AuPS/ASB Meeting - Adelaide 2010

Free communications: Energy metabolism in exercise

Monday 29th November 2010 - Broughton Room - 08:30

Chair: Rod Snow

Reduced mitochondrial biogenesis activation during exercise after short-term training

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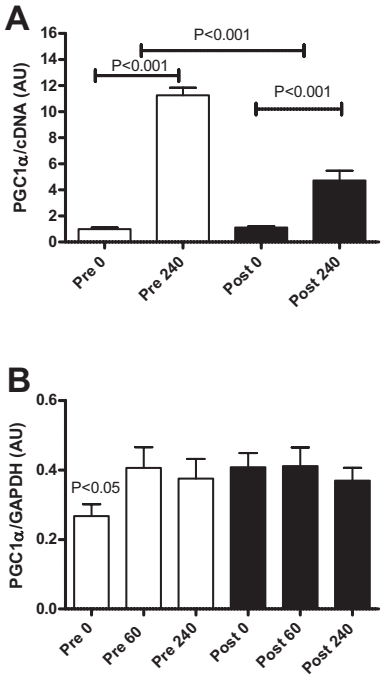
Mitochondrial biogenesis and function are important for energy production in cells and tissues. Aberrant mitochondrial function, specifically reduced volume not function, has been implicated as a cause or at least a contributor to lifestyle diseases including insulin resistance, obesity and diabetes. It is also well established that mitochondrial function and biogenesis is promoted by physical activity and exercise. In this study we investigated whether mitochondrial biogenesis was maintained in response to acute exercise after 10d of intensive cycle training despite the reduction of AMPK activity. Nine untrained, healthy participants (mean ± SEM; 23 ± 5 years of age, BMI: 24.9 ± 1 $kg.m^{-2}$ VO_{2peak} 44.1 ± 7.2 $ml.kg^{-1}.min^{-1}$) provided written informed consent. These participants performed a 60 min bout of cycling exercise at 164 ± 9 W (~70% pre-training VO_{2peak}), muscle biopsies were taken from the *vastus lateralis* muscle under local anesthesia at rest, immediately and 3h after exercise. Within 7 days the participants then underwent 10d of intensified cycle training including 4 days of high-intensity interval training. Three days after the final training session participants repeated the pre-training exercise trial with biopsies at the same absolute work load (~164 W). Protein and mRNA were extracted from muscle for analysis by immunoblotting or RT QPCR respectively. AMPK Thr172 phosphorylation increased by 15 fold and 4 fold during exercise before and after training respectively (p<0.05). PGC1-α gene expression was increased by 11 and 4 fold (p<0.001; Figure A) 3 h after the exercise bout before and after training.



PGC1-α protein expression increased 1.5 fold (p<0.05; Figure B) in response to exercise pre-training with no further increases occurring after the post-training exercise bout. COXIV gene expression was increased by training only (1.6 fold; p<0.0001). On the other hand COXIV protein expression increased (1.5 fold; p<0.05) but demonstrated a 20% reduction (p<0.01) in response to acute exercise before and after training. The nuclear co-repressor RIP140 and COXI protein expression was influenced by acute exercise only. Specifically, protein expression of RIP140 increased by ~5.5 fold (p<0.01) and COXI decreased ~2 fold (p<0.01) in response to acute exercise before and after training. These data demonstrate that short-term intensified training promotes gene and protein expression for mitochondrial biogenesis, and that acute exercise after training at the same absolute intensity results in reduced gene expression responses.

