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_IO_2 15.48%, ~3000 m). Whole body oxygen consumption ($\dot{V}O_2$) 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 ± 0.4 $W.kg^{-1}$, and 2-min 'all-out' to determine total work and $\dot{V}O_{2peak}$. A vastus lateralis muscle biopsy was taken at rest and after a standardised 2-min submaximal (5.6 ± 0.4 $W.kg^{-1}$) 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, $\dot{V}O_{2peak}$ fell after LHTL (7%, $P<0.05$). Submaximal $\dot{V}O_2$ 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 $\dot{V}O_2$ 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}H]-ouabain binding sites). Na^+,K^+ATPase activity was decreased by 2.9±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±1.5%, respectively). Plasma [K^+] during exercise was unchanged by LHTL; muscle Na^+,K^+ATPase content was unchanged with LHTL or exercise. $\dot{V}O_{2peak}$ 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_IO_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 [Cl^-] increased (LHTLc 1.5±1.8; LHTLi 1.9±1.5%, P<0.05) and the plasma strong ion difference decreased (LHTLc –4.3±8.5; LHTLi –7.0±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 [^{3}H]-ouabain binding analysis. Dr. Sally Clark assisted with Study 2 muscle β_m analysis".

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ABBREVIATIONS

SUBSCRIPTS		
i	Intracellular	
e	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-*O*-MFP	3-*O*-methylflourescein phosphate
3-*O*-MFPase	3-*O*-methylflourescein phosphatase
3-*O*-MF	3-*O*-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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CHAPTER 1. INTRODUCTION

In skeletal muscle, the Na^+,K^+ATPase enzyme is mainly located in the sarcolemma and t-tubular system, is critical in maintaining trans-sarcolemmal [Na^+] and [K^+] gradients and membrane excitability, and thus has been linked with fatigue (Fowles *et al.*, 2002b; Fraser *et al.*, 2002; Clausen, 2003). The importance of Na^+,K^+ATPase for muscle contractility is emphasised when Na^+,K^+ATPase activity is inhibited. Incubation of rat muscle in ouabain, a specific Na^+,K^+ATPase inhibitor, or low [Na^+] and high [K^+] buffers increased the Na^+ / K^+ leak to pump ratio, depressed muscle tetanic force, accelerated muscle fatigue, and reduced recovery rate and M-wave area (Clausen, 2003). Under fatiguing conditions, skeletal muscle intracellular [Na^+] (Sjøgaard *et al.*, 1985; Juel, 1986; Sejersted & Sjøgaard, 2000) and extracellular [K^+] (Sjøgaard *et al.*, 1985; Juel, 1986; Hallén, 1996; Green *et al.*, 2000e; Sejersted & Sjøgaard, 2000) can double causing inactivation of voltage-dependent Na^+ channels (Ruff, 1999), and reducing the muscle membrane potential (Sjøgaard *et al.*, 1985; Sejersted & Sjøgaard, 2000). The skeletal muscle Na^+,K^+ATPase counteracts the [Na^+] and [K^+] changes with fatiguing exercise, although it appears that exercise may depress maximal Na^+,K^+ATPase activity (Fowles *et al.*, 2002b; Fraser *et al.*, 2002), and thus be important in muscle fatigue. Little is known about the effect of acute intense exercise on Na^+,K^+ATPase function or content, especially in well-trained endurance athletes.

In addition to acute exercise, hypoxia appears to be a depressor of Na^+,K^+ATPase in human skeletal muscle (Green et al., 1999b; Green et al., 2000a; Sandiford et al., 2004), and alters acid-base balance (Mizuno et al., 1990; Saltin et al., 1995a) Paradoxically, endurance athletes commonly use hypoxic exposure through simulated altitude exposure or living high:training low (LHTL) to improve subsequent exercise

performance. Endurance athletes also typically include short periods of high-intensity training in their normal training regimes. The effects of acute intense exercise, LHTL, and short periods of high-intensity training on muscle Na^+,K^+ATPase in well-trained athletes are unknown. This thesis will refer primarily to adaptation to chronic hypoxia, or altitude acclimatisation, unless otherwise stated. Additionally, since chronic Na^+,K^+ATPase activity regulates plasma $[K^+]$ and hypoxia also alters acid-base balance, the effect of LHTL on plasma ion, and plasma and muscle during is also unknown. Therefore, this thesis examined the effects of acute intense exercise, LHTL and HIT on muscle Na^+,K^+ATPase and plasma ions and acid-base in well-trained athletes.

CHAPTER 2. REVIEW OF LITERATURE

2.1 Introduction

This thesis investigated the effects of three types of acute exercise, simulated altitude and high intensity training, on skeletal muscle Na^+,K^+ATPase, buffering capacity and plasma electrolytes in well-trained endurance athletes. It is important to first set the scene for this review by briefly discussing the type of training and competition that these atheletes regularly engage in. The nature of this training and competition dictates that these athletes have already reached a high level of physiological adaptation, and therefore may be both somewhat protected from fatigue, and offer blunted responses to the interventions of simulated altitude exposure or high intensity training. The nature of fatigue and physiological change to an intervention must therefore be interpreted within this framework, with small physiological changes potentially very important to atheletes and is therefore comprehensively discussed in the following review.

2.2 Performance of elite endurance cyclists

Elite endurance cyclists can cover up to 35,000 km annually during training and competition, and regularly compete in events of ~ 200 km or more in length (Lucía et al., 2001). Cycling is a team sport, and involves a variety of disciplines, including both short and long time-trial performance, flat and mountainous road stages and multi-day events of up to three weeks duration (Padilla et al., 2000; Padilla et al., 2001). Therefore cycling teams consist of specialists in each of these disciplines, each specialist with specific physiological characteristics. Short time-trials are typically 6-7 km in length, and short time-trial specialists produce high absolute maximal power outputs, and spend the majority (> 80%) of competition at a high intensity, at or above lactate threshold (Fig 2.1, Padilla et al., 2000). Long time-trials can be up to 60 km in length, with long time-trial specialists also producing high absolute maximal power outputs, but only

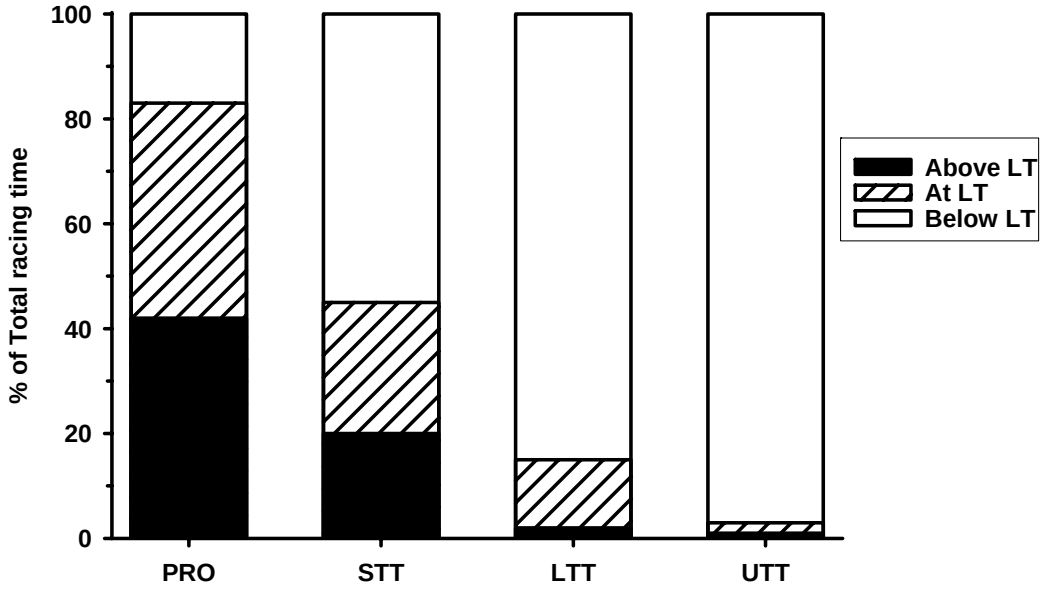
spending ~ 20% of competition at high exercise intensities (Padilla et al., 2000). Flat terrain races are typically ~200 km in length, with large periods of time spent at low intensity with flat terrain specialists producing high absolute power outputs, and a corresponding high absolute oxidative capacity (Lucía et al., 2001). Mountain stages involve up to 200 km of racing and sometimes up to five or six mountain passes. Mountain stage specialists have a high relative power output and oxidative capacity (Padilla et al., 1999; Lucía et al., 2000; Lucía et al., 2001; Padilla et al., 2001). Compared to cyclists with a lower training status, elite cyclists have reached a high level of physiological adaptation, with a higher oxidative capacity, develop higher peak and sustained power outputs (Mujika & Padilla, 2001), and are more efficient (Coyle et al., 1991; Lucía et al., 2002). Elite cyclists do, however, strive for further physiological and performance enhancement, with further increases of even 1.5 % important for improved sporting performance (Hopkins et al., 1999). Two interventions used by elite cyclists to further enhance performance include altitude / hypoxic exposure and replacing a portion of their training volume with high intensity training (HIT).

2.3 Interventions used to enhance endurance performance

2.3.1 Altitude training or hypoxic exposure

Altitude training or chronic hypoxic exposure has been used for many decades by a variety of athletes in order to elicit positive performance gains. Whilst anecdotal evidence points to the success of these training regimes, scientific support has been sparse. Many studies have lacked a suitable control group performing matched training at or near sea-level, making it difficult to determine if any changes reported were from a training effect, or from hypoxia per se. Altitude training protocols have varied enormously, with altitude itself (real or simulated), time at altitude, rate of ascent and descent, activities undertaken under hypoxic conditions and training status of subjects differing between studies.

Figure 2.1 Percentage of total racing time below, at or above lactate threshold spent by Professional cyclists for very short (Prologue, PRO), short (STT), long (LTT) or uphill (UTT) time-trials adapted from (Padilla *et al.*, 2000).



2.3.1.1 Traditional approach

Traditionally, athletes lived and trained at altitude when attempting to improve subsequent sea-level performance (Rusko, 1996b). Despite many years of scientific investigation, the performance enhancing properties of altitude remain contentious (Cerretelli & Hoppeler, 1996; Rusko, 1996b; Saltin, 1996; Wolski et al., 1996; Bailey & Davies, 1997; Böning, 1997; Fulco et al., 2000).

2.3.1.2 Live high: train low

In the 1990's the concept of "Living High: Training Low' (LHTL) gained support as a method of acquiring the perceived benefits of altitude or hypoxic exposure without the inherent problems (Levine & Stray-Gundersen, 1997). It was postulated that training intensity would not be compromised when training at or near sea level, but the hypoxic exposure from living high would allow positive haematological changes to occur (Levine *et al.*, 1991).

The LHTL practice typically involves training at or very near sea-level, and 'living' at a moderate altitude between 2100 and 3000 m. The initial study involved college level runners living at 2500 m, and training at 1250 m (Levine & Stray-Gundersen, 1997). The sea-level 5000 m run times of these subjects improved after LHTL. In follow-up studies by the same group, similar results were obtained in elite level runners (Stray Gundersen *et al.*, 1998; Stray-Gundersen *et al.*, 2001).

There are very few countries in the world that LHTL can be undertaken using natural geography. This problem was first overcome by a group of Finnish researchers who constructed a facility at sea level that allowed athletes to sleep and rest under conditions of simulated altitude (Rusko, 1996b). A small 'altitude house' has been in operation in Australia since 1997, and a series of studies have been conducted using well-trained athletes (Hahn *et al.*, 2001).

The performance enhancing effects of LHTL are now well-established, and are summarised in Table 2.1. At first glance these reported performance enhancements with LHTL seem small. However, an increase of even 1.5% in already elite atheletes has a large practical importance for success in sporting competition (Hopkins *et al.*, 1999).

Table 2.1 Effects of LHTL on athletic performance.

Athletes	Ref	Sex	h.day^{-1} exposure	Altitude (m)	Days	Performance task	Change after LHTL (%)
Road cyclists	1	F	8-11	2650	12	4-min 'all-out' ergometer test	2.3
Kayakers	1	M/F	8-11	2650	11	4-min 'all-out' ergometer test	2.4
Elite runners	1	M	8-11	2650	3 x 5 each separated by 2 nights recovery	1500 m time-trial	1.1
College runners	2	M/F	20-22*	2500-2700	30	5000 m time-trial	2.5
Elite runners	3	M/F	20-22*	2500-2700	30	3000 m time-trial	2.5
Elite runners	4	M	9-12	2650	4 x 5 each separated by 2 nights recovery	Submaximal running at 14, 16 and 18 km.h^{-1}	3.3
							Mean Δ: 2.4

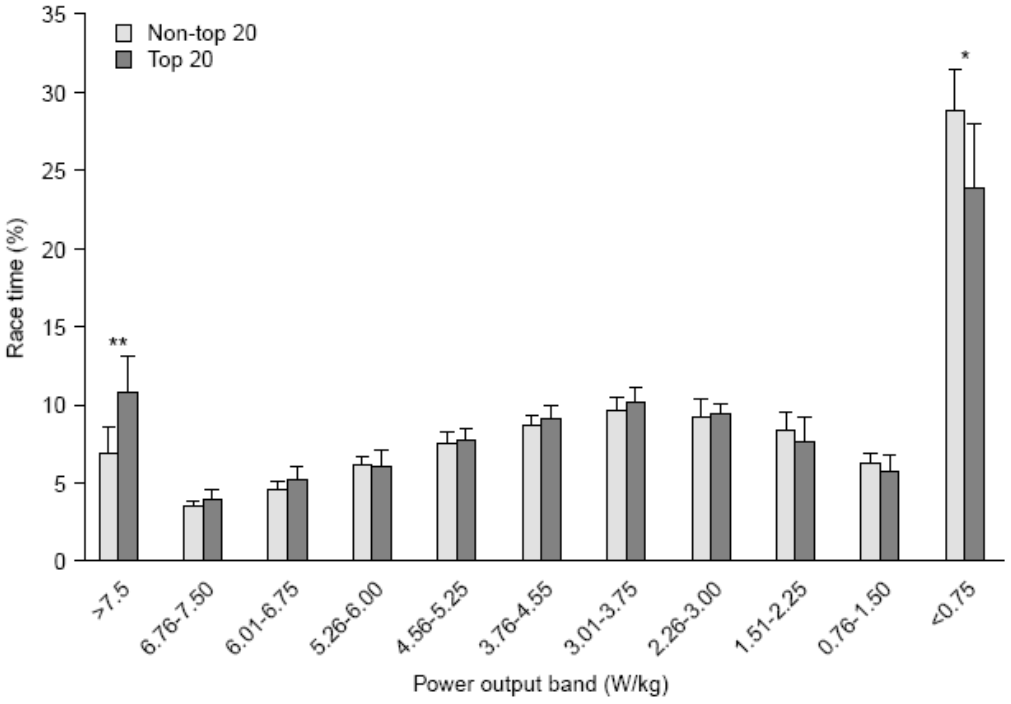
References 1: Hahn et al., 2001; 2: Levine & Stray-Gundersen, 1997; 3: Stray-Gundersen et al., 2001; *cited in (Levine, 2002; Levine & Stray-Gundersen, 2005) and 4: Saunders et al., 2004.

2.3.2 High intensity interval training (HIT)

The physiological demands of elite cycling performance are such that cyclists must produce high power outputs intermittently throughout competition (Martin et al., 2001). More successful elite female cyclists monitored during the 1999 World Cup competition spent a greater amount of time at both high (>7.5 W.kg^{-1}) and low workrates <0.75

W.kg^{-1}) than their less successful colleagues(Fig. 2.2, Martin et al., 2001). Coaches and athletes attempt to mimic these competition demands in training, achieved most effectively through high intensity interval training.

Figure 2.2 Mean power output distribution during the 1999 World Cup rounds 1 and 2. Data are divided into 0.75 W.kg^{-1} power bands, expressed as a percentage of total race time (mean ±SD).



Data were collected from Australian National Team female road cyclists, divided into top 20 placed riders (n = 5) and non–top 20 placed riders (n = 7). * indicates difference between top 20 and non–top 20 ($p < 0.05$); ** indicates difference between top 20 and non–top 20 ($p < 0.01$) (Martin *et al.*, 2001).

High intensity interval training (HIT), or transition training, consists of a number of high-intensity exercise bouts alternated with short rest periods at a slower pace (Hawley *et al.*, 1997; Laursen & Jenkins, 2002). HIT aims to expose the athlete to short periods of high-intensity exercise and also minimise fatigue, thus providing an effective matching of training to racing demands (Hawley *et al.*, 1997).

The effects of HIT on cycling performance of already well-trained athletes have been well documented, and are summarised in Table 2.3 below.

Table 2.2 Effects of HIT on athletic performance.

Athletes	Ref	HIT	Performance task	Change after HIT (%)
Road cyclists	1	6 sessions of 6-8 repetitions of 5-min at ~80% $\dot{V}O_{2peak}$ over three weeks	40_{TT}	3.5
			PPO	4.1
			TF_{150}	16.7
Road cyclists	2	6 sessions of 6-8 repetitions of 5-min at ~80% $\dot{V}O_{2peak}$ over three weeks	40_{TT}	2.3
			PPO	4.7
Road cyclists	3	6 sessions of 6-8 repetitions of 5-min at ~80% $\dot{V}O_{2peak}$ over three weeks	40_{TT}	2.1
			PPO	3.2
			TF_{150}	18.2
Road cyclists	4	6 sessions of either 12 x 30 s, 12 x 60 s, 12 x 2-min, 8 x 4-min or 4 x 8-min cycling at either 175, 100, 90, 85 or 80% $\dot{V}O_{2peak}$	40_{TT}	~2.5
			PPO	
Road cyclists	5	Four sessions (20 x 60 s at PPO, 120 s recovery)	PPO	4.3
Elite runners	6	4 wks of 2 session per week	3000 m TT	3.9

40_{TT} = simulated 40 km time-trial; PPO = peak power output in an incremental exercise test to exhaustion; TF_{150} = time to fatigue cycling at a workrate coinciding with 150% $\dot{V}O_{2peak}$; 3000 m TT = 3000 m running time-trial.References 1: (Lindsay *et al.*, 1996); 2: (Westgarth-Taylor *et al.*, 1997); 3: (Weston *et al.*, 1997); 4: (Stepto *et al.*, 1999); 5: (Laursen *et al.*, 2002); 6: (Smith *et al.*, 1999).

2.4 Physiological responses in well-trained athletes: Metabolic, haematologic, VO_2 and acid-base measures

Investigation of the mechanism (s) responsible for the performance enhancing effects of altitude, hypoxic, or live high:train low (LHTL) interventions and high-intensity interval

training (HIT) have traditionally focused on metabolic, cardiovascular and respiratory changes.

2.4.1 Physiological response and adaptation to hypoxic or LHTL interventions

When examining the effects of hypoxic or LHTL exposure, it is important to delineate between an acute effect, and adaptation to the hypoxic stimulus. The following sections will review metabolic, cardiovascular and respiratory responses to acute hypoxia, and adaptation or acclimatisation to that stimulus.

2.4.1.1 Metabolic response and adaptation to hypoxia or LHTL

2.4.1.1.1 Muscle ATP and PCr after hypoxia or LHTL

There does not appear to be an effect of acute hypoxic or altitude exposure on muscle adenosine 5' triphosphate (ATP) or Phosphocreatine (PCr) contents in resting muscle, or their utilisation during exercise. Two studies both found no change in muscle ATP or PCr stores after 10-min exposure to a simulated altitude of ~ 3400 m (Linnarsson *et al.*, 1974) or 4000 m (Knuttgen & Saltin, 1973). Similarly, prolonged exposure to hypoxia during a 40-d progressive sojourn to a simulated altitude of 8848 m also resulted in no change in muscle ATP or PCr contents (Green, 1992b). Acute exposure to hypoxia had no reported effects on either muscle ATP or PCr hydrolysis during the early stages of exercise (Haseler *et al.*, 1999; Hogan *et al.*, 1999).

2.4.1.1.2 Muscle glycogen storage and cabohydrate utilisation during exercise after hypoxia or LHTL

Muscle glycogen content in resting muscle appears to be unaffected by hypoxic exposure, but the effects on glycogen utilisation remain equivocal. Muscle glycogen content was unchanged after acute exposure of ~10-min to 3400 m (Linnarsson *et al.*, 1974) or 4000 m (Knuttgen & Saltin, 1973). Acute hypoxia caused an increased reliance on carbohydrate use during exercise, with an increased muscle glucose uptake (Katz & Sahlin, 1987; Brooks *et al.*, 1991). There was an associated decreased reliance on fat as

a substrate after acclimatisation to chronic hypoxia at 4300 m (Roberts *et al.*, 1996a). However, the effects of hypoxia on muscle glycogen utilisation remain equivocal. Glycogen utilisation during exercise at 85% of $\dot{V}O_{2peak}$ after a progressive increase to an altitude of approximately 8000 m was unchanged from that in normoxia (Young *et al.*, 1992). Glycogen utilisation during exercise at the same absolute workrate (50% $\dot{V}O_{2peak}$) was unchanged in one study using acute hypoxia (Linnarsson *et al.*, 1974), but 30-60% lower in another after gradual exposure to chronic hypoxia at ~8000 m (Green, 1992a).

The response of lactate after altitude exposure is, however, of interest with a so-called 'lactate-paradox' reported after altitude exposure in humans. The lactate paradox is of interest as it is assumed that increased lactate production should be related to a lower oxygen availability at altitude. However, at a given workload high altitude natives, acclimatized to high altitude conditions had a lower blood lactate concentration than lowlanders (Hochachka, 1988), and even after 6 weeks of 'de-acclimatisation' at sea-level were unable to match the lowlanders for lactate formation. The 'lactate paradox' was explained as being the result of two mechanisms: (1) a decrease in the maximum substrate flux through oxidative phosphorylation, due to a decrease in $\dot{V}O_{2peak}$ in hypoxia; and (2) alterations in the metabolic control of glycogenolysis and glycolysis at the cellular level, resulting from a reduction in adrenergic drive during acclimatisation (Kayser, 1996). More recent investigation has cast some doubt on the second suggested mechanism, as adrenergic drive is not lower, and in fact increases with both high altitude exposure (Hansen & Sander, 2003) and short periods of hypoxia (Xie *et al.*, 2001; Tamisier *et al.*, 2005). Importantly, the effects of LHTL on muscle metabolism are yet to be investigated, and were therefore examined in Chapter 3 of this thesis.

2.4.1.1.3 Muscle enzyme activities after hypoxia or LHTL

There is some uncertainty as to the effect of altitude exposure on skeletal muscle enzyme activities, but the effect seems to be independent of hypoxic stimulus. The activity of key aerobic regulatory enzymes succinate dehydrogenase (SDH) and 3-hydroxl-Co-A dehydrogenase (HAD) were unchanged after 40 days of exposure to a simulated altitude that progressively reached approximately 8000 m (Green *et al.*, 1989). In contrast, SDH and HAD activities were between 10-20% higher after only two weeks exposure to 2000 m (Saltin *et al.*, 1995a). Further adding to the uncertainty, SDH and HAD activities were found to be reduced by ~50% after 5 weeks exposure to at least 5200 m (Boutellier *et al.*, 1983), or ~10% after 6 - 8 weeks at 5000 – 8600 m (Howald *et al.*, 1990). Care must be taken when interpreting these results, however, as the study by Green et al (1989) was performed in a hypobaric chamber, with associated reductions in physical activity, conditions obviously quite different from those experienced by participants during high altitude climbing expeditions.

The effect of acute or chronic exposure to hypoxia on the activity of the glycolytic enzymes hexokinase (HK), phosphofructokinase (PFK), glyceraldehyde phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) is similarly uncertain. In one study, there were no reported changes in HK, PFK, GAPDH or LDH activities after a climbing expedition to Mt Everest (Howald et al., 1990). Conversely, PFK activity was decreased, while HK increased after 3 weeks residence at 4300 m, (Green *et al.*, 1989). In an elegant study, using one-legged training, PFK activity increased by a similar magnitude under normobaric hypoxic conditions (32%) compared to normoxic training (23%) (Terrados, 1990).

2.4.1.2 Haematologic responses and adaptation to hypoxia or LHTL

An increased synthesis and concentration of circulating erythropoietin are the first steps in red blood cell production (Ratcliffe et al., 1996). A consistent finding after acute

hypoxic or LHTL exposure is an increased serum erythropoietin concentration ([EPO]) (Ashenden et al., 2001). The increase in [EPO] has no ergogenic effects unless it concomitantly increases red blood cell production and/or red cell mass (RCM) (Ashenden et al., 1999b). The effects of adaptation to hypoxia or LHTL on red cell mass remain equivocal. Several studies report an increase in red cell mass after 3-4 weeks of moderate 2500 m altitude exposure (Levine *et al.*, 1991; Harper *et al.*, 1995; Laitinen *et al.*, 1995; Levine & Stray-Gundersen, 1997), and each of these studies used a crticised subjective supravital staining technique for the measurement of reticulocytes. However, when the carbon-monoxide (CO) re-breathing method was applied, other researchers were able to detect similar increases (Friedmann *et al.*, 1999; Heinicke *et al.*, 2003; Friedmann *et al.*, 2005; Heinicke *et al.*, 2005). Whilst other studies contrasted these results, reporting no change or a decrease in red cell mass after between 12 days and 4 weeks of moderate altitude 1760 – 2650 m or LHTL exposure, also quantifying red cell mass with the carbon monoxide re-breathing technique (Klausen et al., 1992; Telford et al., 1996; Svedenhag et al., 1997; Gore et al., 1998; Ashenden et al., 1999b; Friedmann, 1999). What is clear, however, is that adaptation to moderate altitude exposure, may not necessarily include changes in red cell mass.

It has also been suggested that the reason several studies fail to report increases in RCM is that these studies fail to reach the mimium dose of 12 $h.d^{-1}$ for 3 weeks at an altitude or equivalent hypoxia of at least to 2100 – 2500 m required to elicit such changes (Levine, 2002; Rusko *et al.*, 2003). Nevertheless, based on these conflicting results, it is apparent that performance gains in athletes from hypoxic exposure or LHTL may be independent from an increase in red cell mass and therefore oxygen carrying capacity of the blood. Given the multifactorial nature of athletic performance, this should not be surprising. Accordingly, alternative mechanisms for performance enhancement with LHTL are investigated in this thesis.

2.4.1.3 $\dot{V}O_{2peak}$ response and adaptation to hypoxia or LHTL

It is well established that the maximum oxygen consumption during exercise falls at altitude. The rate of decrease has been estimated as about 9% for every 1000 m above 1050 m (Robergs *et al.*, 1998), and may be more pronounced in athletes than non-athletes (Gore, 1996). There is still debate as to the effect of adaptation to hypoxic exposure on $\dot{V}O_{2peak}$ measured subsequently at sea level (Rusko *et al.*, 2003). Given the uncertainty that positive haematological changes occur with hypoxia or LHTL exposure, it is equally uncertain as to whether these interventions elicit positive gains in $\dot{V}O_{2peak.}$

Several studies have reported that $\dot{V}O_{2peak}$ is increased by ~ 3% at sea level after 3- 4 weeks exposure to moderate altitude of between 2300 and 3000 m (Adams *et al.*, 1975; Levine, 1992; Burtscher *et al.*, 1996; Stray-Gundersen *et al.*, 2001). Conversely, three studies utilising altitudes of between 4000 and 6194 m reported decreases of between 2 and 9 % in sea-level $\dot{V}O_{2peak}$ after at least 3 weeks altitude acclimatisation to between 4000 and 6194 m (Hochachka *et al.*, 1991; Green *et al.*, 2000d; MacDonald *et al.*, 2001) whilst two other studies reported no change after acclimatisation to 4300 m (Brooks *et al.*, 1991; Roberts *et al.*, 1996a). From these studies, it is possible that low-moderate hypoxic or altitude exposure elicits positive gains in subsequent sea-level $\dot{V}O_{2peak}$, whilst higher doses do not.

The effect of LHTL on sea-level $\dot{V}O_{2peak}$ is similarly equivocal. After a 4-week lead in period training at sea-level, 4-weeks of LHTL improved both subsequent sea-level $\dot{V}O_{2peak}$ and 5000 m running performance in college level runners (Levine & Stray-Gundersen, 1997). Further, elite runners also showed increased $\dot{V}O_{2peak}$ and improved

3000 m running performance after 27 days of LHTL (Stray-Gundersen *et al.*, 2001). Further, in another study, only those subjects who had an increased RCM also showed an increased sea-level $\dot{V}O_{2peak}$ and running performance. In sharp contrast, 11 to 23 days of LHTL failed to increase sea-level $\dot{V}O_{2peak}$ (Ashenden *et al.*, 1999a; Ashenden *et al.*, 1999b; Hahn *et al.*, 2001) despite improvement in 4-min cycling performance (Roberts *et al.*, 2003). It has been suggested that as for positive haematological change, a minimum dose of ~ 12 $h.d^{-1}$ for 3 weeks at an altitude or equivalent hypoxia of at least 2100 – 2500 m is required for positive changes in $\dot{V}O_{2peak}$. Given the large discrepancies in these findings, this thesis examined the effects of 23 nights of LHTL at a simulated altitude of 3000 m on the subsequent $\dot{V}O_{2peak}$ of well-trained athletes.

2.4.1.4 $\dot{V}O_2$ during submaximal exercise, mechanical efficiency and adaptation to hypoxia or LHTL

Two studies have reported a reduction of between 3-5 % in $\dot{V}O_2$ during submaximal exercise, after three weeks exposure to between 4300 and 6194 m (Hochachka *et al.*, 1991; MacDonald *et al.*, 2001). Another study reported an improved running economy and decreased submaximal $\dot{V}O_2$ of ~ 3% in well-trained runners after 20 nights LHTL at ~ 2650 m (Saunders *et al.*, 2003). Two other studies have reported no change in submaximal $\dot{V}O_2$ after between 3-4 weeks exposure to 2000-2700 m (Telford *et al.*, 1996; Levine & Stray-Gundersen, 1997). Efficiency, or mechanical efficiency, is a measure of effective work and is expressed as the percentage of energy expended that produces mechanical work (Elia & Livesey, 1992; Moseley *et al.*, 2004). Mechanical efficiency (%) can be calculated as: (Work performed per minute ($kJ.min^{-1}$) / energy expended per minute ($kJ.min^{-1}$)) x 100. Where work = (Workrate (W) x 60) / 1000 and

energy expenditure = $\dot{V}O_2$ x ((4.868 + (RER – 0.707) / 0.293) x 0.361) x 4.184 (Elia & Livesey, 1992; Gore *et al.*, 2001). Hypoxia, fatigue, muscle shortening velocity, fibre type and temperature, gear ratio and pedalling cadence all affect cycling efficiency (Moseley *et al.*, 2004; Faria *et al.*, 2005). An improvement in efficiency results in a decrease in the oxygen cost of a given amount of work. Potential mechanisms for improved efficiency are the amount of ATP produced per mole O_2 used increases, and/or the amount of ATP necessary for a given amount or work decreases, or a combination of both of these mechanisms (Green *et al.*, 2000d). Therefore, a change in efficiency is possible without a change in the slope for oxygen cost versus workrate (Saunders *et al.*, 2004). A growing number of studies have shown that various forms of altitude exposure can increase submaximal efficiency (Hochachka *et al.*, 1991; Green *et al.*, 2000d; MacDonald *et al.*, 2001; Katayama *et al.*, 2003; Saunders *et al.*, 2004). These exposures are diverse in nature, and include 20-nights exposure to 2,650 m simulated altitude (Saunders *et al.*, 2004), 3-wk exposure to intermittent hypobaria of 4,500 m (Katayama *et al.*, 2003), long-term residence at 4,200 m (Hochachka *et al.*, 1991) and mountain climbing over 3 wk to 6,194 m (Green *et al.*, 2000d; MacDonald *et al.*, 2001).

Improved running economy of 3.3% in elite runners occurred following 20-d of simulated moderate altitude exposure of 2,650 m (Saunders *et al.*, 2004). A 21-d expedition to 6,194 m resulted in an improvement in net efficiency of ~5% in experienced mountain climbers (Green *et al.*, 2000d). Importantly, no studies have investigated the effects of LHTL on submaximal O_2 and mechanical efficiency in well-trained cyclists. An improved mechanical efficiency evident through a decreased submaximal $\dot{V}O_2$ during exercise could potentially be an important factor in improved athletic performance after LHTL, and this was therefore investigated in this thesis.

2.4.2 Physiological response and adaptation to HIT

There is a great deal of information available on the effects of HIT in previously untrained, or recreationally active individuals (for recent review see Laursen & Jenkins, 2002). These individuals are likely, however, to have much greater responses to this type of training than already well-trained participants. This section of the review of literature will therefore concentrate on responses to HIT of already well-trained athletes.

2.4.2.1 Metabolic response and adaptation to HIT

Many studies have investigated the effects of HIT on muscle metabolism in untrained, or recreationally active participants. In contrast, relatively few studies have investigated the effects of HIT in well-trained athletes on muscle metabolism and the activity of related metabolic enzymes.

2.4.2.1.1 Muscle PCr and glycogen content after HIT

After 48 h recovery from a single training session incorporating 6 min of cycling each hour for 16 h, muscle PCr and glycogen contents each increased, in previously untrained participants (Green *et al.*, 2000c). Similarly, in untrained participants, ~30% increases in both muscle PCr and glycogen contents were evident after two weeks HIT (Rodas *et al.*, 2000). The effects of HIT on muscle PCr and glycogen in well-trained athletes have yet to be investigated.

2.4.2.1.2 Muscle carbohydrate utilisation during exercise after HIT

Previously untrained participants have an increased glycogenolytic capacity post training evidenced by a greater phosphorylase activity (Tabata *et al.*, 1996). Well-trained athletes engaging in short periods of HIT had lowered rates of carbohydrate oxidation, higher fat oxidation and lower blood lactate accumulation in one study (Westgarth-Taylor *et al.*, 1997). Conversely, neither carbohydrate nor fat oxidation were changed during exercise at 65% of pre intervention $\dot{V}O_{2peak}$, but in exercise at 85% of $\dot{V}$

O_{2peak} carbohydrate oxidation was lowered and fat oxidation increased (Clark *et al.*, 2004b). In this study, HIT sessions involved exercise at 85% of $\dot{V}O_{2peak}$, and thus the fuel utilisation response seems training intensity specific (Clark *et al.*, 2004b). In another study HIT in well-trained athletes resulted in a greater use of fatty acids (Billat, 2001), which is supported by the findings of a reduced respiratory exchange ratio during exercise following HIT (Westgarth-Taylor *et al.*, 1997; Laursen *et al.*, 2002).

2.4.2.1.3 Muscle enzyme activities after HIT

In untrained participants, the effect of HIT on muscle enzymes has been extensively investigated. In these participants, HIT resulted in increased activity of CS (Simoneau *et al.*, 1987; Keith *et al.*, 1992; Linossier *et al.*, 1993; MacDougall *et al.*, 1998; Parra *et al.*, 2000; Rodas *et al.*, 2000), PFK (MacDougall *et al.*, 1998; Parra *et al.*, 2000; Rodas *et al.*, 2000), LDH (Simoneau *et al.*, 1987; Linossier *et al.*, 1993; Rodas *et al.*, 2000) and HAD (Simoneau *et al.*, 1987; Keith *et al.*, 1992; Parra *et al.*, 2000; Rodas *et al.*, 2000).

In one study in well-trained athletes, 3-weeks of HIT did not alter the activities of HK, PFK CS, nor HAD, despite increases in peak power output, time to fatigue during intense sprint exercise and simulated 40 km time-trial performance (Weston *et al.*, 1997). However, in contrast to the findings in that study, an increase of 18% in CS was reported after training consisting of repeated 500 m runs at ~120% $\dot{V}O_{2peak}$ five times per week (Shepley *et al.*, 1992).

2.4.2.2 Ventilation and $\dot{V}O_2$ adaptation to HIT in well-trained athletes

In non-athletes, a variety of HIT interventions each increased $\dot{V}O_{2peak}$ (Tabata et al., 1996; MacDougall et al., 1998; Ray, 1999; Rodas et al., 2000). However, in already well-trained athletes, HIT did not lead to changes in either maximal ventilation ($\dot{V}_E$), or

$\dot{V}O_{2peak}$ (Lindsay et al., 1996; Westgarth-Taylor et al., 1997; Weston et al., 1997; Stepto et al., 1999). The athletes utilised in these studies typically would already have reached a high level of adaptation for $\dot{V}O_{2peak}$ and therefore tests used may not be sensitive enough to detect any further small changes. Further, the lack of change in $\dot{V}O_{2peak}$ suggests an uncoupling of $\dot{V}O_{2peak}$ from performance in these well-trained athletes after HIT.

HIT in well-trained athletes leads to increased cycling performance (Table 2.2) in the absence of major changes in muscle metabolism, fuel utilisation or $\dot{V}O_{2peak}$. Subsequently, further investigation of other mechanisms for improved cycling performance is required. Elite human cycling performance is a complex multifactorial phenomenon. The remainder of this review of literature, will focus on the role of the Na^+,K^+ATPase in mediating changes in potassium and sodium ions and in fatigue. Further, the effects of both LHTL and HIT in well-trained athletes on the Na^+,K^+ATPase and acid-base balance will be reviewed, and investigated in this thesis.

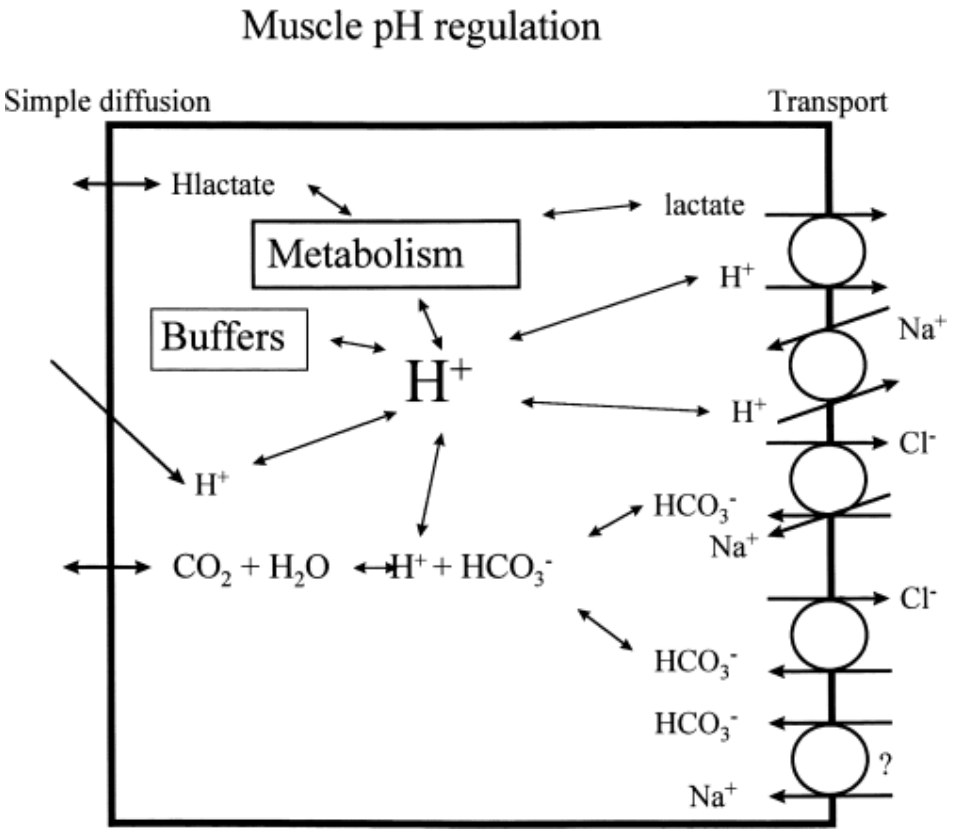
2.4.3 Skeletal muscle acid-base balance and muscle performance

For many years the regulation of skeletal muscle acid-base balance or hydrogen ion concentration ($[H^+]$) was thought to be vital for athletic performance. During fatiguing stimulation of single muscle fibres muscle, the cell $[H^+]$ increased and acidification reduced the maximal isometric force and shortening velocity (Donaldson et al., 1978; Metzger & Moss, 1987). Furthermore, correlations between human muscle fatigue and acidification had also been established (Miller et al., 1988; Cady et al., 1989). However, more recent studies performed at or near physiological temperatures have seriously questioned the role of acidification in muscle fatigue (Ranatunga et al., 1987; Pate et al., 1995; Wiseman et al., 1996; Westerblad, 1997). Muscle force production, or shortening velocity, do not seem to be greatly affected by a rise in $[H^+]$ (for review see

(Westerblad, 1999)). However, the importance of muscle pH regulation for muscle performance should not be completely dismissed (See Section 2.4.3.1).

Regulation of muscle [H^+] requires the complex interaction of metabolism, intracellular buffers and ion regulation, including the activity of numerous membrane bound transport systems (Figure 2.3) (Juel, 1998). The regulation of muscle pH has been recently extensively reviewed (Juel, 1998), and therefore is not a focus of this thesis. At least two studies have, however, shown an increased muscle buffering capacity of ~16% (β_m) after training correlated with improved performance (Bell & Wenger, 1988; Weston *et al.*, 1997). Importantly, one of these studies reported an increased β_m and performance after HIT in well-trained athletes (Weston et al., 1997). Further, in two studies β_m was elevated by ~6% after altitude exposure (Mizuno *et al.*, 1990; Saltin *et al.*, 1995a). The effects of HIT and LHTL on β_m are therefore of interest and investigated in this thesis. The following sections begin with a review of HIT and LHTL effects on β_m, and then a wider review of muscle acid-base balance.

Figure 2.3 A schematic model of muscle pH regulation including mechanisms of simple diffusion and active transport (Juel, 1998).



2.4.3.1 Muscle buffering capacity

High intensity exercise results in the production of large amounts of lactate and hydrogen ions within skeletal muscle, with early literature reporting a prominent role for intramuscular acidosis in the development of muscle fatigue (Hill, 1955; Donaldson *et al.*, 1978). Even allowing that acidosis is most likely only directly responsible for at most 10% of fatigue (Westerblad, 1999), an increase in skeletal muscle buffer capacity (β_m) with training may be beneficial in attenuating this small component of fatigue, especially in elite athletes regularly requiring maximal performance.

An index of β_m can be obtained by either titrating a muscle homogenate through a given pH range with a known acid or base [in-vitro buffering ($\beta_{in\text{-}vitro}$)], or by allowing the muscle to 'self-titrate' with metabolic acids and then measuring the change in metabolic acid concentration relative to the delta muscle pH [in-vivo buffering ($\beta_{in\text{-}vivo}$)] (Mannion *et al.*, 1993). The major buffering constituents of human skeletal muscle are protein,

inorganic phosphate (P_i) and the histidine containing di-peptides carnosine and anserine (Parkhouse & McKenzie, 1984).

2.4.3.1.1 Training effects on muscle buffering capacity

The effects of training on β_m are equivocal. Muscle *in-vitro* β_m increased ~ 16% after both one-legged sprint training (Bell & Wenger, 1988), and after 3 weeks of HIT in already well-trained cyclists (Weston *et al.*, 1997). In contrast, *in-vitro* β_m was unchanged after isokinetic knee training (Mannion *et al.*, 1994; Pilegaard *et al.*, 1999) or seven (Harmer *et al.*, 2000) or eight (Nevill *et al.*, 1989) weeks of sprint training.

2.4.3.1.2 Hypoxia effects on muscle buffering capacity

Two studies have also reported an increase in *in-vitro* β_m of ~6% following altitude exposure (2000-2700 m) (Mizuno *et al.*, 1990; Saltin *et al.*, 1995a). The effect of LHTL on β_m are also unknown, and was thus examined in this thesis.

2.4.3.1.3 Muscle buffering capacity and performance

The relationship between an increase in β_m and increased performance remains controversial. An ~16% increase in β_m was correlated with an ~18 % increase (Table 2.2) in simulated 40 km time-trial performance and time to fatigue during sprint exercise after a short period of high-intensity interval training (Weston et al., 1997). Furthermore, in another study utilising one-legged high-intensity training, an ~16% increase in β_m was also correlated with improved performance (Bell & Wenger, 1988). However, after a period of isokinetic training which increased total work, there was no change in β_m, and there was no correlation between β_m or anaerobic performance (Sahlin & Henriksson, 1984).

2.4.3.2 Stewart model of muscle [H^+]

The regulation of muscle [H^+] can alternately be described via physicochemical principles (Stewart, 1981; Lindinger & Heigenhauser, 1990; Heigenhauser, 1995). This

model reports that there are three main independent variables which contribute to the [H^+] of exercising muscle: a) the strong ion difference ([SID]) (Lindinger & Heigenhauser, 1990; Johnson et al., 1996); b) pCO_2; and (c) total concentration of weak acids and bases ([A_{tot}]) (Lindinger, 1995).

2.4.3.3 Origins of H^+ ions in skeletal muscle

2.4.3.3.1 Muscle [SID]

The strong ion difference ([SID]) (Johnson et al., 1996) can be calculated as ([SID]) = (∑ [strong base cations]) - (∑ [strong base anions]) (Stewart, 1981). Strong ions are electrolytes completely dissociated into ions when disolved in H_2O at physiological pH (Stewart, 1981). The physiologically important inorganic ions are Na^+, K^+, Cl^-, SO_4^{2-}, Mg^{2+}, and Ca^{2+}, whilst the strong organic ions are Lac-, PCr^{2-}, NH_4^- and ketones (Heigenhauser, 1995). As [SID] became more positive from rest during exercise, [H^+] is consequently decreased; if the [SID] lowered, then [H^+] is increased (Heigenhauser, 1995).

Muscle ions, and, therefore, by implication the [SID] are tightly regulated during exercise. At the onset of especially high intensity exercise, water, Cl^- and Na^+ ions move from the plasma into the muscle, and K^+ and Lac^- ions exit (Sahlin et al., 1978; Sjøgaard et al., 1985; McKenna, 1992; Lindinger et al., 1994). For more detail on Na^+ and K^+ ion movements, see sections 2.7.2 and 2.7.3 of this review. The main changes which contribute to a decreased muscle [SID] of up to 31% during intense exercise are a decreased [PCr^{2-}], an increased intracellular [Lac^-] and an increased efflux of K^+ from the muscle (Kowalchuk et al., 1988; Lindinger & Heigenhauser, 1988; Lindinger & Heigenhauser, 1990; Lindinger, 1995). The decrease in [PCr^{2-}] tends to increase [SID] and therefore has an alkalinising effect on muscle (Lindinger, 1995), which is rapidly reversed during recovery from exercise and re-synthesis of PCr (Lindinger & Heigenhauser, 1990, 1991).

The decrease in $[K^+]_i$ and increase in $[Lac^-]_e$ both decrease the [SID] and thus increase muscle $[H^+]$. Muscle $[K^+]$ changes are tightly regulated, notably by the Na^+,K^+ATPase as discussed in detail later in this review. The lactate anion cannot cross the plasma membrane by free diffusion, and requires a specific transport mechanism, the lactate (monocarboxylate) transporter (MCT) (Poole & Halestrap, 1993; Stallknecht, 1998; Halestrap & Price, 1999; Juel & Halestrap, 1999). The MCT is a proton symport, stereoselective for Lac^- (Bonen, 2000). The removal of lactate and H^+ from the cell contributes to the defence of intracellular pH, as well as allowing lactate to be taken up by other active skeletal and cardiac muscle, for use as a substrate (Roth, 1991; Juel, 1997). Changes in the other ions in muscle are small, or tend to cancel each other out (Lindinger, 1995).

2.4.3.3.2 Muscle PCO_2

CO_2 is produced in muscle from both Krebs cycle activity and the carbonic anhydrase catalysed reaction of H^+ with HCO_3^- (Gros & Dodgson, 1988). Each carbon entering the Kreb's cycle results in the production of one molecule of CO_2 (Gros & Dodgson, 1988). In plasma, an increased CO_2 plays a large role in plasma H^+ production, but in muscle CO_2 is likely to only play a small role in the increased intracellular $[H^+]$ ($[H^+]_i$) which occurs during high intensity exercise, as muscle pCO_2 rapidly equilibrates with plasma pCO_2 and due to the high intracellular protein buffering of H^+ (Lindinger & Heigenhauser, 1990).

2.4.3.3.3 Muscle $[A_{tot}]$

The $[A_{tot}]$ is the total weak acid and base concentrations (Heigenhauser, 1995). The main contributing factors to $[A_{tot}]$ in human muscle are the histidine groups of proteins, Cr, P_i and ATP (Lindinger & Heigenhauser, 1990).

During high intensity exercise, ATP and PCr hydrolysis, as well as degradation of muscle glycogen and production of lactate, are the predominant energy-yielding

pathways (Withers et al., 1991). Muscle ATP levels have been shown to be reduced by between 10 and 40% (Cheetham *et al.*, 1986; Lindinger *et al.*, 1987b; Söderlund & Hultman, 1991), and in single muscle fibres by up to 90% (Karatzaferi et al., 2001). A reduction in ATP, PCr and an increase in Pi results in a net increase in $[A_{tot}]$ which in turn causes a rise in $[H^+]$ (Lindinger, 1995).

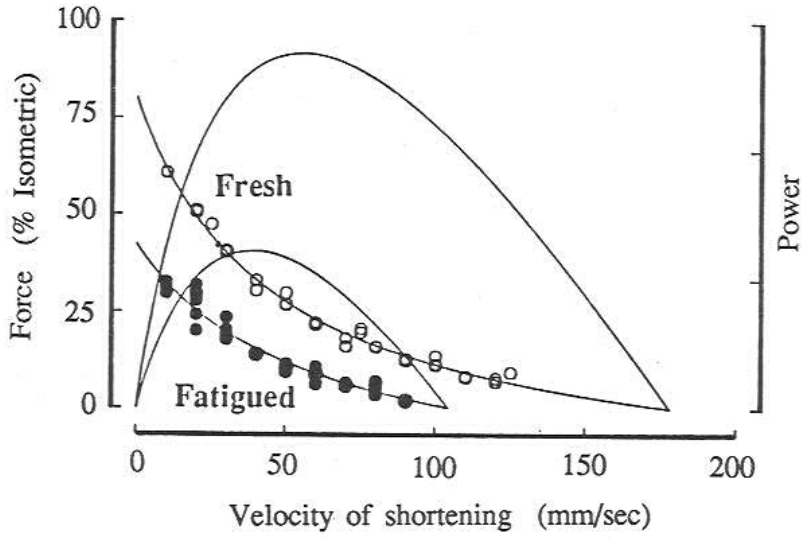
2.4.3.4 Concluding remarks on acid-base balance

Acid-base balance during exercise occurs through a complex interaction of muscle metabolism, buffers and ion regulation. Pertubations of acid-base balance are now not thought to be a major contributor to fatigue during exercise. However, even a small physiological adaptation via either LHTL or HIT could be of high practical significance for an elite athlete. There are some intriguing questions unanswered as to the effects of LHTL or HIT on acid-base balance in well-trained athletes, and these were therefore investigated in this thesis.

2.5 Muscle fatigue

A major factor affecting performance in highly trained endurance athletes is muscle fatigue. An early definition of fatigue stated that fatigue represented a decrease in muscle force generating capacity (Vøllestad & Sejersted, 1988). This definition concentrates solely on the force generating ability of a muscle, and belies the complex nature, and differing types of fatigue. A more encompassing definition of fatigue needs to include reference to both the decline in force and velocity of shortening and therefore the power of a muscle contraction (Figure 2.4). Muscle fatigue can therefore be defined as a reduction in muscle power output during exercise, which is reversible during recovery (McKenna, 2003). The importance of each facet of fatigue is dependent on the intensity and duration of the stimulus or exercise, and training status (Fitts, 1996a). The following sections provide an overview of fatigue, and detail current views on the specific role of skeletal muscle ion regulation in fatigue.

Figure 2.4 Decreased maximum force and velocity of shortening, and thus muscle power output of rat medial gastrocnemius muscle prior to (Fresh) and after fatiguing (Fatigued) contractions (From (de Haan et al., 1991).



2.5.1 Central fatigue versus peripheral sites of fatigue

Fatigue can be described within two broad categories, central and peripheral. Central fatigue refers to fatigue in one or more of neural input to and within the higher brain centres, recruitment of alpha motor neurones, the alpha motor nerves, and the neuromuscular junction (Gandevia, 2001). Peripheral fatigue refers to fatigue within the muscle and may occur as a result of changes to membrane excitability, excitation-contraction coupling, metabolic energy supply and/or other processes (Fitts, 1996a). A combination of both central and peripheral fatigue is proposed to induce a decrease in muscle power generating capacity (Gandevia, 1998). This section gives a brief overview of central fatigue.

The underlying mechanisms of central fatigue are still incompletely understood. It was reported that a slowing of motor neuron firing rates occurs during fatigue, and postulated that this was due to increased feedback from muscle group III & IV afferents (Bigland-Ritchie *et al.*, 1986b). A progressive reduction in voluntary drive to motor

neurones during isometric contractions has also been demonstrated (Gandevia, 2001). The role of higher brain centres in central fatigue was confirmed when transcranial magnetic stimulation of the motor cortex increased muscle force output during fatigue (Taylor *et al.*, 2000; Gandevia, 2001).

The interpolated twitch method has also confirmed the existence of central fatigue during repeated isometric contractions (McKenzie *et al.*, 1992; Gandevia, 2001). This technique involves superimposing a brief electrical stimulation during a maximal voluntary contraction. If the electrical stimulation causes an increase in muscle force output in fatigued muscle, then central fatigue has occurred (Gandevia, 2001).

The magnitude of contribution of central fatigue to total fatigue is likely to be small. In one study, the reported a loss of power elicited by electrical stimulation ascribed to central fatigue, was calculated to account for only ~ 20% of total power decrement (James *et al.*, 1995). It is important to note however, that central fatigue can occur during both brief (McKenzie *et al.*, 1992; Taylor *et al.*, 2000; Nordlund *et al.*, 2004) and prolonged exercise (Bigland-Ritchie *et al.*, 1986a; McKenzie *et al.*, 1992; Bilodeau *et al.*, 2001; Millet *et al.*, 2003a). Importantly for elite cyclists, when measured via twitch interpolation technique central fatigue did not occur after prolonged cycle racing (Millet *et al.*, 2003b). Most research, however, points to fatigue occurring predominantly beyond the neuromuscular junction (Fitts, 1996a). The following section will give a detailed overview of peripheral fatigue, focussing where possible on human skeletal muscle.

Peripheral fatigue involves a complex interaction of factors involved in excitation-contraction coupling and the energetics of these and other processes (Fitts, 1994; McKenna, 2003). The involvement of muscle ions in membrane excitability and muscle fatigue is discussed in detail in the following sections. Other potential factors in peripheral fatigue include Ca^{2+} release, Ca^{2+} re-uptake and Ca^{2+}ATPase activity which

are impaired with fatiguing exercise (for review see (Fitts, 1994; Allen *et al.*, 1995; Allen *et al.*, 1998; Favero, 1999; Westerblad, 1999; Allen & Westerblad, 2001)). Metabolic factors such as PCr degradation during high intensity exercise (McCartney *et al.*, 1986) or muscle glycogen depletion during prolonged exercise (Bergström *et al.*, 1967; Hermansen *et al.*, 1967) potentially interfere with the function of ATPases and therefore may play a large role in fatigue, but these will not be reviewed for this thesis.

