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Chair: Tony Luff

Opposing effects of acute and chronic high intensity exercise on Na^+K^+ATPase activity in skeletal muscle

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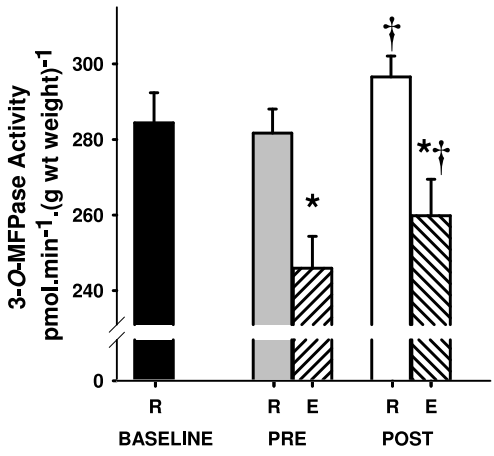
The Na^+K^+ATPase enzyme is critical in maintaining muscle trans-sarcolemmal [Na^+] and [K^+] gradients and membrane excitability. However, repeated maximal muscle contractions reduce maximal Na^+K^+ATPase activity (Fraser *et al.*, 2002). High-intensity interval training is commonly used by endurance athletes to improve endurance performance, but the effects of acute high-intensity interval exercise on muscle Na^+K^+ATPase activity are not known. Furthermore, although sprint training increases muscle Na^+K^+ATPase content (McKenna *et al.*, 1993), the effects of high-intensity interval training on muscle Na^+K^+ATPase activity are unknown. We therefore examined the possible contradictory effects of acute and chronic high-intensity interval exercise on the muscle Na^+K^+ATPase activity in seven male endurance-trained athletes.

A vastus lateralis muscle biopsy was taken at rest, 3-wks prior to (Base), at rest and immediately after exercise prior to (Pre) and after (Post) 3-wks of training. Muscle samples were analysed for maximal *in vitro* Na^+K^+ATPase (K^+ stimulated 3-*O*-MFPase) activity. Performance was characterised by incremental $\dot{V}O_2$ and peak power output (PPO); and during a simulated 40km time-trial, by mean power output (MPO). A t-test was used for resting 3-*O*-MFPase activity to determine reproducibility (Base – Pre) and to compare change scores (Base–Pre, & Pre-Post) to identify a change with training. A two-way ANOVA with repeated measures was applied to test for main effects of exercise (Rest, Ex) and training (Pre, Post).



Resting muscle 3-*O*-MFPase activity did not differ between Base and Pre. Acute high-intensity interval exercise depressed muscle 3-*O*-MFPase activity by 12.7±1.2% (mean±SEM, Exercise main effect, $P<0.05$, *****, see figure). In contrast, training increased 3-*O*-MFPase activity by 4.9±0.7% (training main effect, $p<0.05$, †, see figure). Resting 3-*O*-MFPase activity was increased after training by 5.4±1.0% (Pre – Post change score, $P<0.05$). Neither $\dot{V}O_2$ (Base 64.4±1.6; Pre 64.3±1.5; Post 65.8±1.9), PPO (Base 368±12; Pre 374±13; Post 379±14), or MPO in the 40km time-trial (Base 279±12; Pre 303±15; Post 303±13) differed significantly after training.

In conclusion, Na^+K^+ATPase measures were reproducible in resting muscle. Acute and chronic high-intensity interval exercise had converse effects on Na^+K^+ATPase activity. The small rise in Na^+K^+ATPase activity with training was insufficient to improve exercise performance.

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McKenna, M.J., Schmidt, T.A., Hargreaves, M., Cameron, L., Skinner, S.L. & Kjeldsen, K. (1993) *Journal of Applied Physiology,* **75,** 173-180.

