Physiological changes in pH alter markers of mitochondrial biogenesis after a single bout of high-intensity exercise in rats

A.J. Genders, J. Kuang, E. Marin, X. Yan, J. Broatch, N. Saner and D.J. Bishop, Institute of Sport, Exercise & Active Living, Victoria University, P.O. Box 14428, Melbourne, VIC 8001, Australia.

Exercise is known to stimulate mitochondrial biogenesis, and to increase mitochondrial function and content (Holloszy, 1967; Granata *et al*, 2016). However, during high intensity exercise muscle pH can decrease below pH 6.8, and we have observed that minimising the decrease in muscle pH that occurs during high-intensity physical activity appears to promote greater activity-induced adaptations of the mitochondria (Edge *et al.*, 2006; Bishop *et al.*, 2010). This raises the intriguing possibility that intracellular pH may affect the cellular signalling pathways and genes that regulate mitochondrial biogenesis - which we have previously demonstrated to be up-regulated by physical activity (Little *et al.*, 2011). The aim of this study was to investigate the effects of altering pH on acute, activity-induced genes that are involved in the regulation of mitochondrial biogenesis in skeletal muscle. Male Wistar rats were acclimatised to the treadmill over three days and then their exercise capacity was assessed using an incremental exercise test. The incline for both the incremental exercise and acute exercise session was set at 10 degrees. The average top speed during the incremental exercise test was 0.48 ± 0.01 m/s. Animals were removed from the treadmill when they could no longer keep up with the speed despite encouragement. At least 72 h after the test all animals were given orally *via* gavage placebo, 0.05 g/kg $NaHCO_3$ or 0.05 g/kg NH_4Cl. 15 min after gavage treatment animals were exercised at 80% of their top speed (approximately 0.38 m/s) for seven 2 minute intervals interspersed with 1 min rest. Prior to, immediately post, and 3 h after the completion of the exercise protocol, rats were humanely killed using 90 mg/kg i.p. pentobarbitone and the *soleus* and superficial white portion of the medial *gastrocnemius* was removed. This study was approved by the Victoria University Animal Ethics Committee. Gene expression was assessed using qRT-PCR. PGC-1α increased 1.6 fold 3 h after exercise compared to the non-exercise control group, this effect was enhanced with administration of $NaHCO_3$ prior to exercise with expression increasing 2.4 fold at 3 h. NH_4Cl administration decreased PGC-1α expression 3 fold immediately following exercise. Although $NaHCO_3$ administration did not alter cytochrome c expression after exercise, NH_4Cl administration prior to exercise decreased expression by half. COX-IV expression did not change with pH manipulation. In conclusion sodium bicarbonate administration enhances exercise-induced increases in PGC-1α gene expression, whereas ammonium chloride administration appears to have a negative impact suggesting blood and in turn muscle pH during exercise can impact upon mitochondrial biogenesis.

Bishop DJ, Thomas C, Moore-Morris T, Tonkonogi M, Sahlin K, Mercier J. (2010) *Am J Physiol Endocrinol Metab* **299**, E225-E233

Edge J, Bishop D, Goodman C. (2006) *J Appl Physiol* **101**, 918-25

Granata C, Oliveira RS, Little JP, Renner K, Bishop DJ. (2016) *FASEB J* **30**, 959-970

Holloszy JO. (1967) *J Biol Chem* **242**, 2278-2282

Little JP, Safdar A, Bishop D, Tarnopolsky MA, Gibala MJ. (2011) *Am J Physiol Regul Integr Comp Physiol* **300**, R1303-10