The role and regulation of skeletal muscle microRNAs in response to exercise

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MicroRNAs (miRNAs) are newly discovered regulators of transcriptional and translational activity in the cell. MiRNAs can be highly and specifically enriched in certain tissues (Sood *et al.*, 2006). Skeletal muscle enriched miRNAs are referred to as myomiRs and include miR-1, miR-133a, miR-126, miR-133b, miR-206, miR-208, miR-208b, miR-486 and miR-499 (McCarthy & Esser, 2007; Callis *et al.*, 2008). Although their role in muscle cells has only been partially investigated, skeletal muscle miRNAs play an essential regulatory role in muscle proliferation, differentiation, regeneration and metabolism in response to internal and external stimuli (Güller & Russell, 2010). MiRNA expression is sensitive to both endurance and resistance exercise; however, our understanding of how miRNA expression and activity is regulated by exercise is rudimentary.

In two independent studies, we investigated the changes in skeletal muscle miRNAs following acute endurance exercise and acute resistance exercise. For the acute endurance exercise study, nine young subjects performed a single bout of endurance exercise and muscle biopsies were collected before and after exercise. The expression levels of myomiRs as well as specific miRNAs known to be upregulated in several myopathies were measured. In the 3 h period following exercise, miR-1, -133a, -133-b and miR-181a increased while miR-9, -23a, -23b and -31 decreased. Although the precise molecular targets of these miRNAs are unknown, prediction softwares identified several potential miRNA gene targets that have been shown to be regulated by exercise and which are known to influence mitochondrial biogenesis and metabolism. A luciferase reporter assay demonstrated that in muscle cells, miR-31 directly interacts with and down regulates HDAC4, a component of the MAPK pathway, as well as with NRF1, which is involved in mitochondrial biogenesis and metabolism. In addition, negative correlations were observed between miR-31 and HDAC4 protein and NRF1 protein 3 h after exercise. These results suggest an important role for miR-31 in the early adaptation to exercise.

For the acute resistance exercise study, 10 young and 10 old subjects completed an acute bout of resistance exercise. Muscle biopsies were collected before and 2 h after exercise and the expression of 748 miRNAs was measured in muscle samples using the TaqMan® Array Human MicroRNA Cards Set v3.0 (Applied Biosystems). 247 of these miRNAs (34%) were significantly expressed in muscle tissue. Statistical analysis revealed that 20 miRNAs were significantly up- or down-regulated in young and/or old subjects following exercise. Additional bioinformatics analysis identified 4 of these miRNAs to potentially target genes likely to be involved in protein synthesis and known to be responsive to resistance exercises.

MiRNAs provide another level of complexity in transcriptional and translational regulation. These studies show that many miRNAs are sensitive to the stress induced by both endurance and resistance exercise. Identifying miRNAs regulated by exercise combined with bioinformatics analysis allows the identification of potential exercise-regulated pathways that control the adaptive response to exercise. However, the precise genes and proteins targeted by these miRNAs, as well as the molecular processes involved in muscle adaption to exercise, remain to be established. These studies also illustrate two different approaches to measure skeletal muscle miRNA expression. Modulating the expression of specific miRNAs *in vitro* and *in vivo* in rodents will increase our understanding of the function of miRNAs in skeletal muscle health.

Callis TE, Deng Z, Chen J-F & Wang D-Z. (2008) *Experimental Biology and Medicine* **233:** 131-38.
Güller I & Russell AP. (2010) *Journal of Physiology* **588:** 4075-87.
McCarthy JJ & Esser KA. (2007) *Journal of Applied Physiology* **102:** 306-13.
Sood P, Krek A, Zavolan M, Macino G & Rajewsky N. (2006) *Proceedings of the National Academy of Sciences of the United States of America* **103:** 2746-51.