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Free communications: Exercise physiology

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Chair: Gordon Lynch

Effect of high-intensity, interval exercise on signalling proteins and gene expression in human skeletal muscle

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Short duration, high-intensity interval exercise is typically associated with strength or resistance training and linked to pathways that stimulate skeletal muscle growth. However, repeated sessions of such high-intensity, interval exercise induce rapid changes in skeletal muscle phenotype that are similar to those induced by traditional endurance exercise training, *i.e.* increased skeletal muscle oxidative capacity without marked hypertrophy (Burgomaster *et al.*, 2005). To investigate the effects of such high-intensity, interval exercise on signalling proteins and gene expression in human skeletal muscle, six untrained men (23 + 2 yrs) completed four 30s bouts of "all out" cycling exercise, separated by four min rest. Muscle (*v. lateralis*) samples were obtained by percutaneous needle biopsy before, after the first and fourth bouts and after three hours of passive recovery. Activation of signalling pathways was assessed by Western blotting using phosphospecific antibodies to AMP-activated protein kinase (AMPK), calcium/calmodulin-dependent protein kinase (CaMKII), p-38 mitogen-activated protein kinase (MAPK), protein kinase B (PKB) and 70-kDa S6 protein kinase (S6K). Skeletal muscle expression of selected genes (GLUT4, PGC1, PDK4 and COXIV) was assessed on muscle samples obtained before exercise and after three hours of recovery using real-time RT-PCR with specific primers and SYBR Green detection (BioRad). Phosphorylation (p-) of AMPK, CaMKII, p38 MAPK, PKB and S6K were unchanged after the first bout; however, p-AMPK and p-p38 MAPK were increased ($p < 0.05$) ~40% after the fourth bout, while p-PKB was reduced ($p < 0.05$) by ~50%. No changes in p-CaMKII, p-S6K or 4E binding protein phosphorylation were observed after exercise. PGC1 and PDK4 mRNA were increased ($p < 0.05$) ~3-fold after three hours of recovery, with no significant change in GLUT4 and COXIV mRNA. These data suggest that high-intensity, interval exercise activates signalling pathways known to contribute to mitochondrial biogenesis, with no activation of pathways responsible for growth-related protein synthesis. Such activation may explain the previously observed changes in muscle oxidative capacity and aerobic exercise performance following high-intensity, interval exercise training.

Burgomaster KA, Hughes SC, Heigenhauser GJF, Bradwell SN & Gibala MJ (2005) *Journal of Applied Physiology*, **98:** 1985-90.

The effects of hydration and aerobic fitness on physiological strain and endurance performance

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Introduction. Despite the widely accepted notion that dehydration of 1-2% body mass impairs endurance performance, there is no convincing evidence that athlete performance is suboptimal when fluid is consumed *ad libitum*; a rate that typically causes hypohydration of ≥ 2%. Regular endurance training stimulates physiological and psychological adaptations that enable athletes to better tolerate exercise stress. Given that prolonged endurance training is generally associated with at least mild dehydration (1-3%), it is conceivable that aerobically-trained people may also be better adapted to tolerate hypohydration. Therefore, the purpose of this study was to examine the hypothesis that aerobic fitness attenuates mild hypohydration-augmented physiological strain and associated performance decrement in a temperate environment.

