

Effects of chronic intermittent hypoxia, acute and chronic
exercise on skeletal muscle Na^+ , K^+ ATPase, buffering
capacity and plasma electrolytes in well-trained athletes

Submitted by

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ABSTRACT

Endurance athletes may use hypoxic exposure, and high intensity interval training to improve subsequent endurance performance. Research on the physiological adaptation of athletes to these interventions has tended to focus on metabolic, haematological and respiratory measures. Consequently, relatively little is known, in well-trained athletes, about the effects of chronic intermittent hypoxia, acute and chronic exercise on skeletal muscle Na^+ , K^+ ATPase, buffering capacity and plasma electrolytes. Thus the effects of acute exercise and these interventions in well-trained athletes are the focus of this thesis.

Study 1-Part I

This study investigated whether hypoxic exposure increased muscle buffer capacity (β_m) and mechanical efficiency during exercise in male athletes. A control (CON, $n = 7$) and a live high:train low hypoxic group (LHTL, $n = 6$) trained at near sea level (600 m), with the LHTL group sleeping for 23 nights in simulated moderate altitude ($F_{\text{I}}\text{O}_2$ 15.48%, ~3000 m). Whole body oxygen consumption ($\dot{V}\text{O}_2$) was measured under normoxia before, during and after 23 nights of sleeping in hypoxia, during cycle ergometry comprising 4 x 4-min submaximal stages, 2-min at $5.6 \pm 0.4 \text{ W}\cdot\text{kg}^{-1}$, and 2-min 'all-out' to determine total work and $\dot{V}\text{O}_{2\text{peak}}$. A vastus lateralis muscle biopsy was taken at rest and after a standardised 2-min submaximal ($5.6 \pm 0.4 \text{ W}\cdot\text{kg}^{-1}$) bout, before and after LHTL, and analysed for β_m and metabolites. After LHTL, β_m was increased (18%, $P < 0.05$). Although work was maintained, $\dot{V}\text{O}_{2\text{peak}}$ fell after LHTL (7%, $P < 0.05$). Submaximal $\dot{V}\text{O}_2$ was reduced (4.4%, $P < 0.05$) and efficiency improved (0.8%, $P < 0.05$) after LHTL, probably because of a shift in fuel utilisation. Hence, hypoxic exposure, per se, increases muscle buffer capacity. Further, reduced $\dot{V}\text{O}_2$ during

normoxic exercise after LHTL suggests that improved exercise efficiency is a fundamental adaptation to LHTL.

Study 1-Part II

Athletes commonly attempt to enhance performance by training in normoxia but sleeping in hypoxia (live high and train low, LHTL). However, chronic hypoxia reduces muscle Na^+, K^+ ATPase content, whilst fatiguing contractions reduce Na^+, K^+ ATPase activity, which each may impair performance. This study examined whether LHTL and intense exercise would decrease muscle Na^+, K^+ ATPase activity; whether these effects would be additive and sufficient to impair performance or plasma K^+ regulation. Subjects and experimental conditions were as per Study 1-Part I. A standardised incremental exercise test was conducted before and after LHTL. A vastus lateralis muscle biopsy was taken at rest and after exercise, before and following LHTL or CON and analysed for maximal Na^+, K^+ ATPase activity (K^+ -stimulated 3-*O*-methylfluorescein phosphatase, 3-*O*-MFPase); and Na^+, K^+ ATPase content ($[^3\text{H}]$ -ouabain binding sites). Na^+, K^+ ATPase activity was decreased by $2.9 \pm 2.6\%$ in LHTL ($P < 0.05$) and was depressed immediately after exercise ($P < 0.05$), similarly in CON and LHTL (-13.0 ± 3.2 ; and $-11.8 \pm 1.5\%$, respectively). Plasma $[\text{K}^+]$ during exercise was unchanged by LHTL; muscle Na^+, K^+ ATPase content was unchanged with LHTL or exercise. $\text{VO}_{2\text{peak}}$ was reduced in LHTL ($P < 0.05$) but not in CON, whilst exercise work was unchanged in either group. Thus LHTL had a minor effect on, and incremental exercise reduced Na^+, K^+ ATPase activity. However, the small LHTL-induced depression of Na^+, K^+ ATPase activity was insufficient to adversely affect either K^+ regulation, or total work performed.