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Acute intense exercise upregulates Na^+,K^+-ATPase isoform mRNA, but not protein expression in human skeletal muscle

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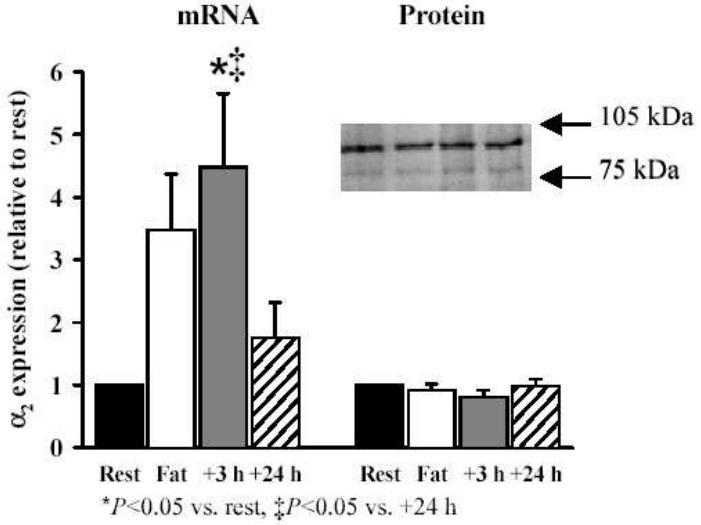
The Na^+,K^+-ATPase comprises a catalytic α subunit (~100-112kDa) and a glycosylated β subunit (˜40-60kDa), and belongs to a multi-gene family, with different genes encoding for four α (α_1, α_2, α_3, α_4) and three β isoforms (β_1, β_2, β_3). Characterisation of the Na^+,K^+-ATPase isoforms expressed in healthy human skeletal muscle, and consequently also the acute exercise effects on these isoforms remains incomplete. We therefore investigated Na^+,K^+-ATPase isoform expression in human skeletal muscle, and the effects of a single bout of intense exercise on the mRNA and protein expression of these isoforms.

Fifteen healthy subjects (eight males, seven females) performed a single bout of isokinetic one-legged knee extensor exercise, continued until fatigue (time to fatigue 352 ± 69 s; mean ± SD). A vastus lateralis muscle biopsy was taken from each subject at rest, fatigue (Fat), 3 h (+3 h) and 24 h (+24 h) post-exercise and analysed for mRNA and protein expression using Real-Time RT-PCR and immunoblotting, respectively. The Na^+,K^+-ATPase isoforms probed were α_1, α_2, α_3, β_1, β_2 and β_3. To ensure maximal recovery of Na^+,K^+-ATPase enzymes, all immunoblots were conducted on crude muscle homogenates.

Muscle from each individual expressed gene transcripts and protein bands for each of the Na^+,K^+-ATPase α_1, α_2, α_3, β_1, β_2 and β_3 isoforms. These were also expressed in a skeletal muscle cell culture. Exercise immediately increased α_3 and β_2 mRNA expression (P<0.05), whereas upregulation of α_1 and α_2 (see the Figure) isoform mRNA (P<0.05) occurred at 24 h and 3 h post-exercise, respectively. Although significant differences between times were not found for β_1 and β_3 mRNA due to variable time-dependent responses, the peak post-exercise mRNA expression of these isoforms was elevated (P<0.05). In contrast to the dramatic mRNA upregulation, exercise had no significant effect on the crude muscle homogenate protein expression of any of the α_1-α_3 and β_1-β_3 isoforms.



*P<0.05 vs. rest, ‡P<0.05 vs. +24 h

In conclusion, the vastus lateralis muscle from healthy humans expresses each of the Na^+,K^+-ATPase α_1-α_3 and β_1-β_3 isoforms at both the transcription and protein levels. Further, only 6 min of exercise was sufficient to dramatically increase the mRNA expression of each of these six isoforms. In contrast, this exercise bout had no effect on isoform protein expression, suggesting this was an insufficient stimulus for Na^+,K^+-ATPase upregulation in muscle. These findings indicate different transcriptional and post-transcriptional Na^+,K^+-ATPase regulation with exercise in human skeletal muscle.

Increased Na^+K^+ATPase content is associated with improved potassium regulation during maximal exercise after sprint training in non-diabetics, but not in type 1 diabetes mellitus

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Sprint training attenuates the rise in plasma $[K^+]$ ($\Delta[K^+]$) during intense exercise (Harmer *et al.*, 2000) and increases Na^+K^+adenosine triphosphatase (Na^+K^+ATPase) content (McKenna *et al.*, 1993) in non-diabetics. In type 1 diabetes mellitus (T1D), Na^+K^+ATPase content has been reported to be higher than normal (Schmidt *et al.*, 1994). However, plasma $[K^+]$ may also be higher if subjects with T1D are hyperglycaemic (Shalwitz *et al.*, 1991). The effects of intense exercise and training on plasma $[K^+]$ regulation and Na^+K^+ATPase content in T1D have never been examined.