30 days of normobaric hypoxia increases mitochondrial respiration

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Contrasting increases in cytochrome c oxidase and decreases in mitochondrial volume have been reported in response to stays at high altitude (Hoppeler & Vogt, 2001). However, none of these studies directly measured mitochondrial respiration and all can be criticised for a lack of control for changes in physical activity. The purpose of this study was to investigate the effects 30 days of hypoxia on directly-measured, mitochondrial respiration. Twenty Wistar rats were randomly assigned to 30 days of either normobaric normoxia (CON; 21% O_2) or hypoxia (HYP; 10% O_2). Both submaximal (0.1 mM ADP) and maximal (2 mM ADP) ADP-stimulated mitochondrial respiration were determined on both isolated mitochondria (from lungs) and permeabilised muscle fibres from the left (LV) and right ventricle (RV), and the *soleus* (SOL) and EDL. Results were analysed using one-way ANOVA ($p<0.05$). Both submaximal and maximal ADP-stimulated respiration was significantly greater in HYP for SOL and LV, and tended to be higher for RV ($p=0.06$). There were no significant differences for the EDL. The significantly greater mitochondrial respiration in the LV of HYP (26%; $p<0.05$) was similar to a previous study (16%, ns) (Novel-Chaté *et al.*, 1998). The non-significantly greater mitochondrial respiration in the RV of HYP is also consistent with previous research (Novel-Chaté *et al.*, 1998) and can probably be attributed to significantly greater mass of the RV. We have shown for the first time however, that there is a greater mitochondrial respiration in the *soleus* of rats exposed to 30 days of hypoxia.

Hoppeler, H. & Vogt, M. (2001). Muscle tissue adaptations to hypoxia. *Journal of Experimental Biology* **204,** 3133-3139.

Novel-Chaté, V., Mateo, P., Saks, V.A., Hoerter, J.A. & Rossi, A. (1998). Chronic exposure of rats to hypoxic environment alters the mechanism of energy transfer in myocardium. *Journal of Molecular and Cellular Cardiology* **30,** 1295-1303.

3-day oral N-acetyl-cysteine supplementation alters metabolism but not performance of high intensity aerobic exercise in trained cyclists

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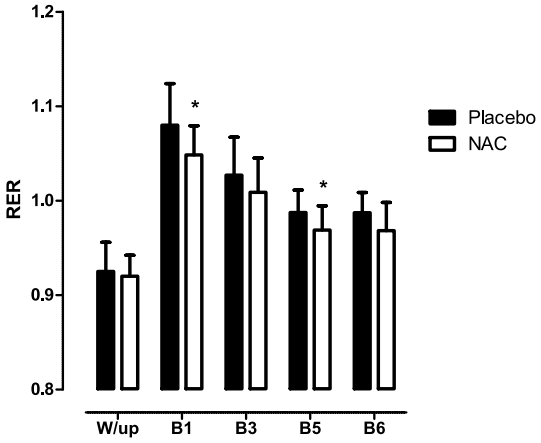
Redox homeostasis is essential for proper functioning of biological systems. Oxidative stress impairs contractile activity in skeletal muscle, and contributes to muscular fatigue during heavy exercise (Barclay & Hansel, 1991; Reid *et al.*, 1992). Accordingly, antioxidant supplements may assist endogenous antioxidants to prevent deleterious effects associated with oxidative stress (Medved *et al.*, 2004; Kelly *et al.*, 2009). In this study we investigated the effect of oral N-acetyl-cysteine (NAC) supplementation on metabolism and high intensity cycling performance. Nine well-trained male cyclists (mean ± SD; 27 ± 6 years of age, VO_{2peak} 69.4 ± 5.8 $ml.kg^{-1}.min^{-1}$) provided written informed consent. In a randomized, double-blind crossover design, subjects performed a 6 × 5 min High Intensity-Interval Training (HIT) cycling session at 82.5% of peak sustained power output, followed by a 10 minute self-paced Time Trial (TT) on two occasions 7 d apart. Prior to one session subjects consumed 5 × 750ml doses (2 × 2 d, 2 × 1 d, 1 × 1 hr pre-trial) of sports drink each containing 100$mg.kg^{-1}$ NAC, which was repeated for the other session, but without NAC. Metabolic, electromyographic (EMG), performance data, and blood/plasma samples were collected for analysis before, during, and after the 6 × 5 min HIT bouts and subsequent TT. Respiratory Exchange Ratio (RER) was decreased in the NAC condition throughout HIT exercise, and was significant at bouts 1 and 5 ($p < 0.05$) as shown in The Figure. Compared to placebo, NAC decreased blood lactate during TT and recovery ($p < 0.05$). Both pH ($p < 0.01$), and HCO3 ($p < 0.05$) were reduced throughout exercise and recovery with NAC. In contrast NAC resulted in higher blood glucose concentration during HIT ($p < 0.05$). EMG median frequency of the *vastus lateralis* decreased in HIT bout 6 in the NAC condition ($p < 0.05$). No significant difference was observed in the total work performed in the 10-min TT ($p = 0.16$). These data indicate that NAC does not change performance in a self-paced 10-min TT, but induces a shift in muscle fibre-type recruitment and alters metabolism during high intensity interval exercise, which may provide a glycogen-sparing effect during prolonged exercise.



