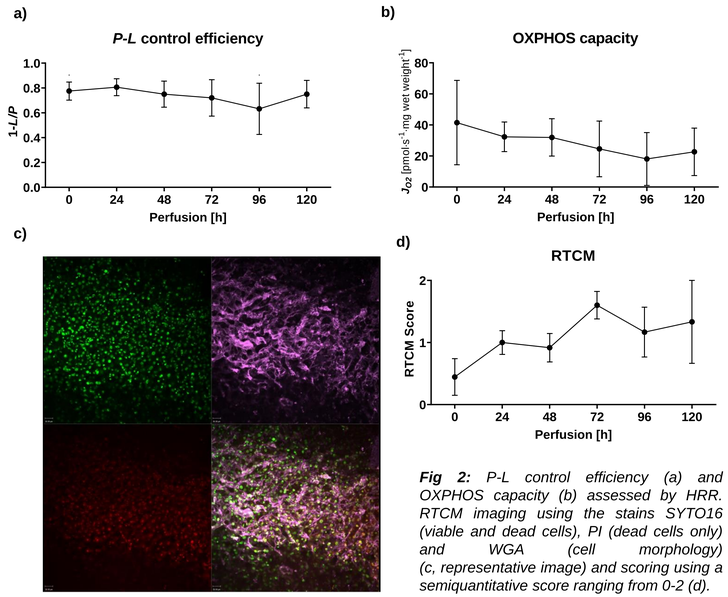

Fig 2: *P-L control efficiency (a) and OXPHOS capacity (b) assessed by HRR. RTCM imaging using the stains SYTO16 (viable and dead cells), PI (dead cells only) and WGA (cell morphology) (c, representative image) and scoring using a semiquantitative score ranging from 0-2 (d).*

Further prolongation of NMP revealed **doubling** of the proportion of **LEAK respiration** (Fig. 3a), indicating oxidative damage to the inner mitochondrial membrane. In line, a significant **increase of cytochrome *c* control efficacy** (Fig. 3b) shows an impaired integrity of the outer mitochondrial membrane.

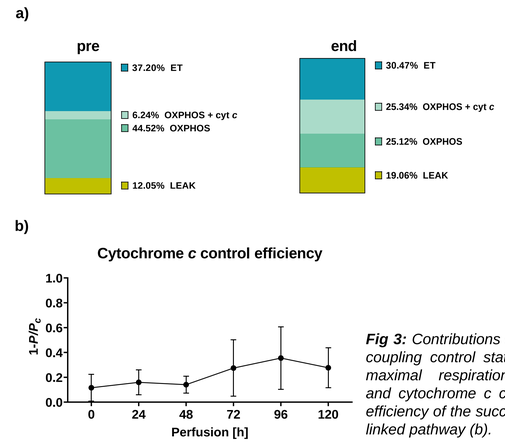

Fig 3: *Contributions of the coupling control states to maximal respiration (a) and cytochrome c control efficiency of the succinate-linked pathway (b).*

CONCLUSIONS

Real-time coupling control analysis in mitochondria may serve as a sensitive marker for the assessment of organ function during liver NMP.

The loss of mitochondrial bioenergetic function may **not** be an early **limiting factor of organ function** during prolonged *ex vivo* machine perfusion.

There is a **time-dependent** shift in the **coupling control.**

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