Methods. *Participants* were six untrained and six trained, non-heat acclimated males of similar age (31 ± 9 *vs.* 25 ± 6 y). Trained participants had higher training frequency (6.0 ± 1.3 *vs.* 1.0 ± 0.8 d•wk^{-1}) and VO_{2peak} (64 ± 8 *vs.* 45 ± 4 ml•kg^{-1}•min^{-1}), but lower body mass (71.8 ± 4.3 *vs.* 78.6 ± 9.7 kg) and adiposity (8 ± 2 *vs.* 16 ± 5% body fat) than Untrained. The study was approved by the University of Otago Human Ethics Committee, and participants provided their informed consent. *Procedure:* After two full familiarisations participants completed two 80-min exercise trials (24.3 ± 0.6 °C, 50% rh, air velocity: 4.5 m•s^{-1}), 1-3 wk apart, in balanced order. Trials comprised of 40-min constant load cycling (70%VO_{2peak}) before a 40-min self-paced performance ride, on an electromagnetically-braked ergometer. On the evening preceding each trial participants undertook 50 min of exercise plus heat stress followed by complete (EUH) or partial (resulting in a 1.5-2% reduction in body mass; HYPO) overnight rehydration. During EUH, 100% of metabolism-corrected mass loss was replaced at 10-min intervals throughout exercise by ingestion of a solution containing NaCl (2.9 g•L^{-1}) and artificial sweetener, whist during HYPO only 20% was replaced during constant load exercise followed by *ad libitum* intake during the performance ride. *Measures:* Substrate oxidation and the 70% work rate were calculated from respiratory gas analysis (15-min intervals) throughout familiarisation and experimental trials. Nude body mass and urine specific gravity (U_{SG}) were measured before and after all sessions. Rectal temperature, heart rate and local sweat rates were recorded throughout trials. Blood volume changes and plasma osmolality were estimated from venous samples drawn at rest, 10, 40 and 80 min. *Analyses:* The inferential analyses were unpaired t-test, and one- to three-way ANOVAs ($\alpha = 0.05$) due to one between-subjects factor (Fitness) and two within-subjects factors (Hydration, Time). Post hoc *t*-tests were Bonferroni corrected.

Results. *Rest*: At baseline in EUH, both fitness groups had comparable change in body mass (*cf.* baselines: Untrained +0.2 ± 0.3, Trained +0.5 ± 0.8%, respectively [mean ± SE]; $p = 0.43$) and plasma osmolality (276 ± 1 *vs.* 278 ± 1 mosmol•kg^{-1}, $p = 0.11$) but Untrained had higher U_{SG} (1.016 ± 0.002 *vs.* 1.009 ± 0.002 g.cm^{-3}; $p = 0.04$). At Baseline in HYPO, both groups had similar ($P > 0.4$) reductions in body mass (both -1.8 ± 0.1%), blood volume (both 3 ± 1%) and raised U_{SG} (1.030 ± 0.002 *vs.* 1.030 ± 0.001) and plasma osmolality (282 ± 1 *vs* 283 ± 2 mosmol•kg^{-1}), all of which differed from EUH ($p < 0.05$). *Exercise:* Heart rate and heart rate drift in constant-load exercise were increased during HYPO compared with EUH for Untrained (71 ± 2 *vs.* 63 ± 3% of heart rate range; $p = 0.02$, and 0.55 ± 0.08 *vs.* 0.41 ± 0.07 beats•min^{-2}; $p = .001$) but not Trained (71 ± 2 *vs.* 70 ± 2%; $p = 0.20$, and 0.23 ± 0.02 *vs.* 0.22 ± 0.02 beats•min^{-2}; $p = 0.24$). Rectal temperature was higher in HYPO compared to EUH during both 40-min phases, but the increase was significant only for Untrained (37.60 ± 0.14 *vs.* 37.34 ± 0.10ºC; $p = 0.02$). Similarly, HYPO effect on rate of T_c rise during constant-load phase was fitness dependent (hydration*fitness, $p = 0.001$); being significant for Untrained (29 ± 4%; $p = 0.001$) but not Trained (4 ± 5%; $p = 0.17$). Untrained but not Trained showed lower whole-body sweat rate and higher forehead sweat threshold in HYPO compared with EUH, but both groups had lower sweating sensitivity and whole-body sweat rate *vs.* plasma osmolality in HYPO than in EUH. Performance trial distance (D) and mean power output (PO) were reduced ($p < 0.05$) during HYPO compared to EUH, with Trained (D = -3 ± 3%; PO = -7 ± 3%) tending ($p = 0.13$) to be less affected than Untrained (D = -7 ± 1%; PO = -13 ± 7%).

Conclusion. Moderate hypohydration of 1.5-2% body mass augments thermal (T_c) and cardiovascular (HR) strain and impairs endurance performance in a temperate environment, but aerobic fitness attenuates some of these effects.

nNOS is necessary for normal increases in glucose uptake during contraction of skeletal muscle

