Muscle-specific expression of tafazzin or spargel (PGC1-alpha) restores exercise tolerance in a Drosophila model of Barth syndrome
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Q&A

Q: PGC-1alpha requires activation through sirtuins, which in turn use NAD+. This can be stimulated with NAD precursors like NMN or NR. Have you tried any of these? If successful they could quite easily be tested in patients
A: Yes, I have tried both NR and NMN and they are successful at increasing baseline endurance.

Q: Nice work. I am the farthest thing from a fly biologist, but my understanding is that the fly relies mostly on glucose metabolism. If that is true, how does this affect your conclusions from this model.
A: The fly uses both glucose and fatty acid metabolism. Most likely, the deficit in endurance is due to reduced fatty acid metabolism, but we have not measured that yet.

Q: Do Drosophila have both Type I and Type II muscle?
A: Drosophila have both glycolytic and oxidative muscles. The jump muscles are glycolytic and similar to fast-twitch muscles while the indirect flight muscles are oxidative.

Q: Very nice work. Is there any issue using the RU486 (Mifepristone) expression system. RU486 can impact on glucocorticoid responsive receptors.
A: I have done an experiment where I fed my tafazzin mutants RU486 and I see no difference in endurance or ability to adapt to exercise training relative to tafazzin mutants that were not fed RU486. Therefore, I do not believe RU486 is responsible for the rescues I've observed.
Q: Great talk. Have you considered directly measuring cardiolipin remodeling or MLCL:CL levels in your tafazzin rescues vs. WT?
A: Yes, we definitely want to do that. We are looking for a collaborator to help us do so.

Q: Does CL profile improve with rescue?
A: We haven’t analyzed the cardiolipin profile of our rescues yet. We are looking for a collaborator to help us with that.

Q: Great presentation. Can you rule out behavioral effects in the neuronal knockdown?
A: We do not believe the reduced endurance and inability to adapt to exercise training in the neuronal knockdown is due to differences in behavior. The neuronal knockdown flies respond to the dropping stimulus in the same way as wild-type flies, by climbing upwards. Another behavioral effect that may differ between the neuronal knockdown flies and control flies is spontaneous activity. We have not measured that yet. However, in wild-type flies, spontaneous activity does not correlate with exercise adaptations and, therefore, would not be indicative of their ability to adapt to training.

Q: Very nice. In the Spargel rescue, is there an increase in muscle and or mitochondrial mass in muscle?
A: Fly muscles do not undergo hypertrophy, since they have an exoskeleton. Thus, an increase in muscle mass is highly unlikely. However, we have not directly looked at that. We plan at looking at mitochondrial mass and number in future experiments.

Q: Is PGC1alfa able to improve the CL/MLCL ratio?
A: The Schlame lab published a paper detailing that overexpression of PGC-1α in flight muscles of flies improved the CL/MLCL ratio. However, we have not tested that yet, but plan to in the future once we find a collaborator.

Q: I was wondering about the x-axis of the exercise graphs... the endurance seems to be highly variable, in all groups. For example, in early slides, the graph suggests exercise training actually shortens endurance?
A: With training, wild-type exercised flies have better endurance than aged-matched cohorts. However, due to aging, exercised flies do not have improved endurance compared to their younger selves. Flies only improve their endurance after exercise training relative to their younger selves if they are kept at 18 degrees Celsius. At this temperature, the rate of ageing is significantly slowed.
Q: The spare reserve capacity (FCCP) is difficult to interpret given there was no difference in oligo (an indirect measure of actual ATP synthesis). The data would suggest that ATP synthesis is similar between groups.
A: I imagine this question refers to the higher spare respiratory capacity observed upon transient glucose starvation. It is known that short-time glucose starvation induces higher reliance on mitochondrial respiration. So, although glucose levels are lower, cells increase mitochondrial-derived ATP production to sustain viability (Gomes et al, Nature Cell Biology, 2011). Thus, it is not surprising that although lower glucose availability is reduced, starved cells maintain a comparable ATP-coupled respiration measured after oligo. The higher efficiency in mitochondrial respiration during transient glucose starvation is also reflected by the higher maximal respiration after FCCP uncoupling.

Q: Deficits in memory T-cells in some other mitochondrial diseases causes common variable immune deficiency. Does CVID occur in Barth syndrome?
A: Unfortunately, this question is out of my expertise and I would need to turn it to physicians more expert on patients’ status.