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The effect of hemolysis during normothermic machine perfusion of the kidney in RPTEC cells

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CHIRURGIE INNSBRUCK
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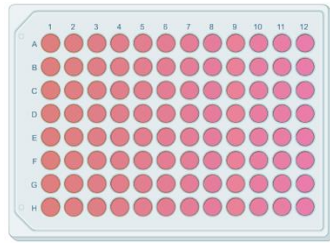
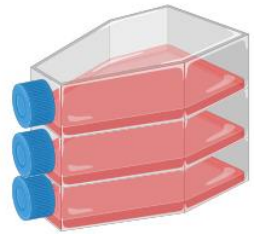
- For kidney normothermic machine perfusion (NMP) mainly red blood cell-based perfusion solutions are used
- Significant hemolysis has been reported in kidney NMP
 - Induction of oxidative stress

How does **hemolysis impact kidney's bioenergetic function** during NMP?

Experimental Design

Renal Proximal Tubular Epithelial Cells (RPTEC)

- Treated with 50 μ M Hemin
- Control with Media



5000 cells/well

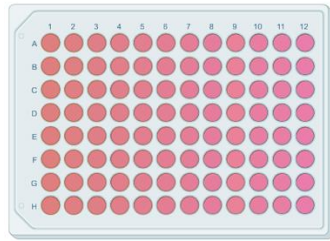
Treatment for 24h



100 μ L of CellTiter-Glow reagent (Promega)



Quantification of ATP



5000 cells/well

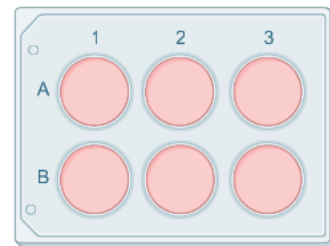
Treatment for 24h



20 μ L of CellTiter96 AQ reagent (Promega)
+ 3,5h incubation

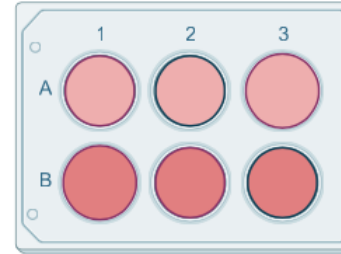


Metabolic activity by MTS assay



1×10^6 cells/well

Treatment for 24h



Preparation of cell suspension
(0.5×10^5 cells/mL) for HRR
measurement

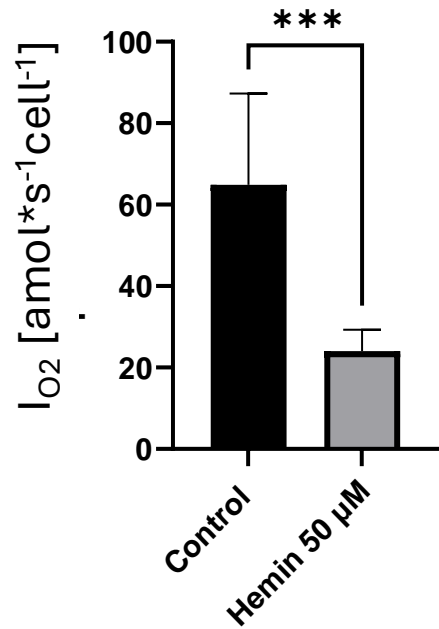


Mitochondrial analysis using High Resolution Respirometry

Results HRR

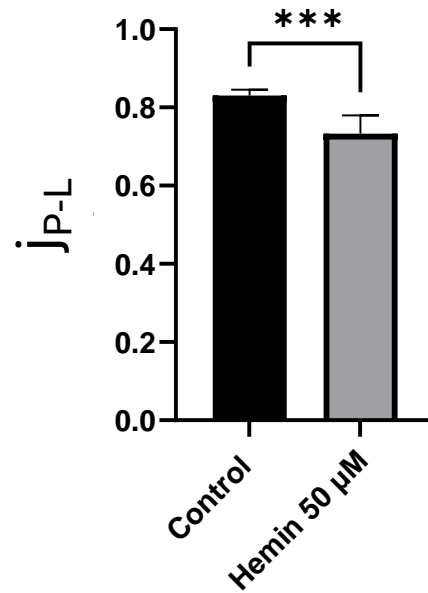
Free hemin leads to **decreased** mitochondrial respiration

OXPHOS capacity



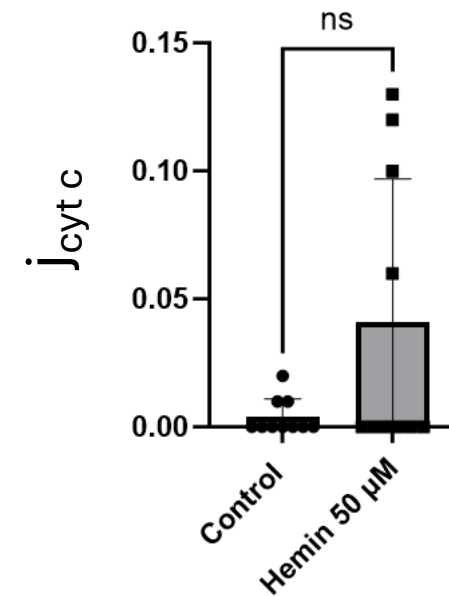
Reduced
OXPHOS
capacity

P-L control efficiency



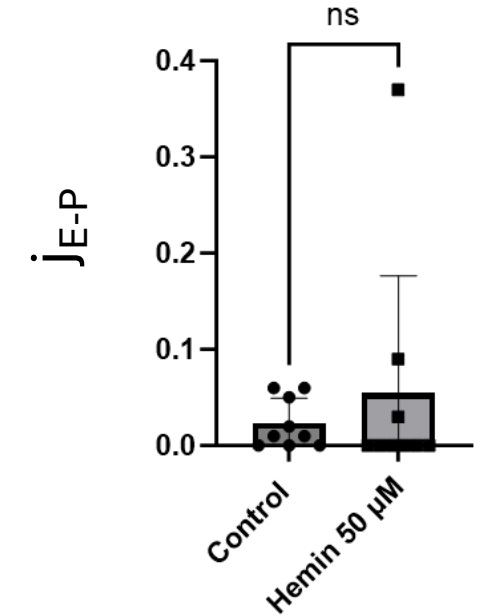
Decreased efficiency
of ATP production
shown by OXPHOS
coupling efficiency

Cytochrome c control efficiency



Outer
mitochondrial
membrane integrity
stayed **intact**

E-P control efficiency



Excess capacity
remained unchanged

This current study revealed...

1. **A potential mitochondrial damage which may lead to impaired kidney function during NMP**
2. **A decreased maximal bioenergetic capacity** in the presence of free hemin
3. **Compromised state of ATP synthesis**
4. **No structural alterations** of the mitochondrial membranes were detected