Species-Specific Mitochondrial Preservation: Porcine but Not Murine Isolated Mitochondria Withstand Cryogenic Stress

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Background

Mitochondrial transplantation is emerging as a bioenergetic therapy.

The need for freshly isolated mitochondria limits its battlefield use. Cryopreservation could enable storage; however, freeze-thaw injury disrupts mitochondrial structure and function. We evaluated controlled-rate freezing to preserve mitochondria.

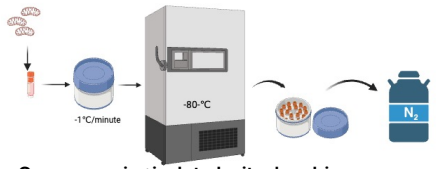
We hypothesized that controlled-rate cryopreservation would preserve mitochondrial oxidative phosphorylation (**OXPHOS**) and membrane integrity, with susceptibility differing by species and tissue.

Methods

Skeletal muscle (**SM**), heart, and kidney were obtained from pigs (n=8) and C57BL/6 mice (n=19). Mitochondria were isolated by differential centrifugation, resuspended in respiration medium (Mir05).

Mitochondrial function was evaluated by measuring uncoupling, oxygen consumption rate (**OCR**), and ATP synthase capacity in both fresh and frozen preparations (OROBOROS).

The same protocol was applied, with NADH substituted for pyruvate/malate, to assess inner mitochondrial membrane damage.



Cryopreserving isolated mitochondria.
Mitochondria underwent controlled-rate freezing (-1°C/min) overnight, then stored in liquid nitrogen for up to 2 weeks.

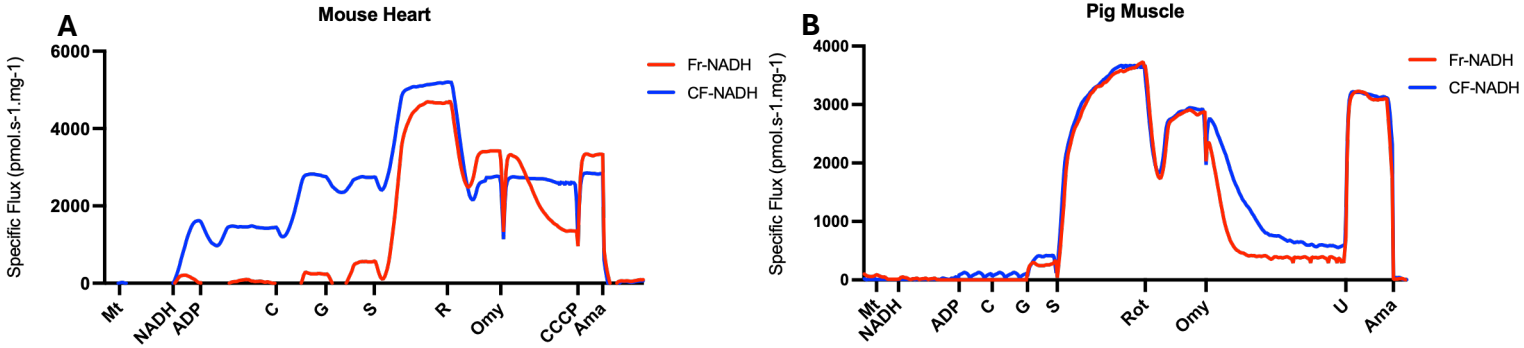


Figure 1: Inner membrane damage. A. NADH stimulated flux increase in mouse frozen mitochondria indicating inner membrane damage . **B.** Pig muscle showed no significant increase in NADH stimulated flux in the frozen mitochondria samples. **Fr:** fresh. **CF:** controlled frozen.