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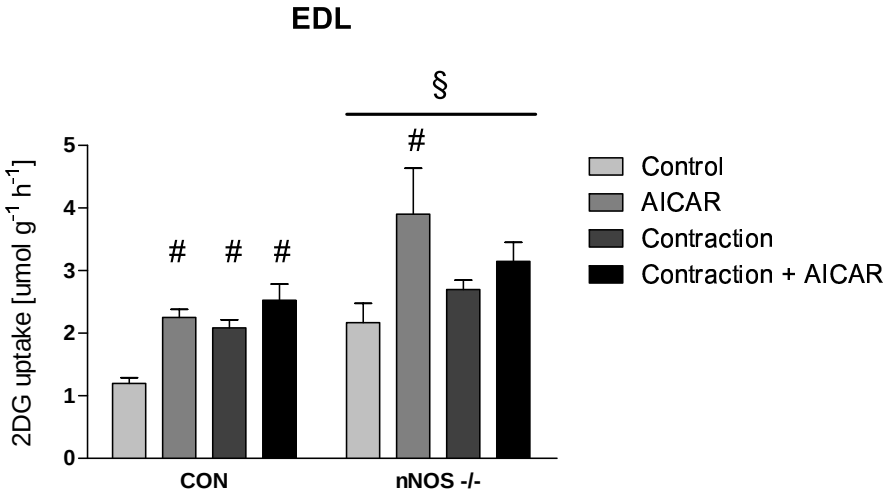
People with type 2 diabetes have reduced skeletal muscle glucose uptake in response to insulin, however, glucose uptake during exercise is normal. The factors regulating skeletal muscle glucose uptake during exercise are unclear. We have evidence to suggest that nitric oxide (NO) plays a role in humans, however, evidence in rodents is conflicting. We examined glucose uptake in response to contraction and in response to the AMP-activated protein kinase (AMPK) activator AICAR (2 mM) in nNOS null mice (nNOS-/-, on a C57 Bl/6 background) and in C57Bl/6 control mice (CON). It has been shown the there is no increase in cGMP content in response to contraction in nNOS-/- mice.

Extensor digitorum longus (*EDL*) and *soleus* muscles from adult CON and nNOS-/- mice (n = 8/group) were surgically excised from deeply anesthetized mice (Nembutal, 60 mg/kg i.p.). Muscles were stimulated to contract for either 10 minutes (*EDL*, 60 Hz, 350 ms, train rate 0.167) or 15 minutes (*soleus*, 60 Hz, 600 ms, train rate 0.167) and 2-Deoxy-D-glucose (2-DG) uptake was examined during contraction. GLUT1 and GLUT4 protein expression was examined in *tibialas* anterior muscle.

Basal 2-DG uptake was elevated ($p < 0.05$) in the nNOS-/- mice compared with the CON mice. There was no difference in GLUT1 protein expression, however, GLUT4 protein expression was elevated ~20% ($p < 0.05$) in the nNOS-/- mice. Contraction increased ($p < 0.05$) 2-DG uptake in both muscles in the CON mice. There was, however, no significant increase ($p > 0.05$) in 2-DG uptake during contraction in either the *EDL* or *soleus* of the nNOS-/- mice. AICAR increased 2-DG uptake similarly in the nNOS-/- mice and the CON mice.

These results indicate that skeletal muscle nNOS is required for normal increases in glucose uptake in response to contraction, but not in response to AICAR. They suggest that NO is a critical regulator of skeletal muscle glucose uptake during contraction.



#, Significantly different than control group of the same genotype ($p < 0.05$)
§, Main effect for genotype ($p < 0.05$).

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Rat skeletal muscle 3-O-MFPase activity is not decreased by fatiguing *in vitro* electrical stimulation

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Maximal 3-O-MFPase activity is a surrogate measure of the Na^+,K^+-ATPase activity and is commonly utilized in human exercise studies involving skeletal muscle biopsy samples. Studies to date (e.g. Fowles *et al.*, 2002; Petersen *et al.*, 2005), using a range of exercise intensities and durations, have shown that an acute bout of fatiguing exercise results in a decrease in maximal 3-O-MFPase activity at the point fatigue of between ~11 to 38%. These observations have been interpreted as evidence that the maximal activity of the Na^+,K^+-ATPase has declined thus contributing to skeletal muscle fatigue. It is speculated that endogenous factors such as reactive oxygen species (ROS) and calcium (Ca^{2+}) may be responsible for this observed reduction in 3-O-MFPase at fatigue. The aim of this study was to investigate whether rat skeletal muscle 3-O-MFPase activity could be reduced further than the above range from human studies by using large isolated skeletal muscles subjected to intense fatiguing *in vitro* electrical stimulation.

