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Free communications: Exercise

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Chair: Mike McKenna

Can the increase in mitochondrial biogenesis in skeletal muscle following acute exercise be prevented by antioxidant supplementation?

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Introduction. Endurance exercise potently stimulates increases in skeletal muscle mitochondrial volume and the increased mitochondrial biogenesis (synthesis) following exercise training is largely attributed to the cumulative effects of each acute bout of exercise (Hood, 2001). Recent evidence suggests that small physiological increases in skeletal muscle reactive oxygen species (ROS) play a role in the regulation of exercise-induced mitochondrial biogenesis. Vitamin C supplementation (a non-specific antioxidant; 0.5g/kg body weight/day) during 6 wk of exercise training in rats prevents improved exercise capacity and completely abolishes increases in several markers of exercise-induced mitochondrial biogenesis in skeletal muscle (Gomez-Cabrera *et al.*, 2008). Therefore, to understand the molecular mechanisms that are regulating the longer term increases in mitochondrial biogenesis following training, the aim of this project was to determine if vitamin C supplementation attenuates mitochondrial biogenesis signalling pathways following acute exercise in skeletal muscle of rats.

Methods. Thirty-two male Sprague Dawley rats (227 ± 2 g) were familiarized to treadmill running 1 wk prior to experimentation. Half the rats were given vitamin C (0.5g/kg body weight/day) in their drinking water for 7 days prior to the experiment as this dose of vitamin C has previously been shown to block increases in ROS levels during exercise (Sastre *et al.*, 1992). Animals were further assigned rest (Rest), or exercise (Ex) groups (8 rats in each group). The exercise groups ran on a motor driven treadmill at 25m/min on a 5% incline for 60 min. Rats were killed immediately after exercise with an intraperitoneal injection of Pentobarbital sodium (170 mg/kg) and the gastrocnemius (Gomez-Cabrera *et al.*, 2008) was rapidly frozen in liquid nitrogen. Mitochondrial biogenesis signalling proteins were examined (phosphorylated p38 MAPK, AMPKα Thr172 and ATF-2) *via* immunoblotting with commercially available antibodies.

Results. Treadmill running significantly (main effect for exercise; $p < 0.05$) increased phosphorylation of p38MAPK AMPKα Thr172 and ATF-2. Vitamin C supplementation did not significantly alter the phosphorylation or protein abundance of p38 MAPK, AMPKα or ATF-2.

Conclusions. Antioxidant treatment *via* vitamin C supplementation does not attenuate the phosphorylation of the mitochondrial signalling proteins, p38 MAPK, AMPKα and ATF-2 following acute exercise. The attenuation of exercise training-induced mitochondrial biogenesis by vitamin C supplementation previously observed in rats (Gomez-Cabrera *et al.*, 2008) does not appear to be due to an altered mitochondrial biogenesis signalling pathway involving p38 MAPK or AMPK.

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Hood DA. (2001) *Journal of Applied Physiology* **90**, 1137-1157.

Sastre J, Asensi M, Gasco E, Pallardo FV, Ferrero JA, Furukawa T & Vina J. (1992) *American Journal of Physiology - Regulatory, Integrative and Comparitive Physiology*, **263**, R992-995.

Aberrant skeletal muscle mitochondrial responses to exercise in overweight women with polycystic ovary syndrome (PCOS)

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Background. Skeletal muscle constitutes 45% of body mass, accounting for 80% of insulin stimulated glucose uptake and is implicated in the pathogenesis of insulin resistance (IR) and type II diabetes mellitus (DM2), although the mechanisms of IR remain unclear. Diminished mitochondrial oxidative capacity and mitochondrial damage have been shown in DM2. Exercise training has been shown to promote mitochondrial biogenesis and function in skeletal muscle, and is currently believed to be a key mechanism for enhancing insulin sensitivity after exercise training. The present study aimed to compare the effects of 12 weeks of exercise training (treadmill) on mitochondrial biogenesis in skeletal muscle of normoglycaemic, overweight women with polycystic ovary syndrome (PCOS; an IR, pre-diabetic condition) to age and weight matched controls.

Materials and Methods. 20 PCOS and 13 control women who met the inclusion criteria began the study, with only 8 PCOS and Control women completing the entire study. These women after a 3 month "wash out" period completed a DEXA scan, VO_{2peak} test and a hyperinsulaemic euglycaemic clamp with *vastus lateralis* muscle biopsies. They then completed 12 weeks of treadmill exercise training (3d wk^{-1}) alternating between 60 min of moderate intensity constant speed exercise and 45-60min high-intensity intermittent exercise. The initial tests were all repeated within 7 d of completing the training. Muscle samples were analysed for gene expression by semi quantitative real-time PCR, protein expression and enzyme activity of key representative mitochondrial proteins.