Barclay, J. & Hansel, M. (1991) Free radicals may contribute to oxidative skeletal muscle fatigue. *Canadian Journal of Physiology and Pharmacology*, **69**, 279-284.

Kelly, M., Wicker, R., Barstow, T. & Harms, C. (2009) Effects of N-acetylcysteine on respiratory muscle fatigue during heavy exercise. *Respiratory Physiology & Neurobiology*, **165**, 67-72.

Medved, I., Brown, M.J., Bjorksten, A.R., Murphy, K.T., Petersen, A.C., Sostaric, S., Gong, X. & McKenna, M.J. (2004) N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. *Journal of Applied Physiology*, **97**, 1477-1485.

Reid, M., Haack, K., Franchek, K., Valberg, P., Kobzik, L. & West, M. (1992) Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue in vitro. *Journal of Applied Physiology*, **73**, 1797.

Actions of the antioxidant *N*-acetylcysteine on cell signaling response to exercise in human skeletal muscle

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Production of reactive oxygen species (ROS) in skeletal muscle is markedly increased during exercise and may be essential for exercise adaptation. We therefore investigated the effects of infusion with the antioxidant *N*-acetylcysteine (NAC) on exercise-induced activation of signaling pathways and genes involved in exercise adaptation in human skeletal muscle. Subjects completed two exercise tests, 7 days apart, with saline (control, CON) or NAC infusion before and during exercise. Exercise tests comprised of cycling at 71% VO_{2peak} for 45 min, then 92% VO_{2peak} to fatigue with *vastus lateralis* biopsies at pre-infusion, after 45 min cycling and at fatigue. Analysis was conducted on the mitogen-activated protein kinase (MAPK) signaling pathways, which are involved in growth, metabolism, differentiation, transcription, translation, and remodeling and also nuclear factor-κB (NFκB) signaling, which is a major stimulator of genes involved in inflammation and muscle protein turnover. We found that exercise increased phosphorylation of the MAP kinases c-Jun N-terminal kinase (JNK), p38 MAPK, and extracellular signal regulated kinases 1 and 2 (ERK 1/2), and that NAC had no effect on these kinases. NF-κB p65 phosphorylation was unaffected by exercise; however it was reduced in NAC at fatigue by 14% (p<0.05) compared to pre-infusion. Additionally, we analysed expression of exercise and/or ROS sensitive genes involved in stress-response (heat shock protein 70, HSP70), inflammation (interleukin-6, IL-6; monocyte chemotactic protein-1), anti-oxidant defense (manganese superoxide dismutase, MnSOD) and mitochondrial biogenesis (peroxisome proliferator-activated receptor coactivator-1α, PGC-1α). Exercised induced mRNA expression was ROS dependent for MnSOD (Figure), but not PGC-1α, interleukin-6, MCP-1, or heat-shock protein 70. These results suggest that inhibition of ROS attenuates some skeletal muscle cell signaling pathways and gene expression involved in adaptations to exercise.