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Analyzing Mitochondrial Bioenergetics to Improve Organ Hibernation Methods.

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Summer Undergraduate Research Program 2023

Analyzing Mitochondrial Bioenergetics to Improve Organ Hibernation Methods.

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BACKGROUND: In the United States there is a shortage of organs that are viable for transplantation due to the small window of viability of only a few hours. Through developments in the study of cellular mitochondria, we can now observe the impact of its mechanics in order to design a method of prolonging the “shelf life” of donated organs.

METHODS

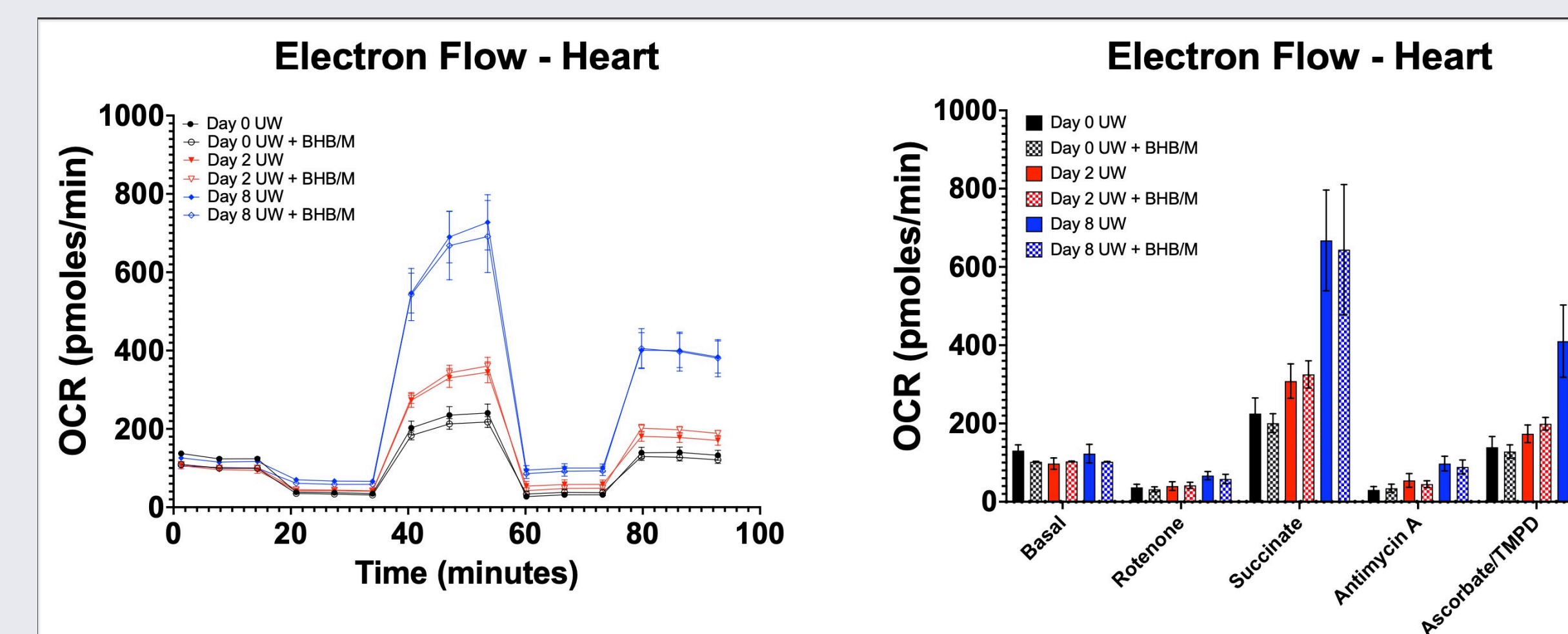
1. Brain-dead donor pigs were infused with either BHB/M or a vehicle control prior to organ harvest.
2. Hearts were harvested under standard conditions and stored in a standard UW solution or a UW+BHB/M solution under normal static cold conditions.
3. Left ventricular wall tissue was sampled by biopsy at harvest, two days, and eight days post-harvest.
4. Mitochondria were isolated from heart tissue by differential centrifugation, quantified, and analyzed on a Seahorse XFe96 Flux Analyzer.

RESULTS

- Electron flow OCR between tissues submerged in UW and UW+BHB/M solutions were the same for all 3 days.
- OCR with the complex I and complex II coupling assays yielded the same results with little to no variation between the UW and UW+BHB/M solutions.
- Electron flow did show a marginal increase from Day 0 to Day 8
- Complex I and II function showed impairment from Day 0 to Day 8.