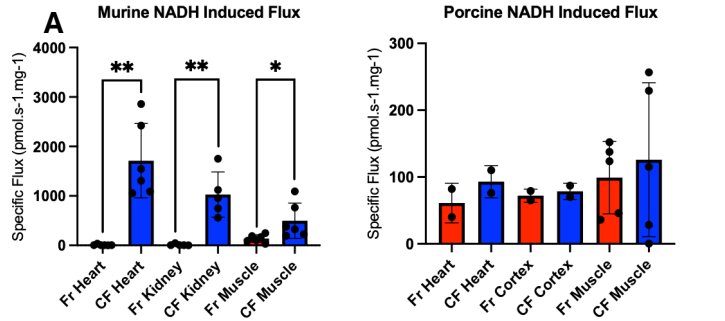


Figure 2: NADH induce flux. A. Murine frozen mitochondria from all tissues had an increase in NADH-stimulated respiration. **B.** Porcine frozen mitochondria from heart, muscle and kidney, showed no significant NADH-stimulated respiration after freezing. **Fr:** fresh. **CF:** controlled frozen.

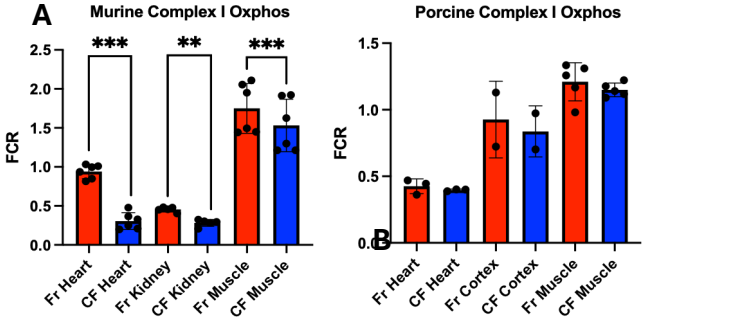


Figure 3: Complex1 OXPHOS capacity in fresh and frozen mitochondria. A. Mouse mitochondria from all tissues showed a mark reduction in complex 1 OXPHOS capacity. **B.** Porcine frozen mitochondria from all tissues maintained complex 1 OXPHOS capacity similar to fresh isolated mitochondria. **Fr:** fresh. **CF:** controlled frozen.

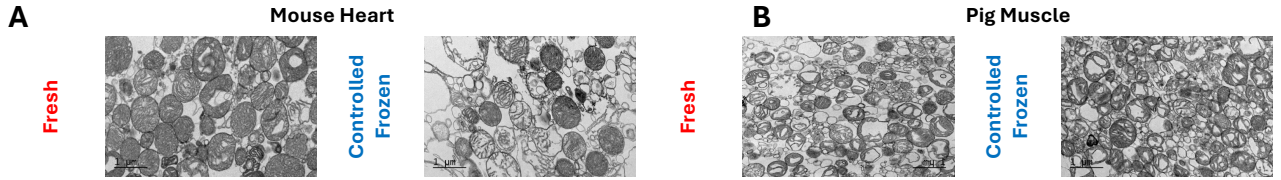


Figure 4: Electron microscopy images of fresh and controlled frozen isolated mitochondria A. Mouse frozen heart mitochondria show significant loss of matrix content. **B.** Pig muscle mitochondria have similar morphology before and after freezing.

Results

Porcine frozen SM, kidney, and heart mitochondria retained uncoupling after uncoupler (CCCP) titration (p=0.031, p=0.031, p=0.0002). The mouse frozen heart mitochondria showed no uncoupling (p=0.062), while SM and kidney mitochondria were 20-30% lower than fresh samples (p=0.03,p=0.02).

Complex I–supported OCR was preserved in all porcine mitochondria after freezing. In contrast, mouse mitochondria from all tissues exhibited marked reductions in complex I–supported respiration (all p<0.0001).

Murine frozen mitochondria from all tissues had an increase in NADH-stimulated respiration, consistent with inner membrane damage.

Porcine frozen mitochondria from SM and kidney, showed no NADH-stimulated respiration.

There was decreased ATP synthase activity for frozen pig mitochondria (SM -3.2% p=0.02, heart-4.8%, kidney-4.1%) and mouse frozen mitochondria (SM -20% p<0.0001, heart -97% p<0.0001, kidney -27% p=0.019).

Conclusion

Controlled-rate cryopreservation preserved OXPHOS and membrane integrity in porcine, but not murine, mitochondria, demonstrating porcine cryo-resilience. These findings establish SM as a robust source of preserved mitochondria and support translation to human donor-derived products. Ongoing efforts focus on lyophilized field-deployable bioenergetic therapeutics for austere environments.

Acknowledgement

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