Eight subjects with T1D and seven non-diabetics (CON) undertook 7 weeks of sprint cycling training. Before training, subjects cycled to exhaustion at 130% $\dot{V}_{O_{2\,peak}}$. After training subjects cycled at the same workrate for the same duration. Subjects with T1D delayed insulin administration until after testing, which was conducted in the fasted state. Vastus lateralis biopsies obtained at rest were assayed for Na^+K^+ATPase content ($[^3H]$ouabain binding). Arterialised venous blood drawn during rest, exercise and recovery was analysed for plasma glucose, $[K^+]$, $[Na^+]$, catecholamines, insulin (IRI), and glucagon (IRG).

Na^+K^+ATPase content (T1D, 328±24; CON, 313±29 pmol•(g ww)$^{-1}$) and $\Delta[K^+]$ with a single bout of maximal exercise did not differ between groups (T1D 1.3±0.1; CON, 1.6±0.3 mmol•l^{-1}). Noradrenaline and the rise in plasma glucose were higher in T1D during exercise (P<0.05). In late recovery in T1D, plasma glucose (P<0.001), $[K^+]$, and IRG/IRI were higher, and plasma $[Na^+]$ lower than in CON (P<0.05). Training increased Na^+K^+ATPase content by 8.2±2.2% and reduced $\Delta[K^+]$ by 21±7% (P<0.05), with no difference between groups. These variables were correlated in CON (r = -0.65, P<0.05), but not T1D.

These findings demonstrate that acute regulation of plasma $[K^+]$ during a single bout of maximal exercise is similar in subjects with T1D who are relatively hypoinsulinaemic versus non-diabetics, however in late recovery, hyperglycaemia-induced hyperkalaemia may be anticipated. Sprint training enhanced plasma $[K^+]$ regulation, associated with increased Na^+K^+ATPase content in CON. Although K^+ regulation was also improved in T1D, the lack of correlation with Na^+K^+ATPase content suggests that other factors, e.g. altered hormonal conditions (higher noradrenaline), may play a significant role during intense exercise.

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Effect of exercise on intracellular insulin signalling in human skeletal muscle

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Exercise enhances skeletal muscle insulin action, and subsequently affects a number of insulin sensitive processes such as glucose uptake and glycogen synthesis. The effects of exercise on insulin action and glucose homeostasis have important implications for the maintenance of good health and in the treatment and prevention of type 2 diabetes. However, the underlying mechanism/s mediating the increase in skeletal muscle insulin action following exercise are equivocal. One hypothesis is that exercise or muscle contraction enhances intracellular insulin signalling events downstream of the insulin receptor. Recently we have demonstrated in mouse skeletal muscle, that prior exercise enhances insulin-stimulated insulin receptor substrate-2 (IRS-2) phosphorylation and associated phosphatidyl-inositol (PI) 3-kinase activity (Howlett *et al.*, 2002). However, no study has examined whether this exercise-mediated effect on IRS-2 signalling also occurs in human skeletal muscle. In light of this, the aim was to examine insulin signalling in human skeletal muscle in response to a hyperinsulinaemic euglycaemic clamp following an acute bout of exercise. Seven untrained males (24 ± 2 yr, 73 ± 3 kg, $\dot{V}_{O_{2\,peak}}$ = 3.63 ± 0.22 l.min^{-1}) were studied at rest and after 60 min of strenuous exercise (75 ± 4% $\dot{V}_{O_{2\,peak}}$). Immediately following rest or exercise, a 120 min hyperinsulinaemic (40 mU.m^{-2}) euglycaemic (5 mM) clamp was performed. Muscle biopsies were obtained at rest, post exercise, and 30 and 120 min of hyperinsulinaemia. Plasma insulin levels were similar during hyperinsulinaemia (Rest, 704 ± 34; Exercise, 691 ± 40 pmol.l^{-1}). Insulin-mediated glucose disposal rates were similar during the final 30 min of the clamp (Rest, 9.1 ± 1.1; Exercise, 8.3 ± 1.0 mg.kg^{-1}.min^{-1}). Insulin had no significant effects on IRS-1 and IRS-2 associated PI 3-kinase activity. Exercise, per se, tended to decrease IRS-1 (0.70 ± 0.13 fold) and IRS-2 (0.71 ± 0.10 fold) associated PI 3-kinase activity. Following exercise, insulin-stimulated IRS-2 associated PI 3-kinase activity tended to increase at 30 min (1.29 ± 0.11 fold) and was further enhanced at 120 min (2.83 ± 0.81 fold, $p<0.05$). In contrast, following exercise insulin-stimulated IRS-1 associated PI 3-kinase activity increased to a peak at 30 min (1.88 ± 0.40 fold, $p<0.05$), although remained elevated above basal at 120 min (1.67 ± 0.18 fold, $p<0.05$). Despite the effect of exercise on these proximal insulin signalling proteins, there was no significant effect of exercise on insulin-stimulated activation of downstream insulin signalling proteins, including phosphorylation of Akt (Ser473) and GSK3-β (Ser9). However, exercise did result in an increase in insulin stimulated phosphorylation of GSK3-α (Ser21). In conclusion, prior exercise increases insulin-stimulated IRS-2 signalling in human skeletal muscle. It appears that insulin-stimulated IRS-1 and IRS-2 signalling in human skeletal muscle may be differentially regulated by exercise.