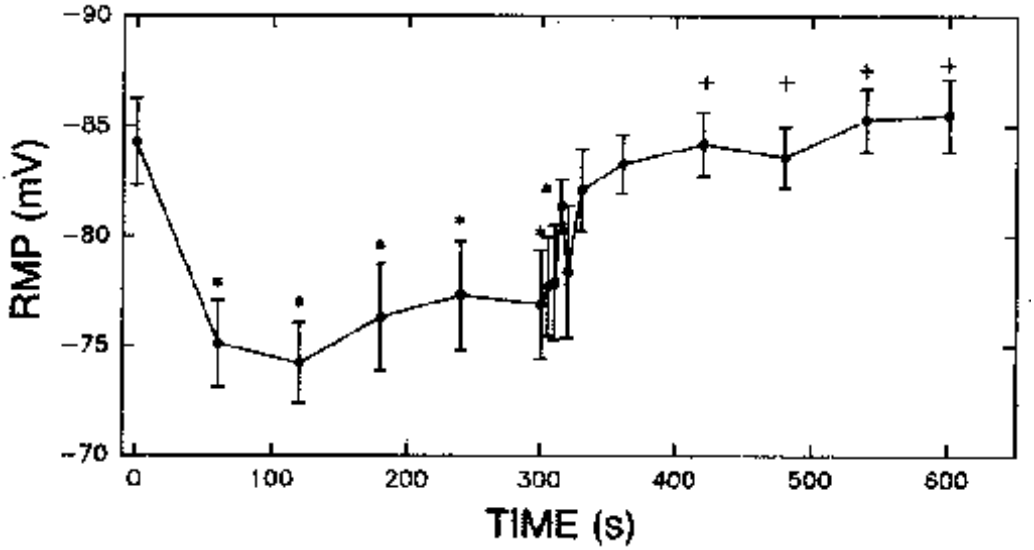
2.6 Muscle ions and membrane potential with exercise

2.6.1 Membrane potential

Resting muscle membrane potential (resting E_m) is influenced by the chloride (Cl^-), Na^+ and K^+ ions, which create a transmembranous electrochemical charge. The major influence is from K^+ (Cunningham et al., 1971). E_m is calculated from the sarcolemmal differences in electrochemical potential and individual permeabilities of the charged ions (Hodgkin & Howowitz, 1959). Under resting conditions, the E_m is approximately -90mV in human skeletal muscle (Cunningham et al., 1971; Sjøgaard et al., 1985; Fitts, 1996b). An action potential is created when the voltage-gated Na^+ channels of the sarcolemma open, resulting in a rapid influx of Na^+ and a depolarisation of the membrane (Ruff, 1996). Within 2-3 msec, the E_m increases to approximately -50mV and in turn facilitates the opening of further Na^+ channels. The E_m peaks at approximately +20 to +35 mV (Balog et al., 1994). The return of the membrane to it's resting potential is triggered once the peak voltage is obtained, through the rapid inactivation of the Na^+ channels, and opening of the the voltage sensitive K^+ (K_v) channels creating the APs (Sejersted & Sjøgaard, 2000), the big conductance Ca^{2+}-dependent K^+ ($BKCa^{2+}$) channels (Jacquemond & Allard, 1998; Mallouk & Allard, 2000), and the ATP-sensitive K^+ (K_{ATP}) channels (Lindinger et al., 2001; Nielsen et al., 2003a) leading to increased K^+ permeability and therefore K^+ efflux (Balog et al.,

1994). The successful propagation of the action potential therefore depends on the maintenance of steep chemical gradients for Na^+ and K^+ (Fitts, 1996b).

Figure 2.5 Depolarisation of the resting membrane potential in electrically stimulated frog semitendinous skeletal muscle (Balog *et al.*, 1994).



2.6.2 Muscle Na^+ concentration, content and fluxes with exercise and relationship to fatigue

Resting muscle intracellular [Na^+] has been reported to range from 12-16 mM in mouse and frog skeletal muscle when measured by ion-sensitive electrodes (Juel, 1986; Balog & Fitts, 1996). Values of between 11-15 mM as measured by flame photometry have been reported in rat skeletal muscle (Nielsen & Clausen, 1997). In a complex study, measurement of total tissue water and extracellular fluid volume and intracellular fluid volume, allowed calculation of $[Na]_I$, which in resting muscle was ~ 6 mM (Sjøgaard *et al.*, 1985). In human skeletal muscle, resting muscle [Na^+] measured by flame photometry of between 3 and 9 mM (Sjøgaard *et al.*, 1985) and 8.9 and 12.7 mM (Sjøgaard, 1983) have been reported, with no differences for Na^+ content found between fast or slow twitch muscle fibres (Sjøgaard, 1983). This wide range of values appears to be method, species and muscle dependent, and not necessarily indicative of measurement error.

With each action potential, there is a net Na^+ influx (Everts & Clausen, 1988; Clausen & Everts, 1989). A net Na^+ influx of approximately 15.6 $pmol.cm^2.impulse^{-1}$ was reported in electrically stimulated single frog muscle fibres (Hodgkin & Howowitz, 1959). In rat skeletal muscle, each action potential produced a Na^+ influx of 2 – 11 $\mu mol.kg\ ww^{-1}$ (Nielsen & Clausen, 1997).

Intracellular sodium concentration ($[Na^+]_i$) increased from 16 mM to 49 mM after electrical stimulation comprising 100-ms trains at 150 Hz , with one stimulus per second for 5-min (Balog & Fitts, 1996). Electrical stimulation at 60 Hz for 960 100 ms stimuli in mouse skeletal muscle increased the intracellular $[Na^+]$ from 12.7 to 23.3 mM (Juel, 1986). During cycling exercise to exhaustion, intracellular Na^+ content increased by ~68%, without alteration in $[Na^+]$, which may be more due to an increase in intracellular water content, rather than actual changes in intracellular sodium content (Sjøgaard, 1983). In another study, $[Na^+]_i$ rose from 6 to 24 mM, without a corresponding decrease in muscle extracellular $[Na^+]$ (Sjøgaard *et al.*, 1985).

A reduction in Na^+ equilibrium potential as a result of Na^+ movement into the muscle and the depolarisation-induced inactivation of Na^+-channels was suggested to contribute to a decrease in the action potential overshoot with fatigue (Balog *et al.*, 1994). In that study, electrical stimulation at 150 Hz for 5 minutes of frog semitendinous muscle, reduced the overshoot from 19.5 ± 1.9 mV at rest, to 7.3 ± 1.9 mV at fatigue (Balog *et al.*, 1994). Importantly though, the overshoot never fell below 0 mV, the threshold value for full activation of the voltage-sensors in the t-tubular space (Shlevin, 1979; Gomolla *et al.*, 1983; Balog *et al.*, 1994). In another study, a reduction in $[Na^+]_o$ to 25 mM by substituting Na^+ with choline reduced tetanic force to ~30% of control in isolated rat soleus muscles (Overgaard *et al.*, 1997). However, in that study the inhibitory effect of low $[Na^+]_i$ was considerably augmented by an increase in $[K^+]_e$ (Overgaard *et al.*, 1997).

Thus, *in-vivo* it is likely that a combination of Na^+ influx and K^+ efflux have important effects on muscle contractility (Overgaard *et al.*, 1997; Overgaard *et al.*, 1999). When a ~40% reduction in extracellular [Na^+] was combined with an ~80% increase in extracellular [K^+], tetanic force in isolated rat soleus muscle decreased by ~50% (Overgaard *et al.*, 1999).

2.6.3 Muscle K^+ concentration, fluxes with exercise and relationship to fatigue

When examining the literature on measuring K^+, the sampling site, sampling and analytical techniques must all be taken into consideration (Sejersted & Sjøgaard, 2000). The three main compartments for K^+ storage in the body are the muscle cell, the interstitial space, and the plasma (Sejersted & Sjøgaard, 2000). Muscle K^+ content can be measured through the use of radio isotopes (Qayyum *et al.*, 1993), or flame photometry, atomic absorption, neutron activation or titration (Fenn *et al.*, 1938; Bergström *et al.*, 1971; Costill *et al.*, 1976; Lindinger & Heigenhauser, 1987; Clausen & Everts, 1991; Lindinger & Heigenhauser, 1991). Interstitial fluid K^+ can be measured directly with ion-selective electrodes (Balog *et al.*, 1994; Balog & Fitts, 1996), or via microdialysis (Green *et al.*, 1999c; Juel *et al.*, 2000b; MacLean *et al.*, 2000). Further, the effect of the depolarisation of the membrane due to changes in potassium can in theory be evaluated from the Nernst equation. However, this equation has important limitations and thus actual measures from the literature were reviewed instead. The first limitation of the Nernst equation is that it assumes that membranes are permeable to only one ion, which is not valid for muscle plasma membranes (Hodgkin & Huxley, 1952; Malmivuo & Plonsey, 1995; Karp, 1996; Moran *et al.*, 1999; Lodish *et al.*, 2004). Second, the Nernst equation underestimated actual K^+ flux through a membrane (Hodgkin & Keynes, 1956; Andersen, 1999). Finally, the Nernst equation fails to take into account that ion channels can concentrate ions and become saturated (Läuger, 1973; Andersen, 1999).

In human skeletal muscle, resting $[K^+]_i$ was ~ 168mM (Sjøgaard *et al.*, 1985), and the resting extracellular K^+ concentration ($[K^+]_e$) was between 3.5 and 5.3 mM (Sjøgaard, 1983; Sjøgaard *et al.*, 1985; Worth, 1985).

During muscle contractile activity, there is an opening of the the voltage sensitive K^+ (K_v) channels (Sejersted & Sjøgaard, 2000), the big conductance Ca^{2+}-dependent K^+ ($BKCa^{2+}$) channels (Jacquemond & Allard, 1998; Mallouk & Allard, 2000), and the ATP-sensitive K^+ (K_{ATP}) channels (Lindinger *et al.*, 2001; Nielsen *et al.*, 2003a) leading to increased K^+ permeability and therefore K^+ efflux (Balog *et al.*, 1994). The magnitude of K^+ efflux during the repolarisation phase of an action potential has been calculated as being approximately 9.6 $pmol.cm^{-2}$ $impulse^{-1}$ in stimulated frog muscle (Hodgkin & Howowitz, 1959). The calculated K^+ efflux in rat skeletal muscle was between 7-11 $nmol.g\ wet.wt^{-1}.contraction^{-1}$ for rat muscle (Everts & Clausen, 1988; Clausen & Everts, 1989). In human skeletal muscle, each repolarisation is associated with K^+ efflux calculated as approximately 1 -2 $\mu mol.kg^{-1}.min^{-1}$ (Hallén, 1996).

As a result of K^+ efflux, intracellular $[K^+]$ ($[K^+]_i$) fell from 142 mM to 97 mM, after fatiguing stimulation of frog muscle at 150 Hz (Balog & Fitts, 1996) and from 168 mM to 129 mM in human muscle after exhaustive cycling exercise (Sjøgaard *et al.*, 1985). Plasma $[K^+]$ increased from approximately 4 $mmol.L^{-1}$ to up to 7-8 $mmol.L^{-1}$ after intense running or cycling (Hermansen *et al.*, 1984; Kowalchuk *et al.*, 1988; McKenna *et al.*, 1997). These studies may have underestimated the rise in $[K^+]_e$ however, as studies employing the microdialysis technique have reported $[K^+]$ of 9 $mmol.L^{-1}$ after one-legged kicking at 50 W (Juel *et al.*, 2000b) and up to 15 $mmol.L^{-1}$ in the extracellular space of human muscle (Green *et al.*, 1999c; Green *et al.*, 2000e; Nielsen *et al.*, 2003b; Nordsborg *et al.*, 2003b; Rosendal *et al.*, 2004). With resting $[K^+]_e$ at ~ 4 mM (Sjøgaard, 1983; Sjøgaard *et al.*, 1985), this represents an approximate 3-fold increase in extracellular K^+, with a concomitant decrease in $[K^+]_i$ (Balog & Fitts, 1996).

Muscle membrane potential is largely determined by the transmembranous $[K^+]$ gradient. Increased $[K^+]_e$, with a 10-20% concomitant decrease in $[K^+]_i$ have therefore been commonly proposed as possible contributors to fatigue. Above $[K^+]_e$ of ~4 mM the membrane depolarises by > 18 mV for a halving of the $[K^+]_e$ / $[K^+]_i$ concentration gradient from the resting state (Cairns et al., 1995; Cairns et al., 1997). This represents a large change in resting E_m of ~30%.

The consequences of the run-down in K^+ gradient during fatiguing exercise are a slow inactivation of Na^+ channels (Lännergren & Westerblad, 1986; Juel, 1988; Cairns & Dulhunty, 1995) and a reduced action potential propagation velocity (Balog & Fitts, 1996). Thus, this rundown in K^+ gradient may also result in muscle fatigue. An increase in $[K^+]_e$ from 3 to 7 mM resulted in a 41% reduction in tetanic force in frog muscle (Bouclin et al., 1995). In another study using isolated mouse soleus and extensor digitorum longus muscles, an increase in $[K^+]_e$ from ~4 mM to 7.5 mM reduced contractile force by only ~5% (Juel, 1988) and a further increase to 10 mM resulted in a 40% reduction in force (Juel, 1988). When extracellular $[K^+]$ was raised to 12.5 mM, there was a 96% reduction in contractile force in rat skeletal muscle (Clausen et al., 1993). Thus, the combined effect of decreased $[Na^+]_i$ and increased $[K^+]_e$ will result in a large reduction in muscle force production.

In summary, muscle K^+ efflux occurs with each action potential. During fatiguing exercise, the accumulation of K^+ in the muscle interstitial space may reach up to 15 mM, which has serious adverse implications for muscle contractility, and may therefore contribute to muscle fatigue.

2.6.4 Muscle water fluxes with exercise

Water content of muscle rapidly increases within seconds of muscle stimulation occurring (Sjøgaard et al., 1985; Lindinger & Heigenhauser, 1988; Lindinger & Heigenhauser, 1991; Watson et al., 1993). The rise in muscle water content is a direct

result of increases in both the extracellular, and intracellular fluid spaces in muscle (Sjøgaard et al., 1985). This rise in muscle total water content may also be exercise intensity and duration specific (Sjøgaard & Saltin, 1982; Sjøgaard et al., 1985; Ward et al., 1996). During submaximal exercise at 50-70% $\dot{V}O_{2peak}$, a rise in the muscle extracellular fluid space was responsible for an ~10% increase in water content (Sjøgaard et al., 1985). However, during high-intensity exercise at 100% $\dot{V}O_{2peak}$, the muscle water content increased by ~21% (Sjøgaard et al., 1985). A ~15% rise in total water content was observed in the human vastus lateralis after 3 minutes of intense cycling (Sjøgaard & Saltin, 1982), while 2 minutes of electrical stimulation at 4 Hz increased the total tissue volume by ~3% in isolated cat muscle (Ward et al., 1996).

The mechanism for increased muscle water content is quite complex. During moderate to intense exercise, blood and plasma volume decrease (Lundvall et al., 1972; van Beaumont et al., 1973; van Beaumont et al., 1981; Harrison, 1985; Freund et al., 1987; McKenna et al., 1997) and fluid moves into both the interstitial and intracellular muscle compartments (van Beaumont et al., 1973; van Beaumont et al., 1981; Kowalchuk et al., 1988). The drive for this movement comes from increases in osmolality of contracting muscle interstitial and intracellular spaces, due to an accumulation of lactate and potassium ions in the interstitium (Juel *et al.*, 2003a) and rapid hydrolysis of intracellular phosphocreatine (Hultman & Sjoholm, 1983).

The effect of this water movement is a dilution of intracellular ions (Sjøgaard, 1983; Sjøgaard *et al.*, 1985; Lindinger & Heigenhauser, 1988) and metabolites, and an increased concentration of extracellular or plasma ions (Sjøgaard, 1983; Vyskocil *et al.*, 1983; Sjøgaard *et al.*, 1985; Green *et al.*, 1999c; Green *et al.*, 2000e) and metabolites (Hermansen *et al.*, 1984). Thus decreasing the intracellular to extracellular gradients for $[K^+]$ and may therefore be influential for fatigue.

2.7 The skeletal muscle Na^+,K^+ATPase

In 1997, the Danish researcher, Jens C. Skou was awarded the Nobel Prize in Chemistry for his discovery in 1957 of the Na^+,K^+ATPase enzyme (Skou, 1998). This important discovery by Skou followed from initial observations that marked Na^+ and K^+ disturbances occur in skeletal muscle with electrical stimulation (Fenn & Cobb, 1936). Experiments in humans indicated that venous K^+ increased by ~25% during exhaustive short-term exercise, with a rapid fall during recovery (Keys, 1937). The concept of active transport developed in the 1940's and 1950's with the 'pump-leak concept' where Na^+ and K^+ leaked down electrochemical gradients, but K^+ had to be actively pumped back into the cell against this gradient (Dean, 1941; Skou, 1957). The discovery that a membrane transport pump could be 'poisoned' by a cardiac glycoside (ouabain) (Schatzmann, 1953; Hodgkin & Keynes, 1956) and was stimulated by Na^+, Mg^{2+} and K^+ led Skou to reason that the ouabain suppressible-pump was in fact the Na^+,K^+ATPase (Skou, 1960)

Since the discovery by Skou, a PubMed search indicated ~19,000 papers have been generated investigating Na^+,K^+ATPase, including research on its ubiquitous nature, isoforms, tissue-, organ- and developmental-expression, short- and long-term regulation and important functions. The following section of this thesis will review the function, structure, locations, quantification, activation and regulation of the Na^+,K^+ATPase, and its role in skeletal muscle fatigue.

2.7.1 Na^+,K^+ATPase function

The Na^+,K^+ATPase is situated in the plasma membrane of virtually all animal cells (Clausen, 1998). The major role of the Na^+,K^+ATPase is to transport Na^+ and K^+ ions against their concentration gradients, and in the case of Na^+, electrical gradient (Clausen, 1998). In each cycle of the Na^+,K^+ATPase, an ATP molecule is split, and 3 Na^+ ions are extruded from the cell in exchange for 2 K^+ ions which re-enter the cell,

thus exerting its electrogenic role in maintaining membrane excitability (Clausen, 1998).

2.7.2 Na^+,K^+ATPase structure

The Na^+,K^+ATPase is a transmembranous protein (Figure 2.6), expressed in excitable cells. The Na^+,K^+ATPase comprises a catalytic α subunit (~100-112 kDa) and a glycosylated β subunit (~40-60 kDa), which is present in an αβ complex, and forms the minimum functional Na^+,K^+ATPase unit (Fig. 2.6) (Jorgensen, 1982; Lingrel, 1992; Levenson, 1994; Blanco & Mercer, 1998). There is also a third (γ) subunit of the Na^+,K^+ATPase, now known to be an FXDY protein (Crambert & Geering, 2003; Geering *et al.*, 2003; Horisberger, 2004).

2.7.3 Quantification of Na^+,K^+ATPase content

Na^+,K^+ATPase content is best quantified by counting the binding of the vanadate-facilitated radio-labelled glycoside [^{3}H]-ouabain, to Na^+,K^+ATPase in the muscle (Hansen, 1979; Nørgaard *et al.*, 1984a). In resting human skeletal muscle values for [^{3}H]-ouabain binding sites of ~300 pmol.(g wet wt)$^{-1}$ are common (McKenna, 1998) but a large range of values from ~220 to ~420 pmol.(g wet wt)$^{-1}$, have been reported (Table 2.2). The highest reported values in human muscle of ~420 pmol.(g wet wt)$^{-1}$ have been found in elite male alpine cross country skiers (Medbø *et al.*, 2001).

Some researchers have also measured Na^+,K^+ATPase activity through the indirect measurement of associated K^+-stimulated phophatase activity, or inorganic phosphate (P_i) production (Nørgaard *et al.*, 1984b) and converted the results to Na^+,K^+ATPase content by assuming a certain molar activity for the Na^+,K^+ATPase (Nørgaard *et al.*, 1984b).

2.7.4 Quantification of Na^+,K^+ATPase activity

One method for measuring Na^+,K^+ATPase activity is via the measurement of radiolabelled ion ^{86}Rb fluxes (Clausen *et al.*, 1987). This technique cannot be used *in-*

vivo or in human skeletal muscle specimens obtained by muscle biopsy (Fraser *et al.*, 2002). An alternative is to measure inorganic phosphate production in purified muscle preparations (Bonting *et al.*, 1961), or the activity of K^+-dependent phosphatases, using the substrate *p*- nitrophenyl phosphatase (Hundal *et al.*, 1994) or 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) (Nørgaard *et al.*, 1984b; Kjeldsen *et al.*, 1988). Measurement of direct Na^+,K^+ATPase activity in skeletal muscle requires a large amount of tissue, and is unreliable (Fowles *et al.*, 2004). The 3-*O*-MFPase assay has been optimised for human muscle samples, and can be used on crude muscle homogenates, ensuring complete quantification of Na^+,K^+ATPase activity (Fraser & McKenna, 1998). The 3-*O*-MFPase assay also has 2-3 times the sensitivity of the *p*-nitrophenyl phosphatase assay, and therefore requires 50-100 times less tissue (Clausen *et al.*, 1987), making it the best available method for studies with human muscle biopsy specimens (Fraser & McKenna, 1998). Values of between ~ 200 and 300 $nmol.min^{-1}$ (g wt weight)$^{-1}$ have been reported in resting human muscle (Fraser *et al.*, 2002; Leppik *et al.*, 2004; Petersen *et al.*, 2005), although this differs with training status (Fraser *et al.*, 2002).

2.7.5 Na^+,K^+ATPase isoforms

The α-subunit consists of a NH_2-terminal segment with 4 transmembrane spanning domains, as well as a carboxy-terminal region containing 6 membrane spanning domains (Blanco & Mercer, 1998). Both the NH_2- and carboxy-terminals of the α-subunit are exposed to the cytoplasm (Blanco & Mercer, 1998). The α-subunit contains the binding sites for Na^+, K^+ and ATP, located in the cytoplasmic middle region, and cardiac glycosides such as ouabain, located between the transmembrane segments 1 and 2 (Blanco & Mercer, 1998).

The β-subunit is characterised by a single transmembrane spanning segment and only a short NH_2-terminal exposed to the cytoplasmic side (Geering, 1991). All of the β-

isoforms share a common basic structure ((Fambrough et al., 1994; Blanco & Mercer, 1998). Each β-isoform is heavily glycosylated, the mammalian β_1-isoform has 3 N-linked

Figure 2.6 Scheme of the membrane topology of the α- and β-isoforms of the Na-K-ATPase. Residues are coloured to indicate the amino acid homology among the different α-isoforms (1, 2, 3, and 4) or β-isoforms (1, 2, and 3) (Blanco & Mercer, 1998).

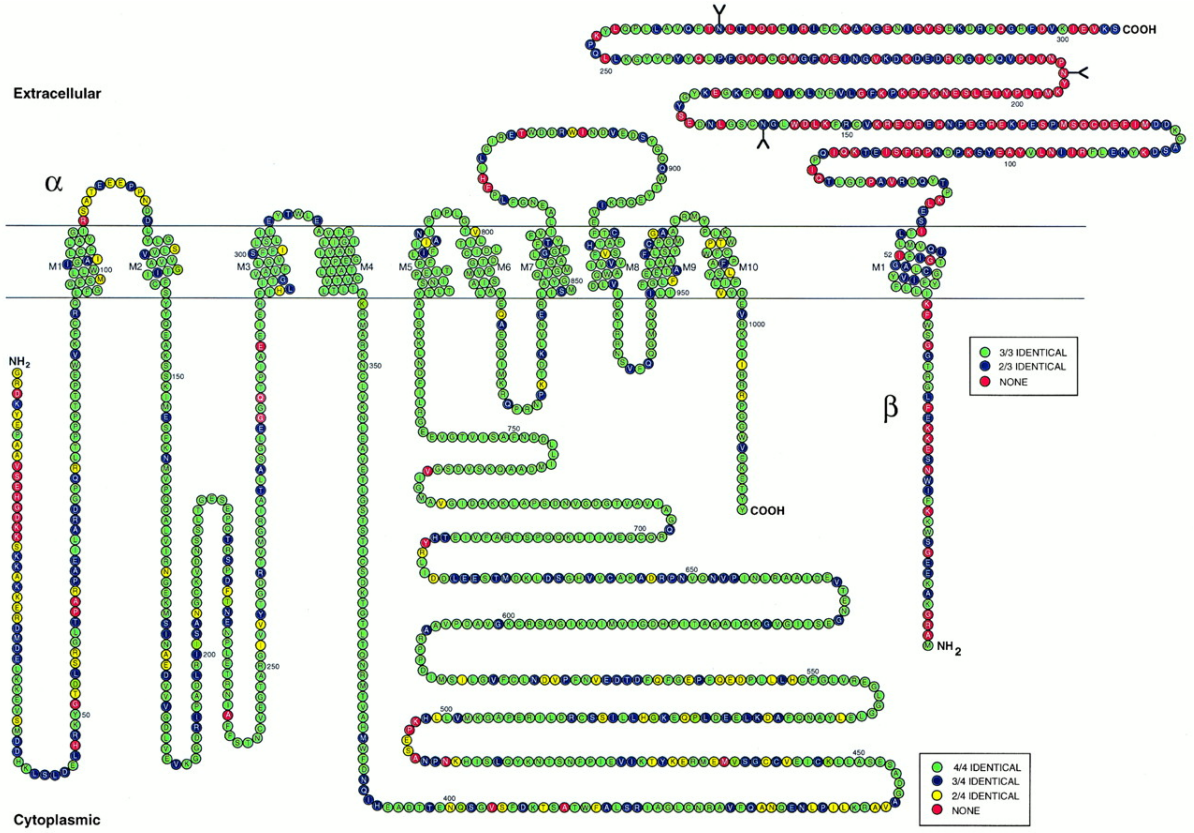


Table 2.3 [^{3}H]-ouabain binding site content in human skeletal muscle biopsies. Data is Mean ±SEM.

Control [^{3}H]-ouabain binding site content pmol.(g wet wt)$^{-1}$	**Reference**
223±13	(Schmidt *et al.*, 1994)
243±13 (Women)	(Green *et al.*, 2001)
258±13	(Gullestad *et al.*, 1995)
258±16	(Dørup *et al.*, 1988)
268±19 (Men)	(Green *et al.*, 2001)
276±19	(Klitgaard & Clausen, 1989)
278±15	(Nørgaard *et al.*, 1984)
281±14 (Women)	(Evertsen *et al.*, 1997)
289±22	(Green *et al.*, 1999)
306±26	(Leivseth & Reikeras, 1994)
306±27	(Leivseth *et al.*, 1992)
307±43	(Madsen *et al.*, 1994)
311±41	(Fraser *et al.*, 2002)
317±37	(Haller *et al.*, 1998)
333±19	(McKenna *et al.*, 1993)
333±20	(Leppik *et al.*, 2004)
339±16	(Green *et al.*, 1993)
343±11(Men)	(Evertsen *et al.*, 1997)
348±12	(Green *et al.*, 2000)
425±11	(Medbø *et al.*, 2001)

glycosylation sites while the number of glycosylation sites for the β_2-isoform is species-dependent (Blanco & Mercer, 1998). Human β_2-isoforms have 8, while the rat β_2-isoform displays 7 sites for glycosylation (Blanco & Mercer, 1998). The glycosylation of the β-subunit is important for protein folding and therefore the formation of a functional Na^+,K^+ATPase unit (Blanco & Mercer, 1998).

The γ-subunit of the Na^+,K^+ATPase is actually a member of the FXYD family (FXYD2) of proteins (Crambert & Geering, 2003; Geering *et al.*, 2003; Horisberger, 2004). The FXYD2 is associated especially with the $\alpha_1\beta_1$ Na^+,K^+ATPase isozymes, and appears to modulate its transport activity in a tissue-specific manner (Crambert & Geering, 2003).

2.7.5.1 Na^+,K^+ATPase isoforms and ouabain sensitivity

Human Na^+,K^+ATPase isoforms have heterogeneous ouabain sensitivities (Blanco & Mercer, 1998). Interestingly, in rat skeletal muscle, the α_1-isoform has an ~100-fold lower apparent affinity for ouabain than the α_2- or α_3-isoforms (Sweadner, 1979, 1985). The implication of this lower affinity is a potential underestimation of Na^+,K^+ATPase content when using the [^{3}H]-ouabain binding sites assay with rat skeletal muscle as the α_2 isoform has been reported to be more abundant than the the α_1 isoform by between 2.5, and 4 to 1 (McDonough & Thompson, 1996), or 3:1 (Hansen, 2001)..

2.7.5.2 Na^+,K^+ATPase isoform Na^+, K^+ and ATP affinities

The Na^+,K^+ATPase α_1- and α_2- isoforms have similar affinities for Na^+ ($K_{1/2}$ = 3.5 – 5mM), whereas the α_3-isoform had a 2 - 3 fold lower Na^+ affinity (Orlowski & Lingrel, 1988; Jewell & Lingrel, 1991). In contrast, the α_3 isoform has a high affinity to the nucleotide ATP compared to α_1 and α_2 (Blanco & Mercer, 1998). The apparent affinities of the α_1- and α_2-isoforms for K^+ do not differ, while the α_3-isoform has a 2.5-fold lower affinity for K^+ (Jewell & Lingrel, 1991).

2.7.5.3 Na^+,K^+ATPase isoform specific functions

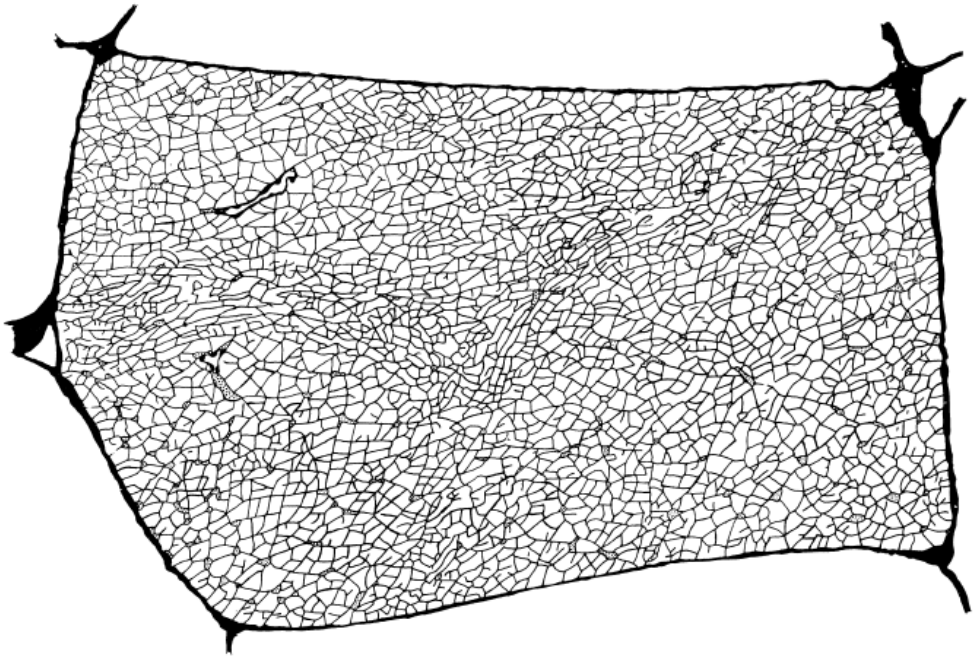
Individual Na^+,K^+ATPase isoforms have differential functions, regulation and transcription factors (Crambert *et al.*, 2000; McDonough *et al.*, 2002; Ng *et al.*, 2003; Green *et al.*, 2004). The Na^+,K^+ATPase α_1- and α_2-isoforms are the most abundant of all Na^+,K^+ATPase isoforms expressed in skeletal muscle (Orlowski & Lingrel, 1988). The α_1 Na^+,K^+ATPase isoform has been thought to regulate basal Na^+,K^+ATPase activity (Blanco & Mercer, 1998). Further, the α_1 Na^+,K^+ATPase isoform expression is upregulated when α_2 isoform expression is lowered by approximately 50% in α_2 gene targeted mice, thus showing the adaptability of the α_1 isoform (He *et al.*, 2001). The α_2-isoform is able to vary its catalytic activity in the presence of fluctuating K^+ availability (McDonough *et al.*, 1992; McDonough *et al.*, 1994). Thus the α_2-isoform is best suited to maintain transmembranous $[Na^+]$ and $[K^+]$ gradients during periods of greater cellular stress such as intense exercise (Blanco & Mercer, 1998), or periods of K^+ deprivation (McDonough *et al.*, 2002). Further, it has been suggested that the Na^+,K^+ATPase α_3 isoform is more likely to be stimulated during fatiguing exercise which lowers muscle [ATP] (Blanco & Mercer, 1998). In gene targeted mice, deletion of half of the Na^+,K^+ATPase α_2 isoforms facilitated an increased muscle force production, possibly through inhibition of the Na^+/Ca^{2+} exchanger, and a greater sarcoplasmic reticulum $[Ca^{2+}]$ (He *et al.*, 2001).

The Na^+,K^+ATPase β-isoforms are necessary for the structural and functional maturation of the α-subunit, localisation to the plasma membrane (Ackermann & Geering, 1990; Noguchi *et al.*, 1990; Hundal *et al.*, 1994), and modulating the activity of the Na^+,K^+ATPase through influencing Na^+,K^+ATPase cation sensitivity (Blanco & Mercer, 1998). It is possible that the β_2-isoform may have an additional role as an adhesion molecule where it is tightly bound to α_2 (Ewart & Klip, 1995).

2.7.6 Na^+,K^+ATPase location

The localisation of Na^+,K^+ATPase in the sarcolemma and t-tubular system skeletal muscle has been determined using giant sarcolemmal vescicles (Juel *et al.*, 2001), isolated membrane preparations, differential centrifugation of muscle homogenates (Caswell *et al.*, 1976) and immunoflourescence labelling (Fambrough & Bayne, 1983; Williams *et al.*, 2001). Measured using the [^{3}H]-ouabain binding sites method, ~80% of skeletal muscle Na^+,K^+ATPase were found to be localised to the plasma membrane, with the transverse-tubules contain the remaining 20% (Venosa & Horowicz, 1981). However, glycerol pre-treatment is unlikely to completely disrupt the t-tubular connections to the sarcolemma causing a gross underestimation of the density of Na^+,K^+ATPase in the t-tubular system (Clausen, 2003). Further, in frog skeletal muscle the amount of Na^+,K^+ATPase in the t-tubular system was 24% greater than the sarcolemma (Jaimovich *et al.*, 1986). Additionally, Na^+,K^+ATPase activity has been reported as being only ~15% higher in sarcolemmal compared to t-tubular muscle fractions (Mitchell *et al.*, 1983; Hidalgo *et al.*, 1986). Importantly, however, the t-tubules represent a surface area 4 - 5 times greater than that of the sarcolemma in a complex arrangement of tubules (Figure 2.7). Therefore, when expressed per unit area the number of Na^+,K^+ATPase within the t-tubules is probably similar in magnitude to the sarcolemma. Therefore, over the entire cell, most Na^+,K^+ATPase are probably in the t-tubules (Clausen, 2003).

Figure 2.7 Cross-section of a single frog muscle fibre, detailing the extensive and circuitous network of the t-tubular system (Peachey & Eisenberg, 1978).



Evidence that the t-tubules contain Na^+,K^+ATPase stems from single muscle fibre studies. Where the sarcolemma is removed and the t-tubule system sealed, concentration gradients for Na^+ and K^+ are maintained, and this response was abolished when Na^+,K^+ATPase were inhibited (Costantin & Podolsky, 1967). Isolated t-tubular membranes also bind [^{3}H]-ouabain (Caswell *et al.*, 1976; Lau *et al.*, 1977).

2.7.6.1 Isoform specific location

Early studies which examined isoform localisation were performed in rat skeletal muscle (Orlowski & Lingrel, 1988; Hundal *et al.*, 1992; Gick *et al.*, 1993). Large quantities of muscle are required to perform the fractionation procedure required to identify Na^+,K^+-ATPase isoform subcellular locations. Furthermore, as little as 0.2% of the plasma membrane can be recovered (Clausen, 1986), thus raising questions about the validity of the procedure (Clausen, 1986; Hansen & Clausen, 1988). In one study,

large quantities of muscle using 20 - 30g of human soleus muscle from amputated limbs were obtained (Hundal *et al.*, 1994). This study utilised a range of techniques including combining fractionation with isoform-specific antibodies, as well as immunocytochemical localisation using antibodies (Hundal *et al.*, 1994). From these techniques, it was found that α_1-isoforms were almost exclusively located in the plasma membrane (Hundal *et al.*, 1994). However, some caution needs to be taken when interpreting this result, as ~4% of the total protein content of the α_1-isoform from the plasma membrane was additionally detected in the so-called intracellular membrane fraction (Hundal *et al.*, 1994). The α_2-isoform was located in both the plasma- and intracellular-membrane fractions with the plasma membrane containing a 25% greater content than the intracellular membrane fraction (Hundal *et al.*, 1994). There was also a large relative abundance of the α_3-isoform in the plasma membrane (Hundal *et al.*, 1994). An immunogold labelling technique was recently applied to demonstrate that the Na^+,K^+ATPase α_1- and α_2-isoforms were co-located with β-spectrin and ankyrin in costameres, structures at the sarcolemma that lie over the Z and M-lines in rat fast twitch muscle fibres (Fig. 2.8, Williams et al., 2001).

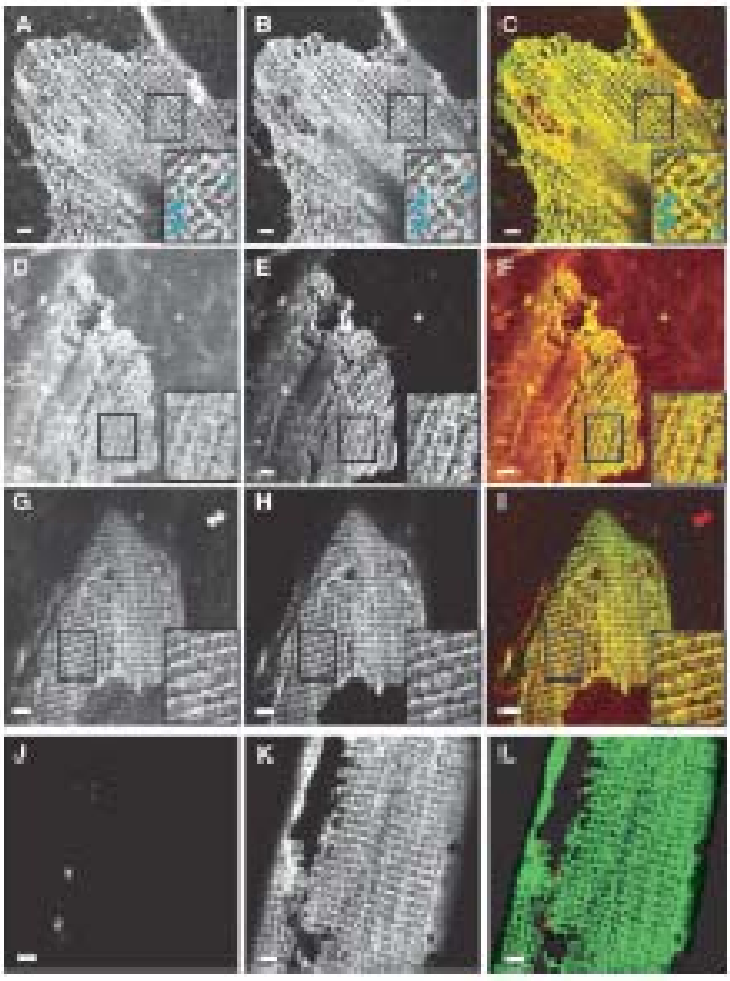
The β_1-isoform was located in both the plasma membrane and intracellular membrane fraction, with ~33% found in the plasma membrane of amputated human muscle (Hundal *et al.*, 1994). In this study the β_2-isoform was not detected (Hundal *et al.*, 1994), however it has since been detected in human vastus lateralis muscle (Juel *et al.*, 2000a). Both α_3- and β_3-isoforms have also since been detected (Murphy *et al.*, 2004), although their exact location isn't known.

2.7.6.2 Fibre-type specific location

It appears that Na^+,K^+-ATPase isoform expression is fibre-type specific in rat skeletal muscle, although there is some controversy regarding this (Hundal *et al.*, 1993;

Thompson & McDonough, 1996; Fowles *et al.*, 2004). The α_1-and α_2-isoforms were equally distributed in both fast-glycolytic and slow-oxidative muscle fibres in two studies (Hundal *et al.*, 1993; Thompson & McDonough, 1996). In apparent contrast, the relative distribution of both α_1- and α_2-isoforms was highest in rat soleus, and red gastrocnemius compared to EDL and white gastrocnemius muscle (Fowles *et al.*, 2004). The expression of β_1-isoforms was ~ 5-fold higher in slow soleus compared to fast-twitch EDL muscle in rats (Hundal *et al.*, 1993; Fowles *et al.*, 2004) and also higher in red gastrocnemius compared with white gastrocnemius muscle (Fowles *et al.*, 2004). In contrast, β_2-isoforms were ~3-fold higher in fast-glycolytic EDL and white gastrocnemius fibres than in slow-oxidative soleus and red gastrocnemius muscle fibres (Hundal *et al.*, 1993; Fowles *et al.*, 2004).

Figure 2.8 Colocalisation of β-spectrin, ankyrin 3 and the α_1 and α_2 subunits of the Na^+,K^+ATPase in costameres.



Individual samples were double labeled with polyclonal rabbit antibodies to the α_1 (A), and α_2 (D) subunits of the Na^+,K^+ATPase, or with monoclonal antibodies to ankyrin 3 (G). Nonimmune rabbit serum was used as a control (J). Composite images were constructed with β-spectrin shown in green, the other antigens in red, and areas with both proteins in yellow (C,F,I,L). The results show that all four proteins codistribute in the rectilinear, costameric lattice. Bars, 5 mm. Insets in A-C: twofold magnifications of the boxed regions indicate the domains of costameres: longitudinal strands (arrowhead), Z line domains (large arrows) and M line domains (small arrow).

2.7.7 Na^+,K^+ATPase synthesis

The mechanism for increased Na^+,K^+ATPase mRNA expression is unknown, although it may be due to increased intracellular Na^+ (Wolitzky & Fambrough, 1986) and / or Ca^{2+} concentrations (Rayson, 1991). Synthesis of the Na^+,K^+-ATPase begins when α- and β-genes are transcribed independently into RNA's, processed into mRNA, translated into nascent subunit proteins, and subjected to a second signal sequence recognition (Jungnickel & Rapoport, 1995) before being translocated into the SR (Bovia & Strub, 1996). The Na^+,K^+ATPase α and β subunits are transcribed independently into RNA's and then processed into mRNA and then co-translationally inserted into the endo(sarco)plasmic reticulum (SR) membrane (Noguchi et al., 1990).

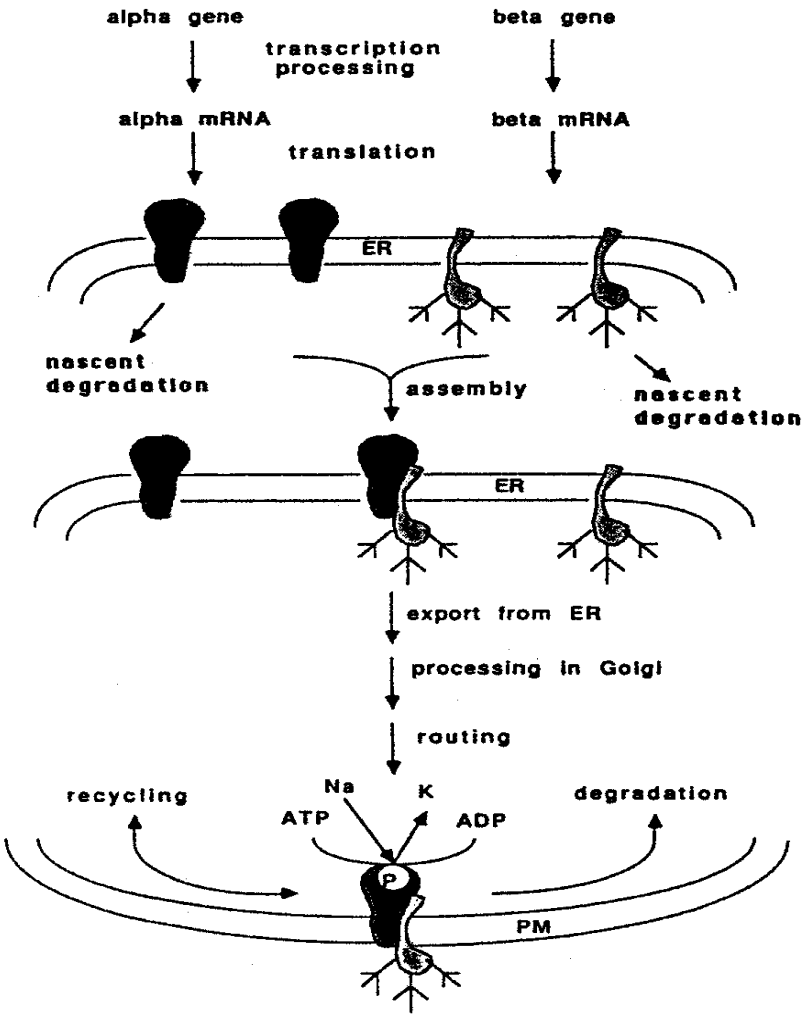
In the SR membrane, synthesis of the α- and β-subunits takes place with subunit specific mRNA and ER membrane locations (Hiatt et al., 1984).

The α-mRNA only achieves correct insertion into the membrane when the β-subunit is present and acting as a receptor or stabiliser (Noguchi *et al.*, 1990). Once assembled, the αβ complex leaves the ER, is processed in the golgi compartment, taking up to 60 min (Tamkun & Fambrough, 1986), and then exported to co-appear at the plasma membrane (Figure 2.9) (Hiatt *et al.*, 1984; McDonough *et al.*, 1990).

2.7.8 Acute activation of the Na^+,K^+ATPase

The Na^+,K^+ATPase is rapidly stimulated at the commencement of muscle contraction, and attenuates the excitation-induced rise in $[Na^+]_i$ and $[K^+]_e$ (Clausen, 1996b).

Figure 2.9 Synthesis, insertion and formation of functional Na^+,K^+ATPase αβ complexes in the endoplasmic reticulum (ER) and plasma membrane (PM) of muscle (McDonough et al., 1990).



Na^+,K^+ATPase activation is facilitated through a combination of electrical, ionic and hormonal factors (Fig. 2.9, Clausen, 1998), as discussed in the following sections.

2.7.8.1 Magnitude of activation

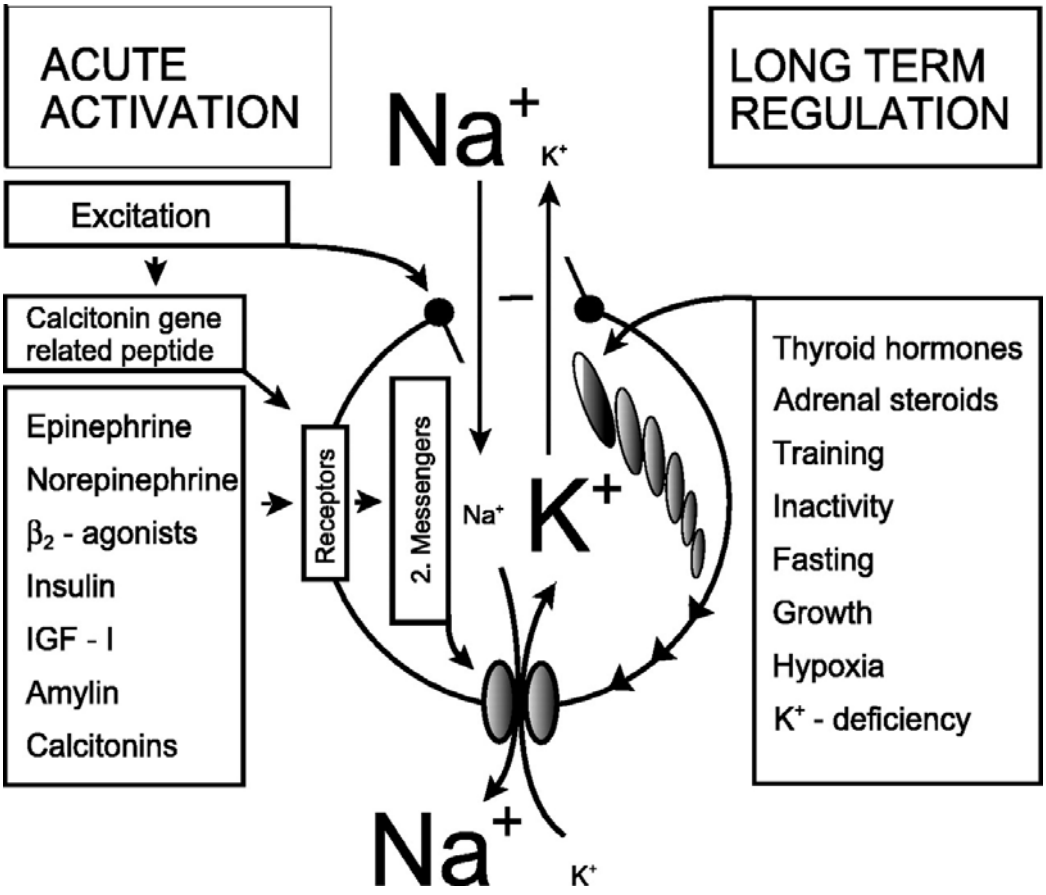
At rest, the Na^+,K^+ATPase is operating at only a few percent of its theoretical maximum activity (Clausen *et al.*, 1987; Clausen & Everts, 1989; Everts & Clausen, 1994). The theoretical maximum Na^+,K^+ATPase activity in muscle has been calculated as being ~17,000 $nmol.min^{-1}(g\ wet\ wt)^{-1}$(Plesner & Plesner, 1981). Electrical stimulation of the

Na^+,K^+ATPase creates the largest acute Na^+,K^+ATPase activation, much greater than that occurring through hormonal activation (Clausen & Nielsen, 1994; Clausen, 1996a), as discussed in the following section.

2.7.8.2 Electrical activation of the Na^+,K^+ATPase

Progressive stimulation of isolated muscle leads to stimulation dependent increases in Na^+,K^+ATPase activity. During 20 minutes of 4 Hz stimulation of isolated perfused dog tibialis cranialis and extensor digitorum longus muscle, Na^+,K^+ATPase activity was increased by ~ 65% (Hazeyama & Sparks, 1979). One minute of 40 Hz stimulation of isolated mouse soleus muscle increased Na^+,K^+ATPase activity ~ 10-fold (Juel, 1986) In isolated rat soleus muscle, only 10 s of stimulation at a frequency of 60 Hz increased the net extrusion of Na^+ by ~12-fold above resting values, which corresponded to ~60% of the theoretical maximum capacity of the Na^+,K^+ATPase (Clausen & Nielsen, 1994; Everts & Clausen, 1994). Further increasing the stimulation frequency to 120 Hz resulted in an ~22-fold increase in Na^+,K^+ATPase activity, which corresponds to ~100% of the expected maximum Na^+,K^+ATPase activity (Clausen & Nielsen, 1994; Everts & Clausen, 1994). This suggests that excitation can induce maximal theoretical Na^+,K^+ATPase activity, at least in isolated rat soleus isolated muscle during unloaded contractions (Clausen, 1996b). Isometric contractions in rat soleus muscle stimulated at 60-120 Hz for 30 s increased Na^+,K^+ATPase activity by ~60% above control, a total increase of 5-10 fold above rest, indicating a lower level of stimulation after isometric contractions (Nielsen & Clausen, 1997). Muscle Na^+,K^+ATPase activity may, therefore attain near-maximal theoretical levels in rat muscle, when stimulated at high frequencies (Nielsen & Clausen, 1997; McKenna et al., 2003). However, motor-unit discharge rates in contracting human muscles are likely to be at lower frequencies (Jones, 1996; Roos et al., 1999) and the Na^+,K^+ATPase probably does not attain its maximum theoretical activity in vivo (Hallén et al., 1994; Sejersted & Sjøgaard, 2000).

Figure 2.10 Diagram of regulatory factors controlling the activity and contents of Na^+,K^+-pumps in skeletal muscle (Clausen, 1998).



2.7.8.3 Ionic activation of the Na^+,K^+ATPase

The $[K^+]_e$ that stimulates the Na^+,K^+ATPase to 50% of its maximum pump rate ($k_{0.5}$) obtained from isolated cells is approximately 0.8-1.5 mM (Cohen *et al.*, 1987). At a normal $[K^+]_e$ of ~ 4.0 mmol, the extracellular K^+ site of the Na^+,K^+ATPase will therefore be approximately 80% saturated, indicating that increased $[K^+]_e$ plays only a small role in Na^+,K^+ATPase stimulation (Sejersted & Sjøgaard, 2000). Furthermore, a rise in the extracellular $[K^+]$ to 20 – 50 mM only induces a 16 - 28% increase in Na^+,K^+ATPase activity (Everts & Clausen, 1994). This $[K^+]_e$ is much higher than recorded *in-vivo* in humans (Vyskocil *et al.*, 1983; Green *et al.*, 1999c; Green *et al.*, 2000e; Nordsborg *et al.*, 2003b). Intracellular $[Na^+]$ is, on the other hand, a strong activator of the Na^+,K^+ATPase (Juel, 1986), although more recently other independent

factors regulating acute activation have also been reported (Clausen, 2003). Under resting conditions with $[Na^+]_i$ ~ 6 mmol.L^{-1}, Na^+,K^+ATPase activity remained low (Everts & Clausen, 1994). When $[Na^+]_i$ was increased by ~60% via a 60 Hz tetanic stimulation, ouabain suppressible Na^+ efflux increased by ~50% (Everts & Clausen, 1994). It was reasoned that the increase in Na^+,K^+ATPase activity with a rise in $[Na^+]_i$ was related directly to the opening of Na^+ channels (Everts & Clausen, 1994). Consistent with this, Na^+,K^+ATPase activation was blocked when Na^+ channels were specifically inhibited by tetrodoxin (Everts & Clausen, 1994). Furthermore, increased Na^+,K^+ATPase activity occurred through the application of the Na^+ ionophore veratridine (Everts & Clausen, 1994). However, in electrically stimulated rat muscle, Na^+,K^+ATPase activity increased greatly, even though $[Na^+]_i$ had returned to resting levels (Nielsen & Clausen, 1997). Maximum stimulation of the Na^+,K^+ATPase can be achieved with $[Na^+]_i$ at just 10 mmol.L^{-1} (Nielsen & Clausen, 1997), indicating that any further rise in $[Na^+]_i$ may not further stimulate the pump (Everts & Clausen, 1994). Furthermore, the net extrusion of Na^+ following 30 s stimulation of rat soleus muscle continued to be high despite $[Na^+]_i$ falling to ~30% below resting levels.

