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Associate editor comment and authors' responses

Effect of selected fluorophores on equine skeletal muscle mitochondrial respiration

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Associate editor: Angela Messina
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Comments from 2023-08-04: *Only major points included.*

Associate editor

The manuscript entitled "Effect of selected fluorophores on equine skeletal muscle mitochondrial respiration" by Michael S. Davis and colleagues is an interesting about the use of specific, commonly used fluorophores in high-resolution respirometry experiments and the production of relative artifacts. The study focalizes on three specific fluorophores and uses two technical approaches. The manuscript is well written, protocols and rationale are clear.

I have only a concern regarding the SUIT protocol here used. Data, indeed, are normalized for the OXPHOS respiration and not for the commonly used maximal capacity, as occurs in many SUIT protocols. Authors should briefly explain this issue.

Authors

We have added a verbiage explaining the omission of an uncoupler in our SUIT protocol. We are incubating the samples at the physiological resting temperature of equine skeletal muscle (38C), and past studies have demonstrated that this induces sufficient intrinsic uncoupling to preclude the need for an uncoupler to produce maximal capacity. Thus, the state produced by the combination of pyruvate, glutamate, malate, succinate, and ADP is simultaneously phosphorylating (because we have not completely destroyed the protonmotive force through addition of an uncoupler) and maximal capacity (because respiration is not limited by the phosphorylating capacity, addition of uncoupler does not typically result in increased respiration.