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Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle

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We have previously shown that a single bout of endurance exercise increases GLUT-4 mRNA in human skeletal muscle (Kraniou *et al.*, 2000), implying an increased rate of transcription. It has also been demonstrated that myocyte enhancer factor 2 (MEF-2) binding activity is necessary for regulation of the GLUT-4 gene in skeletal muscle (Thai *et al.*, 1998). In the basal state, MEF-2 is believed to be inhibited by the class II histone deacetylases (HDACs), an association that is broken by phosphorylation of HDACs and their subsequent nuclear export. Association of MEF-2 with co-activators possessing histone acetylase (HAT) activity is thought to be mediated by the calcineurin/nuclear factor of activated T-cells (NFAT) pathway. Calcineurin dephosphorylates NFAT, resulting in its nuclear translocation where it recruits co-activators possessing HAT activity to MEF-2 allowing maximal MEF-2 DNA binding. While this is sufficient to initiate transcription, the rate of MEF-2 mediated transcription is increased by MEF-2 phosphorylation, with one putative kinase being p38 MAPK. In the present study, we sought to examine whether these various mechanisms may be involved in human skeletal muscle responses to exercise.

Seven healthy, untrained men (27 ± 3 yrs, 83 ± 4 kg, $\dot{V}O_{2\,peak}$ = 47 ± 2 ml•kg^{-1}•min^{-1}, mean ± SD) performed cycle ergometer exercise for 60 min at a power output eliciting 74 ± 2% $\dot{V}O_{2\,peak}$). Muscle samples were obtained from vastus lateralis immediately before and after exercise and quickly (~15 s) frozen in liquid nitrogen for later analysis. Total and nuclear proteins were isolated as described previously (McGee *et al.*, 2003) and quantified by immunoblotting and co-immunoprecipitation. Nuclear HDAC5 content was decreased 54% ($P<0.05$) following exercise, while there was no change in whole cell HDAC5 content. The association of HDAC5 with MEF-2 was reduced by 26% ($P<0.05$). Nuclear NFAT content was similar before and after exercise. Total p38 MAPK phosphorylation increased 4.8 fold ($P<0.05$), while nuclear p38 MAPK phosphorylation increased 1.8 fold ($P<0.05$), with no change in the abundance of either total or nuclear p38 MAPK proteins. Association of p38 MAPK protein with MEF-2 increased 2.7 fold ($P<0.05$) following exercise, while association of phosphorylated p38 MAPK with MEF-2 increased 1.75 fold ($P<0.05$). These results suggest that HDAC5 and p38 MAPK are involved in the regulation of MEF-2 in response to exercise in human skeletal muscle, while the calcineurin/NFAT pathway may be less important under these conditions.

