Measuring mitochondrial respiration *in vivo*: From mouse to humans

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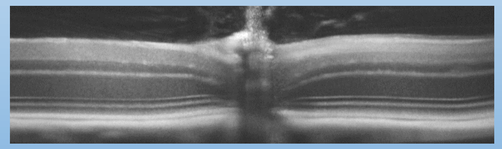


Purpose

- Devastating neurodegenerative diseases often involves dysfunctional mitochondrial respiration.
- Conventional imaging approaches are unable to measure mitochondrial respiration *in vivo* preventing early diagnosis and intervention to mitigate disease.
- Previously, we showed that a common clinical imaging method, optical coherence tomography (OCT) can measure mitochondrial respiration using an uncoupler 2,4 dinitrophenol (DNP, Figure 1) at a non-toxic but higher than clinically indicated doses (PMID: 31923239, 30909602).
- Here we test if OCT has the detection sensitivity to measure mitochondrial respiration using a dose of DNP that could be used in patients (PMID: 30909602).

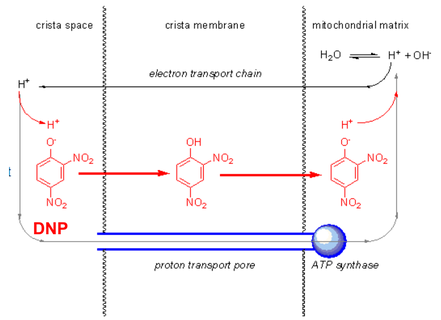
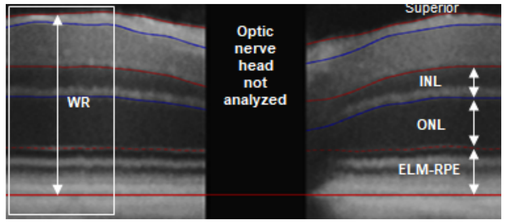


Figure 1: DNP MOA. Schematic showing how DNP (red) reduces the proton gradient across the mitochondrial membrane. This results in increased mitochondrial respriation. Image from Bender, David. *Overheating after Overdosing on E - and Slimming by Taking Dinitrophenol*, david-bender.co.uk/metabonline/energy/ATP/ATP21.html.

Methods

- Baseline scan: Male 2 mo C57BL/6J mice were dark-adapted overnight and then light-adapted for 5 hours before undergoing short-term ketamine / xylazine anesthesia for OCT examination; all mice fully recovered.
- Drug scan: The next day, mice were similarly dark-adapted and again 5 hour light-adapted. They were injected IP with either a clinically-viable dose of DNP (0.5 mg/kg, PMID: 30909602) 1 hour before OCT examination or equal volume of saline vehicle (control).
- Retinal laminae thicknesses were obtained using in-house software with particular attention to the external limiting membrane-retinal pigment epithelium (ELM-RPE) region, which becomes thinner at higher doses of DNP-provoked mitochondrial respiration (Figure 2, PMID: 31923239).



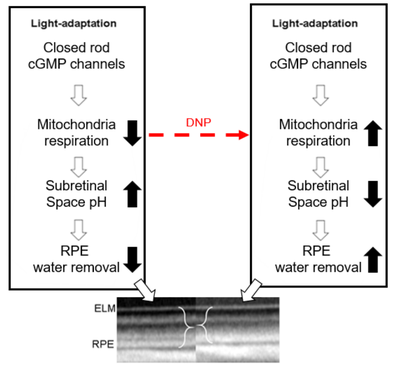


Figure 2: Working model. *Left*: Regions-of-interest are shown on a representative mouse OCT. INL: inner nuclear layer; including outer plexiform layer; ONL: outer nuclear layer; WR: whole retinal. Superior retina indicated. *Right*: A larger ELM-RPE region in the light can be converted to a smaller dark phenotype in the light *in vivo* by increasing mitochondrial respiration with DNP, based on this signaling pathway (PMID: 31923239).

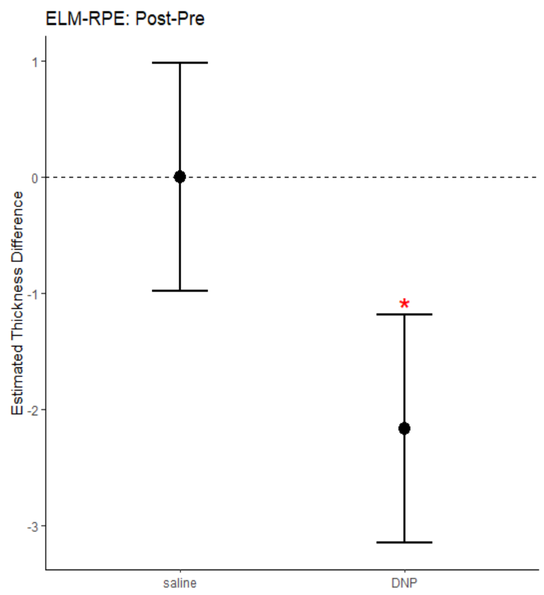


Figure 3: 1 hour post 0.5 mg/kg DNP. Modelled change in ELM-RPE thickness from before (pre) and after (post) DNP or saline. As expected, saline produced no change in ELM-RPE thickness from zero (dashed line). In contrast, DNP caused a significant ($p < 0.05$) thinning of the ELM-RPE region (red star). N = 5-6 per group. Error bars: confidence intervals.

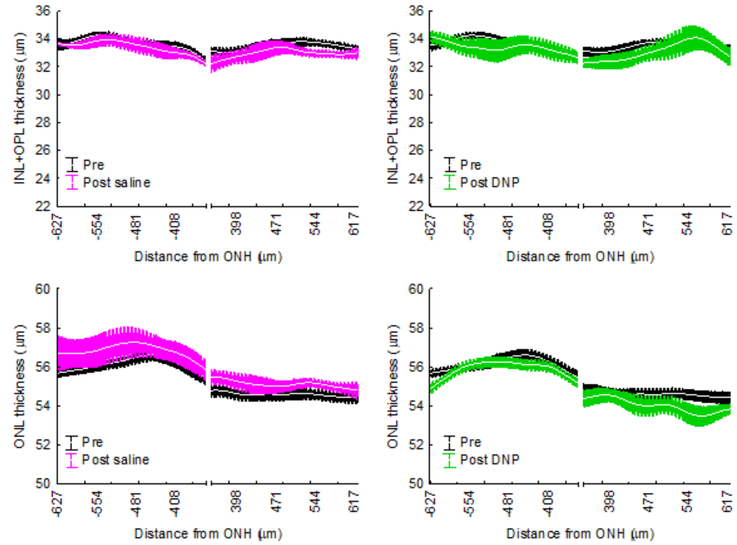


Figure 4: 1 hour post 0.5 mg/kg DNP, other laminae. Data of two other laminae. *Left column*: Comparison of before and after saline (purple) injection; *right column*: comparison of before and after DNP (green) injection. Error bars: SEM. No changes in other retinal laminae are evident with DNP within or between groups.

Conclusion

- The results of this study demonstrate for the first time *in vivo* that increase of mitochondrial respiration through administration of clinical doses of DNP can be
- Application of our novel appr management of mitochondria and potentially other neurode

Supported by the National Eye Institu
NEI Core Grant P30 EY04068, an un
Blindness (Kresge Eye Institute, BAB