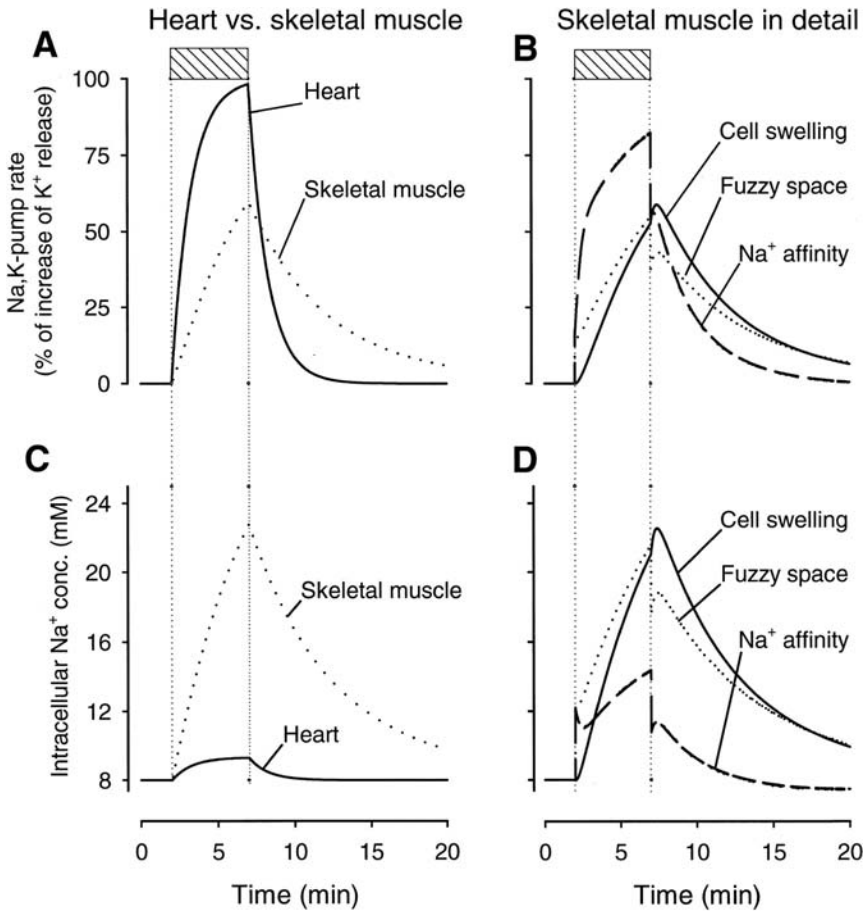
The notion of a sub-sarcolemmal ‘fuzzy space’ has been suggested as contributing to an increased Na^+,K^+ATPase activation in spite of an unchanged $[Na^+]_i$ (Semb & Sejersted, 1996). The explanation of the fuzzy space (Fig. 2.11) is that there are localised areas of high $[Na^+]$ just below the cell membrane where diffusion of Na^+ is slower and pump activity is stimulated. The effects of the ‘fuzzy space’ may be attenuated by an influx of water to the muscle at the onset of exercise (Saltin *et al.*, 1981; Lindinger *et al.*, 1987a), which would in turn attenuate the rise in $[Na^+]_i$ and therefore activation of the Na^+,K^+ATPase (Sejersted & Sjøgaard, 2000). It is also possible that the affinity of the Na^+,K^+ATPase to $[Na^+]_i$ is increased transiently during exercise, again leading to an increased activation (Sejersted & Sjøgaard, 2000).

2.7.8.4 Acute activation by catecholamines

During exercise, an increase in sympathetic nervous system activity stimulates the release of the catecholamines adrenaline and noradrenaline from the the adrenal glands and sympathetic nerve endings (Kjær, 1989; Mazzeo, 1991). Elevated levels of catecholamines increase Na^+,K^+ATPase activity, although this excitatory effect is not additive to the effects of electrical stimulation (Everts *et al.*, 1988). Assessment of catecholamine effects can be made by the use of β-receptor blockade.

Catecholamines stimulate Na^+,K^+ATPase activity via β_2-adrenoreceptor-mediated stimulation of adenylate cyclase, or the second messengers cAMP and protein kinase C (Fig. 2.12) (Clausen & Hansen, 1977). The importance of catecholamine stimulation of Na^+,K^+ATPase activity was highlighted when β-adrenergic blockade exacerbated the exercise-induced hyperkalaemia at the onset of exercise (Hallén et al., 1994). The effect of catecholamine-mediated increases in Na^+ and K^+ transport, measured via $^{86}Rb^+$ uptake, were more pronounced in fast twitch than in slow twitch muscle fibres (Everts et al., 1988). Catecholamine stimulation of the Na^+,K^+ATPase seems important in the transition from rest to exercise, but may be less important as contractile activity continues (Hallén et al., 1996).

Figure 2.11 Simulation of Na^+-K^+ pump activation in heart and skeletal muscle.



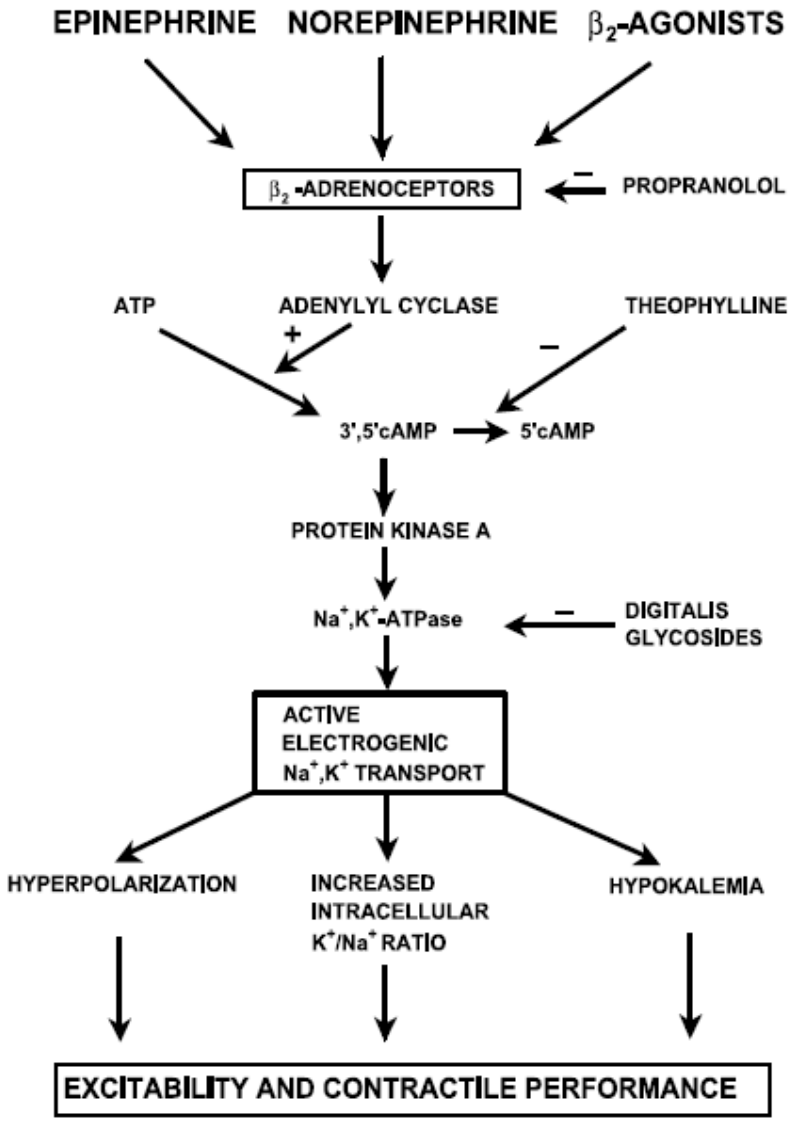
The hatched area marks the period of increased stimulation. A and C: Na^+-K^+ pump rate and intracellular Na^+ concentration ($[Na^+]_c$) during and after a period of increased activity. The mean affinity constant ($k_{0.5}$) was set to 15 mM in the heart and 30 mM in skeletal muscle. In the heart, a small rise of $[Na^+]_c$ (C) was sufficient to activate the pump to match K^+ release (A). In skeletal muscle, a rise of $[Na^+]_c$ of ~15 mM (C) only caused pump rate to match slightly more than 50% of the release rate (A). B and D: Na^+-K^+ pump in skeletal muscle simulated in more detail. The effects of cell swelling and the existence of intracellular Na^+ gradients (fuzzy space) have been successively included in the simulation. Compared with the skeletal muscle simulation in A and C (dotted line), the effects of these two additional factors seem moderate. (Sejersted & Sjøgaard, 2000).

2.7.8.5 Acute activation by insulin and IGF-1

The peptide hormone insulin is released from the pancreas, and increases ouabain-suppressible Na^+ and K^+ transport (Erlij & Grinstein, 1976; Clausen & Kohn, 1977; Clausen et al., 1993). In isolated muscle preparations, insulin increased Na^+,K^+ATPase activity (Clausen & Flatman, 1987) and increased Na^+ efflux by up to 70% (Creese, 1968; Chinet & Clausen, 1984). Insulin also increased radiolabelled ^{42}K influx in rat soleus muscle by ~20% (Flatman & Clausen, 1979). It is thought that insulin acts to increase Na^+,K^+ATPase activity by increasing the Na^+,K^+ATPase affinity for Na^+, or by directly increasing $[Na^+]_i$ (Ewart & Klip, 1995; Sweeney & Klip, 1998). Interestingly, hyperkalaemia increases insulin secretion, which in turn will facilitate K^+ re-uptake by muscle, thus providing an elegant negative feedback mechanism (Clausen & Everts, 1989).

In skeletal muscle, IGF-I has significant structural homology with proinsulin (Clausen, 2003). In isolated rat soleus muscle IGF-1 stimulated ^{22}Na efflux as well as ^{42}K and ^{86}Rb influx, thus indicating increased Na^+,K^+ATPase activity (Dørup & Clausen, 1995). In this study the effects of IGF-1 and insulin were not additive, but those of IGF-1 and adrenaline were (Dørup & Clausen, 1995). In humans, IGF-1 induced hypokalemia, suggesting a role for IGF-1 in regulating Na^+,K^+ATPase activity (Giordano & DeFronzo, 1995).

Figure 2.12 The actions of β_2-agonists and catecholamines on active Na^+,K^+ transport (Clausen, 2003).



2.7.8.6 Activation by calcitonin gene related peptide (CGRF)

Calcitonin Gene Related Peptide (CGRP) is an amino-acid peptide, is present in a variety of neurons, and can be released during electrical stimulation, or exposure to high extracellular [K^+] (Clausen, 2003). CGRP binds to sarcolemmal receptors, and triggers adenylate cyclase activity, leading to a rise in cAMP (Takami et al., 1986). The second-

messenger cAMP mediates the stimulatory effects of catecholamines on the Na^+,K^+ATPase (see Figure 2.12) (Clausen, 2003).

2.7.9 Translocation of Na^+,K^+ATPase

It has been suggested that both insulin administration and a single bout of exercise increased Na^+,K^+ATPase isoform abundance at the plasma membrane in skeletal muscle and thus served as a mechanism for increased Na^+,K^+ATPase exchange (Hundal *et al.*, 1992; Lavoie *et al.*, 1996; Tsakiridis *et al.*, 1996; Juel *et al.*, 2000a; Juel *et al.*, 2001; Al-Khalili *et al.*, 2003).

2.7.9.1 Insulin induced translocation of Na^+,K^+ATPase

In isolated frog sartorius muscle, incubation with insulin increased plasma membrane [^{3}H]-ouabain binding, as well as active Na^+ transport, despite pre-treatment of the basal Na^+,K^+ATPase with ouabain (Grinstein & Erlij, 1974). This suggested a movement of Na^+,K^+ATPase enzymes from an intracellular source to the plasma membrane (Grinstein & Erlij, 1974).

In frog skeletal muscle, the [^{3}H]-ouabain binding capacity of plasma membranes was increased by ~2-fold after pre-treatment with detergent (Omatsu-Kanbe & Kitasato, 1990). Two studies using membrane fractionation have reported an ~2-fold increase in the α_2-isoform relative abundance at the surface membrane, with a corresponding 30–40% reduction in the intracellular α_2-isoform protein levels (Hundal *et al.*, 1992; Marette *et al.*, 1993). Similarly, Na^+,K^+-ATPase β_1-isoform relative abundance rose 2-fold at the plasma membrane with insulin treatment, although no concomitant reduction in the intracellular levels was observed (Hundal et al., 1990). Translocation of Na^+,K^+ATPase α_2- and β_1-isoforms from an undefined intracellular pool to the plasma membrane were reported in response to insulin, as measured by immunogold labelling, but only in slow-twitch muscle fibres (Lavoie *et al.*, 1996).

Insulin-induced translocation of Na^+,K^+-ATPase isoforms to the plasma membrane in skeletal muscle is conceptually similar to the recruitment of the glucose transporter, GLUT-4, by insulin (Marette *et al.*, 1992). However, the Na^+,K^+ATPase α_2-isoform and GLUT-4 were not found to be may be co-localised in the same single intracellular pool (Aledo & Hundal, 1995; Lavoie *et al.*, 1995). Further, using the cell surface biotinylation technique, insulin induced translocation of α_1 and α_2-subunits was noted in both rat epiorchlearis muscle and cultured human skeletal muscle (Al-Khalili *et al.*, 2003).

In contrast to the above findings, rat soleus muscle incubated in 100 mU ml^{-1} insulin for 4 hours did not have a higher number of [^{3}H]-ouabain binding sites compared to control muscles (Clausen & Hansen, 1977). Similarly, Insulin (100 mU ml^{-1}) decreased intracellular Na^+ content by 27 % and increased ^{86}Rb uptake by 23 % in soleus muscles, but [^{3}H]-ouabain binding was unchanged (McKenna *et al.*, 2003). Since the isoforms proposed to be translocated in response to insulin are the α_2- and β_1-isoforms, and α_2-isoforms have a higher ouabain sensitivity it is equivocal as to whether insulin-induced translocation of fully functional Na^+,K^+ATPase units occurs.

2.7.9.2 Contraction- induced translocation of Na^+,K^+ATPase

Three studies have investigated the possibility of contraction-induced Na^+,K^+ATPase translocation (Tsakiridis *et al.*, 1996; Juel *et al.*, 2000a; Juel *et al.*, 2001). In the first study, slow- and fast-twitch muscles were obtained from both control rats and rats exercised for 1 hour of submaximal treadmill running. Na^+,K^+ATPase isoform mRNA and protein contents at the plasma-, crude- and intracellular-membrane fractions of the exercising rats were quantified using fractionation, Northern and immunoblotting and normalised relative to the resting control group. In both red and white muscle fibres, exercise induced an increase in the relative protein abundance at the plasma membrane

of the α_1- (64 and 55% respectively) and α_2-isoforms (43 and 94% respectively) (Tsakiridis *et al.*, 1996). However, no change was observed in the protein contents for these isoforms in the crude or intracellular membranes (Tsakiridis *et al.*, 1996). The authors suggested that the isoforms may have been translocated from a separate, so far undetected intracellular pool (Tsakiridis *et al.*, 1996).

In the second study using humans, just 4.6 min of knee extensor exercise continued until fatigue increased the plasma membrane relative abundance of α_2-and β_1-isoforms by 70 and 26%, respectively; however, no change was detected in α_1 (Juel *et al.*, 2000a). The third study reported a ~30% increase in [^{3}H]-ouabain binding sites in mixed rat muscles after low-intensity treadmill running and a 13–32% increase in the α_1-, α_2-, and β_2-isoforms in sarcolemmal giant vesicles obtained from oxidative muscle fibres, with these increases almost completely abolished after 30-min recovery (Juel *et al.*, 2001). However, in sarcolemmal vesicles only 0.3% of the total amount of Na^+,K^+ATPase present in the tissue were available for quantification, (Juel *et al.*, 2001), raising questions of the representativeness of the sample across the whole muscle (Clausen, 2003).

Recently, the effects of electrical stimulation at a wide variety of frequencies and durations on the total [^{3}H]-ouabain binding sites of isolated rat soleus and EDL muscles were characterised (McKenna *et al.*, 2003). Despite a large percentage of all Na^+,K^+ATPase pumps being activated, no increase in [^{3}H]-ouabain binding sites after electrical stimulation could be detected.

When examining the effects of insulin and contraction on translocation, the principal difficulty in obtaining consistent results lies in the process of membrane fractionation which is required for qualitative measurements of the Na^+,K^+-ATPase isoforms pre- and post-insulin and contraction treatments. Fractionation typically yields only a 0.2 – 8.9%

recovery of all muscle Na^+,K^+ATPase, thereby prompting skepticism over the degree of representation the recovery has on the entire membrane fraction, as well as validity of the results (Clausen, 1986). The muscles utilised in two studies not supportive of translocation had total protein yield meaning a complete recovery of all functional Na^+,K^+ATPase units and thus do not suffer from the criticisms of the fractionation technique (Clausen & Hansen, 1977; McKenna *et al.*, 2003). As supported by immunogold labeling studies, translocation of Na^+,K^+ATPase isoforms may occur in skeletal muscle, but possibly not of functional Na^+,K^+ATPase units, quantifiable with the [^{3}H]-ouabain binding site content assay.

2.7.10 Chronic regulation of the Na^+,K^+ATPase

Numerous factors regulate the Na^+,K^+ATPase content in the longer term, including hormonal influences, physical activity and nutrition levels (see figure 2.9) (Clausen, 2003), and these will be discussed in the following sections of this review.

2.7.10.1 Chronic regulation by thyroid hormones

The effects of thyroid hormones on Na^+,K^+ATPase have been extensively reviewed (Clausen, 1986; Ewart & Klip, 1995). In hyperthyroid rats, Na^+,K^+ATPase content in the soleus muscle was approximately 10-fold larger in hypothyroid rats (Kjeldsen et al., 1986a). The addition of thyroid hormones, or increased thyroid hormone levels in hyperthyroidism increase Na^+,K^+ATPase content in both rat and human skeletal muscle (Lin & Akera, 1978; Nørgaard et al., 1983; Kjeldsen et al., 1984c; Azuma et al., 1993; Riis et al., 2004). Thyroid hormones appear to affect Na^+,K^+ATPase activity in an isoform-specific manner (Azuma et al., 1993). After T3 treatment taking rat skeletal muscle from a hypothyroid to a hyperthyroid state, the α_2- and β_2-isoform relative abundance increased five- and two-fold, repectively, with no change in α_1 or β_1 (Azuma et al., 1993). In rat diaphragm muscle, T3 treatment of 50 micrograms/100 g body wt on 3 alternate days also increased the α_2 isoform relative abundance, without change in α_1

(Haber & Loeb, 1988). Fibre type seems to play a role in determining the magnitude of increase in Na^+,K^+ATPase activity with an increase in thyroid hormone concentration. In rats, slow-twitch skeletal muscle is most responsive to an increase in thyroid hormones, with an increase in both the soleus [^{3}H]-ouabain binding site content and of muscle ^{86}Rb uptake reported in a hyperthyroid state (Clausen et al., 1987).

2.7.10.2 Increase in Na^+,K^+ATPase in response to chronic electrical stimulation

Chronic electrical stimulation increases skeletal muscle Na^+,K^+ATPase content measured by [^{3}H]-ouabain binding sites, in a time-dependent manner (Green et al., 1992; Hicks et al., 1997). In rabbit EDL muscle, chronic low-frequency stimulation (10 Hz) increased [^{3}H]-ouabain binding site content by 40% after four days, and by 86% after 10 days (Green et al., 1992). This result was confirmed when 10 Hz stimulation increased rat fast-twitch muscle [^{3}H]-ouabain binding site content by 60% after 6-days, and by 100% after 20-days (Hicks et al., 1997).

2.7.10.3 Increase in Na^+,K^+ATPase content with chronic exercise (training)

Chronic physical activity, in the form of training increases [^{3}H]-ouabain binding site content in skeletal muscle in both animals and humans (Table 2.4). Seven weeks of cycle sprint training (McKenna et al., 1993) and just 6-days of cycle training at a submaximal workrate (Green et al., 1993) each increased the [^{3}H]-ouabain binding site content of untrained participants ~16% and 13% respectively. The response of Na^+,K^+ATPase to an increased exercise training stimulus is rapid, with an increase in the [^{3}H]-ouabain binding site content of previously untrained participants after just three days of intensified cycle training (Green et al., 2004).

A 15% increase in [^{3}H]-ouabain binding site content was also reported in moderately endurance trained participants as a result of increasing endurance training volume by ~23% (Madsen et al., 1994). Furthermore, [^{3}H]-ouabain binding site content was also elevated in swim (~32%), run (~25%) and strength (~40%) trained older adults, when

compared to age-matched controls (Klitgaard et al., 1989). However, only two studies have characterised the [^{3}H]-ouabain binding site response to training in well trained athletes, with increases of 16% (Evertson et al., 1997) after 5-months hard training and 15% (Medbø et al., 2001) after heavy resistance training. Importantly, in well-trained subjects, no studies have examined the Na^+K^+ATPase response to short-term intensified training. It is therefore unknown if the [^{3}H]-ouabain binding site content increase with training is as rapid (Green et al., 2004) in already well-trained participants, and is therefore examined in this thesis.

2.7.10.4 Training effects on Na^+,K^+ATPase isoforms

Training effects on the Na^+,K^+ATPase isoform transcription and translation in skeletal muscle are unclear. No one study has comprehensively investigated the effects of training in well-trained athletes on each of the α_1, α_2, α_3, β_1, β_2 and β_3 Na^+,K^+ATPase isoforms at either the mRNA or protein levels in human skeletal muscle. Four studies have investigated possible training effects on Na^+,K^+ATPase isoform gene expression or protein abundances in human muscle, but each only probed for α_1, α_2 and β_1 isoforms. Furthermore, these studies used only untrained (Nielsen et al., 2003b; Nordsborg et al., 2003a; Green et al., 2004) and unhealthy participants (Dela et al., 2004). These studies yielded conflicting results for α_1 and β_1 protein abundance (Nielsen et al., 2003b; Dela et al., 2004; Green et al., 2004). HIT for 5.5 weeks did not alter resting α_1, α_2 and β_1 mRNA expression, but blunted the α_1 mRNA upregulation that occurred with acute exercise prior to HIT (Nordsborg et al., 2003a). Thus, the effects of HIT on Na^+,K^+ATPase α_3, β_2 and β_3 mRNA or protein are unknown and are unclear for both α_1, and β_1 protein abundance.

Table 2.4 Effects of training on the [^{3}H]-ouabain binding site content in skeletal muscle, modified from (Clausen, 2003).

Species	**Training**	**Muscle analysed**	**Control [^{3}H]-ouabain binding site content pmol. (g wt weight)$^{-1}$**	**Relative change (%)**	**Ref**
Rat	6-wk prolonged swimming	EDL	288	+43	1
Rat	6-wk prolonged swimming	Gastrocnemius	265	+25	1
Guinea pig	Running	Soleus	258	+25	2
Horse	3 x wk of walking, trotting and running	Gluteus medius	163	NC	3
Horse	5 x mo of daily sprint	Gluteus medius	165	+20	4
Cattle	15 d of 2 h resistance	Semitendinosus	155	+30	5
Human	Resistance and endurance	Vastus lateralis	289	+22	6
Human	Endurance trained versus untrained	Vastus lateralis	311	+18	7
Human	Sprint	Vastus lateralis	333	+16	8
Human	5-mo intensified endurance	Vastus lateralis	343	+16	9
Human	3-mo eccentric strength	Vastus lateralis	425	+16	10
Human	6-wks enhanced endurance	Vastus lateralis	307	+15	11
Human	6-d, submaximal cycling	Vastus lateralis	339	+14	12

1: (Kjeldsen *et al.*, 1986b); 2: (Leivseth *et al.*, 1992); 3: (Suwannachot *et al.*, 2000); 4: (Suwannachot *et al.*, 2001); 5: (Veeneklaas *et al.*, 2002); 6: (Green *et al.*, 1999a); 7: (Fraser *et al.*, 2002); 8: (McKenna *et al.*, 1993); 9: (Evertsen *et al.*, 1997); 10: (Medbø *et al.*, 2001); 11: (Madsen *et al.*, 1994); 12: (Green *et al.*, 1993).

2.7.10.5 Decrease in Na^+,K^+ATPase with inactivity

Inactivity or immobilisation both dramatically reduce the content of Na^+,K^+ATPase in skeletal muscle. Just one week of plaster-induced immobilisation reduced Na^+,K^+ATPase content in rat soleus by ~20% (Kjeldsen et al., 1986b). Similarly, Na^+,K^+ATPase content was reduced by 22% in sheep vastus lateralis muscle also immobilised by plaster (Jebens et al., 1995). Guinea pig plantaris muscle Na^+,K^+ATPase content was reduced by 23% after 3-weeks plaster-induced immobilisation (Leivseth et al., 1992). In humans, deltoid muscle immobilised through shoulder impingement, had a 27% lower Na^+,K^+ATPase content compared to controls (Leivseth & Reikeras, 1994).

2.7.10.6 Effects of total body K^+ content on Na^+,K^+ATPase

Manipulation of total body K^+ content also alters Na^+,K^+ATPase function. K^+ deficiency causes a loss of [^{3}H]-ouabain binding site content of by ~ 70% in animals (Nørgaard *et al.*, 1981; Kjeldsen *et al.*, 1984b) and by ~ 18% in humans (Dørup *et al.*, 1988; Dørup *et al.*, 1993). K^+ supplementation in rats caused an increased [^{3}H]-ouabain binding site content and improved regulation of muscle and plasma K^+ homeostasis (Bundgaard *et al.*, 1997).

2.7.10.7 Effects of hypoxia or LHTL on skeletal muscle Na^+,K^+ATPase

In human skeletal muscle hypoxia induces Na^+,K^+ATPase downregulation, with [^{3}H]-ouabain binding site content lowered by ~14% in experienced mountain climbers after a 21-d sojourn to high altitude (Green et al., 2000a). Furthermore, in untrained subjects, exercise training in hypoxia (Green et al., 1999b) or acute submaximal exercise under hypoxic conditions (Sandiford et al., 2004) decreased [^{3}H]-ouabain binding site content and 3-*O*-MFPase activity by ~ 14% and ~28%, respectively. It is possible that the hypoxic stimulus causes an increase in muscle accumulation of reactive oxygen species (ROS), causing alterations to the lipid membrane where the Na^+,K^+ATPase is

embedded, (Kourie, 1998), which possibly leads to the decreases in Na^+,K^+ATPase. Given that chronic hypoxia downregulates muscle Na^+,K^+ATPase, which could then adversely affect muscle function, it seems somewhat paradoxical that athletes, coaches and physiologists have used altitude exposure for many years in a bid to improve athletic performance. It is not known if well-trained athletes are susceptible to the hypoxia-induced depression in Na^+,K^+ATPase as seen in untrained participants.

As training under even moderate hypoxia compromises training intensity (Brosnan et al., 2000), LHTL may therefore be advantageous by allowing maintenance of training intensity (Levine & Stray-Gundersen, 1992). LHTL has been shown to yield small (0.8-1.3%) improvements in the exercise performance of well trained athletes during a 4-min all-out effort, a 400 m sprint and 3000 m as well as 5000 m runs (Hahn & Gore, 2001). This raises the interesting question about whether the hypoxia-induced depression of Na^+,K^+ATPase is less or absent in LHTL (Green et al., 1999b; Green et al., 2000a). Alternately, if LHTL does reduce muscle Na^+,K^+ATPase this might be expected to adversely affect on muscular performance. Whether hypoxia imposed by LHTL induces a depression in skeletal muscle Na^+,K^+ATPase activity in well-trained athletes has not yet been investigated and is thus examined in this thesis.

2.7.10.8 The effect of gender on Na^+,K^+ATPase

Only one study has reported gender differences in Na^+,K^+ATPase, with highly trained males having an 18% greater [^{3}H]-ouabain binding site content than similarly trained females (Evertsen et al., 1997). Both genders exhibited a similar magnitude increase in Na^+,K^+ATPase content with training (Evertson et al., 1997). This contrasts with previous research which indicated no effect of gender on [^{3}H]-ouabain binding site content (Nørgaard et al., 1984a). Despite a greater $\dot{V}O_{2peak}$, the Na^+,K^+ATPase content reported for well-trained female athletes (Evertsen *et al.*, 1997) is lower than those observed in untrained young males (Green et al., 1993, McKenna et al., 1993, Evertsen

et al., 1997, Green et al., 1999). It is difficult to ascertain the exact effect of gender on Na^+,K^+ATPase, as most studies have utilised male subjects, or failed to report gender differences, if any were indeed apparent.

2.7.10.9 The effects of age on Na^+,K^+ATPase

Muscle [^{3}H]-ouabain binding site content increases with age through to adulthood, independent of training influence, in mice and guinea pigs (Kjeldsen et al., 1984a). Muscle [^{3}H]-ouabain binding site content increased by ~13% in guinea pig muscle from late-gestation before rapidly declining in the post-partum period (Dauncey & Harisson, 1996). In contrast, rat skeletal muscle [^{3}H]-ouabain binding site content increased by approximately five-fold from birth through to four weeks of age, followed by a large subsequent decrease of ~700 pmol (g wt weight)$^{-1}$ (Kjeldsen et al., 1984a).

Few studies have investigated the effects of age on [^{3}H]-ouabain binding site content in humans. One study reported no difference in [^{3}H]-ouabain binding site content in human skeletal muscle obtained post-mortem from 2 months to eight years of age (Kjeldsen & Gron, 1989). Caution should be exercised when interpreting these results as most of the participants died from sudden infant death syndrome (Kjeldsen & Gron, 1989). Young adult (25 yrs) subjects tended to have an ~14% higher [^{3}H]-ouabain binding site content than older (68 yrs) individuals, although these results were not significantly different (Klitgaard & Clausen, 1989). One other study reported no age-related differences in individuals ranging from 25 to 80 yr (Nørgaard et al., 1984a).

2.7.11 The role of the Na^+,K^+ATPase in fatigue

Under fatiguing conditions, there can be a doubling of skeletal muscle intracellular [Na^+] (Sjøgaard et al., 1985; Juel, 1986; Sejersted & Sjøgaard, 2000) and muscle extracellular [K^+] (Sjøgaard et al., 1985; Juel, 1986; Hallén, 1996; Green et al., 2000e; Juel et al., 2000b; Sejersted & Sjøgaard, 2000). The implications of these altered ion concentrations is highlighted as incubation of skeletal muscle in high K^+ solutions (10-

12.5 mM) caused a steady decline in tetanic force, which was reversed upon restoration of low [K^+] (Clausen & Everts, 1989; Clausen et al., 1993). The decline in muscle force was further augmented with a reduction in $[Na^+]_e$ (Overgaard et al., 1997; Overgaard et al., 1999). A rundown of the transmembranous Na^+ and K^+ gradients caused inactivation of voltage-dependent Na^+ channels (Ruff, 1999),. The electrogenic action of the Na^+,K^+ATPase is critical in counteracting these rapid and large ionic movements, in preserving mebrane depolarisation, and thus in protecting muscle membrane excitability.

The rate of fatigue development in isolated rat muscles exposed to high extracellular [K^+] was augmented when Na^+,K^+ATPase was specifically inhibited by ouabain (Clausen & Everts, 1989; Clausen et al., 1993). Further, fatigue was delayed under these conditions and force recovery increased when muscle Na^+,K^+ATPase was stimulated by the β-agonist salbutamol allowing a Na^+,K^+ATPase mediated decline in the fatigue-causing high extracellular [K^+] (Clausen & Everts, 1989; Clausen et al., 1993). The importance of the Na^+,K^+ATPase in attenuating fatigue was further established when stimulation of muscle Na^+,K^+ATPase by insulin or catecholamines overcame ~79% of the loss of force induced by a decreased $[Na^+]_e$ and increased $[K^+]_e$ (Overgaard et al., 1997). This recovery of force could be completely blocked with specific inhibition of the Na^+,K^+ATPase by ouabain (Overgaard et al., 1997). Ouabain treatment of muscle induced a marked reduction in force development in isolated rat muscles, which was reversible with the washout of ouabain (Nielsen & Clausen, 1996).

Acute fatiguing exercise has marked effects upon Na^+,K^+ATPase maximal activity and gene expression in the skeletal muscle. Maximal Na^+,K^+ATPase activity measured by K^+-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase activity) was depressed by ~12-38 % after a single bout of single leg kicking (Fraser *et al.*, 2002), prolonged cycling (Leppik *et al.*, 2004; Sandiford *et al.*, 2004) or repeated isometric muscle

contractions (Fowles *et al.*, 2002b). The depression in maximal Na^+,K^+ATPase activity was not due to decreased Na^+,K^+ATPase content, as measured by [^{3}H]-ouabain binding sites (Fowles *et al.*, 2002b; Leppik *et al.*, 2004).

The acute effects of fatiguing exercise on Na^+,K^+ATPase activity and content in well-trained athletes are unknown and were therefore examined in this thesis.

2.7.12 Mechanism for depressed Na^+,K^+ATPase activity during fatiguing exercise

The exact mechanisms for depressed Na^+,K^+ATPase activity in skeletal muscle during exercise are unknown. Two likely candidates, however, are the increased production of reactive oxygen species (ROS), and/or raised intracellular calcium concentration ($[Ca^{2+}]_i$). Each of these is subsequently discussed below.

2.7.12.1 Effects of reactive oxygen species on Na^+,K^+ATPase

Exercise increases the production of ROS (Davies *et al.*, 1982; Sjödin *et al.*, 1990; Reid *et al.*, 1992). Reactive oxygen species cascade from the superoxide anion radical (for review see (Reid, 2001)). ROS also include hydrogen peroxide and hydroxyl radicals and are produced in contracting skeletal muscle (Davies *et al.*, 1982; Sjodin *et al.*, 1990; Reid *et al.*, 1992). It has been reported that ROS may directly inhibit the Na^+,K^+ATPase, as ROS scavengers inhibited the ischemia-repurfusion-induced reduction in Na^+,K^+ATPase activity in the sarcolemma of guinea-pig cardiac muscle (Kim & Akera, 1987; Kourie, 1998). The inhibitory effect of ROS on Na^+,K^+ATPase may be due to lipid membrane peroxidation, as studied in adult canine cardiac myocytes (Kramer *et al.*, 1984). Further, the intrinsic disulphide bonds of the Na^+,K^+ATPase are susceptible to oxidation (Kourie, 1998) and may reduce the affinity of the Na^+,K^+ATPase for Na^+ and K^+ with a concomitant increased affinity for ATP and ouabain (Mishra *et al.*, 1989).

2.7.12.2 Effects of raised intracellular calcium concentration ($[Ca^{2+}]_i$) on Na^+,K^+ATPase

Intracellular calcium concentration ($[Ca^{2+}]_i$) increases during fatiguing muscle contractions (Allen et al., 1995; Gissel & Clausen, 1999; Gissel, 2000). At μM concentrations, Ca^{2+} inhibits Na^+,K^+ATPase hydrolytic activity and transport activity in muscle and red blood cells (Huang & Askari, 1982; Yingst et al., 1992; Stankovicova et al., 1995). These effects on the Na^+,K^+ATPase may also be augmented by calmodulin, allowing Na^+,K^+ATPase inhibition to occur at μM Ca^{2+} concentrations (Okafor et al., 1997).

2.7.13 Concluding remarks on skeletal muscle Na^+,K^+ATPase

The activity and content of the Na^+,K^+ATPase enzyme are tightly regulated in human skeletal muscle. In isolated muscle the Na^+,K^+ATPase exerts a vital role in skeletal muscular performance (Nielsen & Clausen, 2000; Clausen, 2003), and in maintaining trans-sarcolemmal $[Na^+]$ and $[K^+]$ gradients and membrane excitability (Overgaard et al., 1999; Clausen, 2003). Reduced Na^+,K^+ATPase content in isolated animal muscle preparations (Clausen, 2003) and reduced activity in human muscles with exercise (Fowles et al., 2002b; Fraser et al., 2002; Clausen, 2003) have therefore been linked with fatigue (Clausen, 2003). Elite athletes may already have reached a high level of adaptation for skeletal muscle Na^+,K^+ATPase (Medbø et al., 2001), although the effects of acute exercise on skeletal muscle Na^+,K^+ATPase activity and content of well-trained athletes are unknown, and are therefore investigated in this thesis. Further, the effect of LHTL exposure and short-term intensified training in well-trained athletes were also investigated which may protect against the acute-exercise depression in Na^+,K^+ATPase.

2.8 Aims and hypotheses

2.8.1 Aims

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, and these were investigated in this thesis. Further, this thesis investigated the effects of LHTL on muscle metabolism, β_m and plasma ion regulation and acid-base balance during exercise.

2.8.2 Study 1-Part I (Chapter 3)

The first aim of this thesis was to investigate the effects of LHTL on muscle β_m, and performance in well-trained athletes.

The specific hypotheses tested were that:

1. Merely sleeping in moderate hypoxia (LHTL) would improve β_m.
2. LHTL would improve gross mechanical efficiency during submaximal cycle ergometry conducted in normobaric normoxia.

2.8.3 Study 1-Part II (Chapter 4)

The second aim of this thesis was to investigate the effects of both LHTL and acute exercise on muscle Na^+,K^+ATPase activity and content in well-trained athletes.

Specific hypotheses tested were that:

1. Maximal Na^+,K^+ATPase activity, assessed by maximal *in-vitro* K^+-stimulated 3-*O*-methylflourescein phosphatase (3-*O*-MFPase) activity, would be depressed by acute incremental exercise.
2. Na^+,K^+ATPase content, assessed by [^{3}H]-ouabain binding, would be unchanged by acute incremental exercise.
3. Nightly hypoxia imposed by LHTL would not depress muscle maximal Na^+,K^+ATPase activity, and thus allow enhanced muscle performance.

2.8.4 Study 2 (Chapter 5)

Further aims of this thesis were to investigate consecutive and intermittent models of LHTL at a lower simulated altitude to reflect changed practices of athletes and acute sprint exercise on muscle Na^+,K^+ATPase activity and content and plasma acid-base balance in well-trained athletes.

Specific hypotheses tested were that:

1. Maximal Na^+,K^+ATPase activity would be depressed by acute sprint exercise.
2. An early (5d) depression in maximal Na^+,K^+ATPase activity with hypoxia would be accompanied by a greater plasma $[K^+]$ during exercise.
3. The depression in Na^+,K^+ATPase activity with sprint exercise in well-trained athletes would be augmented by LHTL.
4. Maximal Na^+,K^+ATPase activity in resting muscle would be reduced early in LHTLc with a further decline after 20-n, whereas the greater periods of normoxia during LHTLi would attenuate the hypoxia-induced reduction in Na^+,K^+ATPase with LHTLc.
5. LHTL would not change plasma $[H^+]$ during exercise due to alterations in other strong ions in plasma, which would modify plasma [SID].

2.8.5 Study 3 (Chapter 6)

The final aims of this thesis were to investigate the effects of HIT on muscle Na^+,K^+ATPase activity and content in well-trained athletes.

Specific hypotheses tested were that:

1. Acute high-intensity interval exercise would depress maximal Na^+,K^+ATPase activity which would be associated with increased Na^+,K^+ATPase α-isoform mRNA expression.
2. Even short-term HIT would increase resting muscle Na^+,K^+ATPase content and maximal Na^+,K^+ATPase activity in already well-trained athletes and that these

would consequently enable a higher end-exercise maximal Na^+,K^+ATPase activity.

CHAPTER 3. STUDY 1-PART I: LIVE HIGH:TRAIN LOW INCREASES MUSCLE BUFFER CAPACITY AND SUBMAXIMAL CYCLING EFFICIENCY

3.1 Introduction

Altitude training for improved performance at sea level remains highly contentious (Rusko, 1996b; Saltin, 1996; Wolski *et al.*, 1996) . In part, this may be a consequence of any performance change being small and variable between individuals (Rusko, 1996a) . Recently an alternative approach to enhance athletic performance has been mooted, where athletes live at moderate altitude and train near sea level. This method of using hypobaric hypoxia improved the sea level 5000 or 3000 m run time in both college (Levine & Stray-Gundersen, 1997) and elite level runners (Stray-Gundersen *et al.*, 2001), but enhanced performance is a relatively rare outcome among those studies of altitude training that have used a control (CON) group. Because many countries lack suitable geography, the so-called `live high:train low' (LHTL) approach (Levine & Stray-Gundersen, 1997) has been further refined to include living at simulated altitude under normobaric conditions (Rusko, 1996b). Regardless of whether LHTL or natural altitude sojourns are used by athletes there is some evidence to challenge the traditional paradigm that the key adaptation for any performance benefit is increased red cell mass (Mairbäurl, 1994) and the concomitant increase in maximal aerobic power ($\dot{V}O_{2max}$) that has otherwise been associated with polycythemia (Buick *et al.*, 1980). Two studies have reported that training at altitude (~2000-2700 m) induced a 5-6% increase in skeletal muscle *in-vitro* buffer capacity (β_m) (Mizuno *et al.*, 1990; Saltin *et al.*, 1995a). Furthermore, a carefully conducted study has recently reported a significant (5%) improvement in the net mechanical efficiency of submaximal cycling subsequent to a 21-day mountain ascent (6194 m) (Green *et al.*, 2000d). The mechanism of increased β_m

and mechanical efficiency is unclear, but in both cases hypoxia is a likely candidate. Given the potential importance of anaerobic metabolism (Bulbulian *et al.*, 1986) and efficiency (Snell & Mitchell, 1984) to performance, even in highly trained endurance athletes, further investigation of possible anaerobic adaptations to hypoxia is clearly warranted.

Based on the reported effect of 2 weeks living and training at natural altitude (Saltin *et al.*, 1995a), it was hypothesised that merely sleeping in moderate hypoxia (LHTL) for sufficient duration would improve β_m. Secondly, based on the observation of Green et al. (2000d), it was hypothesised that LHTL of sufficient duration would improve gross mechanical efficiency during submaximal cycle ergometry conducted in normobaric normoxia.

3.2 Methods

3.2.1 Subjects

Thirteen male athletes (nine triathletes, two cross-country skiers and two cyclists) gave written consent to participate in this study, which was approved by the Australian Institute of Sport Ethics Committee. Subjects were ranked according to the power output achieved during the last 2 min of an incremental cycle ergometer test, that also established their peak oxygen consumption (VO_{2peak}). The ranking was used to assign subjects to two fitness-matched groups: the CON group (n=7) and LHTL group (n=6). The physical characteristics of the CON and LHTL groups and their training frequency, intensity and duration did not differ (Table 3.1). The nine triathletes (four CON and five LHTL) trained together and the remaining athletes completed their own sport-specific training schedules.

Table 3.1 Physical and training characteristics.

Variable	LHTL	CON
Age (yr)	25.4±3.6	25.1±5.2
Height (cm)	183.5±10.0	181.2±6.3
Body mass (kg)	73.0±6.7	73.3±6.1
VO_{2peak} ($L.min^{-1}$)	5.08±0.34	4.95±0.45
All-out 2-min power output ($W.kg^{-1}$)	5.74±0.46	5.72±0.31
Training ($sessions.wk^{-1}$)	7.1±2.3	6.8±2.3
Training intensity (Borg units)	13.8±1.1	13.6±1.0
Training ($h.wk^{-1}$)	13.4±3.8	10.6±5.7

The live high:train low group (LHTL, n=6) lived at 3000 m simulated altitude and trained at 600 m (Canberra, Australia), while the control group (CON, n=7) lived and trained in Canberra. The data for peak oxygen consumption (VO_{2peak}) and `all-out' 2-min power output are those achieved during habituation (see Fig. 4.1). Data are mean and (SD). No significant differences were found between groups for any variable

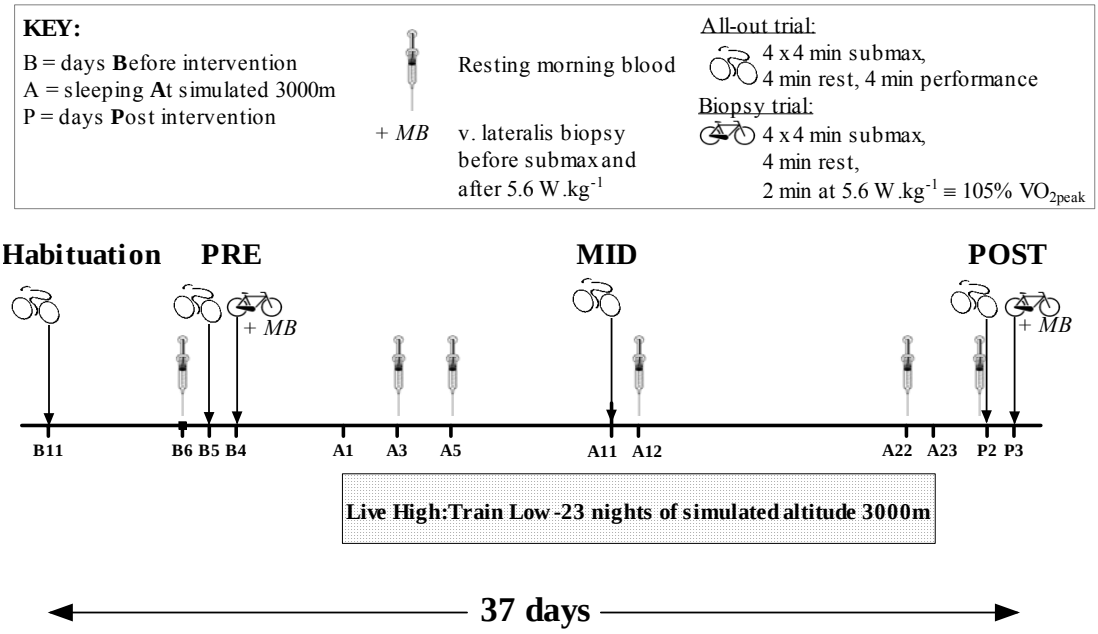
3.2.2 Experimental design

The study was conducted in Canberra, Australia at 600 m altitude, PB ~711 mmHg. The LHTL subjects spent 9.5 $h.night^{-1}$ for 23 consecutive nights in a room where enriched nitrogen produced hypoxia that simulated 3000 m altitude (normobaric hypoxia; O_2 = 15.48%). The CON subjects slept in their own homes under normobaric normoxia. Training and daytime living for all subjects was at an altitude of 600 m.

3.2.3 Submaximal workloads.

After one habituation trial, subjects completed five, four-stage submaximal cycle ergometer tests before, during and after the LHTL group slept at simulated altitude. The timing of these submaximal ergometer tests was 4 and 5 days before (PRE), 2 and 3 days after (POST), as well as after 11 of the 23 nights of simulated altitude (MID) (Fig. 3.1). All tests were completed under normobaric normoxic conditions in Canberra on one ergometer (Excalibur Sport model, Lode, Groningen, Holland) that was dynamically calibrated with a torquemeter. Based on the habituation trial, workloads for each subject were programmed using the Lode `hyperbolic' mode at 1.5, 2.5, 3.5 and 4.5 $W.kg^{-1}$; and for the baseline test (day 5-PRE) these corresponded to an overall group mean of 36, 52, 68, and 84% VO_{2peak}. The workloads programmed on day 5-PRE were replicated for each subject's subsequent tests and their cadence on day 5-PRE was recorded each minute and then matched for the four subsequent tests. Controlling cadence was a necessary precaution when using the `hyperbolic' mode of the Lode ergometer because in this condition power output is constant and cadence independent, and yet cadence can markedly alter the VO_2 of cycling (Woolford *et al.*, 1999).

Figure 3.1 Testing schedule and simulated altitude exposure of control (CON, n = 7) and live high:train low (LHTL, n = 6) groups. Both the CON and LHTL trained in normobaric normoxia in Canberra (600 m altitude), Australia, while LHTL spent 23 nights in normobaric hypoxia.



3.2.4 All-out trials.

On days 5-PRE, 11-MID and 2-POST an `all-out' trial was conducted in which the submaximal ergometer test was followed by 4 min of rest and then by a 4-min maximal effort. The first 2-min was set at an individual load (mean±SD = 5.6±0.4 $W.kg^{-1}$) equivalent to 105% of the workload achieved at VO_{2peak} in the habituation incremental test, and the last 2-min was an `all-out' effort. The 2-min workload at 5.6±0.4 $W.kg^{-1}$ was programmed using the `hyperbolic' mode of the Lode ergometer, after which the 2-min all-out workload reverted immediately to the `linear' mode of the ergometer with the linear factor (gearing) programmed according to individual requirements. In the

`linear' mode, power output on the Lode is cadence dependent and appropriate gearing is important for optimal performance. For each subject, the hyperbolic and linear factors used during the habitation trial were replicated for the three subsequent all-out tests. During the all-out effort, total work, VO_2 and VO_{2peak} were recorded.

3.2.5 Biopsy trials.

On days 4-PRE and 3-POST a 'biopsy trial' was conducted in which each subject had two muscle biopsies (vastus lateralis), one at rest ~30 min before the four-stage submaximal ergometer test and a second biopsy taken immediately (<15 s) after completing 2-min at 5.6±0.4 $W.kg^{-1}$ (Fig. 3.1).

3.2.6 Simulated altitude.

Throughout each of the 23 nights, $\%O_2$ and $\%CO_2$ inside the hypoxic room were measured every 30-min with Ametek (Pittsburgh, PA, USA) O_2 and CO_2 gas analysers (model S-3A and CD-3A, respectively) calibrated every 2-h at two points; with air from outside the laboratory and with one precision grade gas (BOC Gases Australia, Sydney, Australia). The LHTL subjects had their resting heart rate (HR) and blood oxyhaemoglobin saturation (SpO_2) estimated with finger-tip pulse oximetry (model 505-US, Criticare, Waukesha, WI, USA) every 30 min.

3.2.7 Morning resting blood acid-base status.

Resting venous blood was collected under normoxic conditions within 30 min of waking for both LHTL and CON. Samples were taken on the sixth day before entering the altitude house (6-PRE); after 3, 5, 12 and 22 nights at simulated altitude (A3, A5, A12 and A22, respectively), as well as after one night of sleeping in normoxia (2-POST) for determination of acid-base variables (Fig. 3.1). With each subject supine, blood was sampled from a superficial forearm vein via a winged infusion set into a heparinised 2 mL blood-gas syringe. Resting samples were also analysed for red blood cell parameters, with data reported elsewhere (Ashenden *et al.*, 1999a).

3.2.8 Exercise blood sampling.

Before each of the five cycle ergometer tests, a catheter was inserted into a superficial dorsal hand vein and covered with an adhesive plastic dressing and latex glove. After catheterisation, each subject was seated on the cycle ergometer and the catheterised hand was immersed in a water bath (44.5° C) to ensure arterialisation of venous blood. After 10 min in this posture, a 1.5-mL pre-exercise blood sample was acquired via a heparinised 2 mL blood gas syringe. Blood samples (1.5 mL) were taken from a dorsal hand vein during the last 30 s of each of the four submaximal workloads and at 5.6±0.4 $W.kg^{-1}$ during the biopsy trials, and on the days of the all-out trial during the last 15 s of the final 2-min effort.

3.2.9 Blood analyses.

Blood samples were stored on ice (<1 h) until analysis in triplicate for plasma pH and bicarbonate concentration ($[HCO_3^-]$), lactate concentration ($[La^-]_p$), and carbon dioxide partial pressure (PCO_2) using an automated analyser (ABL System 625, Radiometer, Copenhagen, Denmark), which was calibrated daily in accordance with the manufacturer's specifications.

3.2.10 Muscle biopsies and analyses

A needle biopsy sample was taken at rest from the vastus lateralis muscle via one of the two incisions made ipsilaterally under local anaesthesia (Xylocaine, 1%; Astra Pharmaceuticals, Sydney, Australia), with suction applied to the needle. Both biopsies in a trial were taken from separate incisions in the same leg, with the exercise sample taken from an incision ~1.5 cm distal to the rest sample. All biopsies were taken at constant depth by the same, experienced medical practitioner. The second sample was taken immediately after cessation of the 2 min exercise trial at 5.6±0.4 $W.kg^{-1}$, with the subject lying supported on the cycle ergometer. The samples for metabolite and βm analyses were rapidly frozen in liquid nitrogen.

3.2.10.1 Muscle pH, buffer capacity and total protein content.

Before analysis in duplicate, the samples were freeze-dried (Modulo, Edwards, Crawley, UK) and dissected free of connective tissue, blood and fat. The sample was diluted 1:200 in 5 mM NaIAA, 145 mM KCl, and 10 mM NaCl, pH 7.0 and then homogenised (Omni 1000, Omni International, Warrenton, VA, USA) on ice for 60 s. Muscle homogenate pH (expressed as $[H^+]$) was measured at 37°C under magnetic stirring with a glass microelectrode (MI-145, Microelectrodes, Bedford, TX, USA). The *in-vitro* buffer capacity (β_m) was then measured by titration of the homogenate from pH 7.1 to 6.1 and expressed relative to muscle dry mass (μmol H^+ g muscle dm^{-1} pH^{-1}). Total protein content was determined spectrophotometrically (Lowry *et al.*, 1951). The reliability of the duplicate measures was calculated as the within subject standard deviation or typical error of measurement (TEM) (Hopkins, 2000). The TEM for β_m was 3.6 μmol H^+ g muscle dm^{-1} pH^{-1} or 1.9% of the mean, and the corresponding values for total protein were 0.016 mg (mg muscle)$^{-1}$ equivalent to 1.2% of the mean.

3.2.10.2 Muscle metabolites.

The muscle lactate (La^-_m), adenosine triphosphate (ATP), phosphocreatine (PCr), creatine (Cr) and glycogen contents were measured in triplicate on freeze-dried muscle using standard fluorometric techniques (Lowry & Passoneau, 1972). Muscle ATP, PCr and Cr contents were corrected to the total Cr content. Muscle anaerobic ATP production was estimated from the rest to end-exercise changes (Δ) in ATP, PCr and La^-_m, and calculated as $\Delta ATP + \Delta PCr + 1.5\Delta La^-_m$. Because of technical diffculties, the sample size for these measures was n=4 and n=6 for the LHTL and CON, respectively. The respective TEMs for La^-_m, ATP, PCr and glycogen were 1.5, 0.6, 1.5 and 14 mmol.kg dm^{-1} equivalent to 2.0, 2.5, 5.8 and 2.5% of the mean values.

3.2.11 Oxygen consumption and mechanical efficiency

During each cycle ergometer test $\dot{V}O_2$, carbon dioxide output ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$) and respiratory exchange ratio (RER) were measured continuously and results were displayed every 30 s. Data from the last 60 s of each of the four 4-min submaximal workloads were used to indicate the `steady-state' level, and $\dot{V}O_{2peak}$ was determined as the highest value recorded in any 60-s interval during the last 4 min of the all-out trial. The open-circuit indirect calorimetry system comprised Ametek O_2 and CO_2 gas analysers as well as two chain-compensated gasometers and has been described previously (Pierce *et al.*, 1999). The analysers were calibrated before, and checked for drift after, each test using three α grade gases (BOC Gases Australia). The average TEM for $\dot{V}O_2$ was 0.12 and 0.09 L min^{-1}, respectively, for the duplicated PRE (5- and 4-PRE) and POST (2- and 3-POST) four stages of submaximal ergometry. At any submaximal workload the mean difference between either of the two repeat tests was $<\pm$52 mL min^{-1} for both CON and LHTL groups. The corresponding PRE and POST TEMs for $\dot{V}_E$ during submaximal ergometry were 5.0 and 4.7 L min^{-1}, equivalent to 6.1 and 4.9% of the respective mean values. Gross mechanical effciency (%) was determined from the ratio of power output (kJ min^{-1}) to energy expended (kJ min^{-1}), as calculated from $\dot{V}O_2$ and RER (Elia & Livesey, 1992).