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Effect of exercise on Ca^{2+}-sensitive protein kinases in human skeletal muscle

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There is evidence in rodents that PKC (Richter *et al.*, 1989; Chen *et al.*, 2002) and CaMKII (Tavi *et al.*, 2003) activities are higher in contracting skeletal muscle, and that these kinases may regulate skeletal muscle function, including metabolism, during exercise. To investigate this in humans, healthy men (*n*=8, 24 ±5 yr, 23 ±2 $kg \cdot m^{-2}$, $\dot{V}O_{2\ peak}$ = 51±6 $ml \cdot kg^{-1} \cdot min^{-1}$) performed cycle ergometer exercise for 40 min at 76±1 % $\dot{V}O_{2\ peak}$ with skeletal muscle samples taken at rest and after 5 and 40 min of exercise. PKC and CaMKII expression and activities were examined by immunoblotting and *in vitro* kinase assays. There were no differences in maximal ($+Ca^{2+}$/CaM) CaMKII activity during exercise compared with basal. Autonomous ($-Ca^{2+}$/CaM) CaMKII activity was 9 ±1% of maximal at rest, unchanged at 5 min, and increased to 17 ±1% (P<0.01) at 40 min. There were no differences in CaMKII expression (P>0.1). There were no changes in cPKC or PKCθ activities (P>0.1), however aPKC activity was ~70% higher (P<0.05) at 5 and 40 min and total PKC activity was slightly higher at 40 min in an enriched membrane fraction (P<0.05).

The activities of these kinases were also examined in response to maximal aerobic exercise. Healthy men (*n*=9, 25 ±5 yr, 24 ±2 $kg \cdot m^{-2}$, 52 ±9 $ml \cdot kg^{-1} \cdot min^{-1}$) performed cycle ergometer exercise for 10 min at 50 %$\dot{V}O_{2\ peak}$, after which the workload was increased to elicit 100 %$\dot{V}O_{2\ peak}$ with muscle samples taken at rest and at volitional fatigue. Autonomous CaMKII activity was increased by 74 ±17% (P<0.001) with no change in maximal CaMKII activity. There were no changes in total PKC, PKCδ, PKCθ, or aPKC activities.

These data demonstrate that CaMKII and aPKC are activated in contracting skeletal muscle, and thus may represent key signalling proteins potentially regulating skeletal muscle function and metabolism during exercise in humans.

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The effect of repeated bouts of level and downhill treadmill walking on plasma interleukin-6

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The increase in plasma interleukin-6 (IL-6) appears dependent on factors such as exercise intensity, duration, muscle mass recruited, and mode (concentric vs eccentric) (for review see Febbraio & Pederson, 2002). Particularly during the later stages of prolonged endurance exercise the increase in plasma IL-6 is most pronounced. The late appearance in the plasma following prolonged exercise suggests that a component of the rise in plasma IL-6 may be via damage to skeletal muscle. A single bout of eccentric exercise produces significant muscle damage. However, the same eccentric exercise bout performed some weeks afterward shows minimal damage to the active muscle (McHugh *et al.* 1999). Therefore, the aim of this experiment was to investigate the plasma IL-6 response with a single eccentric exercise bout, and 5 weeks later, with another identical "repeated" bout of eccentric exercise, to assess the contribution of skeletal muscle damage to the IL-6 response.

Fifteen inactive males volunteered for this study. Following ethical approval subjects were randomly assigned to a concentric exercise group (CON; n=7) or an eccentric exercise group (ECC; n=8). Subjects performed two bouts of walking exercise separated by 5 weeks (B1 & B2) on a motor driven treadmill at a constant speed (5 $km.hr^{-1}$) for 90 min. CON walked on the flat (0° decline) while ECC walked downhill (14° decline). Forearm venous samples were collected at regular intervals Pre-, during and post-exercise for determination of plasma IL-6 (R&D systems ELISA kit). Maximal voluntary isometric contraction (MVC) of the quadriceps muscle group, and delayed onset muscle soreness (DOMS) were determined at the same time points, except during exercise. A 2-way ANOVA for repeated measures and Student-Newman-Keuls *post hoc* test was used to assess significance with $p<0.05$.