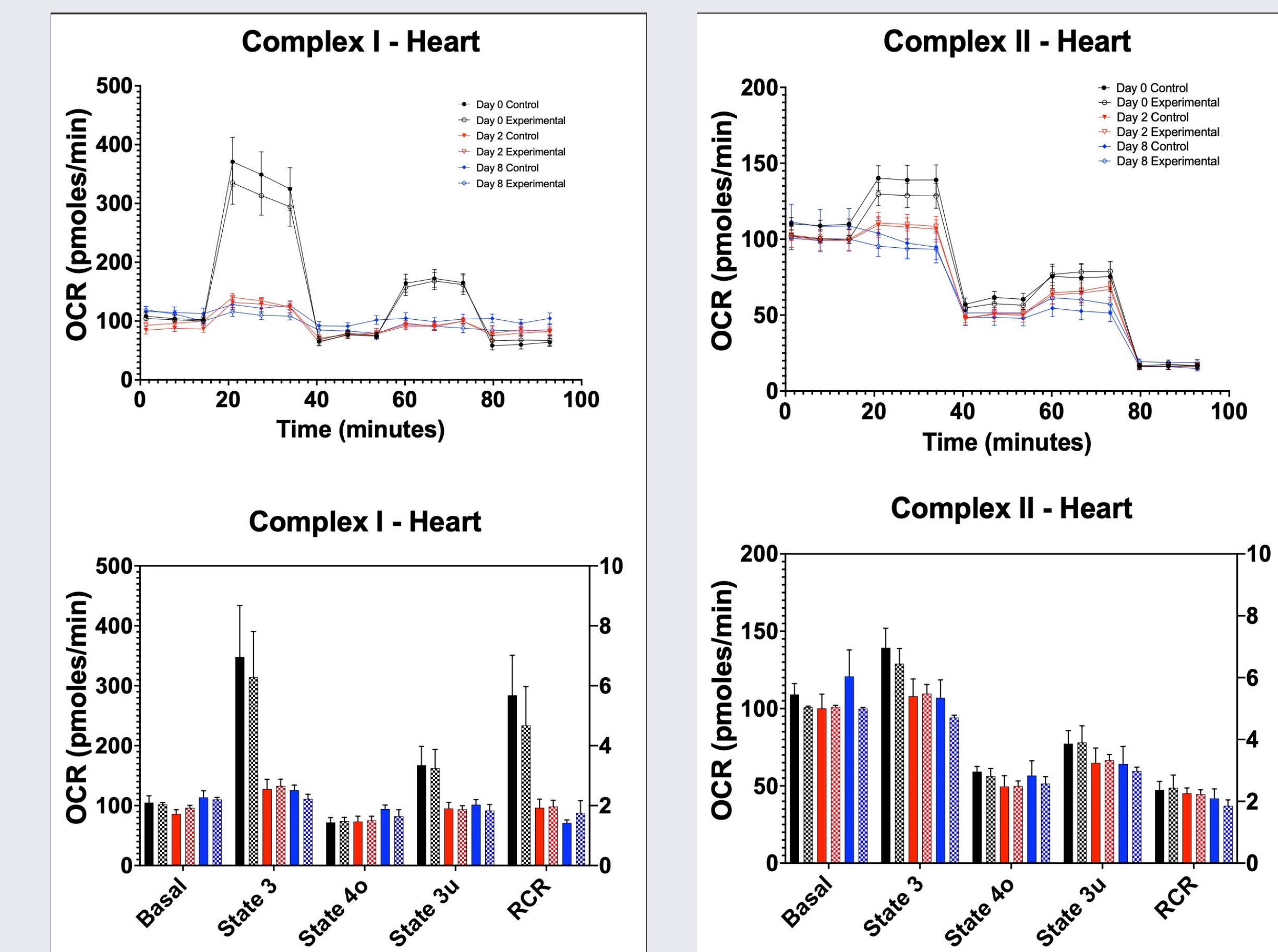
β-hydroxybutyrate and Melatonin May Be the Key to Prolonged Organ Hibernation.

Electron Flow in Heart Mitochondria Shows an Increase in the Oxygen Consumption rate from Day 0 to Day 8.



Oxygen consumption between Day 0 and Day 8 shows a very significant increase that would indicate that the ETC is still functional after 8 days in cold storage. But, there appears to be little to no difference between tissues submerged in the UW solution and the UW+BHB/M solution. This leads us to suspect that the UW+BHB/M solution has no significant effect on ATP production.

Complex I and II Mitochondrial Functions Show an Uncoupling Effect Leading to a Decrease in Oxygen Consumption From Day 0 to Day 8.



Complex I and Complex II of the mitochondria followed a trend of uncoupling leading to a sharp decrease in OCR in complex I from Day 0 to Day 8 and a gradual decrease in OCR from Day 0 to Day 8 in complex II.

CONCLUSIONS

- The UW+BHB/M solution did not yield positive results for this test.
- Tissue damage does not significantly occur during ischemia.
- Most tissue damage is the result of an influx of free radicals during reperfusion.
- Future testing is expected to show the preserving effects of BHB/M during post ischemia reperfusion.