Results. Exercise resulted in a 17% ($p < 0.05$) and 32% ($p < 0.05$) increase in glucose infusion rate in PCOS and Control women respectively. This was accompanied by a 23% ($p < 0.05$) and 16% ($p < 0.05$) increase in VO_{2peak} in PCOS vs. Controls. Normalised gene expression for Tfam, NRF1, PGC1α and UCP3 remained unchanged or increased to a similar extent in the PCOS and controls in response to training. COX4 gene expression increased to a greater extent in PCOS *vs* Control trained muscle (Fold change; mean ± SEM; 1.9 ± 0.1 *vs* 1.4 ± 0.2; $p < 0.05$). In response to training, protein expression of electron transport chain (ETC) protein complex 2 30 kDa, trended towards an increase in Controls *vs* PCOS muscle (1.8 ± 0.3 fold *vs* 0.9 ± 0.2 fold; $p = 0.06$), with an apparent trend towards this differential effect of exercise seen across the ETC.

Discussion. Markers of mitochondrial biogenesis and function in normoglycaemic, overweight IR women with PCOS showed aberrant gene and protein expression in response to exercise training, compared to age and weight matched controls. Potentially, this is related to the IR state observed in PCOS, impairing mitochondrial response to exercise.

Effects of antioxidant supplementation and exercise training on skeletal muscle antioxidant enzymes and mitochondrial biogenesis

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An acute endurance exercise bout increases free radical production in skeletal muscle. This 'hormesis' stimulates positive adaptations in skeletal muscle, leading to improvements in endurance performance. Nutritional supplement use is popular among athletes, yet paradoxically, antioxidant supplementation may not be beneficial. Vitamin E and α-lipoic acid are lipid and water soluble antioxidants, respectively and it is currently unknown whether these antioxidants inhibit muscular adaptations to endurance training. We investigated the role of redox regulation on changes in skeletal muscle following endurance exercise training.

Male Wistar rats (n=48) were divided into sedentary control (SC), sedentary + antioxidant (SA), exercise (E) and exercise + antioxidant (EA) groups. The antioxidant groups were supplemented with Vitamin E (1000 IU/kg diet) and α-lipoic acid (1.6 g/kg diet) for 14 wk. Exercising animals were treadmill trained (90 min/d, 4 d/wk at 70% VO_2max) during this time. Red gastrocnemius and vastus muscles were excised under general anaesthesia 48 h after the final training bout. Antioxidant enzymes (xanthine oxidase, XO; manganese superoxide dismutase, Mn-SOD; glutathione peroxidase, GPX), antioxidant enzyme gene expression (Mn-SOD and GPX-1), markers of mitochondrial biogenesis (peroxisome proliferator-activated receptor gamma, coactivator alpha, PGC-1α; mitochondrial transcription factor A, mtTFA; citrate synthase) and PGC-1α protein abundance was analysed. All data were tested for normality and a two way ANOVA was performed, with a Tukey *post hoc* analysis. Significance was assumed when $p < 0.05$.

Antioxidant supplementation significantly reduced skeletal muscle PGC-1α mRNA (main effect for antioxidant, $p < 0.05$), whereas exercise training significantly increased PGC-1α mRNA (main effect for exercise, $p < 0.05$). However, there was no significant effect of antioxidant treatment on the exercise-induced increase in PGC-1α mRNA. Furthermore, PGC-1α protein abundance was significantly increased after training (main effect for exercise, $p < 0.01$), although there was no significant effect of antioxidant supplementation. Citrate synthase, mtTFA, GPX and Mn-SOD gene expression was similar between the groups. There were significant ($p < 0.05$) antioxidant × exercise interaction effects for all antioxidant enzymes. When compared to the sedentary group, exercise training suppressed XO activity (E *vs.* SC; -0.3×; $p < 0.01$), whilst antioxidant supplementation reduced XO activity in sedentary group (SA *vs.* SC; -0.5×; $p < 0.001$). GPX activity increased after training in supplemented animals (EA *vs.* SA; 1.4×; $p < 0.01$) but was reduced in the sedentary, supplemented group (SA *vs.* SC; -0.3×; $p < 0.01$). Lastly, exercise training reduced Mn-SOD and total SOD activities (E *vs.* SC; -0.5×; -0.4×; $p < 0.01$, respectively). Antioxidant supplementation reduced Mn-SOD and total SOD activities only in sedentary muscles (SA *vs.* SC; -0.4×; -0.4×; $p < 0.001$, respectively).