Study 2

This study contrasted the effects of consecutive nightly (LHTLc) versus intermittent live high train low (LHTLi) hypoxia and of acute sprint exercise on muscle Na^+ , K^+ ATPase, plasma ions and acid-base. Thirty-three athletes were assigned to Control (CON, n=11), 20-nights (n) LHTLc (n=12) or 20-n LHTLi (4 x 5-n LHTL interspersed with 2-n CON, n=10) groups. LHTLc and LHTLi slept at simulated altitude of 2650 m, ($F_{\text{I}}\text{O}_2$ 0.1627) and lived and trained by day under normoxic conditions; CON lived, trained and slept in normoxia. Standardised sprint exercise was conducted before (Pre), during (d5) and after (Post) intervention, with a quadriceps muscle biopsy taken at rest and immediately after exercise on each day. Muscle was analysed for maximal Na^+ , K^+ ATPase activity and content. Muscle Na^+ , K^+ ATPase activity was reduced ($P<0.05$) after exercise (CON -12 ± 4 , LHTLc -13 ± 5 , LHTLi -12 ± 2 %), whereas muscle Na^+ , K^+ ATPase content was unchanged. Muscle Na^+ , K^+ ATPase activity was reduced (-2.2% , $P<0.05$) after 5-n in both LHTL groups, remained low after 20-n LHTLc, but this effect was reversed after 20-n LHTLi only. Plasma $[\text{Cl}^-]$ increased (LHTLc 1.5 ± 1.8 ; LHTLi $1.9\pm 1.5\%$, $P<0.05$) and the plasma strong ion difference decreased (LHTLc -4.3 ± 8.5 ; LHTLi $-7.0\pm 6.3\%$, $P<0.05$) with LHTL from Pre-d5, with no further change at Post or in CON at any day. In conclusion, LHTL reduced muscle maximal Na^+ , K^+ ATPase activity, whilst the inclusion of additional interspersed normoxic nights reversed this effect despite the same hypoxic exposure. However, the decline in maximal Na^+ , K^+ ATPase activity with acute sprint exercise was not affected by LHTL.

Study 3

Athletes commonly use short periods of high intensity training (HIT) to improve performance and the Na^+ , K^+ ATPase enzyme in skeletal muscle plays an important role in performance. The effects of acute high-intensity interval exercise and HIT on muscle Na^+ , K^+ ATPase maximal activity and content were investigated. Twelve endurance-

trained athletes were tested at 0-wks (Baseline) and 4-wks (Pre) and after HIT (Post). HIT comprised seven sessions over 3-wks, of high-intensity interval cycling exercise, comprising 8 x 5 min at 80% Peak Power Output. Vastus lateralis muscle was biopsied at rest (Baseline) and both rest and immediately post-exercise during the first (Pre-Train) and seventh (Post-Train) HIT session. Muscle was analysed for Na⁺,K⁺ATPase maximal activity and content. Acute high intensity interval exercise decreased maximal Na⁺,K⁺ATPase activity by 12.7±5.1 % ($P<0.05$). HIT increased maximal Na⁺,K⁺ATPase activity by 5.5±2.9% ($P<0.05$) but did not alter Na⁺,K⁺ATPase content. After HIT, the decline in maximal activity with exercise persisted and a higher end-exercise activity was sustained, which may be important in delaying fatigue. Thus, the Na⁺,K⁺ATPase acute response to interval exercise persisted in well-trained athletes after HIT.

Conclusions.

This thesis examined the effects of acute exercise, LHTL hypoxic exposure and HIT on muscle Na⁺,K⁺ATPase in well-trained athletes. The effects of LHTL on muscle metabolism and mechanical efficiency were also investigated. Incremental, sprint and intense interval exercise each depressed maximal Na⁺,K⁺ATPase activity by a similar magnitude, with unaltered Na⁺,K⁺ATPase content. LHTL increased muscle buffering capacity and mechanical efficiency without change in muscle metabolism, Na⁺,K⁺ATPase content or plasma K⁺ regulation. Conversely, LHTL caused a small depression in maximal Na⁺,K⁺ATPase activity, which was reversed with short interspersed periods of normoxia. These findings are important since they demonstrate that a small reduction in muscle maximal Na⁺,K⁺ATPase activity did not affect performance in exercising humans. This may explain why athletes can use LHTL without deterioration in performance. HIT in well-trained athletes increased peak power output, and maximal Na⁺,K⁺ATPase activity. Thus Both LHTL and HIT in already

well-trained athletes caused subtle adaptations in muscle Na^+, K^+ ATPase, showing that even after years of hard training, muscle Na^+, K^+ ATPase is responsive to these interventions.

DECLARATION

“I, Robert J. Aughey, declare that the PhD thesis entitled Effects of chronic intermittent hypoxia, acute and chronic exercise on skeletal muscle Na^+, K^+ ATPase, buffering capacity and plasma electrolytes in well-trained athletes is no more than 100,000 words in length, exclusive of tables, figures, appendices, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work. However, due to the complexity and magnitude of the studies undertaken, considerable collaboration was involved in each of the three studies. Associate Professor Michael J. McKenna, Professor Allan G. Hahn, Dr Christopher J. Gore, and Professor John A. Hawley helped with planning of the studies and conducting some exercise testing. Associate Professor Michael J. McKenna, and Associate Professor Michael F. Carey helped with muscle analyses. Qualified medical personnel performed all muscle biopsies. Mr. Aaron Petersen and Dr. Craig Goodman assisted with some muscle [^3H]-ouabain binding analysis. Dr. Sally Clark assisted with Study 2 muscle β_m analysis”.