3.2.12 Heart rate

Overnight resting heart rate (HR) each night was calculated for the LHTL group as the grand mean from 11:00 PM to 05:00 AM. The HR during cycle ergometry was assessed every 5 s by telemetry (Polar Vantage, Polar Electro OY, Kempele, Finland). The TEMs for HR during the four-stage submaximal ergometer tests at PRE and POST were 3 and 4 beats min^{-1}, equivalent to 2.6 and 3.5% of the respective mean values.

3.2.13 Statistical analysis

All values are reported as mean±SD. The physical and training characteristics of the two groups were assessed with independent t-tests. Three-way analysis of variance (ANOVA) with repeated measures was used to test for interaction and main effects for most of the dependent variables measured during exercise. The three factors were group (CON and LHTL), day (PRE, MID and POST simulated altitude), and stage of exercise (rest, end of exercise and where relevant the four submaximal workloads). When the three-way interaction was not significant, the data of LHTL and CON groups were analysed with separate two-way repeated measures ANOVA for day and stage of exercise. Peak exercise data were analysed with two-way repeated measures ANOVA for group by day. When interactions or main effects achieved statistical significance, Tukey post hoc tests were used to identify differences between cell means. Statistical significance was tested at the $P<0.05$ level using Statistica software (StatSoft, Tulsa, OK, USA). In addition, and as a method to partially circumvent the likelihood of a type II error as a consequence of our small sample size, the effect size [ES= ($mean_1$ ± $mean_2$)/SD] was calculated for selected results that did not achieve significance and the pooled SD was calculated when the SDs were unequal (Cohen, 1988). Cohen's (Cohen, 1988) conventions for effect size were adopted for interpretation, where ES = 0.2, 0.5 and 0.8 are considered as small, medium and large, respectively.

3.3 Results

3.3.1 All-out trials

3.3.1.1 Performance and $\dot{V}O_2$.

The $\dot{V}O_{2peak}$ of LHTL fell significantly by -3.8±1.9% at MID and -7.2±4.1% at POST, whilst CON $\dot{V}O_{2peak}$ was unchanged (Table 3.2). Total $\dot{V}O_2$ in the 2 min all-out effort was also significantly depressed in LHTL at POST compared with PRE, although, the

corresponding work output was not changed in either group (Table 3.2). Total $\dot{V}O_2$ during 2 min at 5.6±0.4 $W.kg^{-1}$ was not different between groups (P>0.20) but tended to be less after 23 nights of sleeping in hypoxia in LHTL (Δ PRE vs. POST -4.0%) than in CON (Δ=1.1%). Cadence at PRE ranged from 90±7 to 102±2 rev min^{-1} for 1.5 $W.kg^{-1}$ and all-out workloads, respectively, for LHTL, and 93±15 to 102±3 rev min^{-1} for CON. No significant differences were found between groups or between different days of exercise.

3.3.1.2 Submaximal $\dot{V}O_2$ and mechanical efficiency.

During the first four stages of the all-out trial, LHTL had a significantly lower submaximal $\dot{V}O_2$ at both MID (-3.1±2.9%) and POST (-4.4±3.3%) compared with PRE (Fig. 3.2). Submaximal $\dot{V}_E$ was significantly increased after 23 nights of sleeping in moderate hypoxia (Fig. 3.2). Although RER of LHTL was not significantly different between days, the effect sizes tended to be large (at 1.5 $W.kg^{-1}$ PRE vs. MID, ES = 2.27; PRE vs. POST, ES = 1.67). Overall, RER for LHTL was 0.88±0.07 PRE and 0.91±0.07 POST. The CON showed no change in submaximal $\dot{V}O_2$, $\dot{V}_E$ and RER for MID and POST vs. PRE (Fig. 3.2). Submaximal efficiency of LHTL was significantly different between days and stage of exercise (P<0.02). Each POST value (16.6±1.5, 19.6±0.8, 20.9±0.7 and 21.5±0.7%) was higher than the corresponding PRE value (15.8±1.4, 18.7±0.9, 20.2±1.0 and 21.0±0.7%) at 1.5, 2.5, 3.5 and 4.5 $W.kg^{-1}$, respectively. Overall, submaximal efficiency of the LHTL group was improved 0.8% from PRE (18.9±2.7%) to POST (19.7±2.4%) (P<0.01).

Table 3.2 All-out trials. Peak and total $\dot{V}O_2$, work, peak HR, and end exercise $[La^-]_p$ and pH for 2-min all-out cycle ergometry. The groups and the intervention are described in Table 3.1 and the timing of tests is illustrated in Fig. 3.1. Data are mean±SD.

Variable	Group	Day of measurement		
		day 5-PRE	Day 11-MID	day 2-POST
VO_{2peak} ($L.min^{-1}$)	LHTL	5.08±0.34	4.90±0.33*	4.78±0.36*
	CON	4.95±0.45	4.92±0.47	4.87±0.44
VO_{2total} in 2 min (L)	LHTL	9.99±0.72	9.63±0.71	9.24±0.66*
	CON	9.60 ±1.09	9.77±0.93	9.64±0.92
Work in 2 min (kJ)	LHTL	50.0±4.2	51.0±3.9	49.2±4.2
	CON	50.5±6.0	51.5±6.5	50.3±5.8
Heart $rate_{peak}$ ($b.min^{-1}$)	LHTL	183±9	185±6	183±6
	CON	189±8	189±9	190±9
$[La^-]_p$ ($mmol.L^{-1}$)	LHTL	15.4±3.3	16.7±2.5†	17.3±2.6†
	CON	17.4±1.2	21.1±3.1*	22.4±1.7*
pH	LHTL	7.26±0.03	7.25±0.03	7.26±0.03
	CON	7.24±0.03	7.23±0.03	7.24±0.02

3.3.1.3 Heart rate

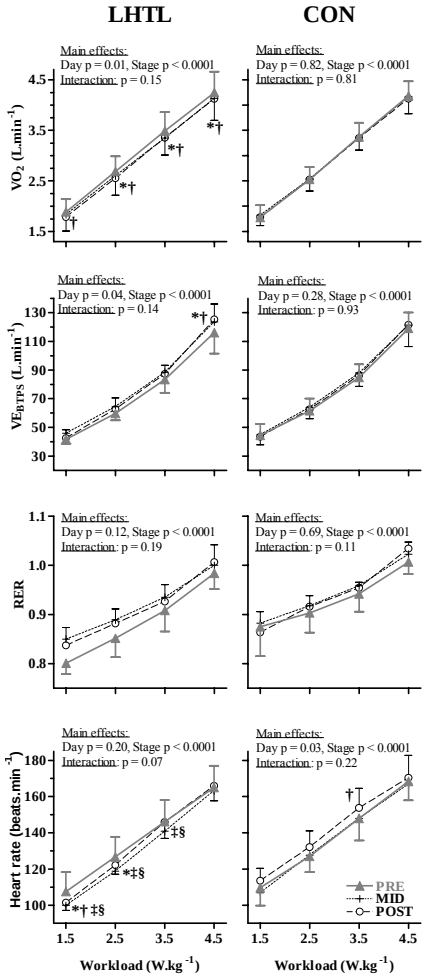
Submaximal HR was significantly different between groups when comparing the three test days and four submaximal stages of exercise (P<0.03). The PRE HR was not different between groups at any workload, however, HR was signifcantly lower for

LHTL than CON at the first three submaximal workloads at both MID and POST (Fig. 3.2). In addition, HR of LHTL during the first two stages of the MID test and the first stage of the POST test were significantly lower (6±8 beats min^{-1}) than at PRE. The HR_{peak} was not different within or between groups for PRE vs. POST (Table 3.2).

3.3.1.4 Blood biochemistry

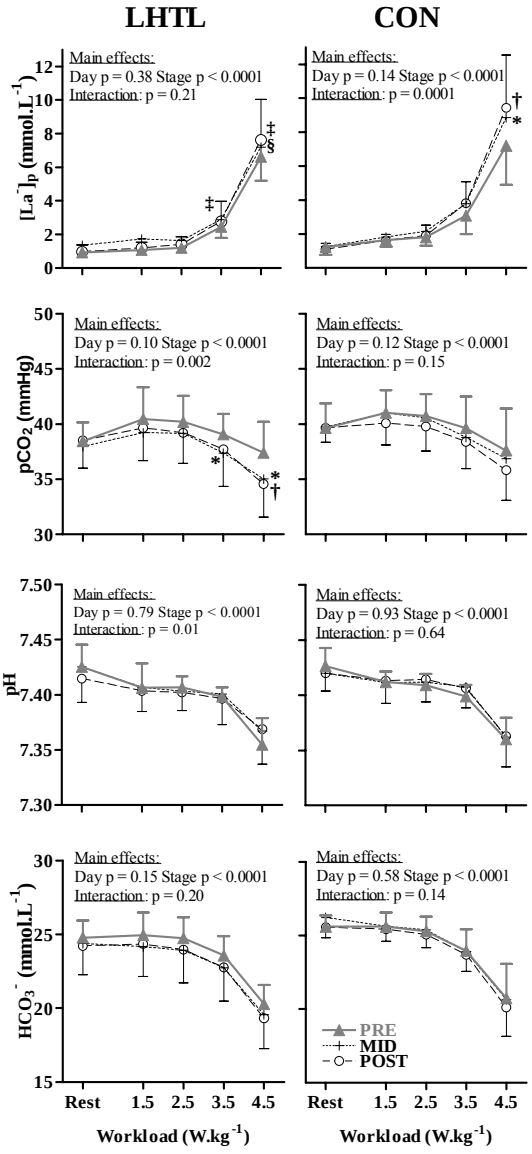
The three-way interaction between groups, test days and the five stages of rest or submaximal exercise was significant for $[La^-]_p$ ($F_{(8,88)}$ = 2.22, P=0.03). During submaximal exercise, $[La^-]_p$ for LHTL was not different between test days, but within CON $[La^-]_p$ at 4.5 $W.kg^{-1}$ was significantly higher than PRE at both MID and POST (Fig. 3.3). Furthermore, $[La^-]_p$ for LHTL was significantly lower than that of CON at MID at 3.5 $W.kg^{-1}$, and at both MID and POST at 4.5 $W.kg^{-1}$. At the end of the 2 min all-out effort, LHTL $[La^-]_p$ was not different between PRE, MID and POST tests, although CON $[La^-]_p$ was significantly higher at both MID and POST than PRE (Table 3.2). The LHTL PCO_2 during MID was lower than at PRE at both 3.5 and 4.5 $W.kg^{-1}$, and at POST was lower than PRE at 4.5 $W.kg^{-1}$ (Fig. 3.3). Although Tukey post hoc tests did not identify differences between cell means, pH at 4.5 $W.kg^{-1}$ tended to be higher compared with PRE at both MID (ES ~0.50) and POST (ES ~0.49). While not significantly different between days, LHTL $[HCO_3^-]$ at rest and during submaximal exercise tended to be lower at both MID and POST compared with PRE (ES ~0.5). At the end of all-out exercise, pH was stable in each group for the three tests (Table 3.2).

Figure 3.2 Oxygen consumption (VO_2), ventilation (V_E), respiratory exchange ratio (RER) and heart rate (HR) for Live High:Train Low (LHTL, n=6, left panels) and Control (CON, n= 7, right panels) groups during submaximal cycle ergometry before (PRE), after 11 nights (MID), and 2 days after (POST) 23 nights of simulated altitude.



Values are mean and SD. Significant differences within groups; *MID vs. PRE, †POST vs. PRE; significant differences between groups at matched time, §MID vs. MID, ‡POST vs. POST. Main effects for Day (PRE, MID, POST), exercise stage (1.5±4.5 $W.kg^{-1}$), as well as the day by stage interaction are indicated in each subpanel.

Figure 3.3 Arterialised venous plasma lactate concentration $[La^-]_p$, CO_2 tension (PCO_2), pH and bicarbonate ion concentration $[HCO_3^-]$ for the LHTL (left panel) and CON (right panel) groups as described in Fig. 3.2.



Values are mean and SD. Significant differences within group; *MID vs. PRE, ‡POST vs. PRE; significant differences between groups at matched time, §MID vs. MID, †POST vs. POST.

3.3.2 Biopsy trials

3.3.2.1 Performance and $\dot{V}O_2$.

The LODE ergometer was programmed to ensure that the amount of work completed during 2 min at 5.6±0.4 W.kg^{-1} was the same on both days (48.2±5.1 kJ). There was a non-significant (P>0.20) trend for total $\dot{V}O_2$ during the 2 min to be lower in LHTL (Δ = -3.5%) but not CON (Δ = -0.2%).

3.3.2.2 Muscle buffer capacity and metabolites

Resting β_m increased significantly in LHTL (17.7±4.9%) but was unchanged in CON (0.5±5.8%, Fig. 4.4). Analysis of β_m in post-exercise samples confirmed this finding of an elevation in the LHTL group only (Appendix B4). The increased β_m was not the result of an increased total muscle protein content, as the latter did not differ between or within groups (Table 3.3). Muscle [H^+] was not significantly different between the groups. The pooled data of both groups indicated no difference in resting muscle [H^+] PRE vs. POST (70.0±4.1 vs. 66.9±4.9 nmol kg dm^{-1}, respectively), although at the end of exercise it tended to be lower POST than PRE [140.2±19.6 vs. 159.7±21.6 nmol kg dm^{-1}; day by exercise interaction (P=0.06)]. The [H^+] accumulation and calculated *in vivo* β_m after exercise at 5.6±0.4 W.kg^{-1} (Δ[H^+]) was unchanged in either LHTL or CON (Table 3.3). Muscle ATP, PCr and glycogen decreased with exercise whereas Cr increased, but these were not different between groups nor affected by 23 nights sleeping in hypoxia (Table 3.3). The La^-_m accumulation (ΔLa^-_m) and estimated anaerobic energy production after exercise at 5.6±0.4 W.kg^{-1} was unchanged from PRE to POST in either group (Table 3.3).

3.3.2.3 Heart rate

No differences between or within groups were found for PRE vs. POST HR at 5.6±0.4 W.kg^{-1} (Table 3.4).

3.3.2.4 Blood biochemistry.

At 55.6±0.4 W.kg^{-1} $[La^-]_p$, PCO_2, pH, and $[HCO_3^-]$ were not different between groups or from PRE to POST (Table 3.4).

3.3.3 Morning blood biochemistry

Morning resting plasma pH was not different between groups at baseline or after one night of sleeping in hypoxia, but was significantly higher at day A5 in LHTL than in CON (Fig. 3.5). Within LHTL, pH at A5 tended to be higher than at baseline (P<0.07, ES = 2.29). Morning resting $[HCO_3^-]$ was not different between groups on any day, although it tended to be lower (at least 1.2 mmol L^{-1}) in LHTL during and 2 days after simulated altitude (Fig. 3.5). The between group effect sizes at days A3 and A22 were 0.51 and 1.04, respectively.

3.3.4 Overnight heart rate and blood saturation

Overnight resting HR of the LHTL group was unchanged across the 23 nights, with a grand mean of 57±11 beats min^{-1}, and the SpO_2 was 91±3% for the 219 h spent in normobaric hypoxia.

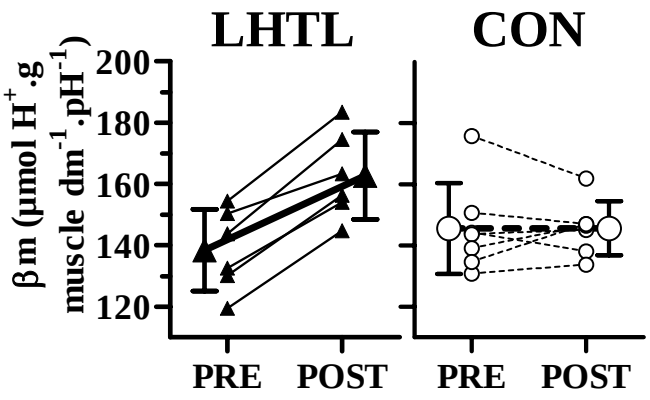
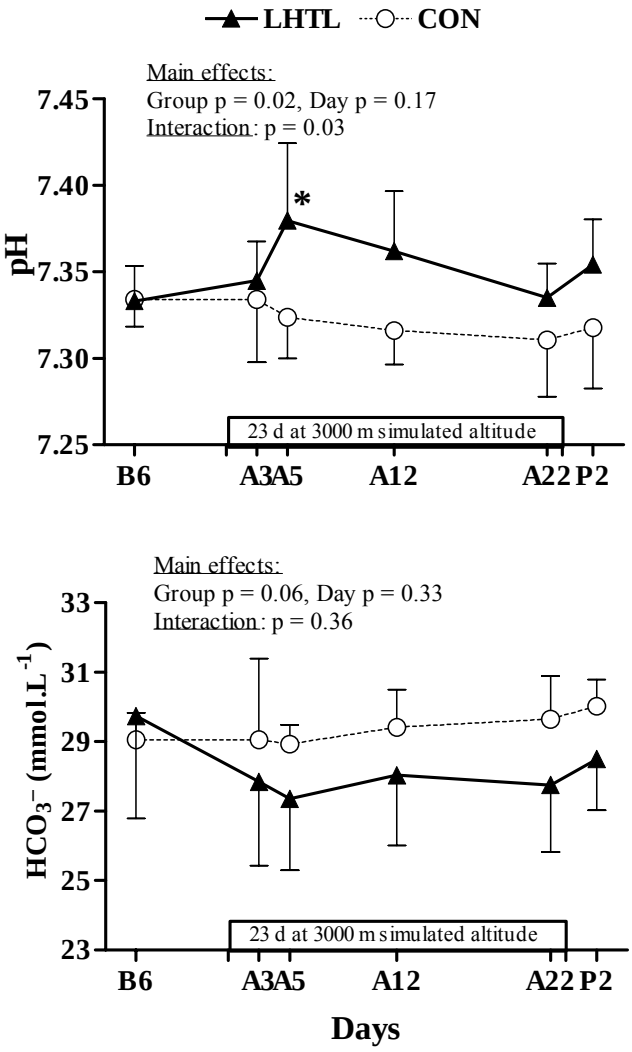


Figure 3.4 Change in resting in-vitro muscle buffering capacity (β_m) PRE and POST 23 nights of simulated altitude. Left panel shows individual data points of LHTL group (n=6) that lived high and trained low with mean±SD data indicated with large symbols. The right panel is for the control (CON, n=7) group.

Table 3.3 Biopsy trials. Muscle protein content, H^+ concentration and metabolites at rest and immediately after 2 min of cycle ergometry at ~ 5.6 $W.kg^{-1}$. The groups and the intervention are described in Table 3.1 and the timing of tests is illustrated in Fig. 3.1. The sample size for ATP, PCr, glycogen, Cr and La^-_m are n=4 and n=6 for the LHTL and CON groups, respectively. Data are mean±SD. Differences within and between groups are not significant

Variable	Condition	LHTL day 4-PRE	LHTL day 3-POST	CON day 4-PRE	CON day 3-POST
Protein ($mg.mg\ muscle^{-1}$)	Rest	0.177±0.010	0.174±0.013	0.168±0.014	0.166±0.015
	Exercise	0.175±0.013	0.174±0.012	0.168±0.012	0.166±0.014
$[H^+]$ ($nmol.\ L^{-1}$)	Rest	71.1±3.8	67.3±5.5	69.0±4.4	66.4±4.8
	Exercise	156.6±22.8	139.9±20.4	162.3±21.9	140.5±20.5
	Δ (Ex – Rest)	85.5±21.7	72.6±19.2	93.3±18.3	74.1±22.5
$\beta_{in\text{-}vivo} = (\Delta H^+/\Delta[La^-]_m)$		105.6±30.0	104.7±40.1	115.4±17.8	136.5±30.0
ATP ($mmol.kg\ dm^{-1}$)	Rest	28.6±0.8	28.6±0.6	28.7±1.2	28.7±1.0
	Exercise	18.0±0.4	18.1±0.5	18.0±0.3	17.9±0.4
PCr ($mmol.kg\ dm^{-1}$)	Rest	87.9±0.7	87.9±0.5	87.9±3.0	89.0±1.9
	Exercise	62.5±0.9	62.3±0.7	62.9±0.7	63.0±1.1
Glycogen (mmol glucosyl units.$kg\ dm^{-1}$)	Rest	576±98	571±79	611±68	599±56
	Exercise	245±15	247±10	235±19	227±27
Cr ($mmol.kg\ dm^{-1}$)	Rest	47.5± 1.4	47.6±1.2	47.7±1.3	47.7±1.3
	Exercise	72.9± 1.0	73.1±1.1	73.8±0.7	73.7±0.9
$[La^-]_m$ ($mmol.kg\ dm^{-1}$)	Rest	5.9±2.9	6.0±2.5	4.9±0.6	4.9±0.5
	Exercise	44.5±7.1	40.1±12.1	47.5±2.5	48.2±3.4
Anaerobic ATP production ($mmol.kg\ dm^{-1}$)	Δ (Rest to end Ex)	93.9±9.7	87.1±18.9	100.7±3.7	101.9±5.8

Figure 3.5 Morning resting plasma pH (top panel) and bicarbonate concentration (bottom panel) of live high:train low (LHTL, n=6) and control (CON, n=7) groups before, during and after LHTL spent 23 nights sleeping in hypoxia. Values are mean and SD. *Significant difference between groups.



3.4 DISCUSSION

The major findings from this chapter challenge conventional concepts of adaptation to chronic hypoxic exposure. The first major finding was that muscle in-vitro buffer capacity was increased after sleeping in hypoxia, and thus can be attributed to chronic hypoxic exposure alone. However, after LHTL this did not coincide with enhanced muscle H^+ regulation, evidenced by an unchanged post-exercise muscle [H^+], or by a

general up-regulation of anaerobic metabolism during intense exercise. The second major finding was that whole body $\dot{V}O_2$ during submaximal cycle ergometry under normobaric, normoxic conditions was significantly lower after 23 nights of sleeping at 3000 m simulated altitude. The finding of reduced $\dot{V}O_2$ at a constant exercise workload, without a corresponding elevation in anaerobic metabolism suggests that 23 nights exposure to moderate hypoxia enhances mechanical efficiency during exercise.

3.4.1 Muscle buffer capacity, anaerobic metabolism and acid-base regulation

This chapter confirms that merely sleeping, rather than living and training, in hypoxia elevates β_m and thus strongly suggests that hypoxia is the key factor in improving β_m. The increase in β_m was not the result of increased muscle protein content but apparently reflected a qualitative change in the buffer capacity of the dipeptides or protein expressed. This may be a consequence of a higher intramuscular carnosine concentration as suggested by others (Saltin et al., 1995a), but the mechanism remains unknown. Elevated β_m after LHTL is consistent with the 5-6% increase reported after training and living at ~2000-2700 m (Mizuno et al., 1990; Saltin et al., 1995a). In contrast, a recent report indicated an unspecified decrease in β_m after living at 2500 m and training at 2200±3000 m (Stray-Gundersen et al., 1999). This is also the first report of the effects of LHTL on skeletal muscle H^+ regulation during exercise.

Surprisingly, there did not appear to be a positive modulation of intramuscular H^+ regulation, with an unchanged post-exercise muscle $[H^+]$ during intense exercise after LHTL, in comparison with the CON group. Such an effect with LHTL would be expected to be evident from the matched work bout used in this study because identical, rather than exhausting, work bouts are a salient method to compare markers of muscle metabolism and ion regulation (Harmer *et al.*, 2000). Further, the calculated *in-vivo* β_m was not enhanced after LHTL, although considerable variability was found in the data.

This suggests that intramuscular H^+ regulation was not improved after LHTL. As $[H^+]$ was measured in dried muscle and CO_2 is lost during the freeze-drying process, the $[H^+]$ values are slightly lower than expected in wet muscle. Nonetheless, both F_ECO_2 (data not shown) and arterialised venous PCO_2 were lower during exercise after LHTL, suggesting that intramuscular CO_2 would also tend be less in the LHTL group. Hence, the CO_2- dependent H^+ accumulation would be lower in LHTL, consistent with conclusions that an increased β_m was not associated with improved muscle H^+ regulation. Thus, the results are incompatible with the concept that the primary importance of increased β_m is to confer benefits for muscle H^+ regulation. In addition to β_m, muscle H^+ regulation during exercise will be affected by the sarcolemmal lactate/H^+ and Na^+/H^+ exchange mechanisms, capillarisation and muscle blood flow (Juel, 1998) and by changes in the intracellular strong ion difference (Kowalchuk *et al.*, 1988). The effects of LHTL on each of these remain unknown. An interesting finding was that the increased β_m in the LHTL group occurred without any corresponding elevation in other markers of anaerobic metabolism, in contrast with the suggestion of others who used natural altitude exposure (Mizuno *et al.*, 1990; Saltin *et al.*, 1995a); although with a small sample size, the analyses performed in this study are prone to type II error. The degradation of muscle ATP, PCr and glycogen during intense exercise were unchanged, as were the intramuscular and blood accumulation of La^- and H^+ ions. Each of these changes was highly reproducible with low TEM, and was identical in the PRE and POST trials in both the CON and LHTL groups. The decline in ATP was most likely because of the 2-min exercise bout at 105% VO_{2peak}. The work completed in this trial (~48 kJ) was similar to that in the last 2 min of the all-out trial (~50 kJ), when subjects were asked to produce as much work as possible. Thus, reductions in ATP are not unexpected with this heavy exercise. The decline in PCr and rise in Cr was surprisingly

small relative to the rise in La^-. This may reflect the usual slight delay in biopsy sampling and a likely rapid PCr resynthesis in these endurance-trained athletes. The anaerobic ATP production may consequently be slightly underestimated, but importantly, this was clearly not enhanced after LHTL.

The typical lactate response to exercise during chronic altitude exposure is an initial elevation in lactate accumulation in arterial and venous blood as well as in muscle, together with elevated muscle lactate release, each of these subsequently decline with acclimatisation (Hochachka, 1988; Brooks *et al.*, 1992; Reeves *et al.*, 1992; Brooks, 1998). The data from this study clearly demonstrates that Lac^- accumulation was not elevated during intense exercise after LHTL, although data in this study was collected under normoxic conditions, and therefore would not necessarily follow typical lactate responses to altitude The muscle and blood lactate data are inconsistent with the premise that lactate production was greater after sleeping in hypoxia, consistent with the conclusion that anaerobic metabolism is not enhanced after LHTL. The $[Lac^-]_p$ also was not increased within the LHTL group after 23 nights spent in hypoxia. The $[Lac^-]_p$ during the latter stages of exercise was lower in LHTL than in CON subsequent to simulated altitude but this was because of an unexpected increase in the CON group. Thus, this data suggest it is unlikely that the typical lactate response to natural altitude occurred after LHTL, possibly because of both the simulated altitude and duration of exposure being insufficient to elicit such a response.

3.4.2 Reduced submaximal oxygen consumption and enhanced efficiency

A clear finding in this study was that under normoxic conditions VO_2 of the LHTL group was depressed and efficiency was increased at each of the four, 4-min submaximal workloads after both 11 and 23 nights of sleeping in hypoxia (Fig. 3.3).

These results challenge the conventional concept that, at sea level, $\dot{V}O_2$ at any given submaximal power output remains unchanged after returning from an altitude or simulated altitude sojourn (Levine & Stray-Gundersen, 1997; Piehl-Aulin *et al.*, 1998). Most other studies have reported no change in submaximal $\dot{V}O_2$ at sea level (Wolfel *et al.*, 1991; Grassi, 1996; Levine & Stray-Gundersen, 1997; Piehl-Aulin *et al.*, 1998). However, the data from this study are consistent with a recent report that $\dot{V}O_2$ was significantly lower (8-10%) during prolonged submaximal cycle ergometry subsequent to a 21-day climb (2160-6194 m) (Green *et al.*, 2000d). Collectively, the data from this study and those of subjects living and climbing at natural altitude (Green *et al.*, 2000d) suggest that one can attribute the increase in mechanical efficiency to hypoxia *per se* rather than hypobaria, cold or the effects of heavy athletic training. Interestingly, the results of this study are consistent with those of several cross-sectional studies that have reported higher exercise efficiency in altitude natives compared with lowlanders (Hochachka *et al.*, 1991; Saltin *et al.*, 1995b). It is unkown why the findings of this study differ from those of most others. However, the indirect calorimetry system used in this study has good precision and identical pedalling cadences were maintained for a subject across all exercise trials. Failure to control cadence may have confounded the work of others (Sutton *et al.*, 1988; Wolfel *et al.*, 1991) as for example, a cadence of 90 vs. 120 rev min^{-1} lowers submaximal $\dot{V}O_2$ by an average of 0.47 $L.min^{-1}$ (Woolford *et al.*, 1999). The reduction in whole body $\dot{V}O_2$ during exercise after LHTL can likely be explained by a shift from fat to carbohydrate oxidation, rather than a shift from oxidative to anaerobic metabolism. In this study, submaximal RER was marginally higher (0.03) POST than PRE, which is sufficient to entirely explain the 0.8% improvement in efficiency of exercise after LHTL. The higher RER is consistent with

the suggestion that at altitude increased carbohydrate and lactate fluxes reflect an overall shift towards carbohydrate utilisation which optimises the available energy for a given oxygen consumption (Brooks, 1998). Preferential use of carbohydrate fuels rather than fats at 4300 m altitude has been shown at rest and during submaximal exercise (Brooks *et al.*, 1992; Roberts *et al.*, 1996b). The findings of this study of a lower $\dot{V}O_2$ at the same absolute workload are consistent with the postulate that altitude acclimatisation improves coupling of ATP demand and supply (Hochachka, 1988). Other mechanisms which might contribute to increased exercise efficiency after LHTL include a reduction of ATP consuming processes within skeletal muscle as shown recently by down-regulation of Na^+,K^+ATPase after an altitude sojourn (Green *et al.*, 2000a), and a reduction in $\dot{V}O_2$ of the respiratory musculature. The latter is unlikely because exercise ventilation was increased after LHTL and during heavy cycle exercise the respiratory muscles consume a significant fraction of the pulmonary $\dot{V}O_2$ (Harms *et al.*, 1997). Lastly, type I fibres are energetically more efficient when cycling (Coyle *et al.*, 1992), and both fibre recruitment and cycling $\dot{V}O_2$ are cadence-dependent (Barstow *et al.*, 1996; Woolford *et al.*, 1999). However, it seems doubtful that the LHTL group may have increased their type I fibre recruitment because all fibres would be expected to be recruited during the maximal work bouts, when $\dot{V}O_{2peak}$ was also subnormal. Reduced submaximal cycling cadence also cannot explain the subnormal $\dot{V}O_2$ as this was maintained constant for each subject in all tests.

3.4.3 $\dot{V}O_{2peak}$ and performance after simulated altitude.

A novel finding in this study was that after LHTL $\dot{V}O_{2peak}$ was depressed by 7%, although total work was unchanged, during 2 min of all-out cycling. It is implausible

that this decrease in $\dot{V}O_{2peak}$ could be explained by detraining of the LHTL group. Even studies of well-trained athletes who completely cease training for 2-3 weeks have reported a decrease in $\dot{V}O_{2max}$ of only 2-7% (Houston *et al.*, 1979; Coyle *et al.*, 1984; LaForgia *et al.*, 1999). Before and during the period of LHTL, most of the CON group trained with the LHTL group and the former maintained a stable $\dot{V}O_{2peak}$ throughout this study. It therefore seems unlikely that the LHTL group which spent 13 h.week^{-1} in athletic preparation at a mean intensity of 13.8 Borg units would have detrained. Finally, the $\dot{V}O_2$ system used in this study has high precision, which suggests that measurement error was not the cause for the observed reduction in $\dot{V}O_2$. In this context it is notable that tests on LHTL and CON subjects were interspersed. Although there is a widespread paradigm that acclimatisation to hypoxia increases red cell mass and consequently $\dot{V}O_{2max}$ (Cerretelli & Hoppeler, 1996; Rusko, 1996b; Levine & Stray-Gundersen, 1997; Rodriguez *et al.*, 1999; Fulco *et al.*, 2000), this view is opposed (Sawka *et al.*, 2000; Hahn & Gore, 2001). The LHTL subjects exhibited no change in haemoglobin mass or reticulocyte indices of accelerated erythropoiesis (Ashenden *et al.*, 1999a) but their $\dot{V}O_{2peak}$ was depressed. The potential mechanisms underlying decreased $\dot{V}O_{2peak}$ require further investigation. $\dot{V}O_{2peak}$ is limited in normoxia by muscle cardiac output and muscle blood flow (Saltin *et al.*, 2006). It is unknown if LHTL affects either cardiac output or muscle blood flow. Total work as an indicator of performance was unchanged in the LHTL group but this may be a problem of insufficient statistical power. There is mounting evidence from three independent groups that LHTL may yield small improvements (0.8-1.3%) in events lasting from ~50 s to 17 min; 400 m sprint (Nummela & Rusko, 2000), 4-min all-out effort (Hahn &

Gore, 2001), as well as 3000 m (Stray-Gundersen *et al.*, 2001) and 5000 m (Levine & Stray-Gundersen, 1997) run times.

3.5 CONCLUSIONS

Chronic nightly hypoxic exposure using the LHTL model for 23 days increased βm by ~18%, but this occurred in the absence of enhanced muscle H^+ regulation during intense exercise. Living high:training low also significantly reduced whole body oxygen utilisation during exercise in normoxia, including during standardised submaximal exercise workloads, and by 7% at $\dot{V}O_{2peak}$. Thus, submaximal cycling efficiency was increased by 0.8%, which could be attributed to increased carbohydrate oxidation. These results suggest that increased β_m may be merely an indicator of adaptation and that greater efficiency may be of more practical importance.

CHAPTER 4. STUDY 1-PART II: CHRONIC INTERMITTENT HYPOXIA AND INCREMENTAL CYCLING EXERCISE INDEPENDENTLY DEPRESS MUSCLE MAXIMAL Na^+,K^+ATPase ACTIVITY IN WELL-TRAINED ATHLETES.

4.1 INTRODUCTION

In skeletal muscle, the Na^+,K^+ATPase enzyme is mainly located in the sarcolemma and t-tubular system, is critical in maintaining trans-sarcolemmal [Na^+] and [K^+] gradients and membrane excitability, and thus has been linked with fatigue (Fowles *et al.*, 2002b; Fraser *et al.*, 2002; Clausen, 2003). Under fatiguing conditions, skeletal muscle intracellular [Na^+] (Sjøgaard *et al.*, 1985; Juel, 1986; Sejersted & Sjøgaard, 2000) and extracellular [K^+] (Juel, 1986; Hallén, 1996; Green *et al.*, 2000e; Juel *et al.*, 2000b; Sejersted & Sjøgaard, 2000) can double causing inactivation of voltage-dependent Na^+ channels (Ruff, 1999), and reducing the muscle membrane potential (Sjøgaard *et al.*, 1985; Sejersted & Sjøgaard, 2000). The importance of Na^+,K^+ATPase for muscle contractility is emphasised when Na^+,K^+ATPase activity is inhibited. Incubation of rat muscle in ouabain, a specific Na^+,K^+ATPase inhibitor, or low [Na^+] and high [K^+] buffers increased the Na^+ / K^+ leak to pump ratio, depressed muscle tetanic force, accelerated muscle fatigue, and reduced recovery rate and M-wave area (Clausen, 2003). In human skeletal muscle hypoxia seems to induce Na^+,K^+ATPase downregulation, with Na^+,K^+ATPase content lowered by ~14% in experienced mountain climbers after a 21-d sojourn to high altitude (Green *et al.*, 2000a). Furthermore, in untrained subjects, exercise training (Green *et al.*, 1999b) or acute submaximal exercise under hypoxic conditions (Sandiford *et al.*, 2004) decreased Na^+,K^+ATPase content and activity by ~ 14% and ~28% respectively. One study measuring only the α_1, α_2 and β_1 Na^+,K^+ATPase subunits did, however, fail to report an effect of hypoxia on Na^+,K^+ATPase (Juel *et al.*,

2003b). Given that chronic hypoxia seems to downregulate muscle Na^+,K^+ATPase, which could then adversely affect muscle function, it seems somewhat paradoxical that athletes, coaches and physiologists have used altitude exposure for many years in a bid to improve athletic performance. It is not known if well-trained athletes are as susceptible to the hypoxia-induced depression in Na^+,K^+ATPase. More recently the practice of living high and training low (LHTL), comprising training in normoxia but sleeping in hypoxia has gained popularity with athletes (Levine & Stray-Gundersen, 1997; Hahn & Gore, 2001; Hahn *et al.*, 2001). As training under even moderate hypoxia compromises training intensity (Brosnan *et al.*, 2000), LHTL may therefore be advantageous by allowing maintenance of training intensity (Hahn & Gore, 2001). LHTL has been shown to yield small (0.8-1.3%) improvements in the exercise performance of well trained athletes during a 4-min all-out effort, a 400 m sprint and 3000 m as well as 5000 m runs (Hahn & Gore, 2001). This raises the interesting question about whether the hypoxia-induced depression of Na^+,K^+ATPase is less or absent in LHTL (Green *et al.*, 1999b; Green *et al.*, 2000a). Alternately, if LHTL does reduce muscle Na^+,K^+ATPase it will be important to determine the effect on muscular performance. Whether hypoxia imposed by LHTL induces a depression in skeletal muscle Na^+,K^+ATPase activity in well trained athletes has not yet been investigated.

Acute single leg kicking (Fraser *et al.*, 2002) or submaximal cycling exercise in untrained humans (Sandiford *et al.*, 2004) and prolonged running in rats (Fowles *et al.*, 2002a) each depresses maximal Na^+,K^+ATPase activity in skeletal muscle. Furthermore, repeated isometric muscle contractions for 30-min at 60% of maximal voluntary contraction, depressed muscle Na^+,K^+ATPase activity by as much as 38%, with corresponding reductions in muscle M-wave area and excitability (Fowles *et al.*, 2002b). It is unknown whether vigorous daily training which involves fatiguing exercise bouts, coupled with nightly hypoxic exposure, as occurs in the well-trained athletes

utilising LHTL practise, would result in a synergistic decline in muscle Na^+,K^+ATPase activity.

This study therefore investigated the effects of a LHTL intervention and of intensive exercise on skeletal muscle Na^+,K^+ATPase activity (K^+-stimulated 3-*O*-methylfluorescein phosphatase, 3-*O*-MFPase activity), content ([^{3}H]-ouabain binding sites), K^+ regulation and exercise performance in well-trained humans. It was hypothesised that nightly hypoxia imposed by LHTL would not depress muscle maximal Na^+,K^+ATPase activity, and thus allow enhanced muscle performance, whereas fatiguing exercise would acutely depress muscle maximal Na^+,K^+ATPase activity.

4.2 METHODS

4.2.1 Subjects

Thirteen male athletes (9 triathletes, 2 cross-country skiers and 2 cyclists) gave written informed consent to participate in this study, which was approved by the Australian Institute of Sport Ethics Committee, and Victoria University of Technology Human Research Ethics Committee. Subjects were ranked according to the work output achieved during the last 2 min of an incremental cycle ergometer test which established their peak oxygen consumption ($\dot{V}O_{2peak}$). Subjects were then assigned into two groups matched for work output achieved; control (CON n = 7) or live high:train low (LHTL, n = 6). Due to limited accommodation in, and access to, the Altitude House facility used for this study, the sample size of the LHTL group was limited to six. The nine triathletes (4 CON and 5 LHTL) trained together whilst the remaining athletes completed their own sport-specific training schedules. The physical characteristics of the CON and LHTL groups and their training frequency, intensity and duration did not differ (Table 5.1). This study is part of a larger investigation that has previously reported on the

effects of LHTL on hematologic variables (Ashenden *et al.*, 1999a), exercise VO_2 and performance, skeletal muscle metabolites, pH and buffering capacity, blood gas and acid base (Chapter 3).

Table 4.1 Subject physical and training characteristics. The live high:train low group (LHTL, n = 6) slept at a simulated altitude of 3000 m (F_IO_2 15.48%) and trained at 600 m (Canberra, Australia), while the control group (CON, n = 7) lived and trained at 600 m. Data are mean±SD. No significant differences were found between groups for any variable.

	LHTL	CON
Age (yr)	25.4±3.6	25.1±5.2
Height (cm)	183.5±10.0	181.2±6.3
Body mass (kg)	73.0±6.7	73.3±6.1
Training (sessions.wk^{-1})	7.1±2.3	6.8±2.3
Training intensity (Borg units)	13.8±1.1	13.6±1.0
Training (h.wk^{-1})	13.4±3.8	10.6±5.7

4.2.2 Experimental design, and exercise tests

Details of experimental design and each exercise test are described in detail in sections 3.2.2, 3.2.3 and 3.2.4 of this thesis. Briefly, this study was conducted in Canberra, Australia. The LHTL subjects spent 9.5 h.night^{-1} for 23 consecutive nights at a simulated 3000 m altitude. The CON subjects slept in their own homes under normobaric normoxia. Training and daytime living for all subjects was at an altitude of 600 m.

4.2.3 Simulated altitude

Ambient air in the 'altitude house' was diluted with nitrogen gas until the desired percentage of oxygen (F_IO_2 15.48%) was obtained, equivalent to an altitude of approximately 3000 m above sea level. Throughout each of the 23 nights, F_IO_2 and F_ICO_2 inside the hypoxic room were measured every 30 min (Chapter 3).

4.2.4 Blood sampling, analyses and calculations

Before each of the cycle ergometer tests, a catheter (20-G, Jelco) was inserted into a superficial dorsal hand vein and covered with an adhesive plastic dressing and waterproof glove. After catheterisation, each subject was seated on the cycle ergometer and the catheterised hand was immersed in a water bath (44.5^{o}C) for 10-min to ensure arterialisation of venous blood. Efficacy of arterialisation was demonstrated since pO_2 > 70 mm Hg (data not shown) (Forster *et al.*, 1972; McLoughlin *et al.*, 1992). Two blood samples (1.5 ml and 2 ml) were taken from a dorsal hand vein during the last 30 s of each workrate, and at 1 and 5-min recovery. Blood samples were immediately analysed for acid-base variables (Chapter 3), plasma potassium concentration ($[K^+]$) and blood hemoglobin concentration ([Hb]), using an automated analyser (ABL System 625, Radiometer, Copenhagen, Denmark). Hematocrit (Hct) was determined in quadruplicate via capillary tubes spun in a micro-centrifuge (Biofuge, Heraesus Instruments, Osterode, Germany). Plasma acid-base variables have been reported previously (Chapter 3). The rise in plasma $[K^+]$ above rest ($\Delta[K^+]$) was calculated for each workrate and also normalised for work performed ($\Delta[K^+].work^{-1}$ ratio, $nmol.L^{-1}.J^{-1}$) as previously described (McKenna *et al.*, 1993; Fraser *et al.*, 2002).

4.2.5 Muscle biopsy sampling and analyses.

A muscle biopsy was taken at rest and immediately post-exercise, both before (Pre) and after (Post) the LHTL or CON intervention. The needle biopsy sample was taken from the vastus lateralis muscle under local anaesthesia (Xylocaine, 1 %), with suction

applied to the needle. The post-exercise sample was taken immediately after cessation of the 2 min exercise trial at 5.6 ± 0.4 $W.kg^{-1}$, with the subject lying supported on the cycle ergometer. Both biopsies in a trial were taken from separate incisions in the same leg, with the exercise sample taken from an incision ~1.5 cm distal to the rest sample. The same, experienced medical practitioner took all biopsies at approximately constant depth. Muscle samples (100-120 mg) were removed, blotted on filter paper to remove blood, with obvious fat and connective tissue removed by dissection. The muscle was then rapidly divided into two portions with one portion immediately frozen and stored for later analysis of [^{3}H]-ouabain binding sites and the other immediately homogenised for maximal 3-*O*-MFPase activity.

4.2.5.1 Maximal 3-O-MFPase activity

Thirty to forty mg of muscle was quickly blotted on filter paper, weighed, then homogenised (5% wt/vol) on ice for 2 × 20 s, 20,000 rpm (Omni 1000, Omni International) in an homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40) (Fraser *et al.*, 2002). The homogenate maximal *in vitro* Na^+,K^+ATPase activity was determined in triplicate using the K^+-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity assay (Fraser & McKenna, 1998; Fraser *et al.*, 2002). The resting muscle 3-*O*-MFPase activity was also contrasted between Pre and Post conditions, for both LHTL and CON, by calculating the Pre-Post change in 3-*O*-MFPase activity and expressed as a percentage of the Pre-intervention value (change score). The muscle 3-*O*-MFPase activity inter-assay variability was 9.9% (n=13), and the intra-assay coefficient of variation (SD/Mean x 100, CV) for 3-*O*-MFPase was 5.6% (n=52). The 3-*O*-MFPase activity was also expressed relative to the muscle homogenate total protein content, which was measured spectrophotometrically (Lowry *et al.*, 1951).

4.2.5.2 [^{3}H]-ouabain binding sites

Na^+,K^+ATPase content was determined in quadruplicate using [^{3}H]-ouabain binding site content as previously described (Nørgaard *et al.*, 1984a; Kjeldsen *et al.*, 1986b; McKenna *et al.*, 1993; Fraser *et al.*, 2002). Muscle samples were cut into 2-5 mg pieces and washed for 2 x 10 min in 37°C vanadate buffer containing 250 mM sucrose, 10 mM Tris, 3 mM $MgSO_4$, and 1 mM $NaVO_4$ (pH 7.2-7.4). This was to thaw the samples and to maintain lower Na^+ and K^+ concentrations to minimise interference with vanadate-facilitated [^{3}H]-ouabain binding. Muscle samples were then incubated for 120 min at 37°C in the above buffer with the addition of [^{3}H]-ouabain (10^{-6} M, 2.0 μCi.ml^{-1}). After incubation, muscle samples were washed for 4 x 30 min in ice-cold vanadate buffer to remove any unbound [^{3}H]-ouabain, blotted on filter paper and weighed before being soaked overnight in vials containing 0.5 ml of 5% trichloroacetic acid (TCA) and 0.1 mM ouabain. The following morning, 2.5 ml of scintillation cocktail (Opti-Fluor, Packard) was added prior to liquid scintillation counting of the [^{3}H]-activity. The content of [^{3}H]-ouabain binding sites was calculated on the basis of the sample wet weight and the specific activity of the incubation medium and samples and expressed as pmol.(g wet wt.)$^{-1}$. Due to the limited availability of muscle tissue, muscle Na^+,K^+ATPase content could not be measured for all subject days and times (CON Rest Pre n=5; Ex Pre n=2; Rest Post n=3; Ex Post n=4; and LHTL Rest Pre n=6; Ex Pre n=2; Rest Post n=6; and Ex Post n=5). Therefore the resting muscle [^{3}H]-ouabain binding site content was contrasted between Pre and Post conditions where a complete set of Pre and Post rest samples, was available LHTL (n=6) and CON (n=3). The effects of exercise on muscle [^{3}H]-ouabain binding content were also examined, but the sample size was too small for meaningful comparisons between groups. Data were also pooled from both groups prior to intervention to examine a possible exercise effect on [^{3}H]-ouabain binding (n=11). The resting muscle [^{3}H]-ouabain binding site content intra-assay CV

was 14.5% (n=51). The [^{3}H]-ouabain binding site content was also expressed relative to muscle protein content.

4.2.6 Statistical analyses

All data are presented as mean±SD. The physical and training characteristics of the two groups were analysed with an independent t-test. Maximal 3-*O*-MFPase activity in resting muscle was normalised to pre-intervention resting levels and the change score contrasted between CON and LHTL with an independent t-test, to account for the small sample size and typical inter-subject, intra and inter assay variability (Huck & McLean, 1975). A three-way ANOVA with independent factor for group (LHTL versus CON) and with repeated measures for sample time (rest, exercise) and day (Pre, Post) was used to test for main and interaction effects for muscle and blood data. Exercise performance data and [^{3}H]-ouabain binding content were analysed with a two-way repeated measures ANOVA for group and day. The Student-Newman-Kuels post hoc test was used to locate differences found in ANOVAs. Statistical significance was accepted at $P<0.05$.

4.3 RESULTS

4.3.1 Performance

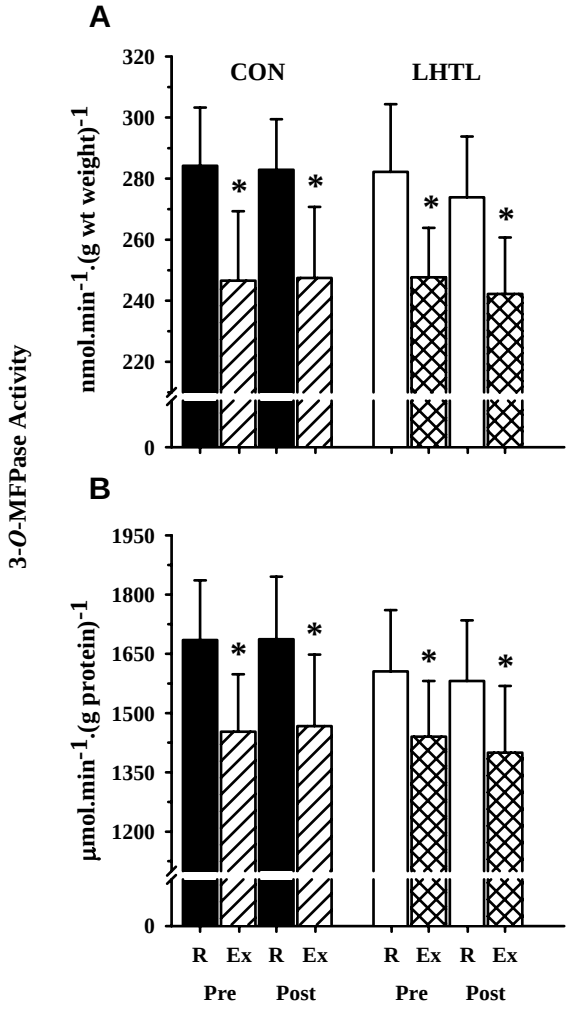
LHTL reduced $\dot{V}O_2$peak by 7.2±4.1% (Pre 5.08±0.34, Post 4.78±0.36 L.min^{-1}, $P<0.05$,) with no change in CON (Pre 4.95±0.45, Post 4.87±0.44 L.min^{-1}, *NS*). Total work did not differ between groups or days (LHTL Pre 50.0±4.2, Post 49.2±4.2; CON Pre 50.5±6.0, Post 50.3±5.8 kJ, *NS*) (Chapter 3).

*4.3.2 Muscle 3-***O-***MFPase activity*

The muscle maximal *in-vitro* 3-*O*-MFPase activity expressed per gram wet weight (nmol.min^{-1}.(g^{-1} wet wt)$^{-1}$) declined by 12.4±4.1% immediately after exercise ($P<0.05$, Fig. 4.1). The muscle 3-*O*-MFPase activity also decreased from Pre to Post (265±27

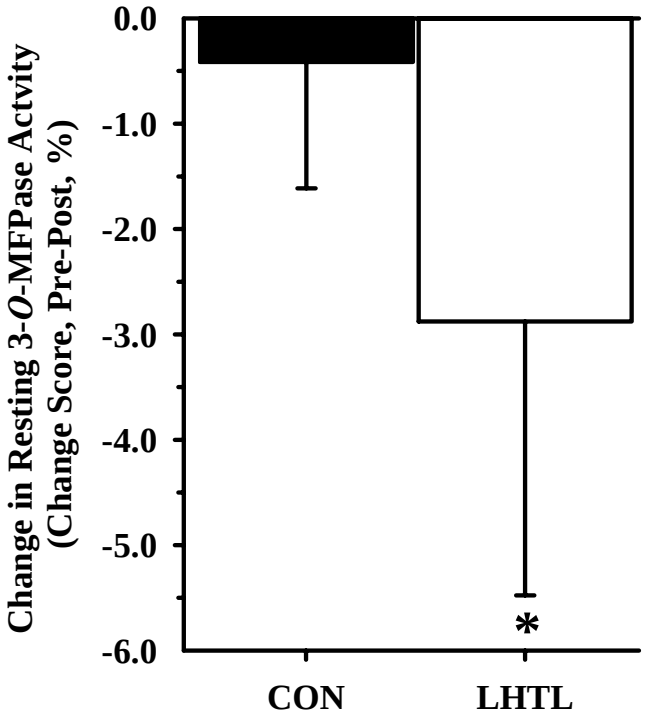
versus 262±26 nmol.min^{-1}.(g wt weight)$^{-1}$, respectively, P<0.05), with no significant difference detected between groups. Maximal 3-*O*-MFPase activity in resting muscle was normalised to pre-intervention resting levels and the change contrasted between CON and LHTL, to account for the small sample size and typical inter-subject, intra and inter assay variability (change score). This indicated that maximal 3-*O*-MFPase activity was decreased by 2.9±2.6% in LHTL (P<0.05), but was unchanged in CON (-0.4±1.2%, *NS*, Fig. 4.2). When muscle 3-*O*-MFPase activity was expressed per gram protein (nmol.min^{-1}.g protein^{-1}), exercise induced a similar depression (-12.3±4.4%, P<0.05, Fig. 4.1).

Figure 4.1 Skeletal muscle maximal *in vitro* K^+-stimulated 3-*O* methylfluorescein phosphatase (3-*O*-MFPase) activity (Na^+,K^+ATPase activity) at rest (R) and end exercise (E) for subjects before (Pre) and after (Post) control (CON) or Live high train low (LHTL) interventions.



Data are expressed as $nmol.min^{-1}.(g^{-1}$ wet wt$)^{-1}$. (A) or $\mu mol.min^{-1}.(g^{-1}$ protein$)^{-1}$ (B); mean ± SD; n=7 for CON, n=6 for LHTL, * End-exercise < Rest ($P < 0.05$, Exercise main effect).

Figure 4.2 Change in resting skeletal muscle maximal 3-*O*-MFPase activity with LHTL and CON.



The resting muscle 3-*O*-MFPase activity was also contrasted between Pre and Post conditions, for both LHTL and CON, by calculating the Pre-Post change in 3-*O*-MFPase activity and expressed as a percentage of the Pre-intervention value (change score). Data are mean ± SD; n=7 for CON, n=6 for LHTL; * LHTL > CON ($P < 0.05$, Group main effect).

