Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle

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We have previously shown that a single bout of endurance exercise increases GLUT-4 mRNA in human skeletal muscle (Kraniou *et al.*, 2000), implying an increased rate of transcription. It has also been demonstrated that myocyte enhancer factor 2 (MEF-2) binding activity is necessary for regulation of the GLUT-4 gene in skeletal muscle (Thai *et al.*, 1998). In the basal state, MEF-2 is believed to be inhibited by the class II histone deacetylases (HDACs), an association that is broken by phosphorylation of HDACs and their subsequent nuclear export. Association of MEF-2 with co-activators possessing histone acetylase (HAT) activity is thought to be mediated by the calcineurin/nuclear factor of activated T-cells (NFAT) pathway. Calcineurin dephosphorylates NFAT, resulting in its nuclear translocation where it recruits co-activators possessing HAT activity to MEF-2 allowing maximal MEF-2 DNA binding. While this is sufficient to initiate transcription, the rate of MEF-2 mediated transcription is increased by MEF-2 phosphorylation, with one putative kinase being p38 MAPK. In the present study, we sought to examine whether these various mechanisms may be involved in human skeletal muscle responses to exercise.

Seven healthy, untrained men (27 ± 3 yrs, 83 ± 4 kg, $\dot{V}O_{2\ peak}$ = 47 ± 2 ml•kg^{-1}•min^{-1}, mean ± SD) performed cycle ergometer exercise for 60 min at a power output eliciting 74 ± 2% $\dot{V}O_{2\ peak}$). Muscle samples were obtained from vastus lateralis immediately before and after exercise and quickly (~15 s) frozen in liquid nitrogen for later analysis. Total and nuclear proteins were isolated as described previously (McGee *et al.*, 2003) and quantified by immunoblotting and co-immunoprecipitation. Nuclear HDAC5 content was decreased 54% ($P<0.05$) following exercise, while there was no change in whole cell HDAC5 content. The association of HDAC5 with MEF-2 was reduced by 26% ($P<0.05$). Nuclear NFAT content was similar before and after exercise. Total p38 MAPK phosphorylation increased 4.8 fold ($P<0.05$), while nuclear p38 MAPK phosphorylation increased 1.8 fold ($P<0.05$), with no change in the abundance of either total or nuclear p38 MAPK proteins. Association of p38 MAPK protein with MEF-2 increased 2.7 fold ($P<0.05$) following exercise, while association of phosphorylated p38 MAPK with MEF-2 increased 1.75 fold ($P<0.05$). These results suggest that HDAC5 and p38 MAPK are involved in the regulation of MEF-2 in response to exercise in human skeletal muscle, while the calcineurin/NFAT pathway may be less important under these conditions.

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