MiRNA expression in skeletal muscle mitochondria following an acute bout of endurance exercise

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Introduction: Mitochondria are primary regulators of energy metabolism, and are crucial for endurance exercise performance. Endurance exercise notably increases mitochondrial biogenesis in skeletal muscle. Regulatory components of the mitochondrial biogenesis pathway, such as *PGC-1α,* increase over 10-fold following a single bout of endurance exercise (Perry *et al.*, 2010). Small non-coding RNAs, particularly miRNAs, have regulatory roles in the modulation of gene expression, and are increasingly implicated in the regulation of mitochondrial biogenesis. Muscle-enriched miRNAs are differentially expressed in human skeletal muscle following endurance exercise, and are increasingly implicated in mitochondrial metabolism (Russell *et al.*, 2013). Furthermore, emerging evidence demonstrates that some miRNAs are transcribed from the nuclear genome but localize within the mitochondria across diverse physiological and pathological states (Barrey *et al.*, 2011). The presence of miRNAs in the mitochondria suggest a novel level of complexity in the regulation of mitochondrial gene regulation. The present study first optimised the isolation of pure and intact mitochondria from human skeletal muscle. Then, the study aimed to determine if miRNAs were expressed, and differentially expressed, in skeletal muscle mitochondria in response to endurance exercise.

Methodology: Twelve males (age 22.9 ± 3.0 y; VO_2peak 44.1 ± 7.5 $ml.min^{-1}.kg^{-1}$) cycled for 60 minutes at approximately 70% VO_2peak. Muscle biopsies were taken from the *vastus lateralis* pre, immediately post, and three hours post exercise. Mitochondria were isolated from whole skeletal muscle and treated with RNase-A to prevent contamination of nuclear and cytosolic RNA. Spectrophotometric determination of citrate synthase activity was used to quantify mitochondrial yield and integrity, whilst *mt-COX1* and nuclear *COX4* expression were quantified using qPCR to confirm mitochondrial purity. The expression of miRs-1, -23a/b, -133a/b, -181c, -206 and -let-7b was quantified using qPCR

Results/Conclusions: High yields of intact mitochondria were successfully isolated from human skeletal muscle. Furthermore, *COX4* mRNA, *18S* and *28S* rRNA were absent from mitochondrial samples, indicating the samples were relatively free from contaminating RNA. Electrophoretic separation of nucleic acids (Agilent Technologies) revealed mitochondrial RNA extracts were enriched in small RNAs, the majority of which aligned to miRNA sequences. MiRs-1, -23a, -133a, -206 and miR-let-7b were detected in human skeletal muscle mitochondria for the first time. We now look to verify the hypothesis that mitochondrial miRNAs are differentially expressed following a single bout of endurance exercise. MiRNA-mediated regulation of the mitochondrial genome is a relatively unexplored field. Ongoing RNA-seq analysis endeavours to identify known and novel miRNAs within skeletal muscle mitochondria. This will further our understanding of mitochondrial gene regulation and allow the identification of novel therapeutic targets.

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