4.3.3 Muscle [^{3}H]-ouabain binding content

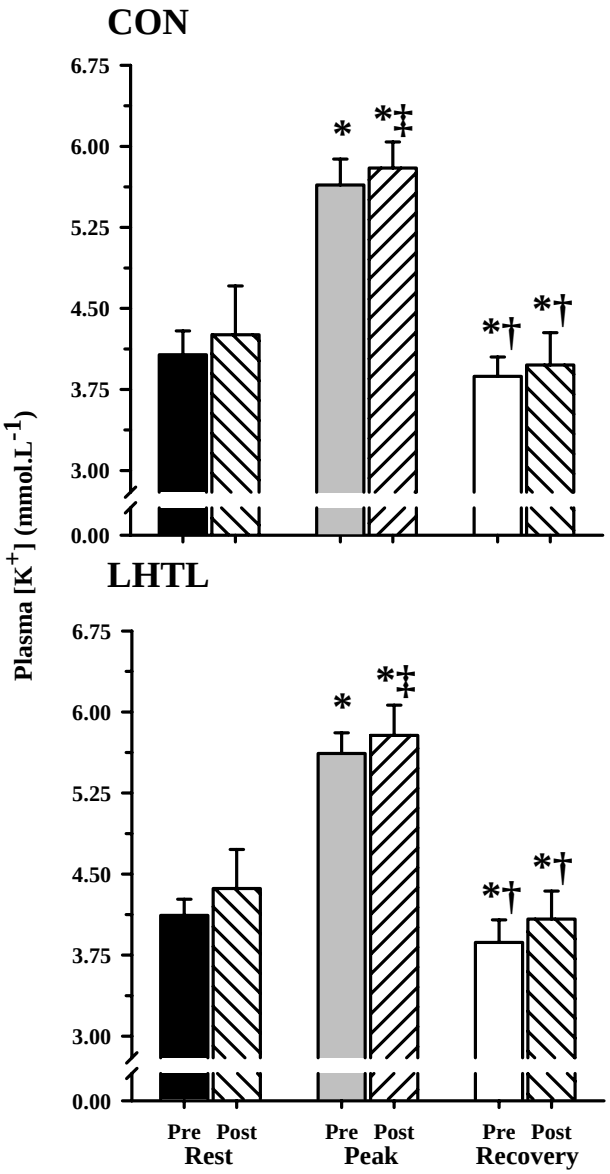
Resting muscle [^{3}H]-ouabain binding content did not differ between group or day when expressed per gram wet weight (CON Pre 307±41, Post 339±54; LHTL Pre 324±52, Post 303±39 pmol.(g wet wt)$^{-1}$), or per gram protein (CON Pre 1746±144, Post 1797 ±252; LHTL Pre 1843 ±311, Post 1751 ±267 pmol.(g protein.)$^{-1}$, *NS*). There was no effect of acute exercise on [^{3}H]-ouabain binding content with data pooled for groups

prior to intervention, when expressed per gram wet weight (pmol.(g wet wt)$^{-1}$) or per gram protein (Rest 1918 ±415; End-Ex 2030 ±611 pmol.(g protein.)$^{-1}$, *NS*).

4.3.4 Change in Plasma [K^+]

Plasma [K^+] increased above rest with each workrate, and fell to below resting levels at 5-min recovery (P<0.05, Fig. 4.3). A small increase in plasma [K^+] was found in the post intervention test (Pre 4.92±0.68 Post 5.11±0.77 mmol.L^{-1}, P<0.05, Fig. 4.3), but plasma [K^+] did not differ significantly between groups. The Δ[K^+] increased above 1.5W.kg^{-1} during exercise at 2.5, 3.5 and 4.5 W.kg^{-1}, remaining elevated at the final 5.6 W.kg^{-1} work bouts (P<0.05, Table 4.2). The Δ[K^+].work^{-1} ratio during the first submaximal workrate (1.5 W.kg^{-1}) was greater (P<0.05, Table 4.2) than for subsequent workrates (2.5, 3.5, 4.5 and 5.6 W.kg^{-1}), was higher Pre for LHTL, and also different to CON Pre (P<0.05, Table 4.2). Plasma [K^+], Δ[K^+] and Δ[K^+].work^{-1} ratio results were almost identical in the biopsy trial and the performance trial (data not shown).

Figure 4.3 Arterialised-venous plasma [K^+] for CON and LHTL obtained at rest, during the peak incremental cycling exercise workrate and at 5 min recovery.



Data are mean ± SD; n=7 for CON, n=6 for LHTL.*, different to rest ($P < 0.05$, exercise main effect), †, different to peak ($P < 0.05$, exercise main effect). ‡, Post > Pre ($P < 0.05$, Day main effect).

Table 4.2 Rise in plasma [K^+] with exercise ($\Delta[K^+]$) and $\Delta[K^+]$ expressed relative to work performed during the biopsy trial ($\Delta[K^+].Work^{-1}$ ratio) for CON (n = 7) and LHTL (n=6). All data are Mean ± SD.* different to 1.5 $W.Kg^{-1}$, †, different to all other workrates ($P<0.05$, Time main effect). ** greater than CON Pre, ‡ LHTL Pre > Post ($P<0.05$, Group by time by day interaction effect).

Variable	Group	Day	Exercise Workrate ($W.kg^{-1}$)				
			1.5	2.5	3.5	4.5	5.6
$\Delta[K^+]$ ($mmol.L^{-1}$)	LHTL	Pre	0.63 ± 0.15	0.80 ± 0.10*	0.87 ± 0.15*	1.47 ± 0.35*	1.63 ± 0.06
		Post	0.53 ± 0.15	0.73 ± 0.25*	0.93 ± 0.21*	1.37 ± 0.31*	1.57 ± 0.12
	CON	Pre	0.49 ± 0.15	0.69 ± 0.16*	0.83 ± 0.21*	1.39 ± 0.16*	1.57 ± 0.27
		Post	0.54 ± 0.19	0.74 ± 0.25*	1.14 ± 0.75*	1.53 ± 0.29*	1.70 ± 0.22
$\Delta[K^+].Work^{-1}$ ratio ($nmol.L^{-1}.J^{-1}$)	LHTL	Pre	26.7 ± 5.5†**‡	11.8 ± 1.6	6.9 ± 1.4	7.0 ± 1.5	5.9 ± 0.9
		Post	19.0 ± 5.6†	9.2 ± 3.3	6.5 ± 1.8	6.4 ± 1.2	5.6 ± 1.0
	CON	Pre	20.4 ± 5.5†	10.6 ± 2.6	6.9 ± 1.8	7.1 ± 1.1	6.4 ± 1.2
		Post	21.9 ± 7.6†	11.1 ± 3.8	8.9 ± 5.2	7.5 ± 1.56	6.7 ± 1.0

4.4 DISCUSSION

4.4.1 LHTL induces only a small decline in resting Na^+,K^+ATPase activity

Hypoxic exposure during either an altitude sojourn or during training depressed skeletal muscle Na^+,K^+ATPase total content (Green *et al.*, 1999b; Green *et al.*, 2000a) and the live high, train low (LHTL) approach of nightly hypoxic exposure could be anticipated to similarly downregulate Na^+,K^+ATPase in skeletal muscle. Therefore, an important finding of this study was that intermittent hypoxia, imposed through 23 nights at 3000 m simulated altitude (LHTL) caused only a very small (~3%) depression in muscle maximal Na^+,K^+ATPase activity, as measured by maximal K^+-stimulated 3-*O*-MFPase activity (Fraser & McKenna, 1998) in already well-trained individuals. This contrasts the ~28% reduction in 3-*O*-MFPase activity recently reported in untrained participants during acute prolonged submaximal exercise in hypoxia (Sandiford *et al.*, 2004). The small reduction in 3-*O*-MFPase activity reported here was accompanied by no change in plasma K^+ regulation during exercise. If this was also true for muscle intracellular and extracellular [K^+] with LHTL, this would suggest no further deterioration in muscle excitability and muscle function with LHTL. Performance, as measured by total work output during high intensity cycling was also unchanged by LHTL. This suggests that the duration, magnitude or intermittent nature of the hypoxic exposure was insufficient to markedly downregulate muscle Na^+,K^+ATPase and adversely affect muscle performance, or that well-trained athletes may have a differential response to imposed hypoxia. Two further important findings were that intensive cycling exercise depressed muscle 3-*O*-MFPase activity in well-trained athletes by ~12%, and that this depression was not exacerbated by LHTL.

No significant change in [^{3}H]-ouabain binding content was found with LHTL, but this may be due to our restricted sample size and the inherent variability of the assay. The [^{3}H]-ouabain binding content tended to be less (~7%) after LHTL, consistent with the

small (~3%) reduction in 3-*O*-MFPase activity. However, this finding contrasts the much larger (~14%) downregulation in Na^+,K^+ATPase content after a 21-d expedition to 6184 m in experienced mountain climbers (Green *et al.*, 2000a), or previously untrained individuals training under hypoxic conditions (Green *et al.*, 1999b). The small reduction in 3-*O*-MFPase activity with LHTL also sharply contrasts the ~28% downregulation of Na^+,K^+ATPase activity following 90-min of submaximal exercise performed in hypoxia (Sandiford *et al.*, 2004), 30-min of repeated isometric muscle contractions in humans (~38%) (Fowles *et al.*, 2002b), and after 30-min of severe acute hypoxia (F_IO_2 7%) in rat liver (~28%) (Mankovskaya *et al.*, 2000). The total time spent under hypoxic conditions, or the degree of hypoxic stimulus alone cannot account for these differing changes in muscle Na^+,K^+ATPase content or activity. This study imposed a large total number of hours of hypoxic exposure (~ 218), albeit with ~14.5 $h.d^{-1}$ spent in normoxia. Untrained participants in the latter training study (Green *et al.*, 1999b) underwent a total of only 12 h of hypoxic exposure, whilst mountain climbers in the altitude sojourn (Green *et al.*, 2000a) underwent ~ 288 h continuously under hypoxic exposure. The reduction in Na^+,K^+ATPase content of ~14% was identical despite the 20-fold difference in exposure time, each greater than the ~3% depression in activity after this LHTL hypoxic exposure. The hypoxic stimulus during the training study (F_IO_2 13.5%) and during the altitude sojourn (within a range of 2160 and 6194 m, equivalent to normobaric hypoxia of F_IO_2 ~ 17% to ~10%) were each greater than in this study (F_IO_2 15.48%), which might contribute to the smaller effect on muscle Na^+,K^+ATPase reported here. These findings are difficult to compare, however, as there may be confounding effects of normobaric versus hypobaric hypoxia, nutritional (Abbate *et al.*, 2002) changes with altitude, training performed under normoxic versus hypoxic conditions and possible effects of cold stress.

It is well established that exercise training in normoxia upregulates muscle Na^+,K^+ATPase content in humans, with increases of between 13 and 29% reported (Green *et al.*, 1993; McKenna *et al.*, 1993; Madsen *et al.*, 1994; Evertson *et al.*, 1997; Green *et al.*, 1999a; Medbø *et al.*, 2001). Participants in this study were highly trained and engaged in strenuous exercise in normoxia for between ten and thirteen hours per week. As little as 6 min strenuous exercise is enough to increase skeletal muscle Na^+,K^+ATPase mRNA (Murphy *et al.*, 2004), suggesting that continual stimuli with repeated training may be important in the elevation in muscle Na^+,K^+ATPase with chronic training. This potential stimulatory effect of exercise training in normoxia could counter-balance the potential decrease in Na^+,K^+ATPase activity and / or content with hypoxia or training in hypoxia, thereby explaining the minor decrease in activity reported with LHTL. When compared to the untrained participants in the hypoxic training study (Green *et al.*, 1999b) or mountain climbers in the altitude sojourn (Green *et al.*, 2000a), participants in this study had a far more extensive normoxic training history which may have further protected against the depressive effect of hypoxia. A further possible explanation is that an initial larger depression in muscle 3-*O*-MFPase activity occurred in the first week of LHTL, or in the first days after LHTL but that this was undetected since the biopsies were taken only before and after the 23-night intervention.

4.4.2 Intense cycling exercise depresses Na^+,K^+ATPase (3-O-MFPase) activity

An important finding of this study was that intensive cycling exercise in well trained athletes depressed muscle maximal 3-*O*-MFPase activity by ~12%. The exercise used in this study comprised submaximal and maximal efforts and thus extends previous work demonstrating a reduction in maximal muscle 3-*O*-MFPase activity after fatiguing single-leg dynamic kicking exercise (Fraser *et al.*, 2002) and fatiguing isometric quadriceps contractions (Fowles *et al.*, 2002b) in humans and after prolonged treadmill

running in rats (Fowles *et al.*, 2002a). An important feature of this study was the reproducibility of this exercise-induced depression. The depression in muscle maximal 3-*O*-MFPase activity was robust, being almost identical in both Pre and Post tests, in both CON and LHTL groups where total work was also unchanged. This further supports the contention that this depression in 3-*O*-MFPase activity is an obligatory response to fatiguing muscle contractions (Fraser *et al.*, 2002).

The reduction in muscle maximal 3-*O*-MFPase activity reported here reflects a reduction in the maximal theoretical 3-*O*-MFPase activity, as measured under *in vitro* conditions. Muscle Na^+,K^+ATPase activity may attain near-maximal theoretical levels in rat muscle, when stimulated at high frequencies (Nielsen & Clausen, 1997; McKenna *et al.*, 2003). However, motor-unit discharge rates in contracting human muscles are likely to be at lower frequencies (Jones, 1996; Roos *et al.*, 1999) and the Na^+,K^+ATPase might not attain its maximum theoretical activity *in vivo* (Hallén, 1996; Sejersted & Sjøgaard, 2000). During exercise Na^+,K^+ATPase activity achieved *in vivo* is insufficient to completely counteract the loss of K^+ from muscle observed during contraction, since large increases in K^+ in circulating plasma and in muscle interstitium occur (Hallén, 1996; McKenna *et al.*, 1997; Green *et al.*, 2000e; Juel *et al.*, 2000b). A 12% decline in the maximal *in vitro* 3-*O*-MFPase activity with exercise cannot be directly equated with a reduced *in-vivo* Na^+,K^+ATPase activity. However, it is likely that *in-vivo* maximal Na^+,K^+ATPase activity would be similarly depressed, which may then exacerbate muscle Na^+ / K^+ fluxes and thereby contribute to muscle fatigue (Clausen, 2003).

The methodology used in this study does not allow determination of the mechanisms underlying the reduction in maximal muscle Na^+,K^+ATPase activity with exercise. Given that the 3-*O*-MFPase activity assay is performed under standardised conditions, the reduction is likely to result from structural changes in the Na^+,K^+ATPase enzyme or changes in the membrane in which it is embedded. Likely candidates for these changes

include increases in muscle accumulation of reactive oxygen species (ROS) (Kourie, 1998) and/or intracellular calcium ($[Ca^{2+}]_i$) (Stankovicova *et al.*, 1995), as previously fully discussed (Fraser *et al.*, 2002). The lack of a cumulative or synergistic depression in muscle Na^+,K^+ATPase activity with LHTL and intense exercise might suggest that different mechanisms are responsible for the exercise- and hypoxia-induced depression in muscle maximal Na^+,K^+ATPase activity.

4.4.3 Depressed Na^+,K^+ATPase activity, yet maintained muscle performance

Intriguing questions addressed here were whether muscle Na^+,K^+ATPase activity was depressed with LHTL and how this could be compatible with reports of increased muscle performance with LHTL (Hahn & Gore, 2001). This possible dissociation could then suggest irrelevance of changes in muscle Na^+,K^+ATPase activity for exercise performance in humans. However, our results were not consistent with this possibility, since only a small decline in muscle maximal Na^+,K^+ATPase activity was evident with LHTL, whilst muscle performance was maintained after LHTL, as measured by an unchanged total work performed in the final two minutes of an 'all-out' test (Gore *et al.*, 2001). A small reduction in muscle maximal Na^+,K^+ATPase activity after LHTL hypoxic exposure may even be beneficial by reducing muscle ATP utilisation which may make a minor contribution to the small reduction in $\dot{V}O_2$ and improvement in gross mechanical efficiency noted after both LHTL (Gore *et al.*, 2001) and an expedition to 6194m (Green *et al.*, 2000b).

4.5 CONCLUSIONS

In conclusion, LHTL hypoxic exposure induced a small but significant depression (2.9 %) in skeletal muscle maximal Na^+,K^+ATPase activity, but with no associated decline in muscle Na^+,K^+ATPase content, performance or plasma K^+ regulation. These findings are important since they demonstrate that a small reduction in skeletal muscle maximal

Na^+,K^+ATPase activity did not affect muscle performance in exercising humans. It is possible that the intermittent nature of the nightly moderate hypoxic exposure and/or daily training in normoxia prevented a more marked deterioration on muscle maximal Na^+,K^+ATPase. This may explain why athletes can use LHTL without deterioration in performance. Acute exercise itself induced a much larger depression in skeletal muscle maximal Na^+,K^+ATPase activity, but there were no cumulative or synergistic effects with LHTL. This depression of maximal Na^+,K^+ATPase activity with vigorous muscular activity was robust and may be an important component of skeletal muscle fatigue.

CHAPTER 5. STUDY 2: EFFECTS OF ACUTE SPRINT EXERCISE AND CONSECUTIVE VERSUS INTERMITTENT NIGHTS OF HYPOXIA ON SKELETAL MUSCLE Na^+,K^+ATPase ACTIVITY, PLASMA IONS AND ACID-BASE

5.1 INTRODUCTION

The Na^+,K^+ATPase enzyme plays an important regulatory role in skeletal muscle by regulating trans-sarcolemmal [Na^+] and [K^+] gradients, membrane excitability and thus contractility (Overgaard *et al.*, 1999; Nielsen & Clausen, 2000; Clausen, 2003). In isolated rat muscle, specific inhibition of Na^+,K^+ATPase activity by ouabain increased muscle Na^+ gain and K^+ loss during muscle excitation, reduced M-wave area during exercise, depressed muscle tetanic force, accelerated muscle fatigue, and reduced recovery rate (Clausen, 2003). This suggests that perturbations that reduce muscle Na^+,K^+ATPase activity in human muscles may have similar effects.

Acute exercise has marked effects upon maximal Na^+,K^+ATPase activity in the skeletal muscle. Na^+,K^+ATPase activity is rapidly increased during contractile activity by up to 18-22-fold in isolated rat muscles, as measured by the rate of net Na^+ extrusion (Nielsen & Clausen, 1997; McKenna *et al.*, 2003). Only two studies have examined in well-trained athletes the effects of fatiguing acute exercise on muscle maximal Na^+,K^+ATPase activity (Fraser et al., 2002; Chapter 4), finding this to be depressed ~13% after both incremental exercise (Chapter 4) and ~6-min of repeated maximal single leg kicking (Fraser *et al.*, 2002). In non-athletes, Na^+,K^+ATPase activity was also depressed after acute prolonged submaximal exercise (Leppik *et al.*, 2004; Sandiford *et al.*, 2004), and submaximal isometric contractions (Fowles *et al.*, 2002b). Sprint exercise involves considerable muscle K^+ efflux (McKenna *et al.*, 1997; Sejersted & Sjøgaard, 2000) which would decrease the intracellular-to-extracellular [K^+] ratio,

reduce sarcolemmal excitability (Sjøgaard *et al.*, 1985; Sejersted & Sjøgaard, 2000) and conceivably contribute to muscle fatigue (Clausen, 2003; Clausen *et al.*, 2004). Hence, inactivation of muscle Na^+,K^+ATPase during acute sprint exercise could augment muscle K^+ disturbances and be an important contributor to muscle fatigue. No studies have investigated whether intense sprinting exercise of ~1-min performed by well-trained athletes might also induce a similar or larger depression in Na^+,K^+ATPase activity. It is recognised that well-trained athletes are likely to have a greater Na^+,K^+ATPase content in skeletal muscle compared to untrained individuals (Klitgaard & Clausen, 1989; Fraser *et al.*, 2002). This upregulation could be a protective adaptation. Therefore, the hypothesis that sprint exercise in well-trained athletes would depress maximal Na^+,K^+ATPase activity was tested.

Elite athletes commonly use hypoxic interventions to improve endurance exercise performance. Typically, this hypoxic training involves living high, and training low (LHTL) (Levine & Stray-Gundersen, 1997; Hahn & Gore, 2001; Hahn *et al.*, 2001). As training under even moderate hypoxia compromises training intensity (Levine & Stray-Gundersen, 1992; Levine & Stray-Gundersen, 1997; Brosnan *et al.*, 2000), LHTL may therefore be advantageous by allowing maintenance of training intensity (Hahn *et al.*, 2001). LHTL has been shown to yield small (0.8-1.3%) but meaningful improvements in the exercise performance of well-trained athletes (Hahn *et al.*, 2001). Hypoxia is also a feature of many clinical conditions, and appears to be a potent stimulus for downregulation of Na^+,K^+ATPase in skeletal muscle, reducing Na^+,K^+ATPase content by ~ 14% (Green *et al.*, 1999b; Green *et al.*, 2000a) and maximal activity by ~28% (Sandiford *et al.*, 2004). In contrast to these large reductions, 23 consecutive nights (n) (LHTLc) hypoxia, equivalent to 3000 m did not alter Na^+,K^+ATPase content with only a small but significant reduction (~3%) in maximal Na^+,K^+ATPase activity (Chapter 4), and 2 or 8 weeks at 4100 m had no effect on α_1, α_2 or β_1 Na^+,K^+ATPase isoforms (Juel

et al., 2003b) . Several factors might explain the unchanged Na^+,K^+ATPase content and small change in maximal activity with LHTL (Chapter 4). First, it is possible that Na^+,K^+ATPase downregulation had occurred early in the 23-n period, but that this was subsequently compensated by an adaptive upregulatory response, perhaps in part consequential to the ongoing, heavy daily training common to well-trained athletes. An early response would be consistent with the large decline in muscle Na^+,K^+ATPase activity during exercise in acute hypoxia (Sandiford *et al.*, 2004). However, the time course of changes in muscle Na^+,K^+ATPase to chronic hypoxia are unknown. The final biopsy in the previous study was taken 3d after cessation of LHTL hypoxia (Chapter 4). Hence, it is also possible that a larger depression in Na^+,K^+ATPase content and activity had in fact occurred, but was missed due to a rapid recovery of muscle Na^+,K^+ATPase upon return to normoxia.

If brief exposures to hypoxia cause a large reduction in Na^+,K^+ATPase activity, then this could be disadvantageous for athletes. Knowledge of whether acute depression and recovery occurs is therefore of considerable practical importance. If such rapid down- and up-regulatory changes in Na^+,K^+ATPase content and activity had occurred with hypoxia, as suggested by others (Sandiford *et al.*, 2004), this might also explain the failed to detect any impairment in plasma $[K^+]$ regulation during exercise with LHTL (Chapter 4). It is commonly thought that the improvement in Na^+,K^+ATPase content with training leads to improved K^+ regulation during exercise (McKenna *et al.*, 1993). This hypoxic intervention therefore also offers a useful model to examine whether reductions in Na^+,K^+ATPase activity are associated with impaired plasma K^+ regulation during exercise. Therefore, the hypothesis that an early depression in Na^+,K^+ATPase activity with hypoxia would be accompanied by a greater plasma $[K^+]$ during exercise was tested.

Acute hypoxia exacerbated the decline in muscle maximal Na^+,K^+ATPase activity during prolonged exercise in untrained individuals (Sandiford *et al.*, 2004). However, there was no alteration in decline in Na^+,K^+ATPase activity with incremental exercise after LHTL (Chapter 4). This may have reflected the delay in not conducting the exercise bout until 3-d after LHTL, as above, or that Na^+,K^+ATPase activity had declined to a greater extent during early exposure to LHTL. Therefore the hypothesis that the depression in Na^+,K^+ATPase activity with sprint exercise in well-trained athletes would be augmented by LHTL was tested.

Recent change to LHTL hypoxic practices for athletes involves interspersing 'blocks' of nightly exposure to hypoxia, with several nights of normoxia (LHTLi), to minimise any adverse psychological impact of LHTL (Saunders *et al.*, 2004). Furthermore, to minimise any altitude sickness, athletes now favor lower altitudes of up to ~2700m (Saunders *et al.*, 2004). The effect of LHTLi on muscle Na^+,K^+ATPase and the time course of this adaptation is not known, especially in well-trained athletes. Therefore the hypotheses that Na^+,K^+ATPase activity in resting muscle would be reduced early in LHTLc with a further decline after 20-n, whereas the greater periods of normoxia during LHTLi would attenuate the hypoxia-induced reduction in Na^+,K^+ATPase with LHTLc were tested. Finally, another adaptation to LHTL in skeletal muscle is an increased muscle buffering capacity (Chapter 3). However, this adaptation was not accompanied by reduced plasma $[H^+]$ during exercise, despite decreased pCO_2 and a trend to reduced plasma lactate concentration (Chapter 3). This suggests possible alterations in other mechanisms of $[H^+]$ regulation with LHTL hypoxia, which may include changes in membrane bound proteins involved in lactate, bicarbonate and H^+ fluxes (Green *et al.*, 2004). Unchanged plasma $[H^+]$ with LHTL hypoxia might reflect altered plasma concentrations of potassium (K^+]), sodium (Na^+]) and chloride ([Cl^-]), since these would in turn modify the plasma strong ion difference ([SID]) (Stewart,

1981; McKenna *et al.*, 1997; Lindinger, 2003). Changes in each of these electrolyte and acid-base variables are likely to be greatest in intense sprinting exercise. However, the effects of LHTL hypoxia on plasma [SID], pCO_2 or $[H^+]$ with sprint exercise remain to be investigated. Therefore the hypothesis that LHTL would not change plasma $[H^+]$ during exercise due to alterations in other strong ions in plasma, modifying plasma [SID] was tested.

5.2 METHOD

5.2.1 Subjects

Thirty-three male endurance-trained athletes (24 cyclists and 9 triathletes) gave written informed consent to participate in the study, which was approved by both the Victoria University Human Research Ethics Committee and the Australian Institute of Sport Ethics Committee. Subjects were ranked according to their initial peak O_2 consumption (VO_{2peak}) and were then assigned into one of three groups; control (CON n = 11), live high:train low consecutive nights (LHTLc, n = 12), or live high:train low intermittent nights (LHTLi, n = 10). This study forms part of a larger study also investigating muscle metabolism, sleep and respiratory responses to LHTL; hence details of subjects, LHTL and some test methodologies have already been presented elsewhere (Townsend *et al.*, 2002; Clark *et al.*, 2004a; Kinsman *et al.*, In press-a). There were no significant differences between groups for age, height or body mass (Table 5.1).

Table 5.1 Subject physical and peak performance characteristics.

	CON	LHTLc	LHTLi
Age (yr)	26.2±4.5	27.2±5.7	26.2±4.5
Height (cm)	177.6±5.4	181.1±8.0	176.8±11.9
Body mass (kg)	71.3±6.0	73.8±10.7	69.6±8.9
$\dot{V}O_{2peak}$ ($L.min^{-1}$)	4.75±0.22	4.66±0.48	4.58±0.50
PPO (W)	362±5	376±14	353±13
Sprint 90% T_f (s)	52±3	46±2	45±5

The LHTLc group slept 8-10 $h.n^{-1}$ for 20 consecutive n in a room enriched with N_2, at a simulated altitude of 2650 m (inspired O_2 = 16.27%; ambient P_B ~948 hPa). The LHTLi group also spent a total of 20-n under the same hypoxic stimulus, however, after every 5th night in hypoxia, subjects underwent 2-n of control. Control subjects slept in their own homes in Canberra or in dormitory style accommodation under normobaric normoxia for 20 consecutive nights. Data are mean±SD. No significant differences were found between groups for any variable. VO_{2peak} = peak oxygen consumption, PPO = peak power output, Sprint 90% T_f = time to fatigue to 90% of peak sprint power output.

5.2.2 Experimental design

The study was conducted at the Australian Institute of Sport, Canberra, Australia, altitude ~ 600 m (PB ~948 hPa). Due to limited accommodation (n = 6) in the altitude house facility used for this study, experimental testing was conducted on four separate occasions over an 11-mo period. The LHTLc spent 8-10 $h.night^{-1}$ for 20 consecutive nights in a room enriched with N_2, at a simulated altitude of 2650 m (F_IO_2 0.1627;

ambient P_B ~948 hPa). The LHTLi group also spent a total of 20 nights under the same hypoxic stimulus, however, after every 5th n in hypoxia, subjects underwent 2-n of control. CON subjects slept in their own homes in Canberra or in dormitory style accommodation under normobaric normoxia for 20 consecutive nights. Subjects maintained their own training during the study and kept a daily log of duration, mode, and frequency of training beginning ~1 wk before and continuing throughout the experimental period (Townsend *et al.*, 2002). All training and daytime living and all exercise tests for all subjects were performed under normobaric normoxic conditions, at an altitude of ~600 m. All exercise tests were performed on the same electromagnetically braked ergometer (Lode, Groningen, The Netherlands), calibrated using a first-principles calibration rig.

5.2.3 Peak power output test

All subjects completed an incremental exercise test to voluntary exhaustion to establish Peak Power Output (PPO) and $\dot{V}O_{2peak}$ at eighteen days prior to altitude exposure, as previously comprehensively described (Clark *et al.*, 2004a). There were no significant differences between groups for $\dot{V}O_{2peak}$ or PPO (Table 5.1).

5.2.4 Sprint exercise test

After one habituation trial, subjects completed a time to fatigue (T_f) trial at 170% PPO determined from the Peak Power Test, at each of 15 and 8-d before LHTL or CON trials. The protocol for individual subjects was pre-programmed into the ergometer to allow a rapid (1 $KW.s^{-1}$) ramp to the desired workrate for the sprint. Prior to the sprint test, subjects underwent a standardised warm-up comprising 10-min at 1.5 $W.kg^{-1}$ and then 2.5-min at 2 $W.kg^{-1}$. During the latter seconds of the warm-up subjects were encouraged to increase cadence to 140 $rev.min^{-1}$ to ensure they were capable of

maintaining pedalling momentum once the desired sprint workrate was reached. The intra-trial (1 and 2) coefficient of variation (CV) for time to fatigue was 13.4% (n=33).

For the experimental sprint tests conducted before and after LHTL intervention or control, time was limited to 90% T_f for each subject. This allowed comparison of changes in Na^+,K^+ATPase activity and plasma ions during matched work bouts, a salient method of detecting training induced changes in muscle metabolism and ion regulation (Harmer *et al.*, 2000). Matched sprint tests were conducted 6-d prior to (Pre); after 5-n LHTL (d5); and at 1-d after 20-n LHTL (Post) altitude exposure or control. Mean cadence and fatigue index (FI) were calculated to verify matching of performance from Pre to Post tests. FI was defined as the percentage decline in mean cadence from the first 5 s to the final 5 s of the test. Prior to intervention, neither starting cadence (Con 142±6; LHTLc 140±4; LHTLi 140±5 rev.min-1) nor 90% sprint time to fatigue (Sprint 90% T_f) differed between groups (Table 5.1).

5.2.5 Simulated altitude

Details of the operation of the altitude house have previously been reported in detail (Townsend *et al.*, 2002; Clark *et al.*, 2004a; Kinsman *et al.*, In press-a). Briefly, ambient air in the 'altitude house' was diluted with nitrogen gas until the desired fraction of oxygen (F_IO_2, 0.1627) was obtained, equivalent to an altitude of approximately 2650 m above sea level.

5.2.6 Blood sampling, analyses and calculations

Before each of the sprint tests, a catheter (20-G, Jelco) was inserted into a superficial dorsal hand vein, and the site was covered with an adhesive plastic dressing and the hand sheathed in a waterproof glove. After catheterisation, each subject was seated on the cycle ergometer and the catheterised hand was immersed in a water bath (44.5ºC) for 10-min to ensure arterialisation of venous blood (McKenna *et al.*, 1997). Efficacy of arterialisation was demonstrated since $pO_2 > 70$ mm Hg (data not shown) (Forster *et al.*,

1972; McLoughlin *et al.*, 1992). Blood was sampled (2 ml) from the dorsal hand vein during the last 30 s of the sprint workrate, and at 1, 3 and 5-min recovery.

Blood samples were immediately analysed for plasma acid-base (pH, pCO_2, bicarbonate concentration ($[HCO_3^-]$) and electrolyte concentrations (potassium $[K^+]$, sodium $[Na^+]$, lactate $[Lac^-]$, chloride $[Cl^-]$), blood haemoglobin concentration ([Hb]) and haematocrit (Hct) using an automated analyser (ABL System 700, Radiometer, Copenhagen, Denmark). The rise in plasma $[K^+]$ above rest ($\Delta[K^+]$) was calculated for each test as previously described (McKenna *et al.*, 1993; Fraser *et al.*, 2002). The plasma strong ion difference ([SID]) (Johnson *et al.*, 1996) was calculated as $[SID] = ([Na^+] + [K^+]) - ([Lac^-] + [Cl^-]$ (McKenna *et al.*, 1997).

5.2.7 Muscle biopsy sampling and analyses.

A muscle biopsy was taken both at rest and immediately after the sprint exercise test, on three separate occasions, before (Pre), after 5-n (d5) and after 20-n (Post) of the LHTL or CON interventions. The needle biopsy sample was taken from the vastus lateralis muscle under local anaesthesia (Xylocaine, 1%), with suction applied to the needle. The post-exercise sample was taken immediately after cessation of the sprint exercise test with the subject lying supported on the cycle ergometer. The two biopsies in each trial were taken from separate incisions on contra-lateral legs. All biopsies were taken at approximately constant depth by an experienced medical practitioner. Muscle samples (100-120 mg) were removed, blotted on filter paper to remove blood, with any obvious fat or connective tissue removed by dissection. The muscle was then rapidly divided into two portions with one portion immediately homogenised for maximal 3-*O*-MFPase activity (Fraser & McKenna, 1998). The other portion was immediately frozen and stored for later analysis of [^{3}H]-ouabain binding sites (Chapter 4) and skeletal muscle buffering capacity.

5.2.7.1 Maximal 3-O-MFPase activity

Maximal Na^+,K^+ATPase activity was measured using the K^+-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity assay (Fraser & McKenna, 1998; Fraser et al., 2002, Chapter 4). Ten to fifteen mg of muscle was quickly blotted on filter paper, weighed, then homogenised (5% wt/vol) on ice for 2 × 20 s, 20,000 rpm (Omni 1000, Omni International) in an homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40) (Fraser & McKenna, 1998). The homogenate maximal in vitro Na^+,K^+ATPase activity was determined in triplicate. The muscle 3-*O*-MFPase activity intra-assay variability was 5.6% (n=52). The 3-*O*-MFPase activity was also expressed relative to the muscle homogenate total protein content, which was measured spectrophotometrically (Lowry *et al.*, 1951).

5.2.7.2 [^{3}H]-ouabain binding sites

Na^+,K^+ATPase content was determined in quadruplicate using [^{3}H]-ouabain binding site content (Nørgaard *et al.*, 1984a), as described in detail in Chapter 4. The effects of exercise on muscle [^{3}H]-ouabain binding content were also examined, with data pooled from all groups prior to intervention, to examine a possible exercise effect on [^{3}H]-ouabain binding (n=20). Due to the limited availability of muscle tissue, muscle Na^+,K^+ATPase content could not be measured for all subject days and times, therefore, the resting muscle [^{3}H]-ouabain binding site content was contrasted between Pre d5 and Post conditions where a complete set of rest samples was available (CON, n=4, LHTLc, n=3 and LHTLi, n=3). For the rest versus exercise comparison, [^{3}H]-ouabain binding was expressed relative to muscle protein content (pmol.(g protein.)$^{-1}$). The resting muscle [^{3}H]-ouabain binding site content intra-assay CV was 17.0% (n=92).

5.2.8 Statistical analyses

All data are presented as mean±SD. The physical and training characteristics of the three groups were analysed with a one-way ANOVA. The Student-Newman-Kuels post

hoc test was used to locate differences found in ANOVAs. Statistical significance was accepted at P<0.05. A three-way ANOVA with independent factor for group (CON, LHTLc and LHTLi) and with repeated measures for sample time (rest, exercise) and day (Pre, d5 and Post) was used to test for main and interaction effects for muscle and blood data. Exercise reproducibility data and [^{3}H]-ouabain binding content were analysed with a two-way repeated measures ANOVA for group and day. Maximal 3-*O*-MFPase activity in resting muscle and plasma [Cl^-] and [SID] data was normalised to pre-intervention resting levels at d5 and Post and the change score contrasted between CON and each LHTL group with a repeated measures (day) ANOVA, to account for the small sample size and typical inter-subject, intra and inter assay variability (Huck & McLean, 1975).

5.3 RESULTS

5.3.1 Sprint exercise

Sprint tests were successfully matched, with no difference for mean cadence between groups or test days (Table 5.2). Whilst the fatigue index was higher at Pre than d5 or at Post (P<0.05), this did not differ between groups (Table 5.2). Thus, changes with exercise in Na^+,K^+ATPase activity and plasma ions and acid-base occurred with an identical exercise stimulus.

5.3.2 Effects of acute sprint exercise on Na^+,K^+ATPase β_m and plasma ion changes

5.3.2.1 Muscle 3-O-MFPase activity

The muscle maximal in-vitro 3-*O*-MFPase activity declined by 12.9±4.0% immediately after sprint exercise when expressed per gram wet weight (nmol.min^{-1}.(g^{-1} wet wt)$^{-1}$) (P<0.05, Fig. 5.1) and declined similarly by 12.0±1.5% when expressed per gram protein (Rest 1545±115; End-ex 1361±117 nmol.min^{-1}.g protein^{-1}).

5.3.2.2 Muscle [^{3}H]-ouabain binding

The reduction in 3-*O*-MFPase activity with acute sprint exercise was not due to a decreased number of Na^+,K^+ATPase, as [^{3}H]-ouabain binding content was unchanged. Data pooled for all groups prior to intervention (n=20) did not differ between rest and exercise whether expressed per gram wet weight (Rest 318±37; End-Ex 327±41 pmol.(g wet wt)$^{-1}$, *NS*) or per gram protein (Rest 1825±212; End-Ex 1879±235 pmol.(g protein.)$^{-1}$, NS).

5.3.2.3 Muscle β_m

There was no effect of LHTL hypoxic exposure on _m (LHTLc, 145.3 ± 18.5 vs. 148.1 ± 18.4; LHTLi, 141.0 ± 14.6 vs. 145.9 ± 15.9; Con, 149.5 ± 20.9 vs. 151.0 ± 22.2, μmol H^+ g muscle dry wt^{-1} pH^{-1}, values are pre- and postintervention, respectively).

5.3.2.4 Plasma ions and acid-base

Arterialised venous plasma [K^+] (Fig. 5.2A), [Na^+] (Fig. 5.2B), and [Cl^-] (Fig. 5.3A) all increased above rest with sprint exercise, and declined by 5-min recovery (P<0.001). Plasma [Lac^-] increased above rest with sprint exercise, and increased further during recovery (Fig. 5.3B, P<0.001). Plasma [H^+] and pCO_2 increased with sprint exercise (P<0.001, Appendix B3), whilst plasma [HCO_3^-] and [SID] decreased with sprint exercise and during recovery (P<0.001, Appendix B3).

5.3.3 Effects of LHTL on Na^+,K^+ATPase and plasma ions

5.3.3.1 Muscle 3-O-MFPase activity

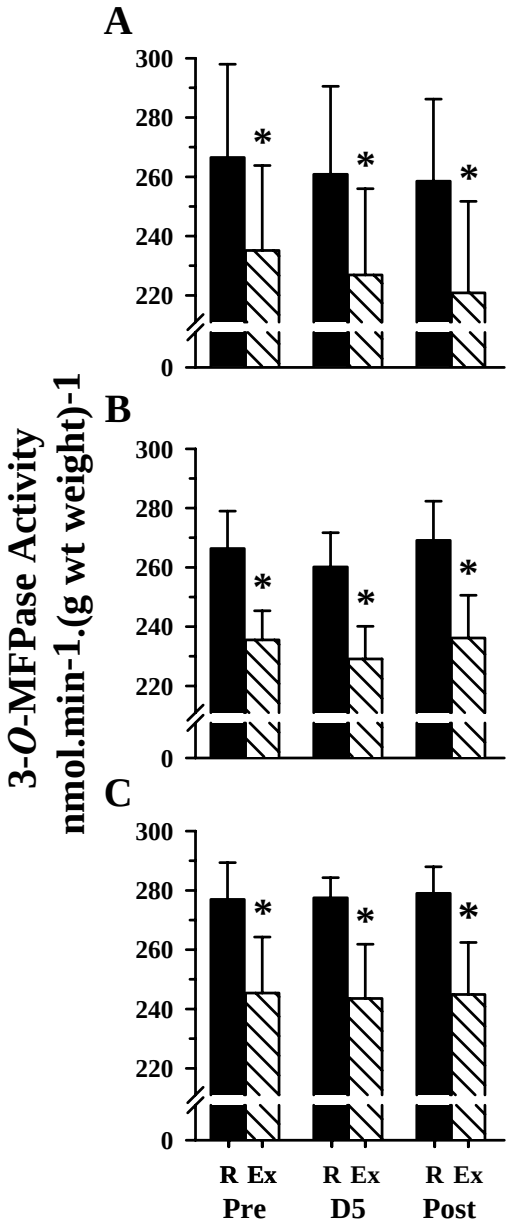
Distinctly different patterns of changes in resting muscle maximal 3-*O*-MFPase activity were seen between the three groups. Maximal 3-*O*-MFPase activity was unchanged at any day in CON (Fig. 5.4). However, change-score analyses (Huck & McLean, 1975) indicated that 3-*O*-MFPase activity decreased by ~ 2% in both LHTL groups at d5

Table 5.2 Matched sprint reproducibility data for CON (n = 7), LHTLc (n = 8) and LHTLi (n = 9). Data is Mean ± SD* Pre > d5 or Post, (P < 0.05, Day main effect).

		Mean Cadence	Fatigue Index
Group	Day	(rev.m^{-1})	(%)
CON	Pre	128±5	41.5±10.0*
	d5	130±8	24.4±18.7
	Post	131±5	19.9±12.3
LHTLc	Pre	125±6	37±10.4*
	d5	125±8	30.6±17.8
	Post	126±8	29.0±18.6
LHTLi	Pre	122±7	44.3±6.7*
	d5	126±6	35.2±6.0
	Post	124±5	31.2±6.2

(*P*<0.05, Fig. 5.4). Maximal 3-*O*-MFPase activity did not change further between d5 and Post in LHTLc and was therefore, reduced Pre-Post in LHTLc compared to CON (*P*<0.05, Fig. 5.4). However, in contrast, a clear divergence was found with LHTLi, with a significant increase in 3-*O*-MFPase activity of ~4% found between d5 and Post, resulting in no Pre-Post reduction evident (*P*<0.05, Fig. 5.4).

Figure 5.1 Skeletal muscle maximal *in vitro* K^+-stimulated 3-*O* methylfluorescein phosphatase (3-*O*-MFPase) activity (Na^+,K^+ATPase activity) at rest (R) and end sprint-exercise (Ex) for tests conducted 6 d prior to (Pre); after 5 n LHTL (d5); and at 1 d after 20 n (Post), Live high train low consecutive (LHTLc) (A); or intermittent (LHTLi) (B) hypoxic or control (CON) (C) interventions.



Data are Mean ± SD; n=7 each for CON, LHTLc and LHTLi, * End-exercise < Rest ($P < 0.05$, Exercise main effect).

5.3.3.2 Muscle [^{3}H]-ouabain binding

Resting muscle [^{3}H]-ouabain binding content did not differ between groups or days as a result of LHTL (Appendix B4).

5.3.3.3 Plasma ions and acid-base

Differences between groups were present for both plasma [Na^+] and [Lac^-] (Figs. 5.2A and 5.3B respectively $P<0.05$), whilst differences between test days were also found for plasma [Lac^-] (Pre 9.2±0.2; d5 8.9±0.3; Post 8.5±0.2 mmol.L^{-1} respectively, $P<0.05$, Fig. 5.3B). However, there was no specific effect of LHTL on these ions or plasma [K^+].

Plasma [Cl^-] increased in both LHTL groups from Pre-d5 ($P<0.05$, Fig. 5.5) with no change in CON, indicating an early change in [Cl^-] with hypoxia. Plasma [Cl^-] did not change in any group from d5-Post. Therefore, plasma [Cl^-] was increased Pre-Post in both LHTLc and LHTLi compared to CON ($P<0.05$, Fig. 5.5).

There was no effect of LHTL on [HCO_3^-], pCO_2 and [H^+]. However, [SID] decreased in both LHTL groups from Pre-d5 ($P< 0.001$, Fig. 5.6) with no change in CON, indicating an early change with hypoxia. Plasma [SID] did not change in any group from d5-Post. Therefore, [SID] was decreased Pre-Post in LHTLc and LHTLi compared to CON ($P<0.001$, Fig. 5.6).

Figure 5.2 Arterialised venous plasma [K^+] (A) and [Na^+] (B) for LHTLc, LHTLi (B) and CON (C) for blood samples obtained at rest, during sprint exercise and at 1-, 3- and 5-min recovery prior to (Pre); after 5 d (d5); and at 1 d after 20 nights (Post) interventions; n=11 for CON, n=12 for LHTLc and n=10 for LHTLi; * different to preceding workrate, (P<0.05, exercise main effect).

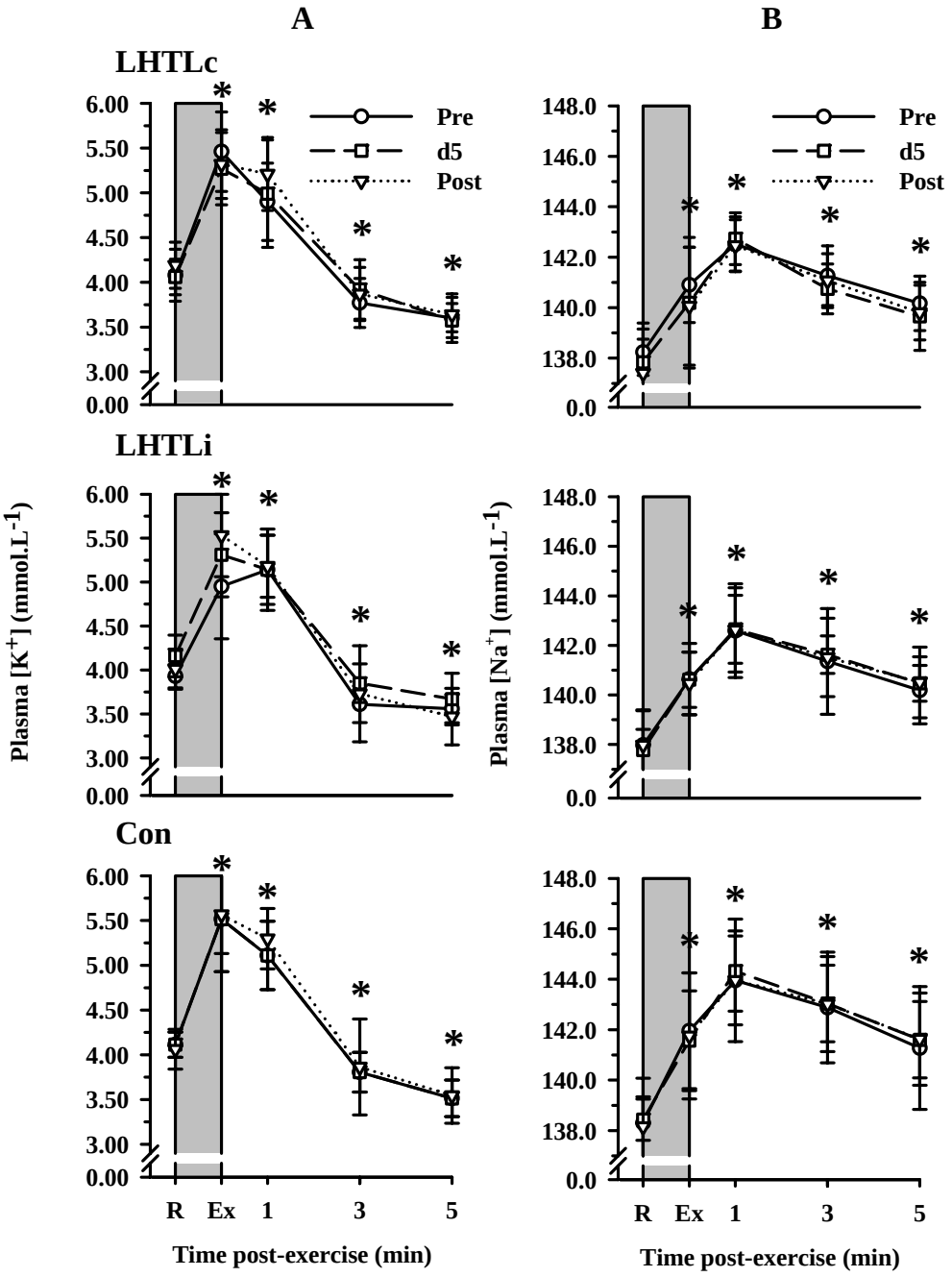


Figure 5.3 Arterialised venous plasma [Cl^-] (A) and [Lac^-] (B) for LHTLc, LHTLi (B) and CON (C) for blood samples obtained at rest, during sprint exercise and at 1-, 3- and 5-min recovery prior to (Pre); after 5 d (d5); and at 1 d after 20 nights (Post) interventions; n=11 for CON, n=12 for LHTLc and n=10 for LHTLi; * different to preceding workrate, (P<0.05, exercise main effect).

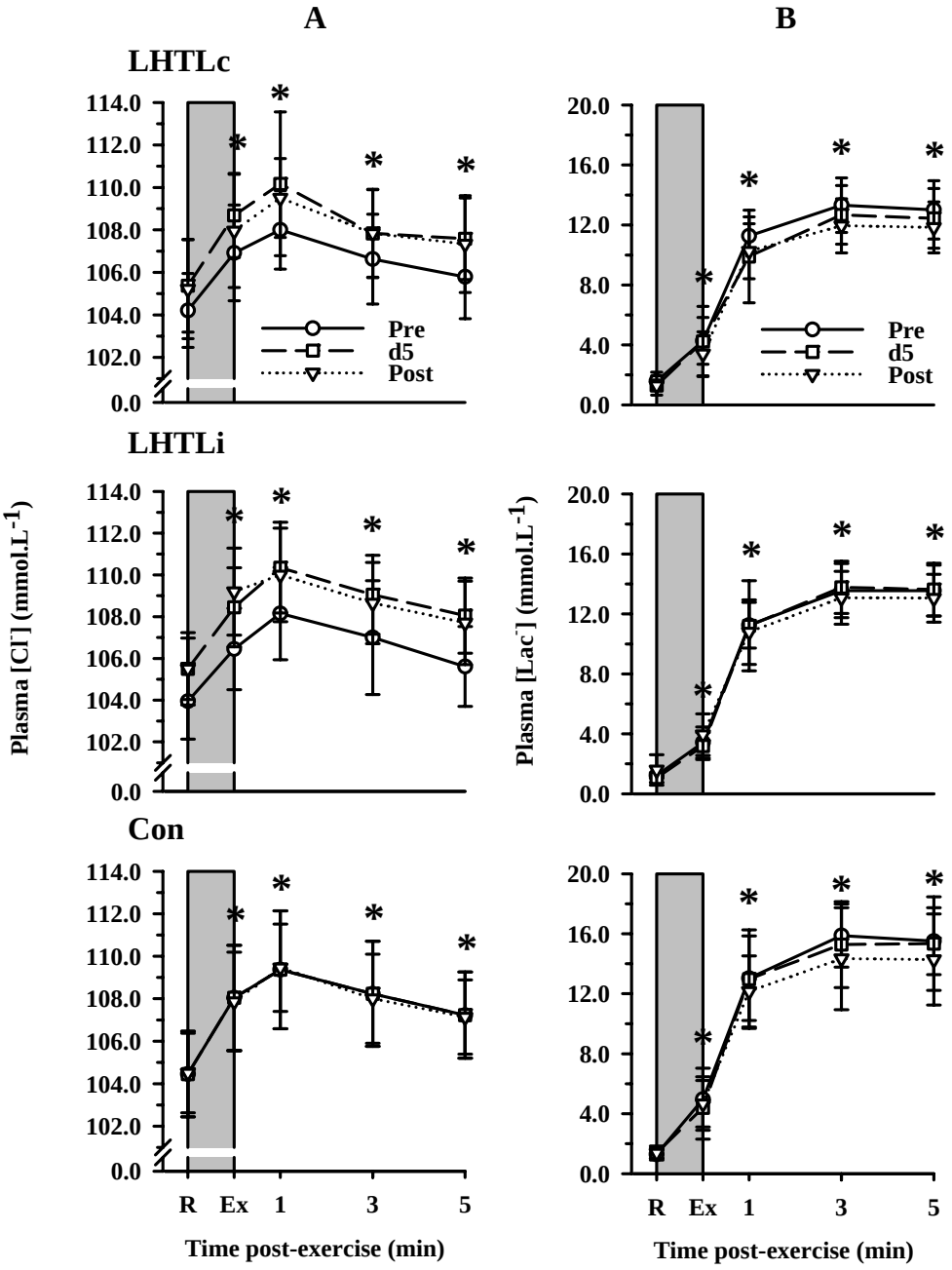
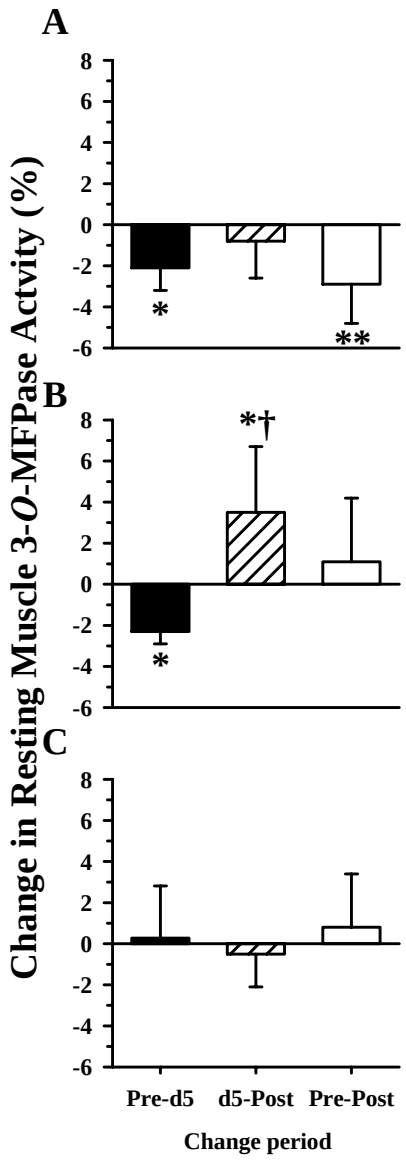
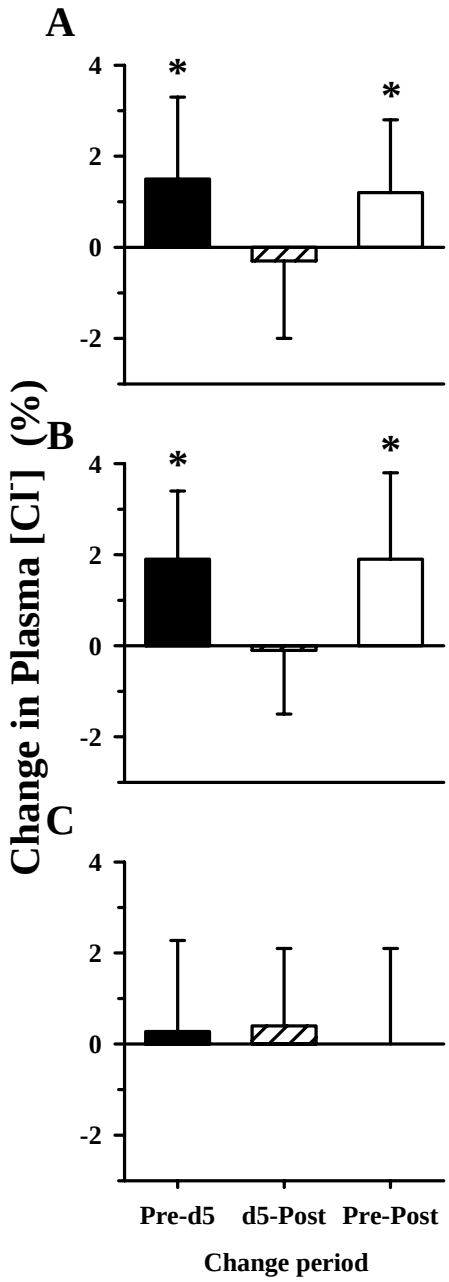


Figure 5.4 Changes in resting skeletal muscle maximal 3-*O*-MFPase activity with LHTL.