Sprague Dawley (260 ± 9g; Mean ± SE) fast twitch *extensor digitorum longus* (EDL) muscles (132 ± 4 mg) were dissected out under anaesthesia (Nembutal; ~85mg/kg) in accordance with Victoria University AEEC procedures and subjected to one of two different stimulation protocols: 1) two bouts of 10s continuous stimulation at a frequency of 100Hz (0.2ms pulse duration) separated by a 1hr recovery period; 2) two bouts of three min intermittent stimulation (1s stimulation at 100Hz followed by 4s recovery) separated by a 1hr recovery period. Tetanic force (500ms, 100Hz, 0.2ms pulse duration) was monitored during recovery. Fatigued muscles and their non-fatigued contra-lateral controls were blotted, weighed, frozen in liquid N_2 and maximal 3-O-MFPase activity analysed (Fraser & McKenna, 1998).

At the end of the first bout of 10s continuous stimulation tetanic force had declined by 51.8 ± 1.8% (n = 8) of initial force. Characteristic of high frequency fatigue, force had recovered to 81.2 ± 2.1% of initial after one min and remained relatively constant over the next hour (87.4 ± 2.6% of initial force at one hour). The second stimulation bout reduced force by 50.3 ± 1.3% of initial force, while 3-O-MFPase activity showed no decline (100.5 ± 3.4%; p = 0.9) compared to the non-fatigued, contra-lateral controls. Three minutes of high frequency intermittent stimulation resulted in tetanic force declining by 87.0 ± 1.0% (n = 8) of initial force. After one hour of recovery, tetanic force had gradually recovered to 62.7 ± 2.1% of initial force. At this time, force-frequency analysis showed the presence of low frequency fatigue with relative force being significantly lower at 10 (39.0 ± 2.1% *vs* 47.0 ± 1.2%; p = 0.005), 30 (44.4 ± 1.4% *vs* 60.7 ± 1.3%; $p < 0.0001$) and 50Hz (76.2 ± 1.4% *vs* 86.7 ± 0.7%; $p < 0.0001$) compared to pre-fatigue force. The second intermittent stimulation bout reduced force by 83.3 ± 1.3% of initial force while 3-O-MFPase activity was not significantly altered (94.4 ± 3.7%; p = 0.2) when compared to the non-fatigued contra-lateral controls.

In conclusion, under these conditions, rat EDL 3-O-MFPase activity was not reduced by either of the two fatiguing *in vitro* electrical stimulation protocols. Thus the decline in muscle force was not related to a depression in maximal 3-O-MFPase activity. Whether this reflects a species difference with resistance to Na^+,K^+-ATPase inactivation in the rat is unclear.

Fowles JR, Green HJ, Tupling R, O'Brien S & Roy BD. (2002) *Journal of Applied Physiology*, **92:** 1585-93.

Fraser SF & McKenna MJ. (1998) *Analytical Biochemistry* 258:63-7. (2006) *Journal of Physiology*, **576:** 279-88.

Petersen AC, Murphy KT, Snow RJ, Leppik JA, Aughey RJ, Garnham AP, Cameron-Smith D & McKenna MJ. (2005) *American Journal of Physiology*, **289:** R266-74.

Iron accelerates skeletal muscle fatigue at 37°C

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The loss of muscle performance during intense or prolonged muscular activity is known as fatigue, and is common in disease states like heart failure. Many factors are thought to contribute to muscle fatigue including a decrease in energy stores and an increase in metabolic by products such as reactive oxygen species (ROS). Recent work from our laboratory has shown that muscle fatigue is accelerated at 37°C due to a loss in calcium sensitivity, which can be prevented by the ROS scavenger Tiron (Moopanar & Allen, 2005) or reversed by the reducing agent DTT (Moopanar & Allen, 2006).

Iron can accelerate the production of the hydroxyl radical ($OH^{\bullet}$) through the Fenton reaction. Given that even the typical gauge 22 metal needle syringe can introduce significant amounts of iron into solution (Buettner, 1990), this study explored the role of iron in muscle fatigue at 37°C.

