MicroRNA expression in female skeletal muscle mitochondria following a single bout of endurance exercise

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Background/Rationale: Endurance exercise produces important cellular stress signals that upregulate signalling networks involved in facilitating positive physiological adaptations within skeletal muscle. A major adaptation includes an increase in skeletal muscle mitochondrial content, which is a key determinant of whole-body metabolism, health and endurance performance (Holloszy, 1967; Nunnari & Suomalainen, 2012). Small non-coding RNA species, called microRNAs (miRNAs), are essential intracellular mediators of gene expression (Zhang, 2009). MiRNA expression is altered in whole skeletal muscle following acute endurance exercise and these changes have been shown to play a role in mediating the increase in mitochondrial biogenesis observed after endurance exercise (Nielsen *et al.*, 2010; Russell *et al.*, 2013). Emerging evidence from human muscle *in-vitro* reveals miRNAs are also expressed in sub-cellular compartments, such as the mitochondria (Barrey *et al.*, 2011). This includes several miRNAs known to respond to exercise at the whole-muscle level and regulate mitochondrial function. It is thus conceivable that miRNA expression might also be altered in skeletal muscle mitochondria following acute endurance exercise. Currently, no studies have investigated the miRNA response to exercise within the mitochondria. As such, this study aimed to first determine whether miRNA localize in female skeletal muscle mitochondria *in-vivo*, and secondly investigate if miRNA expression is altered in skeletal muscle mitochondria following a single bout of endurance exercise.

Methods: Seven healthy females underwent a preliminary VO_{2peak} test whereby participants cycled on a cycle ergometer until volitional fatigue. Following this, participants completed an exercise and muscle biopsy trial. This involved a 60-minute continuous cycle at 70%VO_{2peak} with muscle biopsies taken from the *vastus lateralis* at rest, immediately post-exercise and 3-hours post-exercise. Mitochondria were isolated from whole muscle using the MACS method and analysed for miR-1, -23a, -23b, -133a, -133b and -206 expression. A one way-repeated measures ANOVA was performed to examine differences in miRNA expression at rest, immediately following exercise and 3-hours post-exercise. Mitochondrial extracts were examined for cytosolic marker, COXIV, and mitochondrial markers, COXI, 16s and 12s to determine purity and enrichment.

Results: RT-qPCR analysis of mitochondrial RNA extracts showed high enrichment of mitochondrial markers, COXI, 16s and 12s, and an absence of cytosolic marker COXIV, indicating a high-level of mitochondrial purity. 6 miRNA species (miR-1, -23a, -23b, -133a, -133b and -206) that are regulated by endurance exercise at the whole-muscle level were found to be expressed in mitochondria isolated from human female skeletal muscle in-vivo. However, miR-1, 23a/b, 133a/b, and 206 expression were not altered in female skeletal muscle mitochondria immediately after, and 3-hours after 60-minutes of moderate intensity cycling.

Conclusion: The present study demonstrated for the first time that miRNA can localize in mitochondria isolated from human female skeletal muscle *in-vivo*. Our data shows that no significant changes in miRNA expression were observed in human female skeletal muscle mitochondria following 60-minutes of moderate-intensity exercise. Nonetheless, the detection of miRNA species in human female skeletal muscle mitochondria opens new avenues of research regarding the biological function of miRNA in the mitochondria. Future studies should investigate the exercise-induced response of more mitochondrial miRNA species to varying types, intensities and durations of exercise, in a larger sample size, and at later time points post-exercise.

Barrey E, Saint-Auret G, Bonnamy B, Damas D, Boyer O, Gidrol X. (2011). Pre-microRNA and mature microRNA in human mitochondria. *PloS one* **6**, e20220.

Holloszy, JO. (1967). Biochemical adaptations in muscle effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* **242**, 2278-82.

Nielsen S, Scheele C, Yfanti C, Åkerström T, Nielsen AR, Pedersen BK, Laye M. (2010). Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. *J Physiol* **588**, 4029-37.

Nunnari J, Suomalainen A. (2012). Mitochondria: in sickness and in health. *Cell* **148**, 1145-59.

Russell AP, Lamon S, Boon H, Wada S, Güller I, Brown EL, Chibalin AV, Zierath JR, Snow RJ, Stepto N, Wadley GD, Akimoto T. (2013). Regulation of miRNAs in human skeletal muscle following acute endurance exercise and short-term endurance training. *J Physiol* **591**, 4637-53.

Zhang C. (2009). Novel functions for small RNA molecules. *Curr Opin Mol Ther* **11**, 641-51.