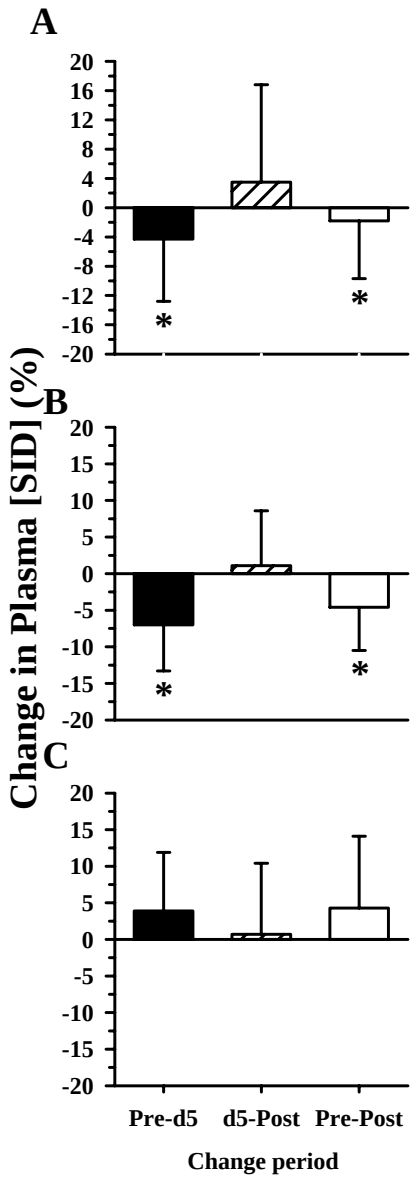
The resting muscle 3-*O*-MFPase activity was contrasted between Pre, d5 and Post, for LHTLc (A), LHTLi (B) and CON (C) by calculating the Pre-d5, d5-Post and Pre-Post changes in 3-*O*-MFPase activity and expressed changes as a percentage of the Pre-intervention value (change score). Data are mean ± SD; n=7 for each group; * different to CON, † different to CON and LHTLc, ** different from CON and LHTLi ($P < 0.05$, group by day interaction effect).

Figure 5.5 Changes in arterialised venous plasma [Cl^-].



Arterialised venous plasma [Cl^-] was contrasted between Pre, d5 and Post, for LHTLc (A), LHTLi (B) and CON (C) by calculating the Pre-d5, d5-Post and Pre-Post changes in 3-*O*-MFPase activity and expressed changes as a percentage of the Pre-intervention value (change score). Data are mean ± SD; n=12 for LHTLc, n=10 for LHTLi and n=11 for CON; * different to CON (P<0.05).

Figure 5.6 Changes in arterialised venous plasma [SID].



Arterialised venous plasma [SID] was contrasted between Pre, d5 and Post, for LHTLc (A), LHTLi (B) and CON (C) by calculating the Pre-d5, d5-Post and Pre-Post changes in 3-*O*-MFPase activity and expressed changes as a percentage of the Pre-intervention value (change score). Data are mean ± SD; n=12 for LHTLc, n=8 for LHTLi and n=11 for CON; * different to CON ($P<0.05$).

5.4 DISCUSSION

5.4.1 Sprint cycling exercise depresses muscle Na^+,K^+ATPase activity, but not content, independent of LHTL.

Intense sprint cycling exercise in well trained athletes depressed muscle maximal Na^+,K^+ATPase activity, by ~12%. This depression occurred despite the subjects being very highly trained athletes performing sport-specific exercise. Depressed maximal Na^+,K^+ATPase activity during intense exercise could theoretically lead to a further increase in transmembranous Na^+ and K^+ fluxes during muscle contraction, thus exceeding the rate of Na^+,K^+ATPase -mediated active ion transport (Clausen *et al.*, 2004), reducing the muscle membrane potential (Sjøgaard *et al.*, 1985; Sejersted & Sjøgaard, 2000), contributing to a decline in muscle excitability and M-wave area (Fowles *et al.*, 2002b), and therefore accelerating muscle fatigue (Clausen, 2003). Given the theoretical importance of maximal Na^+,K^+ATPase activity in the performance of athletes, it is surprising that there appears to be no protective effect of chronic intensive training on this depression. Furthermore, we show that this depression in maximal activity occurred in the absence of any change in skeletal muscle Na^+,K^+ATPase content. This indicates that sprint exercise results in inactivation, rather than loss of Na^+,K^+ATPase units in muscle. The magnitude of the exercise-induced fall in maximal Na^+,K^+ATPase activity is almost identical to that observed after fatiguing single-leg dynamic kicking exercise (Fraser *et al.*, 2002), or intense incremental cycling exercise (Chapter 4). This depression therefore also appears to be independent of training status, adding further strength to the concept that this is an important response during intense exercise. Further studies are required to examine the impact of reduced maximal Na^+,K^+ATPase activity on muscle ion regulation and performance.

5.4.2 Contrasting effects of consecutive nights of LHTL and additional interspersed normoxia on resting skeletal muscle Na^+,K^+ATPase activity

Twenty-three n consecutive LHTL resulted in a small depression in maximal Na^+,K^+ATPase activity (Chapter 4). A novel finding here is that the depressive effects of LHTL on Na^+,K^+ATPase activity occurred within the first 5 n of modest hypoxic exposure. In these well-trained athletes, likely to be already highly adapted for Na^+,K^+ATPase content compared to untrained participants (Klitgaard & Clausen, 1989; Fraser *et al.*, 2002), an early decline in Na^+,K^+ATPase activity with LHTLc was evident. Thus, this confirms the hypothesised rapid onset of hypoxia-induced Na^+,K^+ATPase depression. Importantly, the Post LHTL biopsy here occurred within 2-8 h of hypoxic exposure ending, much earlier than the 3-4 d post-LHTL in the previous study (Chapter 4). Thus the small depression was not due to methodological factors as was potentially the case previously (Chapter 4). These combined results suggest that the time required for restoration of Na^+,K^+ATPase activity after hypoxia may be considerably delayed, and that reduced Na^+,K^+ATPase activity persists for at least several days after return to normoxia.

An important question is why these effects are are so small with LHTL, given the ~14% reduction in Na^+,K^+ATPase content after chronic hypoxia (Green *et al.*, 1999b; Green *et al.*, 2000a) and the ~29% reduction in Na^+,K^+ATPase activity with exercise under hypoxic conditions (Sandiford *et al.*, 2004). The answer might be related to the training undertaken by these athletes. Athletes continue their normal heavy exercise training in normoxia during daytime hours, whilst are exposed to nightly hypoxia in LHTL. Acute exercise of only a few minutes duration can upregulate Na^+,K^+ATPase mRNA (Murphy *et al.*, 2004), whilst just 6 d of training (Green *et al.*, 1993; Green *et al.*, 2004) and chronic training upregulated Na^+,K^+ATPase content (McKenna *et al.*, 1993; Green *et al.*, 1999a). As athletes in this study maintained ~ 15 $h.week^{-1}$ of training (Townsend *et*

al., 2002), it is therefore possible that a potentially larger hypoxia-induced depression in Na^+,K^+ATPase activity was attenuated by the upregulatory impact of daily, ongoing, heavy training.

An important finding was that additional days of normoxia interspersed within the LHTL program (LHTLi) reversed the initial depressive effect of 5 d LHTL hypoxia on muscle Na^+,K^+ATPase activity. This clearly contrasts the effect of both the 20 n consecutive LHTL employed here and the previous finding with 23 n consecutive LHTL (Chapter 4), that each induced a decline in Na^+,K^+ATPase activity. The intermittent LHTL group experienced the same severity and duration of hypoxia as the consecutive LHTL group, yet with different responses in muscle Na^+,K^+ATPase. The time spent living and training in normoxia was the major difference between consecutive and intermittent LHTL. This gives further support to the notion that the stimulatory effect of ongoing heavy training under normoxic conditions (McKenna *et al.*, 1993; Green *et al.*, 1999a) undertaken by the LHTLi group was adequate to reverse the small depressive effect of consecutive LHTL, itself likely attenuated with daily exercise training. The small magnitude of reduction in Na^+,K^+ATPase activity with both LHTL programs clearly differs from the 28% reduction during exercise under hypoxic conditions (Sandiford *et al.*, 2004) and the ~14% reductions in Na^+,K^+ATPase content after an altitude sojourn (Green *et al.*, 2000a) or exercise training under hypoxic conditions (Green *et al.*, 1999b), respectively. None of the total time under hypoxic conditions, the severity of hypoxia nor the training status of participants appear to fully explain the different response to LHTL (Chapter 4). The decline in Na^+,K^+ATPase activity with LHTL occurred without alteration in Na^+,K^+ATPase content, suggesting an inactivation of existing Na^+,K^+ATPase complexes.

In contrast to the greater inhibitory effect of hypoxia on Na^+,K^+ATPase activity during exercise (Sandiford *et al.*, 2004), there was no effect of LHTL exposure on the

reduction in Na^+,K^+ATPase activity with sprint exercise. Furthermore, this effect was not different in LHTLc or LHTLi groups, despite the different effect on resting maximal Na^+,K^+ATPase activity in these groups. Thus there did not appear to be any persistent effects of LHTL on exercise-induced changes in Na^+,K^+ATPase activity.

5.4.3 The effect of LHTL on plasma ions and acid-base balance.

The decrease in maximal Na^+,K^+ATPase activity with LHTL was insufficient to adversely affect plasma [K^+] during exercise and therefore presumably K^+ clearance by both active and inactive muscle. Thus, small reductions in Na^+,K^+ATPase activity in resting muscle do not appear to affect K^+ homeostasis during exercise. Also investigated was whether LHTL affected plasma strong ion concentrations during exercise and specifically the strong ion difference ([SID]), to explain the lack of change in plasma [H^+] after LHTL, despite a lowered pCO_2, [HCO_3^-] and [Lac^-] (Chapter 3). It is demonstrated here that LHTL resulted in a small increase in plasma [Cl^-] and a lowering of the [SID]. An increase in plasma [Cl^-] with LHTL would tend to lower plasma [SID] and thereby elevate plasma [H^+] (Stewart, 1981). Consequently, a lowered [SID] may then reflect a counter-regulatory mechanism of maintaining plasma [H^+] in hypoxia, opposing the lowered pCO_2 and [Lac^-]. Plasma [H^+] was unchanged with LHTL, in accordance with the results in Chapter 3, however, the results for pCO_2 and plasma [Lac^-] differed to these previous findings. Both pCO_2 and plasma [Lac^-] were lower in the LHTL groups than control, although no between group and day effects were found which would indicate specific LHTL effects. These differences might reflect the slightly lower simulated altitude used in this study, which may have been insufficient to perturb plasma ions or acid base to the same extent as in the previous study (Chapter 3). The raised plasma [Cl^-] after LHTL might also offer protection of muscle membrane excitability through attenuation of the depolarisation caused by the exercise-induced run-down of the trans-sarcolemmal K^+ gradient (Cairns *et al.*, 2004).

5.5 Conclusions

In conclusion, intense sprint exercise in well-trained athletes immediately depressed maximal Na^+,K^+ATPase activity, without alteration in Na^+,K^+ATPase content. Many years of specific training of these athletes did not protect against this depression, arguing for an important role of depressed Na^+,K^+ATPase activity in muscle fatigue. LHTL also caused a small, early depression in maximal Na^+,K^+ATPase activity, which was reversed with short interspersed periods of normoxia, but these effects were much smaller than earlier reports of hypoxia-induced depression in muscle Na^+,K^+ATPase in non-athletes. The effects of intense sprinting exercise and LHTL on maximal Na^+,K^+ATPase activity were not synergistic. These are important since they may explain why athletes can use LHTL without deterioration in performance. Furthermore, LHTL elevated plasma [Cl^-], which may act to preserve plasma acid-base balance and potentially protect against a run-down of muscle membrane excitability. Thus, LHTL in already well-trained athletes caused subtle adaptations in skeletal muscle Na^+,K^+ATPase. This demonstrates that even after years of hard training, muscle Na^+,K^+ATPase is responsive to this intervention.

CHAPTER 6. STUDY 3: THE EFFECTS OF HIGH INTENSITY INTERVAL EXERCISE AND TRAINING ON Na^+,K^+ATPase ACTIVITY AND CONTENT IN WELL-TRAINED ATHLETES.

6.1 INTRODUCTION

The Na^+,K^+ATPase enzyme plays an important role in skeletal muscle by regulating trans-sarcolemmal [Na^+] and [K^+] gradients, and thus membrane excitability and contractility (Overgaard *et al.*, 1999; Nielsen & Clausen, 2000; Clausen, 2003). The importance of Na^+,K^+ATPase for muscle contractility is emphasised when Na^+,K^+ATPase activity is specifically inhibited by ouabain. This results in an increased muscle Na^+ gain and K^+ loss during muscle excitation, a reduced M-wave area during exercise, with depressed muscle tetanic force, accelerated muscle fatigue, and reduced recovery rate (Clausen, 2003).

Acute exercise has marked effects upon maximal Na^+,K^+ATPase activity in skeletal muscle. In isolated rat muscle, Na^+,K^+ATPase activity is rapidly increased by up to 18-22-fold during contractile activity, as measured by an increased Na^+ extrusion rate (Nielsen & Clausen, 1997; McKenna *et al.*, 2003). In an apparent paradox in human muscle, however, fatiguing exercise depressed maximal Na^+,K^+ATPase activity, measured *in-vitro* by K^+-stimulated 3-*O*-methylfluorescein phosphatase activity (3-*O*-MFPase). Maximal activity was depressed by ~12-38 % after intense incremental cycling (Chapter 4), sprint cycling (Chapter 5), single leg kicking (Fraser *et al.*, 2002), prolonged cycling (Leppik *et al.*, 2004; Sandiford *et al.*, 2004) or repeated isometric muscle contractions (Fowles *et al.*, 2002b). This depressed activity was not due to decreased Na^+,K^+ATPase content, as measured by unchanged [^{3}H]-ouabain binding sites (Fowles et al., 2002b; Leppik et al., 2004; Chapters 4 & 5).

Both sprint (McKenna *et al.*, 1993) and endurance (Green *et al.*, 1993; Madsen *et al.*, 1994; Green *et al.*, 2004) exercise training increased [^{3}H]-ouabain binding by 9-16%, in untrained and moderately trained participants and after 3-5-months intensified training in already well-trained participants (Evertsen *et al.*, 1997; Medbø *et al.*, 2001). Elite endurance athletes commonly use short periods of high intensity training (HIT) in an effort to improve performance (Stepto *et al.*, 1999; Stepto *et al.*, 2001). We speculated that Na^+,K^+ATPase upregulation would also occur with short-term HIT, even in well-trained athletes. Little is known about training effects on maximal Na^+,K^+ATPase activity. Only six days of training increased muscle maximal Na^+,K^+ATPase activity by a surprisingly large 41%, in previously untrained participants (Green *et al.*, 2004), whereas athletes only tended to a higher (20%, NS) maximal Na^+,K^+ATPase activity compared to untrained controls (Fraser *et al.*, 2002). Reduced maximal Na^+,K^+ATPase activity with acute exercise has been linked with fatigue (Fowles et al., 2002b; Fraser et al., 2002; Leppik et al., 2004; Chapter 4; Petersen et al., 2005) and with Na^+,K^+ATPase gene expression (Petersen *et al.*, 2005). An elevation in resting muscle Na^+,K^+ATPase content and thus maximal activity with HIT could enable a higher end-exercise activity and thereby be important for preserving muscle function. We therefore tested the third hypothesis that even short-term HIT would increase resting muscle Na^+,K^+ATPase content and maximal Na^+,K^+ATPase activity in already well-trained athletes and that these would consequently enable a higher end-exercise maximal Na^+,K^+ATPase activity. Training effects on the Na^+,K^+ATPase isoform transcription and translation in skeletal muscle are less clear. No one study has comprehensively investigated the effects of training in well-trained athletes on each of the α_1, α_2, α_3, β_1, β_2 and β_3 Na^+,K^+ATPase isoforms at either the mRNA or protein levels in human skeletal muscle. Four studies have investigated possible training effects on Na^+,K^+ATPase isoform gene expression or protein abundances in human muscle, but each only probed for α_1, α_2 and

β_1 isoforms. Furthermore, these studies used only untrained (Nielsen *et al.*, 2003b; Nordsborg *et al.*, 2003a; Green *et al.*, 2004) and unhealthy participants (Dela *et al.*, 2004). These studies yielded conflicting results for α_1 and β_1 protein abundance (Nielsen *et al.*, 2003b; Dela *et al.*, 2004; Green *et al.*, 2004). HIT for 5.5 weeks did not alter resting α_1, α_2 and β_1 mRNA expression, but blunted the α_1 mRNA upregulation that occurred with acute exercise prior to HIT (Nordsborg *et al.*, 2003a). Thus, the effects of HIT on Na^+,K^+ATPase α_3, β_2 and β_3 mRNA or protein are unknown and are unclear for both α_1, and β_1 protein abundance. We tested the final hypothesis that HIT in well-trained athletes would increase the mRNA expression and protein abundance of each of the Na^+,K^+ATPase isoforms .METHODS

6.1.1 Subjects

Twelve well-trained male cyclists / triathletes who were riding at least 350 $km.wk^{-1}$ and had not performed any interval training for at least 6-weeks gave written consent to participate in this study, which conformed to the Declaration of Helsinki and was approved by the Human Research Ethics Committees of Victoria University of Technology and of RMIT University. All subjects refrained from vigorous exercise, caffeine and alcohol consumption, and consumed a controlled diet for 24 h prior to each of the exercise tests. This study forms part of a larger study that also investigated muscle metabolism responses to high-intensity training; hence details of subjects, and of some test methodologies have already been presented elsewhere (Clark *et al.*, 2004b). The physical characteristics of the subjects are presented in Table 6.1.

Table 6.1 Subject physical characteristics. Data are mean±SD.

Variable	Mean±SD
N	12

Age (yr)	31±3
Height (cm)	177±6
Body mass (kg)	
Baseline	75.7±4.5
Pre-Training	75.3±2.1
Post-Training	74.1±2.8

6.1.2 Experimental design

In this current study subjects acted as their own controls. To achieve this, each subject underwent a resting muscle biopsy, and performed a peak power output test (Baseline). Subjects then maintained their normal endurance training for a further 4 weeks, before undergoing pre-training testing (Pre-Train), followed by the high-intensity training program (HIT), and post-training testing (Post-Train). An untrained control group could not be included in this study, as untrained subjects are unable to ride for greater than 15 min at the same relative intensity sustained by well-trained subjects (Yu *et al.*, 1997). Each exercise test was performed on the same electromagnetically braked ergometer (Lode, Groningen, The Netherlands).

6.1.3 Peak Power Output Test

Subjects completed a peak power output (PPO) test at each of Baseline, Pre-Training and Post-Training. Each test commenced with subjects cycling at a workrate equivalent to lactate threshold, as previously detailed (Clark *et al.*, 2004b), followed by 25 W increments each 150 s until voluntary exhaustion, defined as the inability to maintain pedal cadence above 60 rev.min^{-1}. PPO was calculated as $W_f + (T_i / 150) \times 25$, where, W_f = final completed workrate; T_i = time at incomplete workrate; 150 = time spent at each workrate; and 25 the size of the step in workrate. The calculated PPO was used to

determine the subsequently described high-intensity interval training session.VO_{2peak} was also determined during this PPO test, as previously detailed (Clark *et al.*, 2004b).

6.1.4 High-Intensity Interval Exercise

Each high intensity interval exercise session comprised a standardised warm-up at 58% PPO for 20-min, followed by eight 5-min intervals performed at a workrate corresponding to 80% PPO, each interspersed with 1-min recovery cycling at 100 W, equivalent to ~ 1.3 $W.kg^{-1}$. The 80% PPO workrate of is equivalent to ~ 85% VO_{2peak} (Stepto *et al.*, 2001).

6.1.5 High-Intensity Interval Training

The interval exercise session described above was repeated on a further six occasions in a 21-d period, with 3 in week one, 2 in week two and 2 in week 3, which constituted high intensity interval training (HIT). The PPO was re-determined after the third session to enable percentage workloads for HIT to be adjusted for sessions 5 and 6. This HIT regime has been previously shown to improve performance in well-trained subjects (Stepto *et al.*, 2001). The workrates used for sessions 1 (Pre-Train) and 7 (Post-Train) were identical. The use of matched high-intensity interval exercise bouts Pre- and Post-Training enabled greater precision in detecting possible training effect on the exercise-induced changes in Na^+,K^+ATPase maximal activity, as previously shown for muscle metabolites and plasma ion regulation (Harmer *et al.*, 2000).

6.1.6 Muscle Biopsy Sampling and Analyses.

An initial resting muscle biopsy was taken at baseline with further biopsies taken at rest (Rest) and immediately post-exercise (Exercise), during the Pre-Train and Post-Train testing sessions. The needle biopsy sample was taken from the vastus lateralis muscle under local anaesthesia (Xylocaine, 1 %), with suction applied to the needle (Evans *et al.*, 1982). The post-exercise sample was taken immediately after cessation of the final

exercise bout, with the subject lying supported on the cycle ergometer. Serial biopsies were taken from separate incisions in the same leg, with the exercise sample taken from an incision ~1.5 cm proximal to the rest sample. Muscle samples were removed, rapidly frozen and stored in liquid N_2 for subsequent analysis of Na^+,K^+ATPase content and maximal activity. Muscle was also analysed for AMPK response to exercise, as reported elsewhere (Clark *et al.*, 2004b), which restricted our ability to complete all analyses for each subject at each timepoint. Consequently, the sample size for each Na^+,K^+ATPase analysis was as follows, Na^+,K^+ATPase content, n=7; and maximal activity, n=7.

6.1.6.1 [^{3}H]-ouabain binding sites

Na^+,K^+ATPase content was determined in quadruplicate using the vanadate-facilitated [^{3}H]-ouabain binding site content (Nørgaard *et al.*, 1984a). Muscle samples were cut into 2-5 mg pieces and washed for 2 x 10 min in 37°C vanadate buffer containing 250 mM sucrose, 10 mM Tris, 3 mM MgSO4, and 1 mM NaVO4 (pH 7.2-7.4). This was to thaw the samples and to maintain lower Na^+ and K^+ concentrations to minimise interference with vanadate-facilitated [^{3}H]-ouabain binding. Muscle samples were then incubated for 120 min at 37°C in the above buffer with the addition of [^{3}H]-ouabain (10-6 M, 2.0 mCi.ml-1). After incubation, muscle samples were washed for 4 x 30 min in ice-cold vanadate buffer to remove any unbound [^{3}H]-ouabain, blotted on filter paper and weighed before being soaked overnight in vials containing 0.5 ml of 5% trichloroacetic acid (TCA) and 0.1 mM ouabain. The following morning, 2.5 ml of scintillation cocktail (Opti-Fluor, Packard) was added prior to liquid scintillation counting of the [^{3}H]-activity. The content of [^{3}H]-ouabain binding sites was calculated on the basis of the sample wet weight and the specific activity of the incubation medium and samples and expressed as pmol.(g wet wt.)$^{-1}$. The resting muscle [^{3}H]-ouabain binding site content was contrasted between Baseline, Pre and Post conditions. The resting muscle [^{3}H]-ouabain binding site content intra-assay CV was 11.0% (n=20). The

[^{3}H]-ouabain binding site content was also expressed relative to muscle protein content, which was measured spectrophotometrically (Lowry *et al.*, 1951). Post-exercise [^{3}H]-ouabain binding was not analysed due to tissue limitations and since this is not changed by brief exercise (Fowles et al., 2002b; Leppik et al., 2004; Chapters 4 & 5).

6.1.6.2 Maximal 3-O-MFPase activity

Approximately 5 mg of muscle was thawed, quickly blotted on filter paper, weighed, then homogenised (5% wt/vol) on ice for 2 × 20 s, 20,000 rpm (Omni 1000, Omni International) in an homogenate buffer containing 250 mM sucrose, 2 mM EDTA, and 10 mM Tris (pH 7.40). The homogenate maximal *in vitro* Na^+,K^+ATPase activity was determined in triplicate using the K^+-stimulated, 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity assay (Fraser & McKenna, 1998; Fraser *et al.*, 2002). The muscle 3-*O*-MFPase activity intra-assay CV was 1.0% (n=34). The 3-*O*-MFPase activity was also expressed relative to the muscle homogenate total protein content.

6.1.6.3 Statistical analyses

All data are presented as mean±SD. Differences between Baseline and Pre-Train for performance and resting muscle data; as well as training effects on exercise performance and on muscle [^{3}H]-ouabain binding content was analysed with a paired t-test. A two-way ANOVA with repeated measures for sample time (Rest, Exercise) and day (Pre-Train and Post-Train) was used to test for main and interaction effects for muscle 3-*O*-MFPase activity. Significant differences in the paired samples t-test and ANOVA were located with a Student-Newman-Kuels post hoc test. Statistical significance was accepted at $P<0.05$.

6.2 RESULTS

6.2.1 Stability from Baseline to Pre-Train

These athletes had very stable exercise PPO (Baseline, 363±12 and Pre-Train 366±10 W, n=12, CV 1.2%, *NS*) and $\dot{V}O_{2peak}$ (Baseline, 4.98±0.24 and Pre-Train 4.96±0.23 $L.min^{-1}$, n=5, CV 2.2%, *NS*) prior to commencing the high-intensity training (HIT) intervention.

Neither maximal 3-*O*-MFPase activity (Baseline, 284±7 and Pre-Train 282±6 $nmol.min^{-1}.(g\ wet\ wt)^{-1}$), n=7, CV 1.0%, *NS*), nor [^{3}H]-ouabain binding (Baseline, 350±36 and Pre-Train 367±28 $pmol.(g\ wet\ wt)^{-1}$), n=8, CV 11%, *NS*), were significantly different in these well-trained athletes from Baseline to Pre-Train.

6.2.2 Acute high-intensity interval exercise effects on muscle Na^+,K^+ATPase

6.2.2.1 Maximal 3-O-MFPase activity

The muscle maximal *in-vitro* 3-*O*-MFPase activity expressed per gram wet weight ($nmol.min.(g^{-1}\ wet\ wt)^{-1}$) declined by -12.5±5.1% immediately after acute high intensity interval exercise ($P<0.05$, Fig. 6.2). A similar reduction of -12.3±5.9%, in Na^+,K^+ATPase activity was observed when expressed per g homogenate protein ($P<0.05$).

6.2.2.2 Correlations between acute exercise induced changes in muscle Na^+,K^+ATPase mRNA expression and 3-O-MFPase activity

The changes in Na^+,K^+ATPase isoform mRNA expression with acute exercise (Appendix C1) were not significantly correlated to either the absolute or relative change in maximal *in-vitro* 3-*O*-MFPase activity at either Pre-Train (α_1, r = 0.20; α_2, r = 0.41; α_3, r = -0.18; β_1, r = -0.11; β_2, r = 0.27; and β_3 r = 0.38, n=7, *NS*), or Post-Train (α_1, r = 0.11; α_2, r = 0.12; α_3, r = 0.41; β_1, r = 0.06; β_2, r = 0.04; and β_3 r = -0.09, n=7, *NS*).

6.2.2.3 Training effects on performance

In these already well-trained athletes, HIT increased PPO by 3±3 % (Pre-Train 369±35; Post-Train 379±39, $P<0.05$) with a maintenance of VO_{2peak} (Pre-Train 4.96±0.56; Post-Train 5.08±0.62 L.min.^{1}).

6.2.2.4 Training effects on muscle Na^+,K^+ATPase

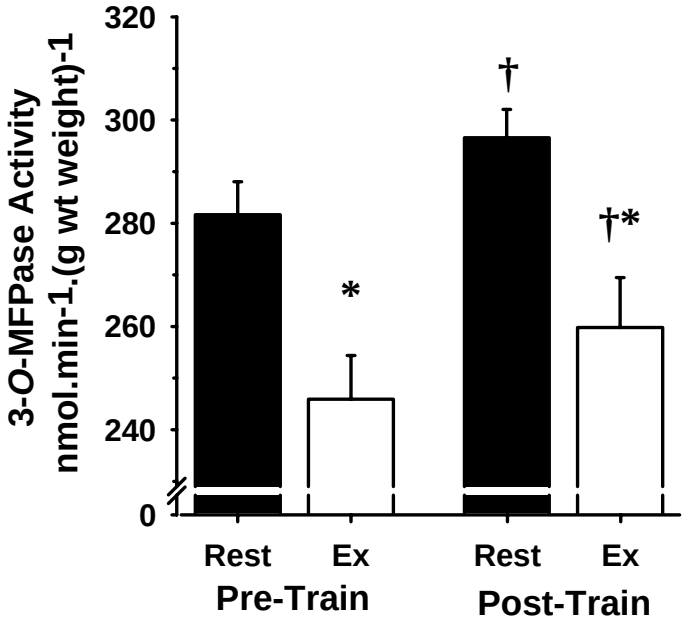
6.2.2.4.1 [^{3}H]-ouabain binding sites content

The muscle [^{3}H]-ouabain binding site content was not significantly altered after training (Pre-Train 355±80; Post-Train 337±124 pmol.(g wet wt)$^{-1}$, *NS*).

6.2.2.4.2 Maximal 3-O-MFPase activity

HIT increased maximal 3-*O*-MFPase activity in resting muscle by 5.4±2.9% and in muscle sampled post-exercise by 5.6±3.4% ($P<0.05$, Fig. 6.2). These training effects were also present when maximal Na^+,K^+ATPase activity was expressed per gram protein for resting muscle (Pre-Train 1653±93; Post-Train 1748±67 nmol.min^{-1}.(g protein)$^{-1}$); and post-exercise muscle (Pre-Train 1443±129; Post-Train 1536±148 nmol.min^{-1}.(g protein)$^{-1}$).

Figure 6.1 Acute exercise and HIT effect on skeletal muscle maximal Na^+,K^+ATPase activity measured *in vitro* by K^+-stimulated 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity. Activity was measured at Rest (solid bar) and immediately after high-intensity interval-exercise (Ex, open bar) for tests conducted prior to (Pre-Train); and after (Post-Train) high-intensity interval training. Mean ± SD; n=7. * Exercise < Rest, $P < 0.05$ (Exercise main effect), † Post-Train > Pre-Train, $P < 0.05$ (Training main effect). Exercise x Train interaction effects were not significantly different.



6.3 DISCUSSION

This study investigated the effects of acute high-intensity interval-exercise as well as short-term high-intensity training (HIT) on skeletal muscle Na^+,K^+ATPase activity in already well-trained athletes. The athletes used in this study had stable performance, and almost identical resting muscle physiology from Baseline to Pre-Train, indicating a stable training regime. Therefore, we were able to examine the expected subtle responses of well-trained athletes to HIT, which increased performance by 3%, which is a practically important increase for these already well-trained athletes (Hopkins *et al.*, 1999). Acute high-intensity interval exercise depressed maximal Na^+,K^+ATPase (3-*O*-MFPase) activity in well-trained athletes, and immediately increased mRNA expression of the three α-isoforms, (Appendix C1) although no significant correlations were found between these events. Importantly, HIT did not modulate any of these acute exercise-induced changes. However, the end-exercise Na^+,K^+ATPase activity was higher after HIT, suggesting a possible training induced protective adaptation.

6.3.1 Acute high intensity interval exercise effects on muscle Na^+,K^+ATPase in well-trained athletes

An intriguing finding was that the magnitude of decrease in maximal Na^+,K^+ATPase activity with high-intensity interval exercise was qualitatively similar to that previously reported after a range of different exercise modalities and in subjects from widely different training backgrounds (Fraser et al., 2002; Leppik et al., 2004; Sandiford et al., 2004; Chapters 4 & 5). This finding strengthens evidence for the decline in maximal Na^+,K^+ATPase activity as being highly reproducible and as potentially an important acute exercise response. Depressed maximal Na^+,K^+ATPase activity could theoretically further exacerbate transmembranous Na^+ and K^+ fluxes during muscle contraction. Passive cellular Na^+ influx (Sjøgaard *et al.*, 1985; Juel, 1986; Sejersted & Sjøgaard,

2000) and K^+ efflux (Sjøgaard *et al.*, 1985; Juel, 1986; Hallén, 1996; Green *et al.*, 2000e; Juel *et al.*, 2000b; Sejersted & Sjøgaard, 2000) already exceed the rate of Na^+,K^+ATPase mediated active ion transport (Clausen *et al.*, 2004), causing slow inactivation of voltage-dependent Na^+ channels (Ruff, 1999). This reduces the muscle membrane potential (Sjøgaard *et al.*, 1985; Sejersted & Sjøgaard, 2000), which may contribute to a decline in muscle excitability (Fowles *et al.*, 2002b), therefore accelerating muscle fatigue (Clausen, 2003). If this decline in maximal Na^+,K^+ATPase activity is important in muscle fatigue, then training which enhances performance might then be anticipated to alleviate this decline with exercise. Although the decline in activity was not altered by HIT, the end-exercise activity was higher (see below).

In contrast to recent findings in untrained or moderately trained participants (Petersen *et al.*, 2005), the acute exercise-induced change in maximal Na^+,K^+ATPase activity was not correlated with the change in Na^+,K^+ATPase isoform mRNA expression (Appendix C1). This might suggest a training status and exercise type specific response of the Na^+,K^+ATPase. It is, possible that the blunted response we report was due in part to only sampling muscle immediately post exercise, and not after further recovery, where a larger mRNA response may have occurred (Pilegaard *et al.*, 2000; Murphy *et al.*, 2004). Alternately, it may suggest that there is no such link between depressed Na^+,K^+ATPase activity and increased Na^+,K^+ATPase gene expression with exercise.

6.3.2 HIT increases maximal Na^+,K^+ATPase activity in resting muscle, but not content

HIT over a three-week period increased maximal Na^+,K^+ATPase activity by approximately 6% in well-trained athletes. In contrast, functional Na^+,K^+ATPase units were not more prevalent, as total [^{3}H]-ouabain binding site content was unchanged. This finding contrasts the well-known upregulatory effects of training on Na^+,K^+ATPase content in untrained participants (Green *et al.*, 1993; McKenna *et al.*, 1993) or

moderately trained subjects (Madsen *et al.*, 1994). Insufficient exercise does not appear to account for this discrepancy, since only 3 days of training was sufficient to increase Na^+,K^+ATPase content in previously untrained participants (Green *et al.*, 2004). Interestingly, in that study a further 3 days of training was required for an increase in Na^+,K^+ATPase activity (Green *et al.*, 2004). Here, maximal Na^+,K^+ATPase activity was increased despite no elevation in Na^+,K^+ATPase content. Participants in this study were already highly endurance trained, and thus likely to have already experienced upregulation of Na^+,K^+ATPase content compared to untrained individuals (Klitgaard & Clausen, 1989; Fraser *et al.*, 2002).

The only other study to find increased Na^+,K^+ATPase content with intense exercise training in well-trained endurance athletes did so after 5-mo intensified endurance training (Evertsen *et al.*, 1997), which represents a much greater increase in training load than used here. Changes in Na^+,K^+ATPase content with short-term HIT are therefore likely to be subtle, and may be missed with the [^{3}H]-ouabain binding site assay, which in this study had a higher variability than the 3-*O*-MFPase assay. Therefore the training status, the training intensity utilised, the short duration of intensified training, coupled with measurement variability, likely explain why [^{3}H]-ouabain binding site content was unchanged with HIT. This might also suggest an independent mechanism is responsible for the increased maximal Na^+,K^+ATPase activity observed with HIT.

6.3.3 HIT increased resting muscle maximal Na^+,K^+ATPase activity, failed to prevent the decline with exercise, but increased the end-exercise activity.

In these well-trained athletes HIT upregulated maximal 3-*O*-MFPase activity but did not attenuate the exercise-induced acute depression in maximal 3-*O*-MFPase activity. This is consistent with the contention that a depression in maximal 3-*O*-MFPase activity occurs as a necessary component of muscle fatigue, possibly as a muscle-protective

strategy (Fraser et al., 2002; Chapter 4). Possible mechanisms for this Na^+,K^+ATPase enzyme inactivation with exercise include an increase in production of reactive oxygen species (Kourie, 1998), or raised intracellular calcium concentration (Stankovicova *et al.*, 1995). In this context, it is of interest to determine whether HIT reduces reactive oxygen species production, or modulates cytosolic [Ca^{2+}], but these have not been investigated.

Whilst training did not attenuate this acute depressive effect of exercise, consistent with earlier findings (Fraser *et al.*, 2002), a significantly higher maximal activity was found at the conclusion of exercise after HIT. This increase likely resulted from an elevated Na^+,K^+ATPase activity in resting muscle, suggesting that this might be an important functional consequence of the well described upregulation of Na^+,K^+ATPase content in muscle with training, although the change in Na^+,K^+ATPase content was not apparent in this thesis.

6.4 Conclusions

In conclusion, acute fatiguing high-intensity interval exercise in well-trained athletes depressed maximal Na^+,K^+ATPase activity, In contrast, HIT increased maximal Na^+,K^+ATPase activity observed in resting muscle. The Na^+,K^+ATPase activity depression in response to acute exercise persisted after HIT. This suggests an obligatory response of Na^+,K^+ATPase activity to fatiguing intense exercise in well-trained athletes. Furthermore, a higher maximal Na^+,K^+ATPase activity was sustained in exercised muscle after HIT, suggesting an important functional role for Na^+,K^+ATPase upregulation.

CHAPTER 7. GENERAL DISCUSSION AND CONCLUSIONS

7.1 Introduction

This thesis investigated the effects of acute exercise, live high train low (LHTL) hypoxic exposure and high intensity training (HIT) on skeletal muscle Na^+,K^+ATPase, buffering capacity (β_m) and plasma ions and acid-base, in well-trained athletes. The results of each chapter have already been discussed in detail; therefore this section will focus on an integrated general discussion of the major results of the thesis.

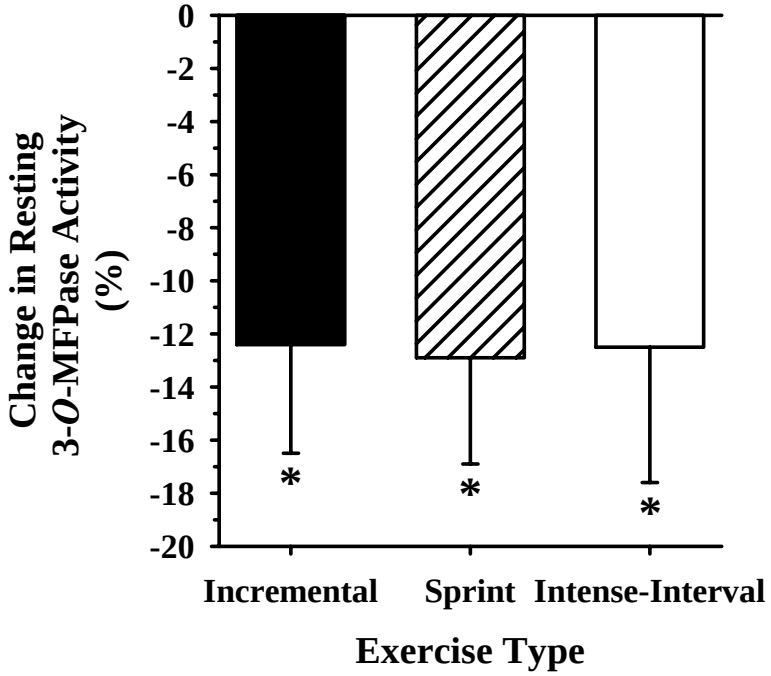
7.2 Acute exercise effects

7.2.1 Acute exercise depresses muscle maximal Na^+,K^+ATPase activity but not content

In these well-trained athletes acute incremental exercise caused a 12.4% depression in maximal muscle Na^+,K^+ATPase (3-*O*-MFPase) activity (Fig. 7.1, Chapter 4). Intriguingly, both sprint exercise (Fig. 7.1, Chapter 5) and intense interval exercise (Fig. 7.1, Chapter 6) both resulted in a similar depression of 12.9%, and 12.5% respectively. Thus, the hypotheses that these three different exercise modalities would depress maximal Na^+,K^+ATPase activity, even in well-trained athletes, were accepted. The magnitude of the exercise-induced fall in maximal Na^+,K^+ATPase activity was also very similar to that after fatiguing single-leg dynamic kicking exercise (Fraser et al., 2002), or prolonged cycling (Leppik et al., 2004).

The depression in Na^+,K^+ATPase activity was seemingly independent of exercise duration and intensity, being present after ~50 s of intense sprint at ~ 170% of peak power output (PPO, Chapter 5), ~ 22 min of intense incremental (Chapter 4), or ~70 min of repeated intense interval exercise at ~85 % PPO (Chapter 6).

Figure 7.1 Change in skeletal muscle maximal in vitro K^+-stimulated 3-*O* methylfluorescein phosphatase (3-*O*-MFPase) activity (Na^+,K^+ATPase activity) from rest (R) to end incremental, sprint or intense interval exercise. Data are expressed as %; Mean ± SD; n=13 for incremental, n=27 for sprint, and n=7 for sprint exercise, * End-exercise < Rest ($P < 0.05$, Exercise main effect).

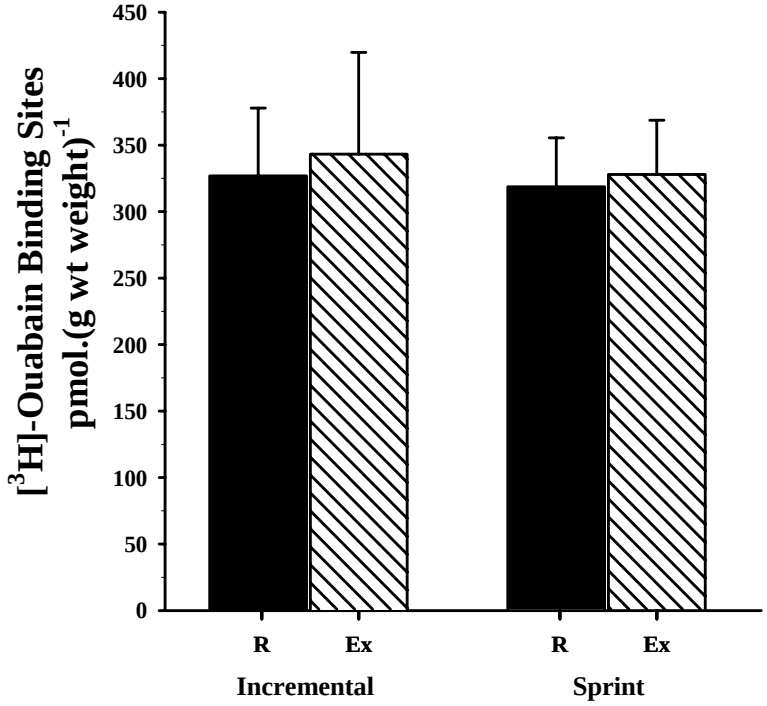


During fatiguing exercise, fluid shifts into working muscle (Sjøgaard et al., 1985; Lindinger & Heigenhauser, 1988; Lindinger & Heigenhauser, 1991; Watson et al., 1993). The exercise-induced depression in 3-O-MFPase activity is unlikely to be a result of fluid shifts into working muscle, contamination of the sample from fluid as a result of suction or blood, as the depression was still evident when expressed relative to muscle protein content (Chapter 4). Given the theoretical importance of maximal Na^+,K^+ATPase activity in muscle performance of athletes, it is surprising that there appears to be no protective effect of chronic intensive training on this depression. This depression appeared to be independent of training status, being of a very similar magnitude in well-trained athletes (Fig. 7.1) and to results from an earlier study which

investigated untrained, endurance trained or resistance trained participants (Fraser et al., 2002), and in untrained participants in another (Leppik et al., 2004).

The methodology utilised in this thesis does not allow the determination of the mechanisms for the exercise-induced depression in Na^+,K^+ATPase activity. It has been suggested, however, that the depression is likely a result of increased intracellular reactive oxygen species (Kourie, 1998), and / or calcium (Stankovicova et al., 1995). The depression in 3-*O*-MFPase activity in this thesis, was not, however, a result of a reduced [^{3}H]-ouabain binding site (Na^+,K^+ATPase) content. Muscle [^{3}H]-ouabain binding was not changed as a result of acute incremental (Chapter 4) or sprint exercise (Chapter 5). This result confirms that the depression in maximal Na^+,K^+ATPase activity was not a result of a decrease in the number of functional pumps, but rather may be indicative of a change in the activation of the Na^+,K^+ATPase. Furthermore, a lack of change in [^{3}H]-ouabain binding site content with exercise argues against an exercise-induced translocation of Na^+,K^+ATPase isoforms from an intracellular pool to form additional functional Na^+,K^+ATPase complexes at the plasma membrane (Tsakiridis et al., 1996; Juel et al., 2000a; Juel et al., 2001). If additional functional Na^+,K^+ATPase complexes were being formed, [^{3}H]-ouabain binding should have been greater after exercise, and this was not the case (Chapters 4 & 5, Fig. 7.2). It is possible, however, that there could be an inrease in Na^+,K^+ATPase subunits at the plasma membrane, but not functional pumps.

Figure 7.2 Skeletal muscle [^{3}H]-ouabain binding site content (Na^+,K^+ATPase content) before (R) and after (Ex) exercise. Data are expressed as pmol.(g wet wt.)$^{-1}$; mean ± SD, n=11 for incremental exercise, n=20 for sprint exercise.



7.2.2 Muscle metabolites, acid-base and buffering capacity (β_m)

Acute incremental exercise (Study 1-Part I) caused degradation of muscle ATP, PCr and glycogen and accumulation in skeletal muscle of Lac^- and H^+ ions. The decline in ATP would be expected with this type of heavy exercise (Sahlin *et al.*, 1981). However, the decline in PCr and rise in Cr was surprisingly small relative to the rise in Lac^-, which may reflect the usual slight delay in biopsy sampling and a likely rapid PCr resynthesis in these endurance-trained athletes (Miller *et al.*, 1988; Söderlund & Hultman, 1991; Karatzaferi *et al.*, 2001). As expected, maximal in-vitro buffering capacity (β_m, Chapter 3) was unaffected by intense incremental exercise.

7.3 LHTL decreased but HIT maintained $\dot{V}O_{2peak}$

The LHTL and HIT interventions employed in this thesis had contrasting effects on $\dot{V}O_{2peak}$. Following 23-n of LHTL at ~3000 m, $\dot{V}O_{2peak}$ was depressed by 7%, without reduction in total work during 2 min of all-out cycling (Chapter 3, $P<0.05$). HIT failed to increase $\dot{V}O_{2peak}$, which is consistent with findings employing a very similar training regime and pre-exisiting highly endurance trained status (Lindsay et al., 1996; Westgarth-Taylor et al., 1997; Weston et al., 1997; Stepto et al., 1999), although contrasts the results of another group showing an increase of ~6% in well trained swimmers (Truijens *et al.*, 2003)

The decrease in $\dot{V}O_{2peak}$ after LHTL was not a result of detraining of the LHTL group, as training volume was similar to the control group. Further, when well-trained athletes completely cease training for 2-3 weeks, decreases in $\dot{V}O_{2max}$ are only 2-7% (Houston et al., 1979; Coyle et al., 1984; LaForgia et al., 1999).

The potential mechanisms underlying the decreased $\dot{V}O_{2peak}$ evident in this thesis are unclear. Interestingly, however, the LHTL subjects exhibited no change in haemoglobin mass or reticulocyte indices of accelerated erythropoiesis (Ashenden et al., 1999a) but their $\dot{V}O_{2peak}$ was depressed. This is in sharp contrast to reported increases in red cell mass and consequently $\dot{V}O_{2max}$ (Cerretelli & Hoppeler, 1996; Rusko, 1996b; Levine & Stray-Gundersen, 1997; Rodriguez *et al.*, 1999; Fulco *et al.*, 2000) after hypoxic exposure. The lack of change in haemoglobin mass reported in this thesis may also reflect the relatively short period of time spent under hypoxic conditions by these subjects (Rusko et al., 2003).

7.4 LHTL maintained but HIT improved performance

Only one of the two LHTL studies in this thesis employed a performance test (Chapter 3). Total work, measured as an indicator of performance was unchanged in the LHTL group, but this be due to the small sample size employed and represent a type II error. There is mounting evidence from three independent groups that LHTL may yield small improvements (0.8-1.3%) in events lasting from ~50 s to 17 min; 400 m sprint (Nummela & Rusko, 2000), 4-min all-out effort (Hahn & Gore, 2001), as well as 3000 m (Stray-Gundersen et al., 2001) and 5000 m run times (Levine & Stray-Gundersen, 1997). Running economy was increased by 3.3% in elite runners after 20-n LHTL (Saunders *et al.*, 2004). Further, LHTL has been shown to improve the maximal aerobic power (8.4%) and $\dot{V}O_{2max}$ (7.1%) of elite runners after 18 days sleeping at 2500 and 3000 m, with the positive changes persisting 15 days after exposure ceased (Brugniaux *et al.*, 2005). One recent study has, however, found no performance gain with LHTL, in elite swimmers (Robach *et al.*, 2005). The main aims of the two LHTL studies in this thesis were to investigate mechanisms that may be responsible for positive performance adaptations, rather than demonstrating the existence of such adaptations. This, and the large battery of tests already undertaken by the participants in these studies precluded an in-depth analysis of performance adaptation to LHTL. Indeed the nature of the demanding study design employed, especially in Chapter 5, with its allied investigations (Townsend *et al.*, 2002; Clark *et al.*, 2004a; Townsend *et al.*, 2004; Kinsman *et al.*, In press-a; Kinsman *et al.*, In Press-b) may have actively worked against positive performance gains.

7.4.1 Submaximal $\dot{V}O_2$ and efficiency

This thesis measured the $\dot{V}O_2$ of highly trained athletes after LHTL interventions. As hypothesised, the submaximal $\dot{V}O_2$ of the LHTL group was depressed and efficiency

was increased at each of the four, 4-min submaximal workloads after both 11 and 23 nights of sleeping in hypoxia (Chapter 3, $P<0.05$). These changes were not as a result of measurement error, and were performed with cadence matched for each test (Barstow, 1990; Woolford et al., 1999), or a reduction in $\dot{V}O_2$ of the respiratory musculature (Harms et al., 1997), and unlikely to be associated with altered fibre type recruitment (Coyle et al., 1992) which could each alter cycling efficiency.

These results challenge the conventional concept that after returning from an altitude or simulated altitude sojourn, $\dot{V}O_2$ at any given submaximal power output remains unchanged at sea level (Levine & Stray-Gundersen, 1997; Piehl-Aulin *et al.*, 1998). The results presented in this thesis are consistent with a significantly lower $\dot{V}O_2$ (8-10%) during prolonged submaximal cycle subsequent to a 21-day climb to 6194 m (Green et al., 2000d). Collectively, data from this thesis and those of subjects living and climbing at natural altitude (Green et al., 2000d) suggest that one can attribute the increase in mechanical efficiency to hypoxia per se rather than hypobaria, cold or the effects of heavy athletic training. Interestingly, the results of this thesis are consistent with those of several cross-sectional studies that have reported higher efficiency during exercise in altitude natives compared with lowlanders (Hochachka et al., 1991; Saltin et al., 1995b).

The reduction in whole body $\dot{V}O_2$ during exercise after LHTL can likely be explained by changes in substrate utilisation during exercise (Brooks, 1998), and is in agreement with the preferential use of carbohydrate fuels rather than fats at 4300 m altitude at rest and during submaximal exercise (Brooks et al., 1992; Roberts et al., 1996b).

The findings of this thesis of a lower $\dot{V}O_2$ at the same absolute workload are consistent with an improved coupling of ATP demand and supply (Hochachka, 1988). Another mechanism which might contribute to increased exercise efficiency after LHTL include

a reduction of ATP consuming processes within skeletal muscle such as a depression of skeletal muscle Na^+,K^+ATPase activity after hypoxic exposure (Green et al., 1999b; Green et al., 2000a, Chapters 4 & 5).

7.5 LHTL increased muscle buffering capacity (β_m) and subtly altered plasma [SID], without change in muscle or blood [H^+]

7.5.1.1 Muscle [H^+] and Muscle buffing capacity (β_m)

Muscle in-vitro buffer capacity (β_m) was increased after LHTL (~3000 m) but this did not coincide with enhanced muscle H^+ regulation, or upregulation of anaerobic metabolism during intense exercise (Chapter 3). LHTL did not change plasma [H^+], despite a lowered pCO_2, [HCO_3^-] and [Lac^-] (Chapter 3). An elevation in β_m after LHTL is consistent with the ~6% increase reported after training and living at ~2000-2700 m (Mizuno et al., 1990; Saltin et al., 1995a), and is as hypothesised in this thesis. The increase in β_m was not the result of increased muscle protein content (Chapter 4) but presumably reflected an increased buffer capacity of the dipeptides or protein expressed. This may be a consequence of a higher intramuscular carnosine concentration as suggested by others (Saltin et al., 1995a), but the mechanism remains unknown. Interestingly, the observed changes in β_m at 3000 m (Chapter 4) were not evident after 20-n LHTL at ~2650 m (Chapter 5). This lack of change in β_m at 2650 m, may be due to a combination of the ~ 17% higher simulated altitude utilised in Chapter 4, and the regular normoxic exposure with LHTL that is not a feature of the previous two studies (Mizuno *et al.*, 1990; Saltin *et al.*, 1995a).

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7.5.1.2 Plasma ions and strong ion difference ([SID]).

The decrease in maximal Na^+,K^+ATPase activity (Chapters 4, 5 and 6) was insufficient to adversely affect plasma [K^+] during exercise and therefore presumably K^+ clearance

by both active and inactive muscle. Also investigated was whether LHTL affected plasma ion concentrations during exercise and specifically the strong ion difference ([SID]). Consecutive nights of LHTL at a simulated altitude of ~ 2650 m resulted in a small increase in plasma [Cl^-] and a lowering of the [SID]. A lowered [SID], may reflect a counter-regulatory mechanism of maintaining plasma [H^+] in hypoxia.

The raised plasma [Cl^-] after LHTL may also offer protection of muscle membrane excitability through attenuation of the depolarisation caused by the exercise-induced run-down of the trans-sarcolemmal K^+ gradient (Cairns et al., 2004).

7.6 Contrasting effects of LHTL and HIT on muscle Na^+,K^+ATPase

LHTL induced a small but significant depression in muscle maximal Na^+,K^+ATPase activity after 20 to 23-n of consecutive LHTL, at a simulated altitude of 2650 m (Chapter 4, $P<0.05$) or 3000 m (Chapter 5, $P<0.05$), respectively. HIT, however, resulted in a small but significant increase in maximal Na^+,K^+ATPase activity. As hypothesised, the depressive response of the Na^+,K^+ATPase to the hypoxia of LHTL was rapid, with the reduction in Na^+,K^+ATPase evident after just 5-n LHTL (Chapter 5, $P<0.05$)

Importantly, the depression in maximal Na^+,K^+ATPase activity was reversed when 2-n of normoxia was interspersed in LHTL, as a small increase in 3-*O*-MFPase activity was observed after an additional 15-n LHTL in the intermittent LHTL model (Chapter 5, 4 x (5-n hypoxia, 2-n normoxia); $P<0.05$). The results of Chapters 4 & 5 suggest that the time required for restoration of maximal Na^+,K^+ATPase activity after LHTL may be lengthy, as the reduced Na^+,K^+ATPase activity persists for at least several days after return to normoxia. Thus, even in well-trained participants undergoing ongoing heavy daily training, two nights of normoxia after each 5-n of hypoxic exposure can overcome the depressive effect of nightly hypoxia as used with LHTL. This provides one rationale for athletes to utilise intermittent instead of consecutive LHTL.

