Exercise-training increases skeletal muscle mitochondrial biogenesis despite inhibition of xanthine oxidase

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Reactive oxygen species (ROS) increase in skeletal muscle during exercise and there is some evidence that such small, physiological increases in ROS are required for normal increases in mitochondrial biogenesis following exercise (Gomez-Cabrera *et al.*, 2008). Indeed, vitamin C, a non-specific antioxidant, prevents increases in mitochondrial biogenesis markers during exercise-training in rats (Gomez-Cabrera *et al.*, 2008). Although several ROS producing sites have been identified in skeletal muscle during contraction, there is a lack of knowledge regarding the source(s) of ROS responsible for initiating pathways culminating in mitochondrial biogenesis in skeletal muscle during exercise. A recent study has identified the enzyme xanthine oxidase (XO) as a potential source of ROS responsible for initiating skeletal muscle mitochondrial biogenesis during exercise (Gomez-Cabrera *et al.*, 2005). p38 MAPK is a putative mitochondrial biogenesis signalling molecule (Akimoto *et al.*, 2005) and the inhibition of the XO-mediated ROS production during acute exercise *in vivo* with the XO inhibitor, allopurinol, prevented increases in gastrocnemius p38 MAPK phosphorylation immediately after exercise (Gomez-Cabrera *et al.*, 2005). However, key mitochondrial biogenesis proteins were not analysed and the effect of allopurinol during exercise-training has not been examined. Therefore the aim of this investigation was to determine whether inhibiting XO-induced increases in ROS with allopurinol during exercise training attenuates key proteins involved in mitochondrial biogenesis.

Male Sprague-Dawley rats aged 5 weeks were randomly assigned into four groups: [1] Sedentary (SedWater) (n=6); [2] Exercise-trained (ExWater) (n=6); [3] Sedentary and treated with 0.25 mg/mL of allopurinol in drinking water (SedAllo) (n=6); and [4] Exercise-trained and treated with 0.25 mg/mL of allopurinol in drinking water (ExAllo) (n=6). Pilot testing revealed this treatment to inhibit phosphorylation of p38 MAPK during acute exercise. Trained rats were exercised 5 days/week on a treadmill at a 5% incline for 6 weeks. By the end of the sixth week trained rats were required to run for 90 minutes at a treadmill speed of 30 m/min. Sedentary rats were placed on a stationary treadmill for an identical period of time as the trained rats throughout the investigation. Rats were killed *via* an intraperitoneal injection of pentobarbital sodium (180 mg/kg) 48 hours after the last training bout. Gastrocnemius muscles were rapidly dissected and frozen in liquid nitrogen. Mitochondrial biogenesis markers were examined using commercially available antibodies for PPAR-γ co-activator-1α (PGC-1α), mitochondrial transcription factor A (Tfam) and cytochrome c.

Exercise-training for 6 weeks significantly increased PGC-1α, Tfam and cytochrome c protein levels in gastrocnemius muscle of ExWater and ExAllo rats ($p < 0.05$). Interestingly, allopurinol did not alter the exercise-induced increases in these key mitochondrial biogenesis markers. No difference was observed in PGC-1α, Tfam and cytochrome c protein levels in SedAllo animals when compared to the SedWater controls.

The fact that inhibition of XO-induced production of ROS with allopurinol failed to attenuate the increase in mitochondrial biogenesis markers with exercise-training suggests that XO may not be an essential source of ROS responsible for the stimulation of skeletal muscle mitochondrial biogenesis with exercise-training. Given that vitamin C has been shown to attenuate the increase in mitochondrial biogenesis with exercise-training (Gomez-Cabrera *et al.*, 2008), further studies examining other ROS producing sites within skeletal muscle are required to determine which sources of ROS are involved with regulating skeletal muscle mitochondrial biogenesis during exercise-training.

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