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MITOCHONDRIAL RESPIRATION IN HUMAN SKELETAL MUSCLE: THE IMPACT OF OXYGEN AVAILABILITY

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Mitochondria act as the powerhouse of the cell, utilizing cellular respiration to produce energy essential to the various bodily functions. The electron transport chain, the final step in the process of cellular respiration, creates a proton gradient between the intermembrane space and the mitochondrial matrix, which is then used to power the enzyme ATP synthase, producing a significant amount of ATP, the main source of energy in the body. Activity in the electron transport chain of skeletal muscle can be measured by assessing oxygen (O_2) consumption and hydrogen peroxide (H_2O_2), an index of reactive oxygen species (ROS) production, in either isolated mitochondria or permeabilized fibers. Of note, oxygen availability can have a significant effect on skeletal muscle mitochondrial respiration.

Human skeletal muscle samples were harvested with the needle biopsy technique (n=4), taken from the right vastus lateralis muscle. The samples were prepared for experimentation using one of two methods. In the first, the permeabilized fiber approach, the muscle sample was manually "teased" apart, whereby the surface area of the muscle was increased, after which it was soaked in a saponin filled bath, followed by a double wash in MIRO5, to permeabilize the cell membranes and remove all cellular structures nonessential to the measurement. The second method, the isolated mitochondria approach, included a gentle homogenization into a soup-like mixture and centrifugation to yield pellet of pure mitochondria. The pellet was then re-suspended in solution.

The samples obtained from both processes, were introduced into 4 separate 2 mL chambers of the Oroborus respirometers (O_2k) containing MIRO5, at 37 degrees Celsius, to maintain the function of the mitochondria. After the mitochondria were given adequate time to equilibrate to the chamber, CI+CII state 3 (maximal) respiration was induced by introducing excess malate (5 uL), glutamate (10 uL), succinate (20 uL), and ADP (10 uL) to each chamber. Initial O_2 levels inside the chamber were either kept at normal levels, or artificially elevated by 100% O_2 injection such that respiration with each approach, isolated mitochondria and permeabilized fibers, was assessed in normal and elevated O_2 conditions. Partial pressure of O_2, indicative of respiration, and H_2O_2 levels, indicative of ROS production, were monitored and recorded using high-resolution respirometry, until PO_2 approached 0 mmHg, indicating the cessation of respiration.

Results are preliminary, pending further subject participation, but even these preliminary analyses yield interesting findings. Specifically, maximal respiration was similar in both high and normal PO_2 environments for both isolated and permeabilized samples. The PO_2 critical point, or the level of PO_2 at which respiration is compromised, in isolated mitochondria, was not

altered by initial PO_2. However, in contrast, the PO_2 critical point for mitochondria assessed in permeabilized fibers was affected by initial PO_2. In permeabilized fibers, mitochondria in the high oxygen conditions exhibited higher PO_2 critical points. Furthermore, PO_2 levels, while not affecting maximal respiration, seem to have a negative effect on overall respiration of the mitochondria assessed in permeabilized fibers.

It is expected that this research will provide valuable insight into the link between oxygen availability and mitochondrial function. This will lead, ultimately, to a better understanding of the link between mitochondrial dysfunction, disease states, and aging.

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