MVC and DOMS showed no muscle damage in either B1 or B2 in CON. However, there was significant muscle damage in ECC post B1 only (not B2). Similarly, plasma IL-6 was elevated in ECC only (towards the end of B1), and peaked immediately post-exercise. All changes returned to baseline levels within 7 days.

In this study exercise intensity was not different between B1 & B2 for both CON and ECC. With no muscle damage or change in plasma IL-6 in B2, yet elevated plasma IL-6 in ECC in B1, it appears that exercise-induced muscle damage contributes almost exclusively to the increase in IL-6 reported following under these low intensity walking exercise conditions.

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Determinants of muscle buffer capacity

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Little is known about the stimulus required to increase muscle buffer capacity ($\beta_{in\text{-}vitro}$). It has been hypothesised that it is important that training is: (1) of high intensity; and (2) is performed under conditions of high skeletal muscle hydrogen ion (H^+) accumulation (Weston et al., 1996). We tested this first hypothesis by investigating the effects on $\beta_{in\text{-}vitro}$ of two training protocols of different intensity, but matched for total work. It has previously been shown that increasing the extracellular buffer concentration can reduce the skeletal muscle H^+ accumulation during high-intensity exercise (Costill et al., 1984). We therefore tested the second hypothesis by experimentally manipulating the extracellular buffer concentration during training.

For the first study, 18 untrained females (mean ± SD: age 19 ± 1 y, mass 59.8 ± 5.8 kg) were randomly assigned to high-intensity interval training (INT-5) or moderate intensity continuous (CON-5) training. Training was matched for total work and consisted of 6 - 10 × 2 min intervals (1 min rest) at 130 - 160% of lactate threshold (LT) (INT-5) or 20 - 35 min of continuous cycling at 85 - 95% of LT (CON-5), 3 × per week for 5 weeks. For the second study, 10 untrained females (mean ± SD: age 20 ± 3 y, mass 62.3 ± 10.0 kg) were also randomly assigned to one of two training groups, matched for total work. One group (BIC-8) ingested sodium bicarbonate ($NaHCO_3$, 0.4 $g \cdot kg^{-1}$) while the control group (INT-8) ingested a placebo (NaCl, 0.2 $g \cdot kg^{-1}$) prior to each training session. Training consisted of 6 - 12 × 2 min intervals (1 min rest) at 130 - 180% of LT, 3 × per week for eight weeks. Muscle biopsies (vastus lateralis) were taken at rest to determine muscle lactate ($[La^-]_m$), pH_m and $\beta_{in\text{-}vitro}$.

Training responses are summarised in the table. All training programs resulted in a significant improvement in $O_{2\,peak}$ and LT with no significant difference between groups. However, relative to CON-5, INT-5, INT-8 and BIC-8 had a significantly greater improvement in $\beta_{in\text{-}vitro}$. The pooled data revealed a significant negative correlation between initial $\beta_{in\text{-}vitro}$ and percent change with training ($r=0.58$; $P<0.05$).

Training	Peak O_2		LT		$\beta_{in\text{-}vitro}$	
	Pre	Post	Pre	Post	Pre	Post
CON-5	41.3 ± 7.3	45.6 ± 5.7*	137 ± 33	152 ± 29*	123 ± 32	125 ± 19
INT-5	42.8 ± 6.6	48.1 ± 7.4*	141 ± 27	149 ± 27*	126 ± 15	150 ± 19*
INT-8	40.7 ± 5.6	47.7 ± 6.1*	113 ± 18	130 ± 21*	140 ± 32	161 ± 19*
BIC-8	35.2 ± 7.1	43.0 ± 6.4*	109 ± 21	137 ± 20*	129 ± 32	156 ± 19*

* significantly different to pre-training ($p<0.05$)

Despite similar changes in aerobic fitness, INT-5 had a significantly greater increase in $\beta_{in\text{-}vitro}$ than CON-5. This suggests that it is the intensity of training, not the total work performed, that is the stimulus for change in $\beta_{in\text{-}vitro}$. We have also shown that ingesting $NaHCO_3$ and therefore altering the likely accumulation of H^+ during training, did not affect these adaptations.

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