In summary, antioxidant supplementation suppressed gene transcription of the mitochondrial biogenesis marker PGC-1α, but did not alter PGC-1α protein abundance. There was no effect of antioxidant supplementation on the exercise-induced increase in PGC-1α mRNA and protein levels. Antioxidant supplementation decreased the activities of antioxidant enzymes, with a variable effect of endurance training.

Nitric oxide and ROS regulate skeletal muscle glucose uptake during contraction independent of AMPK α2

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Introduction. The pathway(s) by which exercise regulates skeletal muscle glucose uptake are unclear, but unlike the insulin pathway, it is known to be intact in type 2 diabetics. Thus exercise is an effective treatment for type 2 diabetes, however many patients cannot or do not exercise regularly and rely heavily on blood glucose lowering medication. Currently pharmaceutical treatments for type 2 diabetes are limited in their specificity and effectiveness. Therefore, understanding how exercise simulates glucose uptake may lead to the development of new targeted pharmaceutical agents to aid in the treatment and possibly prevention of type 2 diabetes.
There is evidence that AMP-activated protein kinase (AMPK), nitric oxide (NO) and reactive oxygen species (ROS) regulate glucose uptake during exercise/contraction. Indeed, both NO and ROS have been proposed to increase glucose uptake via an AMPK-dependent mechanism, but disassociation also exist between ROS and NO stimulated glucose uptake and AMPK activation. This study investigated whether AMPKα2 is required for ROS and NO mediated glucose uptake during contraction.

Method. Male and female mice overexpressing a muscle specific AMPKα2 dominant negative transgene (AMPK DN) and wild type (WT) litter mates, aged 20-22 weeks were anesthetized and both hindlimb extensor digitorum (EDL) and soleus muscles were excised. Muscles were mounted in an incubation chamber filled with 30°C Krebs-Henseleit buffer (KHB) and oxygenated with 95% O_2 and 5% CO_2 gas. *Contracted muscles:* Following a 40 min incubation period muscles were electrically stimulated to contract for 10 min (350 ms (EDL) and 600 ms (soleus) duration, 60Hz, 12 contractions·min^{-1}) and glucose uptake was measured. During pre-contraction incubation and contraction, muscles were treated with either a NOS inhibitor (N^G-Monomethy-1-L-Arginine; L-NMMA, 100 μM), or a non-specific antioxidant (N-acetyl-L-cysteine; NAC, 20 mM). Contraction force was measured via proximal tendon suture attachment to a force transducer (PanLab, Spain). *Glucose uptake:* KHB was replaced with KHB containing radio labelled 2DG (2-Deoxy-D-glucose) and mannitol. *Muscle Analysis:* Muscles were analysed via western blot for AMPKα Thr^{172} phosphorylation, ACC-β Ser^{212} phosphorylation, total PAS-AS160 phosphorylation and nNOSμ expression.

Results. Contraction increased glucose uptake in EDL and soleus muscles of AMPK DN and WT to a similar extent (1.6-2.0-fold, $p < 0.05$ *vs* basal). In the EDL muscle, both L-NMMA and NAC attenuated the increase in glucose uptake during contraction by 50-60% ($p < 0.05$) in AMPK DN and WT muscles. NAC prevented the increase in glucose uptake in soleus muscles of AMPK DN and WT ($p > 0.05$ *vs* basal) but L-NMMA treatment had no effect ($p > 0.05$ *vs* contraction). Peak contraction force and rate of fatigue of EDL and soleus was similar between genotypes and was not affected by treatment ($p > 0.05$).

