Skeletal muscle mitochondrial ROS emission and antioxidant genes are elevated 3 h after exercise independent of exercise intensity

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Introduction: Acute exercise intensity may be an important factor in modulating the induction of homeostatic perturbations in skeletal muscle to induce adaptive responses and subsequently enhance metabolic health. Reactive oxygen species (ROS) are known to play an important role in potentiating some adaptive responses to exercise (Jackson, 2015), and the rate of generation of total ROS release from whole muscle have been shown to depend on exercise intensity (Bailey *et al.*, 2004). While mitochondria are unlikely to be the main source of ROS during contraction (Sakellariou *et al.*, 2013), mitochondrial ROS may play an important role during the early post exercise recovery period when many adaptive responses occur. Therefore, whether post exercise skeletal muscle mitochondrial ROS production is altered by exercise intensity and whether this is related to the induction of mitochondrial-related redox sensitive targets was investigated.

Methods: Young, healthy individuals (n=8) performed three acute bouts of cycling exercise on separate occasions using a repeated-measures, randomised, crossover design. Pre-trial diet and exercise was standardized. Exercise was moderate intensity (MOD; 30 min continuous, 50% W_{MAX}), high intensity intervals (HIIE, 5 × 4 min, 75% W_{MAX}, work matched to MOD), and sprint (SPR; 4 × 30 s maximal sprint, ~200% W_{MAX}). Muscle biopsies were obtained after an overnight fast at baseline, immediately post exercise (EX), and after 3 h recovery (3H). Mitochondrial respiration and ROS production (H_2O_2 emission) was determined simultaneously in permeabilized muscle fibres using high resolution respirometry (Oxygraph O2k, Oroboros, Austria). Protein abundance and phosphorylation was determined *via* western blotting and mRNA expression assessed *via* RT-PCR. Data were analysed by two-way ANOVA and LSD post hoc tests where main effects were detected.

Results: Exercise led to a decrease in maximal, succinate-driven mitochondrial ROS emission during $CI+II_{LEAK}$ respiration at EX and 3H (P<0.05), regardless of intensity. In contrast, under conditions of OXPHOS respiration, all three exercise intensities led to a ~65% increase in mitochondrial ROS emission at 3H *vs* EX (P<0.05). The elevated ROS at 3H was concomitant with a decrease in the abundance of a key endogenous antioxidant enzyme, PRX1 (P<0.05). In addition, a number of putative redox sensitive exercise responsive protein phospho-sites ($p38^{Thr180/Tyr182}$, $AMPK^{Thr172}$, $HSP27^{Ser82}$) and genes (*PGC1α, HIF1α, NRF2*), along with mitochondrial antioxidant (*i.e. SOD2, GPX1, UCP3*) and morphology related (*MFN1, DRP1*) genes were increased with exercise (P<0.05), but were not significantly affected by exercise intensity.

Conclusion: In summary, we demonstrate that exercise, regardless of intensity, led to significant disturbances to muscle mitochondrial redox homeostasis and similar positive adaptive molecular signals. Further investigation is required to determine whether these observations are causally related. Though higher exercise intensities did not augment molecular signals relative to moderate intensity, these outcomes were achieved with considerably less exercise time.

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