Given the potential role of a depressed maximal Na^+,K^+ATPase activity in attenuating muscle performance (Clausen *et al.*, 2004), and the performance enhancing benefits of both LHTL, it is somewhat paradoxical that LHTL is deleterious to Na^+,K^+ATPase activity. Even allowing for a very small contribution of Na^+,K^+ATPase to total muscle energy expenditure, a reduction in Na^+,K^+ATPase activity, which does not affect plasma K^+ regulation may be a small part of a mechanism allowing conserved ATP utilisation after LHTL, thus contributing to an improved efficiency.

7.6.1 Intermittent LHTL reversed the depressive effects of consecutive LHTL, and HIT increased maximal Na^+,K^+ATPase activity

Intermittent LHTL, with greater time periods spent training under normoxic conditions reversed the depressive effects of consecutive nightly hypoxia on maximal Na^+,K^+ATPase activity, and HIT increased maximal Na^+,K^+ATPase activity. However, neither intermittent LHTL nor HIT protected against the acute exercise-induced depression in maximal Na^+,K^+ATPase activity, although HIT resulted in a higher post-exercise Na^+,K^+ATPase activity which may represent a positive adaptation.

Muscle [^{3}H]-ouabain binding was not significantly different after LHTL (Chapters 4 & 5) or HIT interventions. This suggests that the depression in Na^+,K^+ATPase activity induced by LHTL, and the elevated maximal Na^+,K^+ATPase activity after HIT were each not a result of a changed Na^+,K^+ATPase content, but rather changed enzyme activity.

The small magnitude of depression in Na^+,K^+ATPase with LHTL differs from reports of decreased Na^+,K^+ATPase content of ~14% after chronic hypoxia (Green *et al.*, 1999b; Green *et al.*, 2000a) and Na^+,K^+ATPase activity by ~29% after exercising under hypoxic conditions (Sandiford *et al.*, 2004). An unchanged [^{3}H]-ouabain binding site content after training similarly contradicts previous reports of increased Na^+,K^+ATPase content in untrained, or moderately trained participants after training (Green *et al.*,

1993; McKenna *et al.*, 1993; Madsen *et al.*, 1994). Participants in this thesis were already highly endurance trained, and thus likely to have already experienced upregulation of Na^+,K^+ATPase content compared to untrained individuals (Klitgaard & Clausen, 1989; Fraser *et al.*, 2002). As the athletes used in the in this thesis maintained heavy ongoing training, changes in Na^+,K^+ATPase with either LHTL or short-term HIT are likely to be very subtle. It is therefore likely, that the training status of the participants in this thesis, and their years of exposure to intense training explains the small changes in Na^+,K^+ATPase with LHTL or HIT interventions.

7.7 Conclusions

The specific conclusions of this thesis are:

1. Sleeping in moderate hypoxia (LHTL) of 3000 m improved β_m, but LHTL of 2650 m did not.
2. LHTL improved gross mechanical efficiency during submaximal cycle ergometry conducted in normobaric normoxia.
3. Maximal Na^+,K^+ATPase activity was depressed by acute incremental, sprint and intense interval exercise.
4. Na^+,K^+ATPase content was unchanged by acute incremental exercise.
5. Consecutive nightly hypoxia imposed by both 3000 m and 2650 m LHTL depressed muscle maximal Na^+,K^+ATPase activity.
6. An early depression in maximal Na^+,K^+ATPase activity occurred with consecutive nightly LHTL of 2650 m.
7. The depression in Na^+,K^+ATPase activity with exercise was not augmented by LHTL.
8. The greater periods of normoxia during LHTLi attenuated the hypoxia-induced reduction in Na^+,K^+ATPase with LHTLc.

9. LHTL did not change plasma [H^+] during exercise, possibly due to alterations in [Cl^-] and therefore [SID].
10. The depression in maximal Na^+,K^+ATPase activity with acute exercise was not associated with increased Na^+,K^+ATPase α-isoform mRNA expression.
11. Short-term HIT increased resting muscle Na^+,K^+ATPase maximal Na^+,K^+ATPase activity but not content and consequently enabled a higher end-exercise maximal Na^+,K^+ATPase activity.

CHAPTER 8. RECOMMENDATIONS FOR FURTHER RESEARCH

Studies conducted in this thesis investigated the effects of acute intense exercise, mild simulated altitude and high-intensity interval training on muscle Na^+,K^+ATPase activity, content, and acid-base balance in already well-trained endurance athletes. This thesis has reported that both LHTL and acute exercise depress maximal *in-vitro* Na^+,K^+ATPase activity.

8.1.1 Acute exercise induced fatigue in well-trained athletes

A reduction in maximal Na^+,K^+ATPase activity may lead to a further increase in transmembranous Na^+ and K^+ fluxes during muscle contractions and therefore accelerate muscle fatigue. The effects of acute intense exercise on altered sarcolemmal ion concentration gradients, Na^+,K^+ATPase activity and membrane excitability have not been investigated in well-trained athletes. The nature, if any, of the relationship between each of these variables is worthy of further investigation.

The methodology employed in this thesis did not allow investigation of the mechanism for a depressed Na^+,K^+ATPase activity in well-trained athletes. This thesis speculated that this depression may be caused by of raised intracellular [Ca^{2+}] or reactive oxygen species. Further research on the exact mechanisms is required. Specifically, measurement of the skeletal muscle cytosolic [Ca^{2+}] of athletes after fatiguing exercise would assist in establishing a relationship between these two events. Further, administration of anti-oxidants appears to improve performance of endurance athletes (Medved *et al.*, 2004), but the specific effects on Na^+,K^+ATPase are unknown.

8.1.2 Mechanisms for improved performance after LHTL and HIT

This thesis investigated the effects of LHTL on muscle metabolites, acid-base and Na^+,K^+ATPase (Chapters 3-5), and HIT effects on Na^+,K^+ATPase (Chapter 6). The subtle changes occurring with LHTL and HIT did not allow definitive determination of

the mechanism for improved performance with each intervention. LHTL induced a depression in Na^+,K^+ATPase activity, subtle alterations in acid-base balance, with maintained performance.

There is increasing evidence that genetic factors may be involved in responses to hypoxia (Jedlickova *et al.*, 2003). Cell adaptation to low oxygen pressures (PO_2), is, in fact, mediated via transcription factors named hypoxia-inducible factors or HIF, that exhibit specific tissue expressions (Semenza, 1999). These hypoxia-inducible factors regulate transcription of more than 70 genes involved in cellular adaptation to low PO_2. Research on the effect of LHTL on HIF genes in well-trained athletes may therefore represent an important step in understanding responses of athletes to LHTL and warrants investigation.

Further research is also required to determine if the depression in Na^+,K^+ATPase activity with LHTL leads to perturbations in sarcolemmal ion concentration gradients and membrane excitability as this has not been investigated in well-trained athletes. Similarly, the effect of LHTL on SR Ca^{2+}ATPase activity has not been investigated, and may represent an important factor in improved performance after LHTL. SR Ca^{2+}ATPase activity is depressed after chronic hypoxic exposure by ~17% (Green *et al.*, 2000b). The magnitude of decreased Ca^{2+}ATPase activity is similar to that of decline in muscle [^{3}H]-ouabain binding site content (~14%) after the same hypoxic exposure (Green *et al.*, 2000a). If would seem paradoxical that LHTL would depress Ca^{2+}ATPase activity, as this would be likely to lead to increased fatigue, and not the performance enhancement reported after LHTL (Levine & Stray-Gundersen, 1997; Hahn *et al.*, 2001; Saunders *et al.*, 2003). In this thesis, maximal muscle Na^+,K^+ATPase activity was depressed without alteration in plasma K^+ regulation (Chapter 4). It may be that SR Ca^{2+}ATPase is similarly depressed, but this depression is not sufficient to impair Ca^{2+}

regulation. Such an adaptive response to LHTL would be energetically efficient and allow maintained or improved performance.

As the performance of intense exercise can be limited by both central and peripheral factors (Fitts, 1994, 1996a; Gandevia, 2001; McKenna, 2003; Allen, 2004), it is likely that these factors will respond to training. Further, the attenuation of fatigue obviously improves the performance of well-trained athletes. Therefore, the effects of short-term HIT in well-trained athletes on central fatigue and SR Ca^{2+} regulation are important avenues for further research.

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APPENDIX A APPENDIX A1 SUBJECT INFORMATION SHEETS

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Study 1-Parts I & II Subject Information Sheet

Project title:
"*The effect of sleeping at simulated altitude on muscle buffering and anaerobic exercise performance*".

Investigators:
Dr Allah Hahn, Dr David Martin, Mr Michael Ashenden, Mr Hamilton Lee
Head of Department of Physiology, Division of Sport Sciences, Australian Institute of Sport, Canberra.
Dr Christopher Gore
Laboratory Standards Assistance Scheme, Australian Institute of Sport, Adelaide.
Dr Michael McKenna and Mr Robert Aughey (Masters student)
Department of Human Movement, Recreation and Performance, Victoria University of Technology, Melbourne
Dr Andrew Garnham (MD)
Department of Human Movement, Deakin University, Burwood, Melbourne

Aims of the study:

The study is designed to determine whether an athlete's performance during short term exercise (such as a 2 minute "all out" effort) is improved by sleeping at a simulated altitude of ~3000 m, compared to sleeping in Canberra (altitude ~600 m above sea level). Additionally, this study aims to examine the importance of several mechanisms which would contribute to improved performance as a consequence of muscle adaptation to sleeping at simulated altitude. These mechanisms include a better ability of your muscles to cope with 'lactic acid' (which makes you fatigue), as well as examining energy sources within the muscle.

The traditional view of training at altitude is that it may enhance performance at sea level as a result of the low oxygen levels at altitude which stimulates the body to make more red blood cells and therefore increases the ability of the body to transport and use oxygen. An alternate but somewhat experimental view of altitude training is that it may

improve performance as a result of changing the ability of an athlete to cope with very high intensity exercise (which causes muscle acidity and fatigue). Finland recently (1993) introduced the concept of using a "nitrogen chamber" to simulate being at altitude by increasing the normal amount of nitrogen from 79% to about 85%. They suggested sleeping at simulated altitude and training near sea level as an optimal method of using altitude – and in relatively flat countries such as Australia this is a far more convenient method to simulate living at altitude than flying overseas to live and train in mountainous areas over 2200 m high.

Through this study we hope to provide a better understanding of the potential benefits of sleeping at simulated altitude for athlete performance at sea level. This may lead to better and more effective use of the nitrogen chamber by our best athletes during the lead up to the Sydney Olympics and beyond.

Subject participation:

As a volunteer, you are free to withdraw from the study at any time, without any adverse effects or reactions.

Overview

You will be required to attend the Physiology Laboratory (Sport Science Building) at the Australian Institute of Sport (AIS) on at least seven occasions. Five visits will be for exercise tests and two visits will be for measuring the total amount of haemoglobin in your body. Two visits for the exercise tests will be within 3 days, one exercise test will be conducted 13 days later and then after a further 2 weeks two more exercise test will be completed within 3 days. During each exercise test samples of blood will be taken from a vein in the back of your hand. On two of the five exercise tests muscle biopsies for this will also be conducted. Details of all procedures are given in the sections below.

One dozen subjects will be recruited for the study and 6 will be randomly assigned to sleep and rest in the Altitude Simulator at the AIS Physiology Laboratory for about 10 hours per day for 25 consecutive days. The simulator comprises three, air-conditioned, small rooms with comfortable single or bunk beds. Those who sleep in the Simulator will be monitored by two staff at all times using measurements of heart rate and blood oxygen levels. Privacy of all subjects is assured by curtains that are under you control since they can only be opened or closed from inside the Altitude Simulator.

Exercise testing procedures:

During your visits to the Laboratory you will be asked to perform a 26 minute exercise test on a bicycle ergometer. This test involves *continuous exercise for 16 minutes* with four, 4 minute easy workloads that will be well below your regular training loads, although the exercise intensity (effort) will be progressively greater. After a *four minute rest* you will be asked to complete 2 minutes of exercise at a workload estimated to be about 105% of the workload that would cause you to reach the maximal rate that you body can use oxygen (VO_{2max}). After a further 2 minute rest you will be asked to *exercise to absolute exhaustion at the highest rate that you can maintain for two minutes* – a 2 minute 'time trial'.

Summary of 26 minute cycling test:

16 minutes of easy cycling	4 minute rest	2 minutes cycling at 105% of maximum	2 minutes cycling to total exhaustion

At rest, during and following the exercise test, blood samples (each about 5 ml or one teaspoon full) will be drawn from a small plastic tube inserted into a vein in the back of your hand (the tube is inserted using a local anaesthetic and will cause minor discomfort). A total of up to 30 ml of blood will be taken during each test – and this compares with ~500 ml if you give blood at the blood bank. In order to get a good blood flow to the hand, your hand will be immersed in a warm water bath (~45^{o}C) up to the wrist which will mean that you can only have one hand on the handle bars whilst cycling. This will feel slightly awkward but will not impair your performance on the bike since all tests must be completed while seated in the saddle. The blood samples will be analysed for acidity (pH), lactate and other metabolites. Venous samples from the catheter cause minimal discomfort and a have small likelihood of bruising or infection. Sterile, single use disposable equipment is used to collect all blood samples and qualified and experienced staff take all samples.

Total Haemoglobin test procedures:

Total haemoglobin is measured by asking you to breathe two very small amounts of carbon monoxide for about 25 minutes while you lie comfortably on a bed. Two blood samples are taken, each with a single use sterile needle and the total volume of blood taken is about 5 ml or approximately one full teaspoon. The amount of carbon monoxide that enters your blood is equivalent to being in a smoky room for several hours or smoking a single cigarette. You can train immediately after this test and would be unlikely to notice any decrease in your performance.

Muscle Biopsy Procedure

Muscle biopsies will be taken before and at after 22 minutes of the 26 minutes of exercise on the bicycle ergometer. You will be given about 15 minutes to rest between the first biopsy sample and when you begin the exercise. The cycling test will finish with the second biopsy sample after 22 minutes on the bicycle ergometer.

Summary of 22 minute cycling test with muscle biopsy before and after cycling:

Resting muscle biopsy	~15 minute rest	16 minutes of easy cycling	4 minute rest	2 minutes of cycling at 105% of maximum	Second biopsy at immediate end of cycling

The muscle biopsies will be taken at the AIS Physiology Laboratory by a doctor from the Deakin University who is highly experienced in this procedure. On two occasions that you perform the 26 minute exercise test there will be biopsy samples and on three occasions you will be asked to exercise with no biopsy. This will allow us to check whether the resting biopsy changes your ability to exercise at a high intensity.

On each of the two occasions, two biopsies will be taken from your thigh muscle. Thus, *a total of four biopsies will be taken in the entire study*. Each biopsy will be performed with a local anaesthetic in your thigh. The muscle biopsy procedure is used to obtain small samples of your muscle tissue for analysis of muscle acidity and energy sources. An injection of a local anaesthetic is made in the skin overlying the muscle in your

thigh, and then a small incision (approx. 0.6 cm long) is made in the skin. The biopsy needle is then inserted into your muscle and a small piece of tissue removed from the muscle. During this part of the procedure you will feel pressure and this will be quite uncomfortable, but will last for only about 1-2 seconds. When the small piece of muscle is removed you may also experience a mild muscle cramp, but this only persists for a few seconds. ***Many people experience pain during this part of the procedure but this lasts only for 1-2 seconds***. The size of muscle removed by the biopsy needle is similar to a medium sized grain of rice. This poses no long term effects for your muscle and will not be noticeable to others apart from a small scar on the skin for a few months. Following the biopsy, the incision will be closed using a Steri-strip and pressure bandage will be applied for about 15 minutes before you begin exercising.

You will then be asked to complete 22 minutes of the 26 minute exercise test (see Summary table above), before a second biopsy sample is taken from the same site as the first biopsy. After the second biopsy you will not have to complete any further exercise. Unlike the day when you cycle without any biopsies you will ***not*** have to complete the last 2 minutes of exhaustive exercise – the 2 minute 'time trial'.

After both biopsies on a each day, the biopsy incision be closed using a steri-strip covered by a transparent waterproof dressing. Then a pressure bandage will be applied which should be maintained for 24-48 hours. Steri-strip closure should be maintained for a few days. You should not exercise for 24-48 hours after biopsies and you should avoid heavy knocks. It is common for subjects to experience some mild soreness in the muscle over the next 2-3 days, however this passes and does not restrict movement. The soreness is due to slight bleeding within the muscle and is best treated by "ice, compression and elevation". An ice pack will be applied over the biopsy site after the biopsy procedure to minimise any bleeding and therefore soreness. In some rare cases mild bruises have been reported, but these symptoms disappear within a week. The whole procedure will be performed under sterile conditions by a qualified medical practitioner. Although the possibility of infection and significant bruising is quite small, if by chance it does eventuate, inform us immediately and then consult your doctor.

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Study 2 Subject Information Sheet

Project title:
"The effect of adaptation to moderate altitude on metabolism and exercise performance in highly trained individuals: Investigation of a new paradigm".

Investigators:

Dr Allan Hahn, Dr Christopher Gore, Dr Kieran Fallon, Australian Institute of Sport
Professor John Hawley and Ms Sally Clark (PhD student) - R.M.I.T. University, Melbourne
Dr Michael McKenna and Mr Robert Aughey (PhD student) - Victoria University of Technology, Melbourne
Dr Chin Moi Chow , **Ms Tahnee Kinsman** (Masters student) **and Mr Nathan Townsend** (PhD student) - The University of Sydney, Sydney.
Professor Alan Roberts – University of Canberra, Canberra.

Aims of the study:

The use of altitude as an aid to preparation for sea level athletic competition has been popular for more than 30 years. Recently several studies have indicated that the best strategy is to live and sleep at altitude but train as close as possible to sea level. This allows adaptation to the altitude environment without the need to compromise training intensity. However, there are few places in the world where "living high, training low" is practical. Consequently, methods have been devised that enable athletes to sleep under conditions of *simulated* altitude at their normal training locations. An altitude simulator (known as the **BOC Altitude House** in recognition of financial and technical support from BOC Gases Pty Ltd) has been operating at the AIS for more than 3 years, and has allowed detailed investigations of the effect of sleeping in reduced-oxygen environments. These investigations have cast doubt on the traditional view that altitude exposure increases the production of red blood cells, thereby enhancing the delivery of oxygen to exercising muscles. Rather, it appears that many of the adaptations to altitude may occur at the muscle level.

This study aims to provide a more complete understanding of the processes of adaptation to sleeping under conditions of moderate altitude (approximately 2700 metres), and to determine how these influence exercise performance. In addition, an

attempt will be made to find out whether it is necessary to sleep at simulated altitude every night for several weeks in order to gain physiological and performance benefits.
It is hoped that the results of the study will enable better and more effective use of the Altitude House by Australian athletes during the lead up to international competitions such as the Olympic Games and World Championships.

Overview of Commitment:

You will be required to attend the Physiology Laboratory (Sport Science Medicine Building) at the Australian Institute of Sport (AIS) for various physiological tests. Some of these tests will involve exercising on a cycle ergometer whilst others will be performed in a resting state. A description of all the test procedures is given below. A timetable indicating when these tests will be conducted throughout the study is attached (Appendix A). Please note that the testing schedule does require a *large commitment* on your behalf.

If you agree to participate in the study you could be assigned to one of three groups:

- Group 1 will sleep and rest in the Altitude House at the AIS Physiology Laboratory for about 10 hours per night for 20 consecutive nights.
- Group 2 will sleep and rest in the Altitude House for 20 nights, made up of 4 blocks of 5 nights separated by intervals of 2 nights of sleeping under normal conditions.
- Group 3 will sleep under normal conditions throughout the study.

The Altitude House comprises three, air-conditioned, small rooms with comfortable single or bunk beds.

How does the BOC Altitude House work?

The altitude is simulated by adding nitrogen gas to the rooms, in order to lower the percentage of oxygen in the air. Normally there is about 21% of oxygen in the air, but typically the Altitude House operates with an oxygen percentage in the range of 17.5 to 15.5% - which effectively simulates altitudes between 2000 and 3000 m. The mechanism for delivering the nitrogen incorporates a series of valves and regulators, so that failure of any single component cannot lead to inappropriate nitrogen input. The multiple valves mean that in the case of a total failure of the nitrogen delivery system, the Altitude House returns rapidly to normal levels of oxygen (21%). In addition to two oxygen sensors used by the automated nitrogen delivery system, the oxygen concentration of the air in the chamber is monitored manually by an independent oxygen analyser. The Altitude House also incorporates a system to prevent carbon dioxide accumulation, an air filtering mechanism, and air conditioning which maintains the rooms at a comfortable temperature.

What does it feel like?

Most athletes comment that they feel absolutely no different from normal when they walk into the Altitude House. Because the Altitude House does not involve any alteration in the barometric pressure there is no sensation of a “sudden change” when

you walk inside. At 2700 m, the altitude simulated is not too different from that to which you are commonly exposed during an international plane flight. However, it possible that you may experience symptoms of altitude sickness.

The symptoms of altitude sickness include:

- headache
- dizziness
- nausea
- shortness of breath
- persistent coughing
- abdominal pain

If you experience any of these symptoms at any time during or after spending time in the Altitude House you must immediately report them to the supervisor. On reporting such symptoms, you will promptly be removed from the Altitude House.

How do you know if you're OK?

In addition to measuring the oxygen and carbon dioxide inside the Altitude House, all athletes are required to wear a small probe on a finger that when taped gently in place allows us to check your heart rate and percentage of blood oxygen while you are asleep. This procedure is repeated at 30 minute intervals and is totally painless. The measurements are taken by shining a small red light through your fingertip. The light is sensed and transmitted by a wire to a machine located immediately outside the Altitude House. This type of machine is commonly used in hospitals for the same purpose – that is, to monitor blood oxygen levels.

Exercise testing procedures:

Maximum Oxygen Uptake ($VO_{2\ Max}$)Test

On your first visit to the lab you will be required to perform a VO_{2max} test. After a warm up (approx 15 min) you will perform a continuous incremental test until exhaustion on a cycle ergometer. The starting workload represents 3.5 watts/kg of your bodyweight. The workload is increased every two and a half minutes until fatigue. This will enable us to determine your peak power output (PPO) from which workloads for subsequent tests will be calculated.

Lactate Threshold Test (including $VO_{2\ max}$)

On two separate occasions you will be asked to perform a submaximal exercise test on a cycle ergometer. This test involves a series of 6-minute workloads separated by 1-minute recovery periods. A blood sample will be taken at the end of each workload. The initial exercise intensity (effort) is very low but it will progressively increase until a lactate of 4mmol/L is reached. After a short rest you will be asked to complete a brief exercise test (~ 10min) to reassess your $VO_{2max.}$

The blood samples collected during this test (each about 3 ml or one teaspoon) will be drawn from a small plastic tube inserted into a forearm vein (the tube is inserted using a local anaesthetic and may cause minor discomfort). A total of up to 30 ml of blood will be taken during each test – and this compares with ~500 ml if you give blood at the blood bank. The blood samples will be analysed for acidity (pH), lactate and other metabolites. Drawing venous samples from the catheter can cause discomfort and there is a small likelihood of bruising or infection. Sterile, single use disposable equipment is used for blood collection and qualified and experienced staff take all samples. For 36 hours prior to the lactate threshold test you will be required to adhere to specific exercise and dietary regimes established by the research team.

Muscle Biopsy Procedure:

Muscle biopsies will be taken before and after a one minute effort on the cycle ergometer which will be performed at 170% of your peak power output (PPO). Your PPO will be determined as the highest workload that you reach during the initial VO_{2max} test. You will be required to perform the 1-minute effort exercise protocol on 3 separate occasions prior to the first biopsy test. This is to ensure that you are familiar with the test protocol and to eliminate any learning effect that may occur. On the days involving collection of muscle biopsy samples you will be given about 15 minutes of rest between the first biopsy sample and the beginning of the 1-minute effort. The second biopsy will be taken immediately after completion of the 1-minute effort.

The muscle biopsies will be taken at the AIS Physiology Laboratory by a doctor who is highly experienced in this procedure. Two biopsies will be collected on each of three different occasions – before, during and after the period in the Altitude House. Thus*, a total of six biopsies will be taken in the entire study.* The muscle biopsy procedure is used to obtain small samples of your muscle tissue for analysis of muscle acidity and energy sources. An injection of a local anaesthetic is made into the skin overlying the muscle in your thigh, and then two small incisions (approx. 0.6 cm long) are made in the skin, one for each biopsy. A sterile biopsy needle is then inserted into your muscle to remove a small piece of tissue. During this part of the procedure you will feel pressure and you may be quite uncomfortable for about 1-2 seconds. After the biopsy you may also experience a mild muscle cramp, but again, this persists for only a few seconds. ***Many people experience pain during the procedure but this lasts only for 1-2 seconds***. The amount of muscle removed during a biopsy is about the size of a grain of rice. This has no long-term effect on the muscle and will not be noticeable to others apart from a small scar on the skin for a few months. Following the biopsy, the incision will be closed using a Steri-strip and pressure bandage will be applied for about 15 minutes before you begin exercising.

After collection of both biopsies on a given day, the incisions will be closed using a steri-strip covered by a transparent waterproof dressing. A pressure bandage will then be applied. You should keep this in place for 24-48 hours and maintain steri-strip closure for a few days. You should not exercise for 24-48 hours after biopsies and you should avoid heavy knocks. You may well experience some mild soreness in the muscle for 2-3 days after biopsy collection, however this passes and does not restrict movement. The soreness is due to slight bleeding within the muscle and is best treated by “ice, compression and elevation”. An ice pack will be applied over the biopsy site after the biopsy procedure to minimise any bleeding and therefore soreness. In some

rare cases mild bruises have been reported, but these symptoms disappear within a week. Although the possibility of infection and significant bruising is quite small, if by chance it does eventuate, inform us immediately and consultation with a qualified medical practitioner will be arranged.

At rest, and following the exercise test, blood samples (each about 3 ml or one teaspoon) will be drawn from a small plastic tube inserted into a vein in the back of the hand (the tube is inserted using a local anaesthetic and will cause minor discomfort). In order to get a good blood flow to the hand, during the warm up period your hand will be immersed in a warm water bath (~45^{o}C) up to the wrist which will mean that you can only have one hand on the handlebars whilst cycling. This will feel slightly awkward but will not impair your performance on the bike since the warm up is at a very light workload. The hand will come out of the water bath for the duration of the 170% PPO test.

Lactate Tracer Procedure:

On two separate occasions (before and after the period in the Altitude House) you will be required to attend the physiology laboratory after an overnight fast. A sterile cannula will be inserted into a forearm vein on one arm and another will be inserted into the back of the hand on the opposite side. One cannula will be used to draw blood. This cannula will have a sterile tap attached to allow rapid, repeated sampling. After each sample the cannula will be flushed with 2-3ml of sterile 0.9% saline solution to prevent clotting. Blood sampling and cannula insertion will be performed by staff who are trained in the correct sterile techniques of blood sampling and venipuncture. The cannula in the opposite arm will be used to infuse a radiolabelled tracer before and throughout exercise. The tracer will be infused continuously at a rate very slow rate from an auto syringe pump. Infusion will commence 90 minutes before exercise and be maintained during 90 minutes of cycling. The first 60 minutes of cycling will be at a workload that represents 58% of your peak power output (PPO) and the final 30min will be at a workload that represents 77% of PPO. The total radiation received over the two tracer procedures will be <10mrem. A total radiation dose of 500 mrem/year is considered safe and acceptable. To ensure the exposure to radiation is kept to minimum you should refrain from being involved in any other radio-labelled tracer studies for the following 12 months, unless these are recommended by a medical officer.
In order to get a good blood flow to the hand, your hand will be immersed in a warm water bath (~45^{o}C) up to the wrist for a 10-minute period before each blood sample is collected. During this period you can only have one hand on the handlebars whilst cycling. This will feel slightly awkward but will not impair your performance on the bike since it is a requirement that you remain seated during all tests. For 36 hours prior to the lactate tracer test you will be required to adhere to specific exercise and dietary regimes established by the research team.

Submaximal Cycle Ergometer Tests

On two separate occasions you will be asked to complete a submaximal ride on the cycle ergometer. The exercise protocol for this test is the same as that is used for the

lactate threshold test. However, only respiratory and heart rate measurements will be collected and there will be no blood sampling.

Responsiveness of Breathing to Low Oxygen Levels (HVR test):

The HVR test is designed to evaluate your sensitivity to a low oxygen environment such as the Altitude House. You will be required to complete the HVR test in the mornings on eight separate occasions (mostly coinciding with morning blood collections). It is important that you arrive at the AIS Physiology laboratory rested and fasted as soon as possible after waking. The test consists of breathing through a mouthpiece at rest for 10 min. During this period, nitrogen will be gradually added to the inspired air via flow control valves. The addition of nitrogen will continue until a simulated altitude of approximately 7000 m is reached. Ventilation is measured as well as arterial oxygen saturation, expired oxygen concentration, and expired carbon dioxide concentration.
You may experience slight discomfort and/or lightheadedness during the last 2-3 min of the test due to hyperventilation. Headache may occur following the test but this is rare.

Sleep Monitoring:

Sleep patterns will be recorded for 2 nights before and 4 nights during, and one night after the period of Altitude House exposure. A portable sleep monitoring system will be used.
Sleep monitoring involves electro-physiological measurement via surface electrodes. Although the monitoring is technically non-invasive there is still a strict, rigorous preparation procedure to follow. Athletes will be subjected to the protocols of skin preparation and electrode attachment (which takes ~15 minutes) before being expected to sleep normally surrounded by numerous monitoring leads. Initially, you may experience some level of discomfort before becoming accustomed to the subtle movement restriction.
To obtain reliable results you will be required to comply with the following procedures:
Training must be consistent for intensity, volume and time for all of the sleep monitoring days. The training for these days will be negotiated with each subject prior to commencing the study.
In addition you will be required to avoid caffeine for six hours prior to sleeping and to avoid any food containing MSG. You will be unable to have any beverage for 90 min before going to bed and will be asked to go to the toilet immediately before going to bed.

Blood Analyses:

Morning blood samples will be taken twice before, six times during and once after the period of Altitude House exposure. This requires a sterile needle to be inserted in a forearm vein to withdraw about 12 ml (approx: 2½ teaspoons) of blood. The samples will be analysed to determine serum erythropoietin concentration, the characteristics of red blood cells, and a range of biochemical parameters. Venous blood sampling seldom causes any problem, although bruising around the sampling site is occasionally

reported. This can be minimised by application of continuous pressure to the site for 3-5 minutes after completion of sampling.

Important: You should not participate in the study if you suffer from any of the following conditions:

- Chronic lung disease
- High Blood Pressure
- Unusual Predisposition to Formation of Blood Clots
- Coronary Artery Disease
- Epilepsy

If you have a blood disorder of any type, you should discuss the desirability of your involvement in the study with a member of the research team.

You should avoid scuba diving or travel by aeroplane within a period of 24 hours after sleeping in a reduced-oxygen environment.

Please note that it is your prerogative to withdraw from the study at any time, and that no explanation is required for such withdrawal.

While the data collected during the study will be published in scientific and coaching journals and presented at conferences, your identity will not be disclosed. Your personal results will be made available to you and your coach but will not be provided to anyone else without your permission.

If you have any queries with respect to the conduct of this study, you may contact the Secretary of the AIS Ethics Committee on 02 6214 1816.

Contact Numbers:

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Study 3 Subject Information Sheet

The effect of short term intensified interval training on skeletal muscle adaptation in well-trained cyclists

Investigators:
Professor John Hawley and Sally Clark (PhD student)
School of Medical Sciences, Division of Exercise Sciences, Exercise Metabolism Group, RMIT University, Bundoora Campus;
Associate Professor Michael McKenna, Robert Aughey (PhD student) and Kate Murphy (PhD student)
School of Human Movement, Recreation and Performance, Victoria University of Technology, Footscray Park Campus

Aims of the study:
Elite endurance athletes show an improved ability to sustain high absolute workrates for prolonged periods compared to less well-trained cyclists. This attenuation of muscle fatigue could be caused by many factors. In recent years, impairments in lactate, sodium, potassium, calcium and hydrogen regulation in skeletal muscle have been identified as important contributors to the fatigue process at several important *sites* these include:

(a) Lactate (lactic acid) produced in the muscle is transported across the muscle membrane by two transporters. The abundance of lactate transporters in skeletal muscle is higher in highly trained athletes compared with untrained individuals. Furthermore, 8 weeks of high intensity training has been shown to increase lactate transporters and lactate/hydrogen transport capacity in untrained individuals. . However it is not known whether a period of intensified training in already well-trained athletes can further increase the abundance of lactate transporters.. Therefore the capacity of the skeletal muscle to take up lactate and utilise it may be a key determinant of endurance performance.

(b) Sodium and potassium are transported across the muscle membrane via an enzyme known as the sodium-potassium pump. This pump allows the electrical impulses that enable muscle contraction to spread into the muscle.

The content of the sodium-potassium pump in skeletal muscle is upregulated after sprint and endurance training in healthy untrained participants. The effects of short-term intensified-interval training on the skeletal muscle sodium-potassium pump and its isoforms in well-trained subjects has not yet been investigated.

Accordingly, we aim to examine the effects of short-term intensified-training on skeletal muscle electrolyte regulation in already well-trained athletes, by measuring the effects on lactate and sodium-potassium regulation.

Subject participation:
Should you decide to participate in this study, you are free to withdraw from the study at any time, without any adverse effects or reactions.

Exercise Testing Procedures:
Should you decide to participate in this study, you will be required to attend the exercise physiology laboratory located in the Robert Magee building at the Bundoora West Campus of RMIT on 16 occasions over a 6-week period. Whilst each test is tiring, you will recover from this very quickly. You will undergo 2 weeks of initial exercise tests, 3 weeks of training and a week of re-testing. There will be a total of 8 exercise tests before, 7 interval training sessions and 5 post training exercise tests for the treatment group. Each exercise test is completed when you become too tired to continue (wish to stop), or unless we stop the test due to you having an abnormal response to exercise, such as an inappropriate heart rate or sweating responses, chest pain or severe shortness of breath. We will closely monitor you and your heart rate during exercise to ensure your safety. The most common event associated with maximal exercise testing is fainting. This will be prevented using our standard laboratory procedures. In the unlikely event of emergency situations, two members of the research team have current CPR (cardio pulmonary resuscitation) qualifications and an ambulance would be immediately called.

Preliminary tests:

1) Height, body mass, skinfolds and Maximal Oxygen Uptake ($\dot{V}O_{2peak}$)

You will perform various baseline tests, including the measurement of your body mass (BM) and anthropometric measures including skinfolds and height. You will then perform a sub-maximal test on a cycle ergometer, to establish your lactate threshold. The initial intensity is very low but it will progressively increase until a lactate of 4 mmol/L is reached. After a short rest, you will perform an exercise test to exhaustion (a max test) on a cycle ergometer. Exhaustion is defined as the point at which you can no longer maintain your cadence above 60 – 70 rpm. From this test the desired work rate (% of peak sustained power output [PPO]) for defining the effort of the subsequent training sessions will be determined. During the test, expired air will be sampled as you breathe into a mouthpiece attached to an automated gas analyser. At the same time, we will also monitor your heart rate using the Polar Sports Tester. The results from these tests will provide you with a $\dot{V}O_{2peak}$, peak sustained power output (PPO), lactate

threshold and maximal heart rate (Hr_{max}). This data is then used to set your personalised training sessions.

2) 40 km Time Trial

Two days later will be required to report to the RMIT University Exercise Physiology Laboratory with your own bicycle, which will be mounted, on an air braked cycle ergometer. After a self-paced warm-up, you will then perform a simulated 40 km time. The only feedback you get is the distance covered.

3) Diet and Training Control

Within the next 48 hrs, you will report to the lab between 1700 and 1800 pm , where we will supervise a moderate training session lasting 60 min. You will then be provided with a special diet for the subsequent 36 hours. During this time, you will be requested to refrain from any further exercise until the next lab session. This diet/training control is undertaken in order to standardise your muscle and liver carbohydrate stores, which are important for the muscle tissue analysis.

4) Lactate Turnover test

36 hrs after the one-hour laboratory-training ride you will report to the lab between 0600 and 0800 after an overnight fast. A sterile cannula will be inserted into a forearm vein on one arm and another will be inserted into the back of the hand on the opposite side. The cannula in the back of the hand will be used to draw blood. This cannula will have a sterile tap attached to it to allow repeated sampling. After each sample, the cannula will be flushed with 2-3 ml of heparinised 0.9% saline solution to prevent clotting. In order to get good blood flow to the hand, your hand will be placed in a waterproof bag and immersed in a warm water bath (~45°C) up to the wrist for a 10-minute period before each blood sample is collected. During this period, you can have only one hand on the handlebars whilst cycling. This will feel slightly awkward but will not impair your performance on the bike since it is a requirement that you remain seated during the test. Blood sampling and cannula insertion will be performed by staff who are trained in the correct sterile techniques of blood sampling and venepuncture.

The cannula in the forearm vein of the opposite arm will be used to infuse a radio-labelled tracer before and throughout exercise. The tracer will be infused continuously at a rate very slow from an auto syringe pump. Infusion will commence 90 minutes before the exercise and be maintained during 90 minutes of cycling. The total radiation received over the two tracer procedures will be <10 mrem. A total dose of 500mrem/year is considered safe and acceptable. This annual limit is the equivalent of the exposure received from 46 return flights between Melbourne and Sydney or from 10 chest x-rays. To ensure the exposure to radiation is kept to a minimum you should refrain from being involved in any other radio-labelled tracer studies for the following 12 months, unless are recommended by a medical officer.

The first 60 minutes of cycling will be at workload that represents 65% of your $\dot{V}O_{2peak}$ and the final 30 minutes will be at workload that represents 85% of $\dot{V}O_{2peak}$.

Blood samples will be taken while you are resting at 15, 30, 60 and 90 min after the start of the infusion, at 5, 15, 30, 45, 59, 65, 75 and 90 min during exercise and at 1, 3 and 5 min post exercise. During the 5 min preceding each blood draw during exercise, you will breathe into a mouthpiece for gas analysis.

Training sessions:

In each of the 7 training sessions you will perform 8 x 5 min @ ~82% of your PPO (~85% $\dot{V}O_{2peak}$), with a 60 s rest between each 5 min bout on the cycle ergometer. Two sessions will be performed in weeks 1 and 3, and 3 sessions in week 2 in the RMIT exercise physiology laboratory. On the days you are not training in the laboratory you are to maintain your normal training rides, but cover 15% less of the distance you were doing before the intensified training. You will be required to log all your training distances in a logbook provided, during the period of the study.

Post-training tests:

48 hours after the training has been completed, the maximal tests, 40 km time-trial and the submaximal ride will be repeated (as described above). These will be conducted on separate days, and will require the same preparatory day before the submaximal ride.

Blood Samples:
A total of 250ml of blood will be sampled during testing, as described in **Section 4** "**Lactate Turnover test"**

Muscle Biopsies
Should you decide to participate in this study, on one visit to the RMIT Exercise Physiology Laboratory, a resting biopsy will be taken from your thigh muscle. On two subsequent visits, a muscle biopsy will be taken from your thigh muscle, at rest, and immediately after you stop the test. **Thus a total of five biopsies will be taken during these three visits.** Muscle biopsies are routinely carried out in this laboratory, with no adverse effects.

The muscle biopsy procedure is used to obtain small samples of your muscle tissue for analysis of enzymes and energy sources. An injection of a local anaesthetic is made in the skin overlying the muscle in your thigh, and then a small incision (approx. 0.6 cm long) is made in the skin. The biopsy needle is then inserted into your muscle and a small piece of tissue removed from the muscle. During this part of the procedure you will feel pressure and this will be quite uncomfortable, but will last for only about 1-2 seconds. When the small piece of muscle is removed you may also experience a mild muscle cramp, but this only persists for a few seconds. Many people experience pain during this part of the procedure but this lasts only for 1-2 seconds. The size of muscle removed by the biopsy needle is similar to a grain of rice. This poses no long-term effects for your muscle and will not be noticeable to others apart from a small scar on the skin for a few months. Following the biopsy the incision will be closed using a steri-strip and covered by a transparent waterproof dressing. Then a pressure bandage will be applied which should be maintained for 24-48 hours. Steri-strip closure should be maintained for a few days.

You should not exercise for 24 hours after biopsies and you should avoid heavy knocks. It is common for participants to experience some mild soreness in the muscle over the next 2-3 days, however this passes and does not restrict movement. The soreness is due to slight bleeding within the muscle and is best treated by "ice, compression and elevation". An ice pack will be applied over the biopsy site after the biopsy procedure to minimise any bleeding and therefore soreness. In some rare cases mild haematomas have been reported, but these symptoms disappear within a week. A qualified medical practitioner will perform the whole procedure. To minimise infection, the biopsy will be conducted under sterile conditions. On very rare occasions, some people have reported altered sensation (numbness or tingling) in the skin near the site of the biopsy. This is due to a very small nerve being cut, but this sensation disappears over a period of a few weeks-to-months.
Although the possibility of infection, significant bruising and altered sensation (for example puss, tenderness, numbness, tingling and/or redness) is quite small, if by chance it does eventuate, please inform us immediately and we will immediately consult the doctor who performed the biopsy.

By signing the informed consent form you are indicating that the tests and procedures have been explained to you and are understood by you. Also, it is accepted by the investigators and by yourself that you are participating voluntarily in the study and that you are free to withdraw from the investigation at any time. Thank you for your co-operation.

Contact Numbers:

Sally Clark	W:	99257670	M:	0417058597
Mr. Robert Aughey	*W:*	*9688 406*		
Ms. Kate Murphy	*W:*	*9688 4066*	*M:*	*0413 862 889*
Professor John Hawley	W:	99257353		
Assoc. Prof. Michael McKenna	W:	9688 4499	H:	(03) 5422-6089

Any queries about your participation in this project may be directed to the researcher (Assoc. Prof. McKenna; ph. 9688 4499). If you have any queries or complaints about the way you have been treated, you may contact the Secretary, University Human Research Ethics Committee, Victoria University of Technology, PO Box 14428 MC, Melbourne, 8001 (telephone no: 03-9688 4710).

Appendix A2 Risk factor questionnaires

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CARDIOVASCULAR RISK FACTOR QUESTIONNAIRE

In order to be eligible to participate in the experiment investigating:
"The effect of short term intensified interval training on skeletal muscle adaptation in well-trained cyclists"
you are required to complete the following questionnaire which is designed to assess the risk of you having a cardiovascular event occurring during an exhaustive exercise bout.

Name: _____ Date: _____

Age: _____ years Weight: _____ kg Height: _____ cms

Give a brief description of your average activity pattern in the past 2 months:
_____
_____
_____

Circle the appropriate response to the following questions.

1. Are you overweight? Yes No Don't know
2. Do you smoke? Yes No Social
3. Does your family have a history of premature cardiovascular problems (eg. heart attack, stroke)? Yes No Don't Know
4. Are you an asthmatic Yes No Don't Know
5. Are you a diabetic? Yes No Don't Know
6. Do you have a high blood cholesterol level? Yes No Don't Know
7. Do you have high blood pressure? Yes No Don't Know
8. Are you on any medication? Yes No
   If so, what is the medication? _____
9. Do you think you have any medical complaint or any other reason which you know of which you think may prevent you from participating in strenuous exercise? Yes No

If Yes, please elaborate _____

I, _____, believe that the answers to these questions are true and correct.
Signed: _____ Date: _____

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MUSCLE BIOPSY QUESTIONNAIRE

NAME: _____

ADDRESS: _____
DATE: _____ AGE: _____ years

1. Have you or your family suffered from any tendency to bleed excessively ? (eg. haemophilia) or bruise very easily ?
   Yes No Don't Know
   If yes, please elaborate..._____
   _____

2. Are you allergic to local anaesthetic?
   Yes No Don't Know
   If yes, please elaborate..._____
   _____

3. Do you have any skin allergies?
   Yes No Don't Know
   If yes, please elaborate..._____
   _____

4. Have you any allergies that should be made known?
   Yes No Don't Know
   If yes, please elaborate... _____
   _____

5. Are you currently on any medication?
   Yes No Don't Know
   If yes, what is the medication? _____
   _____

6. Do you have any other medical problem that should be made known?
   Yes No
   If yes, please elaborate... _____
   _____

To the best of my knowledge, the above questionnaire has been completely accurately and truthfully.

Signature: _____ Date: _____

Appendix B Individual Data

Appendix B1 Subject Physical Characteristics

Table B1.1 Study 1 Control and LHTL subject characteristics (Chapters 3 and 4).

CON						**Skinfolds**					
subject	Age (yrs)	Height (cm)	Mass (kg)	Bicep (mm)	Tricep (mm)	Subsc-apular (mm)	Supra-spinale (mm)	Abdo-minal (mm)	Thigh (mm)	Calf (mm)	Sum (mm)
1	25.2	185.3	77.9	3.2	7.4	8.7	6.8	8.7	11.4	5.8	52.0
2	35.8	189.9	78.3	4.0	7.4	8.3	5.8	7.2	9.1	5.5	47.3
3	25.9	179.7	68.1	3.2	8.2	9.4	4.2	9.2	7.8	5.8	47.8
4	20.2	172.7	64.8	3.4	8.4	8.5	5.9	18.2	11.5	8.0	63.9
5	23.6	177.9	68.4	4.1	8.1	7.4	6.0	8.4	15.9	6.8	56.7
6	20.5	187.0	80.0	4.6	5.5	6.6	5.0	7.7	10.5	6.2	46.1
7	24.8	176.0	75.7	3.8	8.4	9.4	5.4	8.1	16.8	9.0	60.9
n	7	7	7	7	7	7	7	7	7	7	7
mean	25.2	181.2	73.3	3.8	7.6	8.3	5.6	9.6	11.9	6.7	53.5
SD	5.2	6.3	6.1	0.5	1.0	1.0	0.8	3.8	3.3	1.3	7.1
LHTL											
1	28.3	175.7	71.5	3.6	6.5	7.6	4.6	7.6	7.7	6.0	43.6
2	23.7	189.9	78.8	3.8	7.2	7.4	5.9	8.8	8.6	6.6	48.3
3	21.7	197.2	83.2	3.0	7.0	6.8	4.5	5.2	8.0	5.8	40.3
4	27.8	178.5	70.9	3.0	6.9	6.6	5.1	11.2	9.5	7.2	49.5
5	29.5	188.8	68.9	2.3	3.8	5.7	3.6	5.4	5.0	4.5	30.3
6	21.1	171.1	65.0	3.1	5.9	6.2	4.5	9.5	9.0	5.2	43.4
n	6	6	6	6	6	6	6	6	6	6	6
mean	25.4	183.5	73.0	3.1	6.2	6.7	4.7	8.0	8.0	5.9	42.6
SD	3.6	10.0	6.7	0.5	1.3	0.7	0.8	2.4	1.6	1.0	6.9

Table B1.2 Study 2 subject characteristics (Chapter 5).

CON				**LHTLc**				**LHTLi**			
subject	Age (yrs)	Height (cm)	Mass (kg)	subject	Age (yrs)	Height (cm)	Mass (kg)	subject	Age (yrs)	Height (cm)	Mass (kg)
1	29.0	173.0	70.3	1.0	32.8	185.0	74.0	1.0	27.5	173.6	60.9
2	32.0	179.0	64.4	2.0	39.0	164.0	61.3	2.0	26.0	193.0	85.5
3	23.0	173.0	65.2	3.0	27.0	190.0	81.9	3.0	21.1	191.5	80.5
4	24.0	178.0	74.5	4.0	24.0	187.0	86.0	4.0	25.4	173.0	72.0
5	20.7	186.0	75.0	5.0	26.1	180.0	80.0	5.0	18.0	163.0	62.1
6	29.7	175.0	70.0	6.0	19.7	176.0	64.0	6.0	24.6	181.6	73.2
7	23.1	180.5	68.5	7.0	26.6	173.3	68.9	7.0	28.5	162.2	59.0
8	32.1	176.9	78.9	8.0	23.8	181.7	66.0	8.0	31.6	181.0	75.0
9	18.5	185.0	82.0	9.0	37.0	181.0	67.4	9.0	28.8	168.0	64.0
10	29.5	169.0	66.5	10.0	36.1	179.3	67.0	10.0	33.5	176.0	67.5
11	28.0	181.0	70.7	11.0	32.8	183.2	92.7				
				12.0	18.9	174.0	61.0				
n	11	11	11	n	12	12	12	n	10	10	10
mean	26.3	177.9	71.4	mean	28.7	179.5	72.5	mean	26.5	176.3	70.0
sd	4.7	5.2	5.6	SD	6.7	7.0	10.4	SD	4.6	10.7	8.8

Table B1.3 Study 3 subject characteristics (Chapter 6).

subject	Age (yrs)	Height (cm)	Mass (kg)
1	31.0	172.0	70.9
2	27.0	170.0	76.0
3	37.0	180.0	76.5
4	32.0	171.0	71.0
5	31.0	180.0	71.0
6	32.0	180.0	77.0
7	33.0	170.0	77.0
8	25.0	180.0	77.0
9	33.0	190.0	84.5
10	31.0	182.0	82.5
11	34.0	178.0	70.3
12	31.0	173.0	74.2
n	12	12	12
mean	31.4	177.2	75.7
SD	3.1	6.1	4.6

APPENDIX B2 EXERCISE BLOOD DATA

Table B2.1 Study 1 Control subject haemoglobin concentration ([Hb], $g.dL^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	15.05	15.70	15.95	16.00	16.30	16.35	16.65	16.33
2	14.77	14.93	14.85	15.15	15.30	15.60	15.67	15.37
3	17.30	17.66	17.33	17.40	17.90	18.63	18.40	19.30
4	15.95	16.23	16.43	16.65	16.95	17.05	17.00	16.90
5	15.63	16.30	16.50	16.65	16.90	16.73	17.10	16.65
6	14.90	15.35	15.65	15.90	16.45	16.25	16.60	16.25
7	17.60	17.53	17.53	17.75	18.35	18.27	18.05	18.05
n	7	7	7	7	7	7	7	7
mean	15.89	16.24	16.32	16.50	16.88	16.98	17.07	16.98
sd	1.15	1.04	0.94	0.90	1.02	1.10	0.92	1.30
Post								
1	15.55	15.80	15.85	16.10	16.65	17.05	16.80	16.35
2	14.70	16.13	14.70	15.20	15.50	17.05	15.50	15.80
3	15.23	15.70	16.30	16.23	16.45	16.87	16.45	16.30
4	16.25	16.50	16.35	16.45	17.20	17.40	17.15	17.10
5	15.55	16.10	16.15	16.45	16.45	16.90	16.80	16.80
6	16.13	16.15	16.05	16.25	16.65	16.80	16.85	16.30
7	17.00	16.55	16.55	16.70	17.15	17.30	17.25	17.05
n	7	7	7	7	7	7	7	7
mean	15.77	16.13	15.99	16.20	16.58	17.05	16.69	16.53
sd	0.75	0.32	0.61	0.48	0.57	0.22	0.58	0.47

Table B2.2 Study 1 LHTL subject haemoglobin concentration ([Hb], $g.dL^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	15.10	15.30	15.23	15.25	15.75	16.30	15.70	15.70
2	14.30	14.17	14.50	14.67	14.77	14.50	14.70	14.47
3	14.97	15.00	15.65	15.95	16.60	16.75	16.55	16.20
4	14.85	15.80	16.05	15.85	16.43	16.70	16.65	16.10
5	15.90	16.30	16.33	16.35	16.83	16.17	16.57	16.63
6	14.75	15.18	15.55	16.03	16.25	16.25	16.63	16.38
n	6	6	6	6	6	6	6	6
mean	14.98	15.29	15.55	15.68	16.11	16.11	16.13	15.91
sd	0.53	0.73	0.64	0.61	0.75	0.83	0.79	0.77
Post								
1	14.75	14.90	14.90	15.15	15.70	15.95	15.80	15.20
2	14.43	14.83	16.95	15.10	13.35	13.25	15.20	14.90
3	14.90	15.55	15.70	15.90	16.45	16.55	16.75	16.45
4	14.80	15.65	15.30	15.65	16.05	16.20	15.80	16.15
5	14.77	15.65	15.70	15.90	16.25	16.20	16.60	16.20
6	15.45	14.90	15.30	14.75	15.60	15.30	15.65	15.40
n	6	6	6	6	6	6	6	6
mean	14.85	15.25	15.64	15.41	15.57	15.58	15.97	15.72
sd	0.33	0.41	0.71	0.48	1.13	1.21	0.59	0.63

Table B2.3 Study 1 Control subject haematocrit (Hct, %) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	42.0	44.0	44.0	44.0	45.0	46.0	46.0	46.0
2	41.0	41.0	42.0	41.0	43.0	42.0	44.0	43.0
3	43.0	44.0	44.0	46.0	47.0	47.0	47.0	47.0
4	48.0	48.0	48.0	49.0	50.0	50.0	51.0	50.0
5	45.0	48.0	47.0	47.0	47.0	48.0	48.0	49.0
6	43.0	45.0	44.0	45.0	46.0	47.0	48.0	47.0
7	47.0	47.0	47.0	46.0	50.0	50.0	52.0	50.0
n	7	7	7	7	7	7	7	7
mean	44.1	45.3	45.1	45.4	46.9	47.1	48.0	47.4
sd	2.6	2.6	2.2	2.5	2.5	2.7	2.8	2.5
Post								
1	48.0	50.0	48.0	45.0	53.0	54.0	48.0	50.0
2	48.0	50.0	47.0	51.0	49.0	54.0	50.0	48.0
3	45.0	45.0	48.0	50.0	51.0	51.0	54.0	54.0
4	53.0	53.0	57.0	56.0	57.0	59.0	60.0	58.0
5	50.0	50.0	53.0	53.0	55.0	53.0	56.0	56.0
6	50.0	49.0	51.0	52.0	53.0	54.0	43.0	55.0
7	53.0	51.0	51.0	52.0	54.0	51.0	54.0	55.0
n	7	7	7	7	7	7	7	7
mean	49.6	49.7	50.7	51.3	53.1	53.7	52.1	53.7
sd	2.9	2.4	3.5	3.4	2.6	2.7	5.6	3.5

Table B2.4 Study 1 LHTL subject haematocrit (Hct, %) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	42.0	44.0	44.0	44.0	45.0	46.0	46.0	46.0
2	41.0	41.0	42.0	41.0	43.0	42.0	44.0	43.0
3	43.0	44.0	44.0	46.0	47.0	47.0	47.0	47.0
4	48.0	48.0	48.0	49.0	50.0	50.0	51.0	50.0
5	45.0	48.0	47.0	47.0	47.0	48.0	48.0	49.0
6	43.0	45.0	44.0	45.0	46.0	47.0	48.0	47.0
n	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
mean	43.7	45.0	44.8	45.3	46.3	46.7	47.3	47.0
sd	2.5	2.7	2.2	2.7	2.3	2.7	2.3	2.4
Post								
1	48.0	50.0	48.0	45.0	53.0	54.0	48.0	50.0
2	48.0	50.0	47.0	51.0	49.0	54.0	50.0	48.0
3	45.0	45.0	48.0	50.0	51.0	51.0	54.0	54.0
4	53.0	53.0	57.0	56.0	57.0	59.0	60.0	58.0
5	50.0	50.0	53.0	53.0	55.0	53.0	56.0	56.0
6	50.0	49.0	51.0	52.0	53.0	54.0	43.0	55.0
n	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
mean	49.0	49.5	50.7	51.2	53.0	54.2	51.8	53.5
sd	2.7	2.6	3.8	3.7	2.8	2.6	6.1	3.8