Small muscle bundles (2-6 fibres) were isolated from the mouse foot after rapid cervical dislocation. Muscles were fatigued (force reduced to 50%) by repeated short tetani at room temperature, allowed to recover, and fatigued again at 37°C. The time taken to fatigue at 37°C was then normalized to the room temperature fatigue run for analysis. Solutions were heated to 37°C by an insulated heat exchange system consisting of either aluminium or stainless steel piping. Both a non specific (100μM EGTA), and specific iron chelator (250nM-200μM desferrioxamine) were used in separate experiments to bind iron and inhibit Fenton reactions.

Time taken to fatigue at 37°C was reduced to 68% ± 10 (n = 6) of the initial fatigue run at room temperature, using the stainless steel heat exchanger. The accelerated fatigue at 37°C was prevented by the addition of EGTA (106% ± 16, n = 5) and desferrioxamine (108% ± 12, n = 6). No acceleration in fatigue time was observed using the aluminium heat exchanger (125% ± 8, n = 7).

Based on previous work from this laboratory, the accelerated fatigue observed at 37°C using a stainless steel heat exchanger is due to decreased Ca^{2+} sensitivity mediated through ROS. As iron can accelerate the production of $OH^{\bullet}$, and the metal chelators EGTA and desferrioxamine can prevent the accelerated fatigue, it appears likely iron is producing the $OH^{\bullet}$ and is contributing to the accelerated fatigue under these conditions.

Buettner GR. (1990) *Free radical research communications*, **10:** 5-9.
Moopanar TR & Allen DG. (2005) *Journal of Physiology*, **564:** 189-99.
Moopanar TR & Allen DG. (2006) *Journal of Physiology*, **571:** 191-200.

Intra-segmental distribution of emotional sweating

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It is well accepted that glabrous (non-hairy) skin surfaces respond to changes in emotional states (*e.g.* anxiety, fear, cognitive stress) with increased sweat secretion. However, little is known about the participation and regional distribution of non-thermally-mediated sweating in non-glabrous skin regions. We have previously reported increased sudomotor responses at glabrous and non-glabrous skin surfaces of the head during cognitive stress (Machado-Moreira *et al*., 2006). In the current study, we mapped the distribution of this non-thermal sweating over the entire body, and also investigated its intra-segmental distribution. Twenty six healthy and physically-active males and females participated in five experiments. Generalised, thermally-induced sweating, was established through 25 min of passive heating (climatic-controlled chamber: 36°C, 60% relative humidity; and water-perfusion suit: 40°C), which increased mean body temperature from 35.2 ± 0.2°C to 37.0 ± 0.1°C. Subjects were then asked to perform a series of arithmetic calculations, and were challenged and verbally encouraged to solve as many problems as possible within 60 s. Across the five experiments, separated by at least 1 month, sweat rates from the torso (6 sites; N=10), upper and lower arm (5 sites; N = 5), hand (4 sites; N = 10), thigh (6 sites; N = 5), leg (6 sites; N = 5) and foot (5 sites; N = 10) were measured using ventilated sweat capsules (1.40 and 3.16 cm^2). Significant increases in sweating due to the mental stimulus ($P < 0.05$) were observed in 38% of the 32 skin surfaces investigated, with low sudomotor responsiveness verified at the upper arm, thigh and leg. All sites within the forearm, hand and foot showed increased mentally-induced sweat rates ($P < 0.05$), except for the palm ($P > 0.05$) and sole ($P = 0.06$). Within the torso, the abdomen and back regions showed increased emotional sweating ($P < 0.05$), while no significant augmentation of sweat rate was observed for the ventral (chest) and lateral regions of the torso ($P > 0.05$). Changes in sweat rates (peak - baseline) within the torso and hand segments are shown in the Figure.

The current results strongly reinforce our previous observations that mental sweating is not restricted to glabrous skin surfaces (*e.g.* palms and soles), as is often considered. Indeed, many skin surfaces participate in emotional sweating once thermal sweating has been established, and the participation of palms and soles becomes less evident in this condition. Furthermore, although intra-segmental variations in sudomotor responses to mental stress exist, the changes in sweat rates from glabrous and non-glabrous skin surfaces do not differ significantly within each body segment ($P > 0.05$). Our current research foci centre upon examining differences in the recruitment of eccrine sweat glands during thermal and emotional sweating, and evaluating emotional sweating in non-sweating, thermoneutral individuals.

Machado-Moreira CA , Caldwell JN, Meijer AK , Wilmink F & Taylor NAS. (2006) *Proceedings of the Australian Physiological Society*, **37:** 93P.