Robert J.A. Aughey

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ABBREVIATIONS

SUBSCRIPTS

<i>i</i>	Intracellular
<i>e</i>	Extracellular
E_m	Muscle membrane potential

ELECTROLYTES

		Units
K^+	Potassium ion	mmol.l ⁻¹
Na^+	Sodium ion	mmol.l ⁻¹
Ca^{2+}	Calcium ion	mmol.l ⁻¹
Mg^{2+}	Magnesium ion	mmol.l ⁻¹
H^+	Hydrogen ion	nmol.l ⁻¹
Lac^-	Lactate anion	mmol.l ⁻¹
HCO_3^-	Bicarbonate anion	mmol.l ⁻¹
[ion]	Ion concentration	
$\Delta[K^+]$	Change in $[K^+]$	mmol.l ⁻¹
$\Delta[K^+].work^{-1}$	Change in $[K^+]$ relative to work performed	nmol.l ⁻¹ .J ⁻¹

CARDOVASCULAR / BLOOD GASES

		Units
PCO_2	Partial pressure of carbon dioxide	mmHg
PO_2	Partial pressure of oxygen	mmHg
HR	Heart rate	beats.min ⁻¹
$\dot{V}O_2$	Oxygen consumption	l.min ⁻¹
$\dot{V}O_{2peak}$	Peak absolute oxygen consumption	l.min ⁻¹
$\dot{V}O_{2peak}$	Peak relative oxygen consumption	ml.kg ⁻¹ .min ⁻¹
$\dot{V}CO_2$	Carbon dioxide output	l.min ⁻¹

V_E	Pulmonary ventilation	$l.min^{-1}$
RER	respiratory exchange ratio	
MUSCLE		
$Na^+,K^+ATPase$	Sodium-Potassium Adenosine Triphosphatase	
$Na^+,K^+ -pump$	Sodium-Potassium Adenosine Triphosphatase	
3- <i>O</i> -MFP	3- <i>O</i> -methylflourescein phosphate	
3- <i>O</i> -MFPase	3- <i>O</i> -methylflourescein phosphatase	
3- <i>O</i> -MF	3- <i>O</i> -methylflourescein	
ATP	Adenosine 5' triphosphate	
ADP	Adenosine diphosphate	
IMP	Inosine monophosphate	
PCr	Phosphocreatine	
Cr	Creatine	
WORK & POWER		
WR	Work rate	
W	Absolute power	
$W.kg^{-1}$	Relative power	

PUBLICATIONS

The following publications are presented in support of this thesis:

Publications arising directly from this thesis

1. Gore, C. J., Hahn, A. G., **Aughey, R. J.**, Martin, D. T., Ashenden, M. J., Clark, S. A., Garnham, A. P., Roberts, A. D., Slater, G. J. & McKenna, M. J. (2001). Live high:train low increases muscle buffer capacity and submaximal cycling efficiency. *Acta Physiol Scand* 173, 275-286. (Study 1, Part 1; Chapter 3)
2. **Aughey, R. J.**, Gore, C. J., Hahn, A. G., Garnham, A. P., Clark, S. A., Petersen, A. C., Roberts, A. D. & McKenna, M. J. (2004). Chronic intermittent hypoxia and incremental cycling exercise independently depress muscle in-vitro maximal Na^+ , K^+ ATPase activity in well-trained athletes. *J Appl Physiol* 98, 186-192.. (Study 1, Part 2; Chapter 4)
3. **Aughey, R. J.**, Clark, S. A., Gore, C. J., Townsend, N. E., Hahn, A. G., Kinsman, T. A., Goodman, C., Chow, C. M., Martin, D. T., Hawley, J. A. & McKenna, M. J. Effects of acute sprint exercise and consecutive versus intermittent nights of hypoxia on skeletal muscle Na^+ , K^+ ATPase activity, plasma ions and acid-base. (*Submitted to Am J Physiol Regul Integr Comp Physiol, currently under review*). (Chapter 5)
4. **Aughey, R. J.**, Murphy, K. T., Clark, S. A., Hawley, J. A., Garnham, A. P., Hahn, A. G., Gore, C. J., Snow, R. J., Cameron-Smith, D., Christie, J. J. & McKenna, M. J. Muscle Na^+ , K^+ ATPase isoform, content and activity responses to interval exercise and training in well-trained athletes. (*Submitted to J Physiol (Lond) currently under review*) (Chapter 6).

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