nNOSμ was expressed similarly in AMPK DN and WT muscles, with expression being greater in EDL than in the soleus ($p < 0.05$). AMPKα expression was ~2-fold greater in AMPK DN EDL and soleus muscles than in WT ($p < 0.05$ for genotype effect), however, AMPKα Thr^{172} phosphorylation relative to AMPKα expression was greater in WT than AMPK DN ($p < 0.001$ for genotype effect). Contraction increased WT (by ~4-7-fold, $p < 0.05$ *vs* basal) but not AMPK DN ($p > 0.05$ *vs* basal) EDL and soleus AMPKα Thr^{172} phosphorylation. AMPKα Thr^{172} phosphorylation was not affected by NAC or L-NMMA treatment for both muscles of both genotypes ($p > 0.05$). WT had greater ACCβ Ser^{212} phosphorylation than AMPK DN in both the EDL and soleus muscles ($p < 0.001$ for genotype effect). In the EDL contraction increased AMPK DN and WT ACCβ Ser^{212} phosphorylation (by ~1.5-fold, $p < 0.05$ *vs* basal) and NAC treatment prevented this increase in AMPK DN ($p > 0.05$ *vs* basal) but not WT. In the soleus, contraction increased WT (by ~1.5-fold, $p = 0.05$ *vs* basal) but not AMPK DN ACCβ Ser^{212} phosphorylation ($p = 0.17$ *vs* basal), and NAC treatment prevented the increase in WT ACCβ Ser^{212} phosphorylation ($p = 0.17$ *vs* basal). L-NMMA treatment did not affect AMPK DN or WT ACCβ Ser^{212} phosphorylation in either muscles ($p > 0.05$ *vs* basal). Total phosphorylation of PAS-AS160 was not affected by contraction or treatment in both the EDL and soleus muscle of AMPK DN and WT ($p > 0.05$). In the EDL, WT had a greater total phosphorylation of PAS-AS160 than AMPK DN ($p = 0.05$ for genotype effect).

Conclusion. AMPKα2 and AS160 are not essential in regulating skeletal muscle glucose uptake during contraction. NO and ROS appear to be involved in regulating skeletal muscle glucose uptake during contraction via an AMPKα2 independent mechanism.

Iron overload in skeletal muscle; redox stress and exercise capacity

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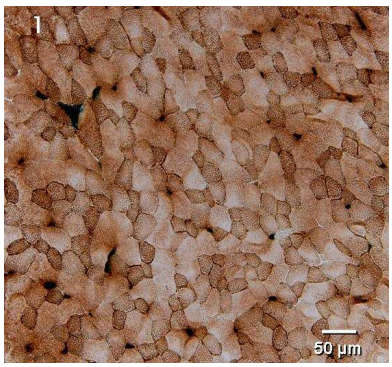
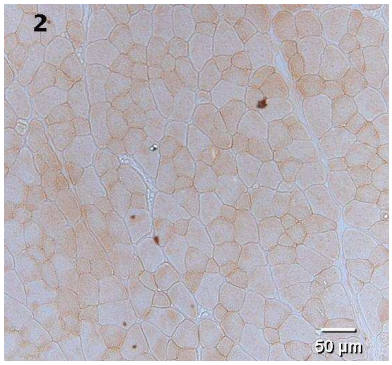
Iron accelerates the production of reactive oxygen species (ROS). Excessive levels of ROS are thought to accelerate skeletal muscle fatigue and contribute to the loss of skeletal muscle mass and function with age and disuse. Increased iron accumulation has been observed in these conditions and it has been proposed that iron may play a role (Jung *et al.*, 2008). Patients with an iron overload disorder frequently report symptoms of weakness and fatigue which is not entirely explained by reduced cardiac function (Davidsen *et al.*, 2007). The contribution of skeletal muscle to these symptoms is unknown.

Recent work from our laboratory has shown that iron accelerates skeletal muscle fatigue at 37°C (Reardon & Allen, 2007). Previous experiments under conditions in which there were trace amounts of iron in the perfusate, demonstrate that iron accelerates skeletal muscle fatigue at 37°C by reducing calcium sensitivity (Moopanar & Allen, 2005). Using a mouse model of iron overload lasting 30 days we determined the extent of iron accumulation in skeletal muscle and the change in the iron storage protein ferritin. The level of oxidative stress, changes in antioxidant enzymes and exercise performance were also assessed. The skeletal muscle analysed in this study was removed following cervical dislocation.

The iron content of the *tibialis anterior* muscle was assessed using inductively coupled plasma mass spectroscopy. Skeletal muscle iron was higher in the iron group (59.5 ± 3.5 μmoles/g dry weight) following the intervention compared to the control group (1 ± 0.1 μmoles/g dry weight) ($p < 0.001$; $n = 7$ per group). Importantly, the intracellular iron component (determined by a DAB enhanced Perls' stain) was also higher in the iron group (Figure 1) compared to the control group (Figure 2) ($p < 0.001$; $n = 6$ per group). The skeletal muscle content of the iron storage protein ferritin light chain was found to be 4 fold higher in the iron group ($p < 0.05$; $n = 5$ per group), indicating an increase in the iron storage capacity of skeletal muscle. The oxidative stress product malondialdehyde was also increased in the iron group compared to the control group ($p < 0.001$; $n = 5$ per group). The *extensor digitorum longus* muscle was used to measure the activity of the anti-oxidative enzymes glutathione reductase (GR) and glutathione peroxidase (GPx). GR activity increased in the iron group compared to the control group by 30% (3.7 ± 0.1 *vs.* 2.8 ± 0.1 nmol/mg/min respectively) ($p < 0.001$; $n = 7$ per group) and GPx activity increased in the iron group compared to the control group by 220% (1.5 ± 0.1 *vs.* 0.7 ± 0.1 nmol/mg/min respectively) ($p < 0.001$; $n = 7$ per group). The increased activity in the GR and GPx enzymes demonstrates that skeletal muscle has the ability to respond to the downstream oxidative stress of iron, but at the same time highlights the incomplete action of ferritin.



Exercise tests were performed before and after iron loading in both groups. Iron overload mice performed less work than the control group on a treadmill test designed to test endurance capacity (7 *vs*. 45 joules respectively) ($p < 0.001$; $n = 7$ per group). Iron overloaded mice produced less force than control mice on a maximal strength test ($p < 0.001$; $n = 7$ per group) and their performance over repeated trials deteriorated more rapidly compared to the control group ($p < 0.01$). Skeletal muscle weight was also lower in the iron group in absolute terms and relative to body weight following the intervention ($p < 0.001$; $n = 7$ per group).



In summary, iron accumulation in skeletal muscle may play a significant role in the reduced exercise capacity seen in iron overload disorders and in ageing, and may play an underlying role in skeletal muscle atrophy.

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The effect of continuous *vs* intermittent exercise on substrate utilization during exercise and recovery in healthy adults

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Introduction. The incidence of obesity and type II diabetes is reaching epidemic proportions in today's society and are associated with long-term ill health and reduced quality of life. As such, it has been recommended that effective weight loss strategies be developed. Carbohydrate and lipids are the primary substrates utilized for energy during exercise. Lipids provide the majority of fuel supply to the exercising skeletal muscle during steady state, moderate intensity exercise (SSMIE), with an increasing supply from carbohydrate sources with increasing intensities (Romijn *et al.*, 2000). However, recent studies have demonstrated an increased capacity for high intensity intermittent exercise (HIIE) to evoke decreases in adiposity compared with SSMIE, without providing metabolic mechanisms (Trapp *et al.*, 2008; Tremblay *et al.*, 1994). Therefore the purpose of this study was to examine plasma and respiratory indicators of lipid and carbohydrate metabolism during and after a single bout of HIIE compared with SSMIE in order to explain decreased adiposity witnessed during HIIE.

Methods. This study obtained approval from Victoria University, Human Research Ethics Committee (HRETH 07/281) and all experiments conformed to the National Statement on Ethical Conduct in Human Research. Participants (8 males and 8 females) performed two exercise bouts, SSMIE (50% VO_2 peak), and HIIE (20s sprint: 40s rest) for 30 minutes on two separate occasions in randomised order. The HIIE bout was designed to be three times the workload of SSMIE, performed for a third of the time, such that the two exercise bouts required the same amount of mechanical work. Blood was taken during exercise and one hour of recovery, and was analysed for glucose, lactate, glycerol and free fatty acids (FFA). Respiratory gas exchange data was also obtained.

Results. There was no significant difference in oxygen consumption between the bouts, indicating similar aerobic requirements of SSMIE and HIIE. Both exercise bouts increased lipid oxidation as measured by increased plasma glycerol concentrations during exercise and in recovery. However, RER values were significantly lower ($p < 0.05$) during recovery after HIIE than SSMIE, indicating an increased reliance on lipid oxidation. HIIE also showed a significant decrease in plasma FFA at the end of exercise ($p < 0.05$), suggesting increased uptake by the muscle to support lipid oxidation. Lactate concentrations rose over the 30 minutes, and were significantly higher in HIIE ($p < 0.05$), mostly due to the anaerobic breakdown of glycogen, as plasma glucose concentrations remained the same.

Discussion. It is feasible that HIIE creates a 'substrate shuttle' whereby there are repeated shifts from anaerobic to aerobic energy sources. ATP and PCr are partly depleted during the high intensity work phases, with their resynthesis during the rest periods occurring via oxidative pathways (Essen *et al.*, 1977), leading to increased energy expenditure during rest periods of HIIE. Glycogen, although depleted, does not appear to be resynthesised during the rest periods, with ongoing diminution over the HIIE session (Bangsbo *et al.*, 1991). As such, restoration of glycogen stores is of high metabolic priority during recovery, contributing to increased energy expenditure and a negative energy balance after HIIE. Differences in metabolism during rest and recovery from HIIE may explain decreases in adiposity observed, and further investigation of exercise specifically designed for fat loss is required.

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