Table B2.5 Study 1 Control subject change in plasma volume (ΔPV, %) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	0.00	-11.07	-12.74	-13.77	-16.85	-17.65	-19.77	-18.13
2	0.00	6.93	4.93	4.30	0.82	0.25	-3.11	-0.82
3	0.00	-3.36	-2.31	-4.85	-10.63	-13.51	-12.74	-16.54
4	0.00	-10.10	-11.46	-14.26	-16.96	-17.13	-18.60	-17.39
5	0.00	-3.79	-2.04	-3.02	-4.98	-6.38	-8.53	-6.54
6	0.00	-3.29	-4.23	-7.11	-11.96	-12.94	-15.23	-12.30
7	0.00	-7.30	-7.11	-6.11	-15.36	-15.36	-18.21	-13.93
n	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
mean	0.00	-4.57	-4.99	-6.40	-10.85	-11.82	-13.74	-12.24
sd	0.00	6.00	6.07	6.39	6.65	6.51	6.10	6.40
Post								
1	0.0	-6.1	-1.5	3.2	-15.6	-18.9	-7.2	-8.3
2	0.0	-12.9	1.3	-9.6	-8.6	-24.1	-8.8	-8.2
3	0.0	-2.3	-10.9	-15.3	-16.8	-20.1	-22.5	-21.1
4	0.0	-1.5	-8.5	-7.7	-13.7	-17.8	-19.0	-15.2
5	0.0	-5.0	-9.3	-11.8	-15.3	-15.1	-19.3	-19.6
6	0.0	2.3	-1.1	-4.2	-7.9	-11.6	9.5	-9.7
7	0.0	6.5	6.8	4.9	-1.8	2.3	-2.8	-3.3
n	7	7	7	7	7	7	7	7
mean	0.0	-2.7	-3.3	-5.8	-11.4	-15.0	-10.0	-12.2
sd	0.0	6.2	6.5	7.5	5.5	8.6	11.3	6.6

Table B2.6 Study 1 LHTL subject change in plasma volume (ΔPV, %) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	0.00	-3.73	5.11	-1.79	-6.90	-13.41	-10.77	-10.19
2	0.00	1.28	-2.31	-11.06	-6.91	-4.88	-7.75	-6.15
3	0.00	-3.83	-8.10	-12.63	-17.70	-19.31	-18.39	-14.56
4	0.00	-14.02	-2.92	-1.81	-6.73	-10.05	-11.07	-7.60
5	0.00	-4.28	-4.17	-8.06	-8.83	-5.81	-9.27	-7.83
6	0.00	-10.24	-7.90	-13.71	-17.20	-15.50	-18.87	-17.51
n	6	6	6	6	6	6	6	6
mean	0.00	-5.80	-3.38	-8.18	-10.71	-11.49	-12.69	-10.64
sd	0.00	5.44	4.83	5.29	5.28	5.64	4.76	4.47
Post								
1	0.00	-4.25	-2.80	-5.53	-14.56	-16.32	-14.67	-14.95
2	0.00	-3.01	-15.33	-5.07	3.94	5.82	-14.99	-12.99
3	0.00	5.82	-10.08	-10.49	-17.45	-6.97	-23.93	-21.78
4	0.00	-9.58	-6.83	-10.92	-14.69	-16.01	-14.51	-18.10
5	0.00	-11.12	-9.58	-11.79	-14.51	-16.05	-19.47	-17.46
6	0.00	1.77	-4.92	3.35	-3.15	-5.87	-4.91	7.09
n	6	6	6	6	6	6	6	6
mean	0.00	-3.40	-8.26	-6.74	-10.07	-9.23	-15.41	-13.03
sd	0.00	6.49	4.43	5.71	8.48	8.78	6.34	10.30

Table B2.7 Study 1 Control subject arterialised venous sodium concentration ([Na^+], $mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	138.0	139.0	139.0	137.0	140.0	141.0	142.0	139.0
2	139.0	138.0	140.0	140.0	141.0	141.0	143.0	140.0
3	137.0	138.0	138.0	139.0	141.0	141.0	141.0	138.0
4	139.0	139.0	140.0	141.0	143.0	143.0	143.0	140.0
5	138.0	140.0	140.0	140.0	141.0	143.0	143.0	140.0
6	137.0	138.0	138.0	139.0	140.0	142.0	142.0	139.0
7	137.0	136.0	136.0	137.0	139.0	140.0	140.0	136.0
n	7	7	7	7	7	7	7	7
mean	137.9	138.3	138.7	139.0	140.7	141.6	142.0	138.9
sd	0.9	1.3	1.5	1.5	1.3	1.1	1.2	1.5
Post								
1	139.0	140.0	140.0	141.0	139.0	143.0	137.0	134.0
2	140.0	139.0	139.0	139.0	141.0	143.0	142.0	138.0
3	138.0	139.0	139.0	140.0	141.0	141.0	142.0	138.0
4	139.0	139.0	140.0	141.0	143.0	144.0	144.0	140.0
5	138.0	139.0	139.0	140.0	141.0	143.0	144.0	139.0
6	140.0	140.0	140.0	141.0	142.0	144.0	145.0	140.0
7	137.0	137.0	138.0	139.0	140.0	141.0	142.0	138.0
n	7	7	7	7	7	7	7	7
mean	138.7	139.0	139.3	140.1	141.0	142.7	142.3	138.1
sd	1.1	1.0	0.8	0.9	1.3	1.3	2.6	2.0

Table B2.8 Study 1 LHTL subject arterialised venous sodium concentration ($[Na^+]$, $mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	137.0	137.0	138.0	138.0	139.0	140.0	139.0	138.0
2	137.0	138.0	138.0	139.0	140.0	141.0	142.0	138.0
3	137.0	137.0	138.0	139.0	141.0	141.0	141.0	138.0
4	137.0	135.0	136.0	137.0	139.0	140.0	141.0	136.0
5	139.0	139.0	140.0	140.0	142.0	142.0	14.0	139.0
6	138.0	138.0	138.0	138.0	140.0	141.0	142.0	139.0
n	6	6	6	6	6	6	6	6
mean	137.5	137.3	138.0	138.5	140.2	140.8	119.8	138.0
sd	0.8	1.4	1.3	1.0	1.2	0.8	51.9	1.1
Post								
1	137.0	138.0	137.0	137.0	140.0	141.0	141.0	137.0
2	138.0	137.0	137.0	138.0	139.0	140.0	140.0	138.0
3	137.0	138.0	138.0	139.0	141.0	141.0	141.0	138.0
4	136.0	137.0	138.0	138.0	140.0	141.0	142.0	138.0
5	140.0	140.0	141.0	141.0	143.0	143.0	144.0	140.0
6	138.0	139.0	139.0	140.0	141.0	142.0	142.0	139.0
n	6	6	6	6	6	6	6	6
mean	137.7	138.2	138.3	138.8	140.7	141.3	141.7	138.3
sd	1.4	1.2	1.5	1.5	1.4	1.0	1.4	1.0

Table B2.9 Study 1 Control subject arterialised venous potassium concentration ($[K^+]$, $mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	4.2	4.6	4.8	4.8	5.4	5.6	5.9	4.1
2	3.8	4.6	4.7	4.9	5.3	5.4	5.8	3.6
3	4.4	4.8	4.9	5.0	5.7	5.5	5.6	3.9
4	4.0	4.4	4.5	4.7	5.4	5.5	5.4	3.8
5	4.2	4.6	5.0	5.1	5.7	6.1	6.2	3.8
6	4.1	4.6	4.8	4.9	5.3	5.8	5.9	4.1
7	3.8	4.3	4.6	4.9	5.4	5.6	5.7	3.8
n	7	7	7	7	7	7	7	7
mean	4.1	4.6	4.8	4.9	5.5	5.6	5.8	3.9
sd	0.2	0.2	0.2	0.1	0.2	0.2	0.3	0.2
Post								
1	4.1	4.6	4.7	4.8	5.6	5.8	6.0	4.0
2	3.8	4.7	5.0	6.6	5.7	5.8	6.0	4.0
3	4.1	4.7	4.8	5.2	5.9	5.7	6.6	4.6
4	4.0	4.3	4.5	4.7	5.4	5.5	5.3	3.8
5	4.4	4.8	5.2	5.3	5.7	6.2	6.2	3.7
6	4.2	4.7	4.7	4.9	5.3	5.6	5.9	3.8
7	5.2	4.7	5.0	5.2	5.8	6.0	6.1	4.0
n	7	7	7	7	7	7	7	7
mean	4.3	4.6	4.8	5.2	5.6	5.8	6.0	4.0
sd	0.5	0.2	0.2	0.6	0.2	0.2	0.4	0.3

Table B2.10 Study 1 LHTL subject arterialised venous potassium concentration ($[K^+]$, $mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	4.2	4.7	4.8	4.9	5.4	5.4	5.5	4.2
2	4.2	4.8	4.9	4.9	5.3	5.5	5.7	3.8
3	3.9	4.9	5.0	5.2	5.8	5.5	5.4	3.6
4	4.1	4.9	5.0	5.0	5.6	5.8	6.1	3.8
5	4.0	4.5	4.8	5.0	5.8	5.6	5.9	4.0
6	4.3	4.9	5.0	5.0	5.4	5.9	6.0	3.8
n	6	6	6	6	6	6	6	6
mean	4.1	4.8	4.9	5.0	5.6	5.6	5.8	3.9
sd	0.1	0.2	0.1	0.1	0.2	0.2	0.3	0.2
Post								
1	4.3	4.5	4.7	4.8	5.3	5.5	5.9	3.8
2	4.6	5.1	5.1	5.3	5.9	5.8	7.0	4.0
3	4.7	5.3	5.3	5.7	6.2	6.1	6.5	4.3
4	4.5	4.9	5.0	5.2	5.6	6.0	6.5	4.2
5	3.7	4.4	4.7	4.8	5.4	5.4	5.7	3.8
6	4.4	4.9	5.1	5.4	5.7	5.9	6.3	4.4
n	6	6	6	6	6	6	6	6
mean	4.4	4.9	5.0	5.2	5.7	5.8	6.3	4.1
sd	0.4	0.3	0.2	0.4	0.3	0.3	0.5	0.3

Table B2.11 Study 1 Control subject rise in plasma [K^+] above rest ($\Delta[K^+]$, $mmol.L^{-1}$) (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
CON	1.5	2.5	3.5	4.5	5.6
1	0.4	0.6	0.6	1.2	1.4
2	0.8	0.9	1.1	1.5	1.6
3	0.4	0.5	0.6	1.3	1.1
4	0.4	0.5	0.7	1.4	1.5
5	0.4	0.8	0.9	1.5	1.9
6	0.5	0.7	0.8	1.2	1.7
7	0.5	0.8	1.1	1.6	1.8
n	7	7	7	7	7
mean	0.5	0.7	0.8	1.4	1.6
sd	0.1	0.2	0.2	0.2	0.3
Post					
1	0.5	0.6	0.7	1.5	1.7
2	0.9	1.2	2.8	1.9	2.0
3	0.6	0.7	1.1	1.8	1.6
4	0.3	0.5	0.7	1.4	1.5
5	0.4	0.8	0.9	1.3	1.8
6	0.5	0.5	0.7	1.1	1.4
7	0.6	0.9	1.1	1.7	1.9
n	7	7	7	7	7
mean	0.5	0.7	1.1	1.5	1.7
sd	0.2	0.3	0.8	0.3	0.2

Table B2.12 Study 1 LHTL subject rise in plasma [K^+] above rest ($\Delta[K^+]$, $mmol.L^{-1}$) (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
LHTL	1.5	2.5	3.5	4.5	5.6
1	0.5	0.6	0.7	1.2	1.2
2	0.6	0.7	0.7	1.1	1.3
3	1.0	1.1	1.3	1.9	1.6
4	0.8	0.9	0.9	1.5	1.7
5	0.5	0.8	1.0	1.8	1.6
6	0.6	0.7	0.7	1.1	1.6
n	6	6	6	6	6
mean	0.7	0.8	0.9	1.4	1.5
sd	0.2	0.2	0.2	0.4	0.2
Post					
1	0.2	0.4	0.5	1.0	1.2
2	0.5	0.5	0.7	1.3	1.2
3	0.6	0.6	1.0	1.5	1.4
4	0.4	0.5	0.7	1.1	1.5
5	0.7	1.0	1.1	1.7	1.7
6	0.5	0.7	1.0	1.3	1.5
n	6	6	6	6	6
mean	0.5	0.6	0.8	1.3	1.4
sd	0.2	0.2	0.2	0.3	0.2

Table B2.13 Study 1 Control subject rise in plasma [K^+] above rest normalised for work performed ($\Delta[K^+]$.work^{-1} ratio, nmol.L^{-1}.J^{-1}) (Chapters 3 and 4).

Pre	Exercise Workrate (W.kg^{-1})				
CON	1.5	2.5	3.5	4.5	5.6
1	14.3	8.0	4.3	5.4	5.0
2	28.5	12.0	7.8	6.7	5.7
3	16.0	7.5	4.8	6.5	4.5
4	17.7	8.3	6.2	7.8	6.8
5	16.3	12.2	7.3	7.6	7.8
6	17.6	9.3	5.6	5.3	6.0
7	26.5	14.1	9.7	8.5	7.6
n	7	7	7	7	7
mean	19.6	10.2	6.5	6.8	6.2
sd	5.6	2.6	1.9	1.2	1.3
Post					
1	17.9	8.0	5.0	6.9	6.1
2	32.1	16.0	19.9	8.5	7.1
3	24.1	10.5	8.8	9.0	6.5
4	13.3	8.3	6.2	7.8	6.8
5	16.3	12.2	7.3	6.6	7.4
6	17.6	6.6	4.9	4.8	4.9
7	31.8	15.9	9.7	9.0	8.0
n	7	7	7	7	7
mean	21.9	11.1	8.9	7.5	6.7
sd	7.6	3.8	5.2	1.5	1.0

Table B2.14 Study 1 LHTL subject rise in plasma [K^+] above rest normalised for work performed ($\Delta[K^+]$.work^{-1} ratio, nmol.L^{-1}.J^{-1}) (Chapters 3 and 4).

Pre	Exercise Workrate (W.kg^{-1})				
LHTL	1.5	2.5	3.5	4.5	5.6
1	28.7	11.5	6.7	6.9	5.4
2	21.0	9.2	4.9	4.8	4.6
3	33.7	13.9	8.7	8.0	5.6
4	31.2	13.2	7.0	7.3	6.6
5	19.9	11.9	8.0	9.0	6.5
6	25.8	11.3	6.0	5.9	6.9
n	6	6	6	6	6
mean	26.7	11.8	6.9	7.0	5.9
sd	5.5	1.6	1.4	1.5	0.9
Post					
1	11.5	7.6	4.8	5.7	5.4
2	17.5	6.5	4.9	5.7	4.2
3	20.2	7.6	6.7	6.3	4.9
4	15.6	7.3	5.5	5.4	5.9
5	27.9	14.9	8.8	8.5	6.9
6	21.5	11.3	8.6	7.0	6.5
n	6	6	6	6	6
mean	19.0	9.2	6.5	6.4	5.6
sd	5.6	3.3	1.8	1.2	1.0

Table B2.15 Study 1 Control subject rise in plasma [K^+] above rest (Δ [K^+], $mmol.L^{-1}$) for 'all-out' trials (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)					
CON	1.5	2.5	3.5	4.5	5.6	'all-out'
1	0.3	0.5	0.6	1.1	1.4	2.5
2	0.5	0.6	0.7	1.0	1.1	2.3
3	0.2	0.3	0.6	1.2	1.1	1.7
4	0.5	0.7	0.8	1.5	1.4	2.3
5	0.5	0.6	0.9	1.4	1.8	3.2
6	0.3	0.5	0.6	0.9	1.4	2.3
7	0.3	0.5	0.8	1.3	1.4	2.4
n	7	7	7	7	7	7
mean	0.4	0.5	0.7	1.2	1.4	2.4
sd	0.1	0.1	0.1	0.2	0.2	0.4
Post						
1	0.5	0.6	0.8	1.4	1.7	2.6
2	0.6	0.7	0.9	1.4	1.6	2.8
3	0.4	0.6	0.9	1.4	1.4	2.3
4	0.4	0.5	0.7	1.7	1.9	2.2
5	0.2	0.4	0.6	1.4	1.4	2.7
6	0.2	0.2	0.5	0.7	1.3	2.2
7	0.3	0.4	1.0	1.5	1.4	2.1
n	7	7	7	7	7	7
mean	0.4	0.5	0.8	1.4	1.5	2.4
sd	0.1	0.2	0.2	0.3	0.2	0.3

Table B2.16 Study 1 LHTL subject rise in plasma [K^+] above rest ($\Delta[K^+]$, $mmol.L^{-1}$) for 'all-out' trials (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)					
LHTL	1.5	2.5	3.5	4.5		5.6
1	0.2	0.4	0.4	1.0	1.3	2.0
2	0.3	0.3	0.4	0.9	1.2	2.3
3	0.4	0.5	0.8	1.4	1.1	1.5
4	0.4	0.6	0.6	1.3	1.4	2.6
5	0.6	0.7	0.9	1.3	1.4	2.4
6	0.6	0.7	0.8	1.4	1.6	2.5
n	6	6	6	6	6	6
mean	0.4	0.5	0.7	1.2	1.3	2.2
sd	0.2	0.2	0.2	0.2	0.2	0.4
Post						
1	0.3	0.6	0.6	1.0	1.3	2.2
2	0.6	0.6	0.7	1.2	1.3	2.5
3	0.1	0.0	0.3	0.9	0.8	0.9
4	0.2	0.4	0.6	1.2	1.3	2.3
5	0.5	0.7	0.9	1.4	1.2	2.2
6	0.6	0.7	0.8	1.4	1.5	2.5
n	6	6	6	6	6	6
mean	0.4	0.5	0.7	1.2	1.2	2.1
sd	0.2	0.3	0.2	0.2	0.2	0.6

Table B2.17 Study 1 Control subject rise in plasma [K^+] above rest normalised for work performed ($\Delta[K^+]$.work^{-1} ratio, nmol.L^{-1}.J^{-1}) for 'all-out' trials (Chapters 3 and 4).

Pre	Exercise Workrate (W.kg^{-1})					
CON	1.5	2.5	3.5	4.5	5.6	'all-out'
1	10.7	6.7	4.3	4.9	6.3	7.5
2	17.8	8.0	5.0	4.5	4.9	6.9
3	8.0	4.5	4.8	6.0	5.5	5.9
4	22.2	11.6	7.1	8.3	7.8	8.8
5	20.4	9.2	7.3	7.1	9.2	10.9
6	10.6	6.6	4.2	4.0	6.2	6.7
7	15.9	8.8	7.1	6.9	7.4	8.4
n	7	7	7	7	7	7
mean	15.1	7.9	5.7	6.0	6.7	7.9
sd	5.4	2.3	1.4	1.6	1.5	1.7
Post						
1	17.9	8.0	5.7	6.3	7.6	7.9
2	21.4	9.3	6.4	6.2	7.1	8.4
3	16.0	9.0	7.2	7.0	7.0	7.8
4	17.7	8.3	6.2	9.4	10.5	8.4
5	8.1	6.1	4.9	7.1	7.1	9.1
6	7.1	2.6	3.5	3.1	5.7	6.4
7	15.9	7.1	8.8	8.0	7.4	7.4
n	7	7	7	7	7	7
mean	14.9	7.2	6.1	6.7	7.5	7.9
sd	5.3	2.3	1.7	1.9	1.5	0.8

Table B2.18 Study 1 LHTL subject rise in plasma [K^+] above rest normalised for work performed ($\Delta[K^+]$.work^{-1} ratio, nmol.L^{-1}.J^{-1}) for 'all-out' trials (Chapters 3 and 4).

Pre	Exercise Workrate (W.kg^{-1})					
LHTL	1.5	2.5	3.5	4.5	5.6	'all-out'
1	11.5	7.6	3.8	5.7	7.5	6.3
2	10.5	3.9	2.8	3.9	5.2	5.8
3	13.5	6.3	5.4	5.9	4.6	3.9
4	15.6	8.8	4.7	6.3	6.8	7.2
5	23.9	10.5	7.2	6.5	7.0	7.0
6	25.8	11.3	6.9	7.5	8.6	7.8
n	6	6	6	6	6	6
mean	16.8	8.1	5.1	6.0	6.6	6.3
sd	6.5	2.7	1.7	1.2	1.5	1.4
Post						
1	17.2	11.5	5.7	5.7	7.5	8.3
2	21.0	7.9	4.9	5.2	5.7	7.3
3	3.4	0.0	2.0	3.8	3.4	2.7
4	7.8	5.9	4.7	5.9	6.3	7.4
5	19.9	10.5	7.2	7.0	6.0	7.5
6	25.8	11.3	6.9	7.5	8.1	9.3
n	6	6	6	6	6	6
mean	15.8	7.8	5.2	5.9	6.1	7.1
sd	8.5	4.4	1.9	1.3	1.6	2.2

Table B2.19 Study 1 Control subject pO_2 (mm Hg) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	68.5	55.5	61.0	65.2	62.0	51.3	68.5	71.8
2	72.0	66.4	69.4	68.1	63.6	79.0	80.5	90.0
3	79.0	50.4	58.7	65.3	68.7	63.5	71.6	88.5
4	85.3	77.5	80.0	77.8	75.9	77.0	87.1	103.4
5	62.5	73.8	72.8	71.5	72.4	71.9	81.3	101.3
6	86.1	85.6	78.7	70.7	73.6	73.6	81.5	87.0
7	69.9	63.4	74.5	72.0	70.0	72.8	81.0	96.0
n	7	7	7	7	7	7	7	7
mean	74.8	67.5	70.7	70.1	69.5	69.9	78.8	91.1
sd	8.9	12.4	8.3	4.4	5.1	9.5	6.4	10.6
Post								
1	68.6	69.5	72.3	67.3	63.0		70.6	79.7
2	71.3	54.5	60.1	62.0	61.6	60.8	67.5	82.6
3	65.1	59.0	59.6	65.0	67.9	58.4	73.5	88.6
4	84.0	57.4	70.4	75.1	73.7	72.6	88.2	90.4
5	107.9	63.4	62.0	65.5	68.0	73.2	76.7	98.5
6	75.3	69.5	74.0	73.3	74.4	72.6	76.1	87.5
7	55.9	75.9	73.7	72.7	73.5	75.2	67.1	83.9
n	7	7	7	7	7	6	7	7
mean	75.4	64.2	67.4	68.7	68.9	68.8	74.2	87.3
sd	16.7	7.8	6.6	5.0	5.2	7.2	7.2	6.2

Table B2.20 Study 1 LHTL subject pO_2 (mm Hg) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	58.1	62.2	55.0	73.1	86.0	57.9	73.6	59.4
2	63.6	65.4	64.0	69.2	73.0	72.0	70.0	86.4
3	74.1	70.6	71.2	68.4	64.9	68.8	84.0	92.3
4	72.0	60.6	70.9	73.2	72.6	70.5	79.0	91.8
5	64.9	64.5	64.5	67.9	68.5	66.0	68.0	91.0
6	78.0	73.9	66.4	73.0	75.0	73.3	80.0	90.2
n	6	6	6	6	6	6	6	6
mean	68.5	66.2	65.3	70.8	73.3	68.1	75.8	85.2
sd	7.5	5.1	5.9	2.6	7.2	5.6	6.2	12.8
Post								
1	57.4	67.6	61.6	73.6	84.9	75.1	80.1	89.2
2	44.3	56.7	68.8	71.9	72.7	69.0	69.6	89.5
3	83.3	75.5	74.6	72.7	72.0	69.8	75.8	88.2
4	73.5	70.1	77.7	73.6	73.4	73.2	76.1	92.7
5	69.5	60.2	61.1	67.5	71.2	64.1	66.8	89.7
6	78.5	69.2	70.1	78.7	73.8	75.5	51.9	95.1
n	6	6	6	6	6	6	6	6
mean	67.8	66.6	69.0	73.0	74.7	71.1	70.1	90.7
sd	14.5	6.9	6.7	3.6	5.1	4.4	10.1	2.6

Table B2.21 Study 1 Control subject pCO_2 (mm Hg) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	40.8	45.5	45.6	44.2	43.5	43.5	43.5	38.3
2	40.0	39.9	40.2	39.6	39.0	35.7	33.9	33.1
3	41.1	43.3	41.9	38.2	35.5	35.0	38.2	32.9
4	36.2	38.9	38.6	37.4	36.1	34.9	34.2	30.1
5	41.8	39.6	38.9	38.0	35.5	36.7	39.4	34.3
6	39.6	39.9	38.7	37.7	36.0	35.8	35.8	35.0
7	38.4	37.4	38.0	36.1	33.4	31.9	32.5	29.8
n	7	7	7	7	7	7	7	7
mean	39.7	40.6	40.3	38.7	37.0	36.2	36.8	33.4
sd	1.9	2.8	2.7	2.6	3.3	3.5	3.8	2.9
Post								
1	40.6	43.1	43.6	43.2	39.5		37.9	33.6
2	39.6	36.1	38.2	39.1	38.0	36.0	36.4	32.0
3	41.4	42.4	40.9	38.9	34.2	35.2	41.9	35.4
4	38.5	40.2	39.8	38.3	36.0	33.7	33.2	31.1
5	38.6	39.8	39.6	37.2	34.9	34.7	35.4	32.1
6	40.2	40.4	38.8	36.9	34.0	34.2	33.4	32.9
7	40.9	34.6	36.3	34.3	31.7	29.1	31.8	28.1
n	7	7	7	7	7	6	7	7
mean	40.0	39.5	39.6	38.3	35.5	33.8	35.7	32.2
sd	1.1	3.1	2.3	2.7	2.6	2.4	3.4	2.3

Table B2.22 Study 1 LHTL subject pCO_2 (mm Hg) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	39.9	38.0	38.4	36.9	33.1	34.0	30.0	33.6
2	40.0	42.2	43.2	41.6	37.7	36.8	37.9	33.1
3	43.2	42.0	42.3	40.5	39.1	37.1	35.8	31.6
4	40.8	40.0	38.8	37.0	34.3	33.8	34.0	29.9
5	32.7	41.6	43.5	43.2	39.9	38.7	41.1	32.5
6	37.5	37.5	39.0	37.3	35.0	34.0	34.3	28.7
n	6	6	6	6	6	6	6	6
mean	39.0	40.2	40.9	39.4	36.5	35.7	35.5	31.6
sd	3.6	2.1	2.4	2.7	2.8	2.1	3.8	1.9
Post								
1	38.5	37.8	38.2	35.8	32.3	32.4	31.6	29.6
2	42.5	41.7	39.6	38.1	34.9	34.8	35.9	30.9
3	42.3	42.9	42.5	39.8	36.7	35.5	37.0	32.4
4	39.1	38.7	39.4	37.5	35.2	34.0	34.9	30.4
5	34.0	43.3	44.2	41.9	39.1	37.7	42.1	33.7
6	34.4	36.1	36.3	35.0	32.6	31.4	35.2	26.1
n	6	6	6	6	6	6	6	6
mean	38.5	40.1	40.0	38.0	35.1	34.3	36.1	30.5
sd	3.7	3.0	2.9	2.6	2.6	2.3	3.4	2.6

Table B2.23 Study 1 Control subject [HCO_3^-] ($mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	25.8	26.6	26.3	25.5	23.1	22.4	19.3	17.8
2	26.3	25.8	25.4	24.4	21.8	19.1	15.8	14.5
3	26.0	26.4	25.7	23.0	18.3	17.4	15.3	13.9
4	23.9	23.9	23.7	22.5	18.8	17.3	15.1	14.3
5	26.5	25.7	25.6	24.1	20.5	19.2	16.2	14.6
6	25.7	25.1	24.9	23.7	21.2	20.0	17.3	16.7
7	25.1	24.1	23.6	21.9	18.3	17.1	15.0	14.0
n	7	7	7	7	7	7	7	7
mean	25.6	25.4	25.0	23.6	20.3	18.9	16.3	15.1
sd	0.9	1.1	1.0	1.2	1.9	1.9	1.5	1.5
Post								
1	25.7	25.8	25.7	24.9	21.2		18.3	16.3
2	26.6	24.9	25.5	24.9	22.5	20.2	18.1	15.0
3	26.6	26.7	26.4	25.0	20.4	19.8	18.0	16.6
4	24.5	23.9	23.5	21.8	17.7	16.4	14.2	13.8
5	25.2	24.9	24.1	21.7	18.5	17.1	14.5	12.6
6	25.6	24.9	24.2	22.7	19.1	17.7	15.0	13.4
7	25.5	23.6	23.4	21.9	17.9	16.6	15.0	13.4
n	7	7	7	7	7	6	7	7
mean	25.7	25.0	24.7	23.3	19.6	18.0	16.2	14.4
sd	0.7	1.1	1.2	1.6	1.8	1.6	1.9	1.5

Table B2.24 Study 1 LHTL subject [HCO_3^-] ($mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	24.9	23.8	23.9	22.7	19.0	18.3	14.9	15.7
2	26.3	26.5	26.3	25.3	21.6	20.5	18.1	15.4
3	24.9	24.4	23.7	21.4	17.8	16.7	14.6	13.1
4	26.6	26.1	25.2	23.4	19.0	17.9	15.0	12.7
5	26.3	27.3	27.6	26.3	22.5	21.6	19.7	17.1
6	23.9	22.9	22.8	21.4	18.4	17.1	15.4	13.2
n	6	6	6	6	6	6	6	6
mean	25.5	25.2	24.9	23.4	19.7	18.7	16.3	14.5
sd	1.1	1.7	1.8	2.0	1.9	1.9	2.1	1.8
Post								
1	23.6	23.5	23.6	22.3	19.1	18.0	15.4	14.3
2	25.8	25.1	24.2	23.4	20.0	19.1	16.1	13.9
3	26.0	24.7	24.0	21.5	16.7	15.7	14.7	13.2
4	25.5	25.5	25.5	24.4	20.8	19.9	17.7	14.7
5	25.8	27.8	27.9	26.5	22.5	21.6	20.0	17.1
6	22.7	22.8	22.7	21.1	17.8	16.3	16.2	12.2
n	6	6	6	6	6	6	6	6
mean	24.9	24.9	24.7	23.2	19.5	18.4	16.7	14.2
sd	1.4	1.7	1.8	2.0	2.1	2.2	1.9	1.7

Table B2.25 Study 1 Control subject pH (arbitrary units) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	7.42	7.39	7.38	7.38	7.34	7.33	7.27	7.29
2	7.43	7.43	7.42	7.41	7.37	7.35	7.29	7.26
3	7.42	7.40	7.41	7.40	7.33	7.32	7.23	7.25
4	7.43	7.40	7.41	7.40	7.34	7.32	7.27	7.30
5	7.42	7.43	7.43	7.42	7.38	7.34	7.24	7.25
6	7.43	7.42	7.42	7.42	7.39	7.37	7.31	7.30
7	7.4	7.4	7.4	7.4	7.4	7.3	7.3	7.3
n	7	7	7	7	7	7	7	7
mean	7.43	7.41	7.41	7.40	7.36	7.34	7.27	7.28
sd	0.01	0.02	0.02	0.01	0.02	0.02	0.03	0.02
Post								
1	7.42	7.40	7.39	7.38	7.35		7.31	7.31
2	7.44	7.45	7.44	7.42	7.39	7.37	7.32	7.29
3	7.42	7.42	7.43	7.43	7.39	7.37	7.25	7.29
4	7.42	7.39	7.39	7.37	7.31	7.31	7.25	7.27
5	7.43	7.41	7.40	7.38	7.34	7.31	7.24	7.22
6	7.42	7.41	7.41	7.41	7.37	7.33	7.27	7.24
7	7.41	7.45	7.43	7.42	7.37	7.38	7.30	7.30
n	7	7	7	7	7	6	7	7
mean	7.42	7.42	7.41	7.40	7.36	7.34	7.28	7.27
sd	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.03

Table B2.26 Study 1 LHTL subject pH (arbitrary units) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	7.41	7.41	7.41	7.41	7.38	7.35	7.32	7.29
2	7.44	7.41	7.40	7.40	7.38	7.36	7.30	7.29
3	7.38	7.38	7.37	7.34	7.28	7.27	7.24	7.24
4	7.43	7.43	7.43	7.42	7.36	7.35	7.27	7.25
5	7.52	7.43	7.42	7.40	7.37	7.37	7.30	7.34
6	7.42	7.40	7.39	7.38	7.34	7.32	7.27	7.29
n	6	6	6	6	6	6	6	6
mean	7.43	7.41	7.40	7.39	7.35	7.34	7.28	7.28
sd	0.05	0.02	0.02	0.03	0.04	0.03	0.03	0.04
Post								
1	7.41	7.41	7.41	7.41	7.39	7.37	7.31	7.31
2	7.40	7.40	7.40	7.41	7.38	7.36	7.27	7.27
3	7.41	7.38	7.37	7.35	7.28	7.27	7.22	7.23
4	7.43	7.44	7.43	7.43	7.39	7.38	7.33	7.31
5	7.49	7.42	7.42	7.42	7.38	7.38	7.30	7.33
6	7.43	7.42	7.41	7.40	7.36	7.34	7.28	7.29
n	6	6	6	6	6	6	6	6
mean	7.43	7.41	7.41	7.40	7.36	7.35	7.29	7.29
sd	0.04	0.02	0.02	0.03	0.04	0.04	0.04	0.03

Table B2.27 Study 1 Control subject lactate concentration ([Lac^-], $mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
CON	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	1.3	1.4	1.4	1.9	4.9	6.1	10.1	8.7
2	1.3	2.5	3.0	5.2	8.0	11.9	16.9	15.9
3	2.2	2.7	2.9	5.3	11.1	11.7	16.6	15.0
4	1.2	1.9	1.9	3.4	9.1	12.0	15.2	13.6
5	1.9	1.7	1.9	3.0	7.1	9.6	15.5	13.1
6	0.9	2.6	2.9	2.8	6.0	8.0	11.8	10.4
7	1.1	2.3	2.9	4.9	10.1	11.6	15.3	14.5
n	7	7	7	7	7	7	7	7
mean	1.4	2.2	2.4	3.8	8.0	10.1	14.5	13.0
sd	0.5	0.5	0.7	1.3	2.2	2.3	2.5	2.6
Post								
1	0.8	1.0	1.1	1.7	4.3		7.5	7.1
2	1.8	1.6	2.1	3.7	8.5	9.4	15.7	15.5
3	1.0	1.1	1.3	3.1	10.5	11.8	17.3	15.2
4	1.0	2.1	2.4	5.0	12.9	13.5	19.2	16.1
5	0.7	1.9	2.7	6.4	11.5	13.9	19.8	18.1
6	0.9	1.7	1.9	2.8	6.2	7.7	10.7	10.5
7	1.0	1.6	2.6	5.2	12.9	14.6	18.6	17.3
n	7	7	7	7	7	6	7	7
mean	1.0	1.6	2.0	4.0	9.5	11.8	15.5	14.3
sd	0.4	0.4	0.6	1.6	3.3	2.7	4.7	4.0

Table B2.28 Study 1 LHTL subject lactate concentration ([Lac^-], $mmol.L^{-1}$) (Chapters 3 and 4).

Pre		Exercise Workrate ($W.kg^{-1}$)					Recovery (min)	
LHTL	rest	1.5	2.5	3.5	4.5	5.6	+1	+5
1	1.5	1.5	1.4	3.3	6.8	7.5	11.8	11.1
2	1.0	1.3	1.4	2.1	6.0	7.6	11.1	12.4
3	1.3	1.6	3.2	5.0	10.6	11.9	14.0	13.5
4	1.1	1.2	1.5	3.5	10.2	12.0	16.7	16.6
5	1.2	1.4	1.2	2.3	7.4	7.8	12.1	10.5
6	1.2	2.1	2.2	4.5	6.9	8.7	11.3	11.0
n	6	6	6	6	6	6	6	6
mean	1.2	1.5	1.8	3.5	8.0	9.3	12.8	12.5
sd	0.2	0.3	0.8	1.2	1.9	2.1	2.2	2.3
Post								
1	1.2	1.1	1.1	1.7	4.4	6.1	9.1	8.5
2	1.9	1.0	1.1	2.0	5.4	6.3	9.8	9.8
3	1.2	2.3	2.7	6.5	14.5	14.9	17.2	15.2
4	0.8	1.0	1.1	2.2	5.9	6.8	9.9	10.0
5	0.9	1.1	0.9	1.8	5.7	6.0	8.9	8.6
6	2.1	2.8	3.3	5.2	9.2	10.7	14.6	15.6
n	6	6	6	6	6	6	6	6
mean	1.4	1.6	1.7	3.2	7.5	8.5	11.6	11.3
sd	0.5	0.8	1.0	2.1	3.8	3.6	3.5	3.2

Table B2. 29 Study 2 Control subject haemoglobin concentration ([Hb], $g.dL^{-1}$) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	14.70	16.20	16.10	16.05	15.70	14.45	15.40	16.00	16.00	15.55	14.80	16.20	16.65	16.25	16.10
2	15.80	16.10	16.20	16.10	15.90	15.35	16.00	16.70	16.50	16.30	14.30	15.15	15.40	15.20	15.15
3	16.80	17.25	17.75	17.65	17.35	16.00	16.85	16.65	17.00	16.80	16.90	17.55	17.85	17.80	17.65
4	15.70	16.50	16.80	16.55	16.65	14.60	15.25	15.80	15.00	15.50	15.00	16.15	16.45	16.40	16.20
5	15.30	16.50	16.90	16.70	16.65	15.20	16.25	16.80	16.45	16.45	15.65	16.80	17.35	17.10	17.05
6	14.20	14.90	15.00	15.95	14.95	14.00	14.75	15.05	15.15	15.20	14.25	14.85	14.70	15.35	15.25
7	15.25	15.59	16.70	16.40	16.30	13.50	14.40	14.90	14.30	14.20	15.10	15.44	16.55	16.20	16.30
8	14.80	15.12	15.55	15.75	15.90	13.85	15.25	15.40	15.10	14.70	14.75	15.17	15.95	16.00	15.75
9	16.50	17.95	17.80	18.10	17.75	16.50	17.60	18.15	17.65	17.60	16.60	17.95	18.05	18.05	17.55
10	14.95	15.95	16.00	16.15	16.10	14.90	15.40	15.45	15.75	15.55	15.25	16.05	16.50	16.75	16.35
11	14.60	15.35	15.95	15.60	15.15	15.10	15.65	15.80	15.70	15.60	15.10	16.00	16.05	15.65	16.00
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	15.33	16.13	16.43	16.45	16.22	14.86	15.71	16.06	15.87	15.77	15.25	16.12	16.50	16.43	16.30
SD	0.81	0.91	0.86	0.78	0.85	0.91	0.92	0.95	0.98	0.96	0.85	0.99	1.00	0.93	0.83

Table B2.30 Study 2 LHTLc subject haemoglobin concentration ([Hb], $g.dL^{-1}$) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	14.45	15.00	14.90	14.90	14.80	14.30	14.95	15.30	14.80	14.70	14.65	15.05	15.40	15.35	15.25
2	14.00	14.70	14.80	14.60	14.55	13.55	13.95	14.20	14.10	14.10	13.90	14.30	14.75	14.60	14.30
3	14.60	15.75	15.35	15.05	15.05	13.90	14.55	14.65	14.70	14.30	14.45	15.20	15.40	15.05	14.95
4	15.10	15.85	16.10	15.90	15.70	14.30	15.00	15.25	15.10	15.00	15.80	16.40	16.85	16.90	16.55
5	14.75	15.45	15.85	15.45	15.45	14.65	15.90	16.15	16.05	15.80	15.30	16.40	16.95	16.70	16.55
6	16.05	16.50	16.85	17.05	16.85	16.25	15.13	14.20	17.65	17.45	16.55	15.70	17.70	17.50	17.25
7	13.60	14.45	14.90	14.80	14.65	14.40	14.66	15.50	15.40	15.00	15.30	15.38	16.05	15.30	15.75
8	14.70	15.60	15.90	15.85	15.70	15.20	15.52	16.45	16.30	16.05	15.00	15.48	16.40	16.30	16.40
9	14.35	15.30	15.45	15.65	15.25	14.85	14.97	15.75	15.90	16.05	15.60	15.88	16.40	16.60	16.55
10	13.20	13.95	14.10	14.10	14.20	13.10	14.15	14.10	14.20	14.25	13.80	13.96	15.30	15.00	14.50
11	15.45	16.25	16.30	16.00	16.25	15.80	16.55	16.90	16.65	16.70	16.70	17.35	17.15	17.75	17.75
12	15.30	15.85	16.10	15.95	15.80	14.65	16.25	16.20	16.00	15.90	15.95	17.30	17.10	16.80	16.70
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	14.63	15.39	15.55	15.44	15.35	14.58	15.13	15.39	15.57	15.44	15.25	15.70	16.29	16.15	16.04
SD	0.79	0.75	0.78	0.79	0.76	0.89	0.80	0.95	1.05	1.05	0.94	1.05	0.91	1.05	1.09

Table B2.31 Study 2 LHTLi subject haemoglobin concentration ([Hb], $g.dL^{-1}$) (Chapter 5).

LHTLi	Pre					d5					Post				
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	15.25	15.25	16.80	16.40	16.45	14.70	15.35	15.30	15.45	15.45	15.20	15.65	15.95	15.95	15.85
2	15.75	16.45	16.70	16.35	15.41	14.50	15.45	15.65	15.80	15.75	14.85	15.85	16.10	15.80	15.65
3	15.10	15.60	16.10	15.70	15.65	15.50	16.00	16.30	16.00	15.95	15.40	15.95	16.60	16.35	16.30
4	15.65	15.95	16.20	16.25	16.40	16.20	16.70	17.25	17.30	17.20	16.40	16.85	17.35	17.05	17.20
5	14.90	15.55	15.75	15.75	15.70	15.10	15.70	16.10	15.90	15.80	15.70	16.15	16.35	16.45	16.40
6	13.80	14.40	15.15	14.75	15.00	13.80	13.98	14.25	14.55	14.50	13.55	14.10	14.20	14.15	14.30
7	14.10	14.55	14.95	14.85	14.70	14.90	15.26	16.70	16.20	16.15	15.35	15.75	16.90	16.35	16.00
8	14.15	14.70	14.70	14.85	14.75	14.85	15.45	15.75	15.65	15.70	15.70	16.00	16.25	16.30	15.95
9	14.55	15.40	15.45	14.30	15.15	15.00	15.80	16.10	15.80	15.70	15.55	16.30	16.45	16.00	16.15
10	14.35	15.00	15.25	15.00	14.85	14.60	15.45	15.40	15.40	15.65	14.90	15.80	16.25	16.15	15.85
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	14.76	15.29	15.71	15.42	15.41	14.92	15.51	15.88	15.81	15.79	15.26	15.84	16.24	16.06	15.97
SD	0.67	0.64	0.73	0.76	0.64	0.63	0.68	0.82	0.69	0.66	0.75	0.70	0.82	0.75	0.73

Table B2. 32 Study 2 Control subject haematocrit (Hct, %) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	45.05	49.55	49.2	49.2	48.1	44.3	47.25	49.05	49	47.7	45.4	49.65	50.95	49.7	49.15
2	48.4	49.25	49.6	49.35	48.7	46.95	49.05	51.1	50.45	50	43.9	46.35	47.2	46.55	46.4
3	51.35	52.75	54.25	53.9	53.1	49	51.55	50.9	52.05	51.45	51.7	53.65	54.65	54.5	53.95
4	48	50.55	51.45	50.7	50.85	44.8	46.75	48.45	45.8	47.35	46	49.5	50.3	50.1	49.6
5	46.85	50.5	51.7	51.2	50.95	46.6	49.75	51.45	50.35	50.4	48.05	51.5	52.95	52.3	52.1
6	43.6	45.75	45.95	45.9	45.8	43.1	45.25	46.2	46.45	46.65	43.65	45.55	45.1	47	46.7
7	46.65	47.73	51.1	50.25	49.9	41.5	42.56	45.7	43.9	43.7	46.25	47.33	50.6	49.7	49.9
8	45.35	46.31	47.7	48.15	48.75	42.45	43.63	47.15	46.2	45.2	45.25	46.51	48.95	48.9	48.15
9	50.55	54.75	54.4	55.25	54.3	50.5	53.85	55.45	53.95	53.8	50.85	54.85	55.1	55.15	53.75
10	45.85	48.9	49.1	49.4	49.25	45.7	47.2	47.4	48.25	47.7	46.65	49.15	50.4	51.2	50
11	44.85	47.1	48.8	47.8	46.45	46.2	47.9	48.45	48.15	47.85	46.25	48.95	49.15	47.9	49
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	46.95	49.38	50.3	50.1	49.65	45.55	47.7	49.21	48.6	48.35	46.72	49.36	50.49	50.27	49.88
SD	2.42	2.71	2.6	2.67	2.57	2.72	3.3	2.84	2.96	2.88	2.57	2.99	2.98	2.82	2.51

Table B2. 33 Study 2 LHTLc subject haematocrit (Hct, %) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	44.40	45.90	45.65	45.65	45.35	43.75	45.85	46.85	45.45	45.05	44.80	46.20	47.20	46.95	46.65
2	42.80	45.10	45.35	44.75	44.60	41.60	42.80	43.55	43.30	43.25	42.75	43.85	45.25	44.80	43.90
3	44.85	48.15	47.10	46.10	46.05	42.60	44.65	45.00	45.10	43.85	44.30	46.65	47.15	46.15	45.80
4	46.20	48.55	49.30	48.75	48.20	43.80	45.90	46.75	46.35	45.95	48.35	50.25	51.50	51.70	50.65
5	45.15	47.35	48.40	47.40	47.30	45.00	48.65	49.40	49.10	48.40	46.90	50.30	51.90	51.20	50.70
6	49.05	50.50	51.55	52.05	51.55	49.70	46.36	43.45	53.90	53.40	50.60	48.11	54.20	53.40	52.80
7	41.75	42.77	45.70	45.40	45.00	44.25	44.99	47.60	47.10	46.05	46.80	47.06	49.10	46.90	48.45
8	45.15	46.23	48.65	48.70	48.05	46.55	47.53	50.35	50.00	49.15	46.05	47.51	50.15	49.90	50.15
9	43.95	45.15	47.30	48.00	46.70	45.50	45.84	48.25	48.70	49.15	47.75	48.59	50.20	50.80	50.75
10	40.40	41.34	43.30	43.00	43.45	40.30	43.50	43.20	43.55	43.65	42.50	42.92	47.00	45.90	44.40
11	47.35	49.65	49.85	48.95	49.80	48.40	50.60	51.75	50.90	48.70	51.15	53.00	52.50	54.25	54.35
12	46.90	48.55	49.40	48.80	48.40	44.95	49.65	49.60	48.95	48.70	48.85	52.90	52.30	51.30	51.15
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	44.83	46.60	47.63	47.30	47.04	44.70	46.36	47.15	47.70	47.11	46.73	48.11	49.87	49.44	49.15
SD	2.42	2.74	2.33	2.43	2.32	2.67	2.37	2.88	3.15	3.00	2.80	3.14	2.75	3.16	3.31

Table B2. 34 Study 2 LHTLi subject haematocrit (Hct, %) (Chapter 5).

LHTLi	Pre					d5					Post				
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	46.75	46.70	51.45	50.10	50.40	45.20	47.05	46.85	47.40	47.35	46.65	47.95	48.80	48.75	48.55
2	48.25	50.35	51.15	49.95	47.22	44.40	47.35	47.90	48.35	48.20	45.55	48.55	49.30	48.35	47.95
3	46.30	47.75	49.20	48.00	47.95	47.45	49.00	49.85	48.95	48.80	47.25	48.85	50.85	50.10	50.00
4	47.90	48.75	49.55	49.75	50.15	49.55	51.10	52.75	52.90	52.60	50.20	51.55	53.05	52.15	52.70
5	45.65	47.75	48.35	48.15	48.05	46.35	48.15	49.30	48.75	48.45	48.10	49.35	50.05	50.35	50.20
6	42.35	43.09	46.55	45.25	46.00	42.45	42.99	43.65	44.65	44.55	41.60	43.40	43.45	43.40	43.95
7	43.10	43.68	45.80	45.40	45.15	45.60	46.70	51.05	49.70	49.40	47.05	48.30	51.65	50.00	48.95
8	43.45	45.00	45.20	45.60	45.25	45.65	47.35	48.20	47.95	48.05	48.10	48.95	49.80	50.00	48.90
9	44.65	47.30	47.45	43.90	46.45	45.95	48.40	49.25	48.40	48.05	47.75	49.85	50.35	48.95	49.45
10	44.00	45.00	46.65	46.00	45.55	44.75	47.30	47.15	47.20	47.90	45.65	48.45	49.75	49.35	48.50
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	45.24	46.54	48.14	47.21	47.22	45.74	47.54	48.60	48.43	48.34	46.79	48.52	49.71	49.14	48.92
SD	2.05	2.30	2.17	2.26	1.91	1.88	2.05	2.49	2.08	1.98	2.26	2.07	2.52	2.28	2.19

Table B2. 35 Study 2 Control subject change in plasma volume (ΔPV, %) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	0	-16.69	-15.59	-15.33	-11.57	0.00	-11.14	-17.39	-17.31	-12.75	0.00	-15.75	-20.15	-16.10	-14.39
2	0	-3.48	-4.74	-3.67	-1.21	0.00	-7.86	-15.27	-13.11	-11.24	0.00	-9.73	-12.61	-10.37	-9.82
3	0	-5.41	-10.99	-9.80	-6.65	0.00	-9.79	-7.48	-11.51	-9.34	0.00	-7.59	-11.10	-10.56	-8.71
4	0	-9.51	-12.75	-10.06	-10.87	0.00	-7.64	-13.71	-4.43	-10.16	0.00	-13.14	-16.08	-15.48	-13.58
5	0	-13.64	-17.73	-15.88	-15.20	0.00	-11.98	-17.74	-14.09	-14.17	0.00	-13.03	-18.31	-15.97	-15.37
6	0	-8.33	-9.28	-14.60	-8.72	0.00	-8.67	-12.04	-13.03	-13.64	0.00	-7.28	-5.56	-12.69	-11.62
7	0	-4.16	-16.30	-13.29	-12.14	0.00	-7.95	-15.90	-9.47	-8.50	0.00	-4.17	-16.15	-12.77	-13.65
8	0	-3.84	-8.92	-10.85	-12.71	0.00	-11.04	-17.41	-14.25	-10.28	0.00	-5.01	-13.77	-13.96	-11.31
9	0	-15.89	-14.52	-17.50	-14.09	0.00	-12.59	-18.18	-13.03	-12.50	0.00	-15.05	-15.99	-16.08	-10.99
10	0	-11.55	-12.17	-13.50	-12.97	0.00	-5.92	-6.58	-9.84	-7.71	0.00	-9.44	-14.07	-16.72	-12.58
11	0	-8.77	-15.02	-11.42	-6.43	0.00	-6.56	-8.43	-7.31	-6.17	0.00	-10.37	-11.00	-6.48	-10.45
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	0	-9.21	-12.55	-12.35	-10.23	0.00	-9.20	-13.65	-11.58	-10.59	0.00	-10.05	-14.07	-13.38	-12.04
SD	0	4.78	3.84	3.81	4.12	0.00	2.25	4.37	3.60	2.55	0.00	3.88	4.00	3.21	2.05

Table B2. 36 Study 2 LHTLc subject change in plasma volume (ΔPV, %) (Chapter 5).

LHTLc	Pre					d5					Post				
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	0.00	-6.27	-5.20	-5.20	-4.03	0.00	-7.92	-11.69	-6.30	-4.97	0.00	-5.13	-9.01	-8.28	-7.15
2	0.00	-8.59	-9.62	-7.38	-6.81	0.00	-4.86	-7.76	-6.70	-6.62	0.00	-4.66	-9.88	-8.20	-4.75
3	0.00	-12.85	-8.77	-5.19	-5.10	0.00	-7.88	-9.09	-9.56	-4.91	0.00	-8.95	-10.97	-7.18	-5.95
4	0.00	-8.89	-11.62	-9.53	-7.40	0.00	-8.23	-11.15	-9.59	-8.31	0.00	-7.20	-11.95	-12.57	-8.78
5	0.00	-8.36	-12.45	-8.45	-8.27	0.00	-13.98	-16.54	-15.53	-13.01	0.00	-12.68	-18.23	-15.80	-14.17
6	0.00	-5.50	-9.42	-11.41	-9.42	0.00	14.52	28.66	-15.62	-13.73	0.00	10.72	-13.31	-10.79	-8.33
7	0.00	-7.53	-14.91	-13.87	-12.35	0.00	-3.08	-12.68	-11.27	-7.10	0.00	-1.01	-8.79	-0.19	-5.87
8	0.00	-7.62	-13.45	-13.26	-11.32	0.00	-3.86	-14.17	-12.77	-9.90	0.00	-5.72	-15.49	-14.54	-15.49
9	0.00	-8.22	-12.67	-14.93	-10.52	0.00	-1.42	-10.47	-12.09	-13.67	0.00	-3.34	-9.34	-11.51	-11.15
10	0.00	-6.87	-10.94	-10.47	-11.80	0.00	-12.38	-11.61	-12.77	-13.23	0.00	-1.87	-16.86	-13.44	-7.97
11	0.00	-9.08	-9.72	-6.37	-9.35	0.00	-8.60	-12.58	-9.70	-5.94	0.00	-7.39	-5.31	-11.89	-12.08
12	0.00	-6.86	-9.82	-7.90	-6.30	0.00	-17.54	-17.21	-15.09	-14.14	0.00	-15.10	-13.02	-9.61	-8.79
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	0.00	-8.05	-10.72	-9.50	-8.56	0.00	-6.27	-8.86	-11.42	-9.63	0.00	-5.19	-11.85	-10.33	-9.21
SD	0.00	1.86	2.55	3.33	2.69	0.00	8.05	12.13	3.17	3.73	0.00	6.49	3.74	4.14	3.37

Table B2. 37 Study 2 LHTLi subject change in plasma volume (ΔPV, %) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	0.00	0.09	-17.24	-12.86	-13.65	0.00	-7.47	-6.81	-8.67	-8.59	0.00	-5.24	-8.54	-8.45	-7.52
2	0.00	-8.14	-10.97	-6.83	4.28	0.00	-3.47	-5.70	-7.40	-6.83	0.00	-11.47	-14.12	-10.85	-9.29
3	0.00	-5.82	-11.28	-6.87	-6.48	0.00	-5.98	-9.25	-5.89	-5.32	0.00	-6.38	-13.56	-10.90	-10.45
4	0.00	-3.48	-6.45	-7.11	-8.69	0.00	-5.97	-12.04	-12.58	-11.51	0.00	-5.31	-10.89	-7.58	-9.44
5	0.00	-7.88	-10.10	-9.75	-9.29	0.00	-7.05	-11.37	-9.28	-8.17	0.00	-5.13	-7.58	-8.70	-8.14
6	0.00	-5.40	-15.55	-11.15	-13.82	0.00	-2.21	-5.18	-8.78	-8.30	0.00	-6.86	-7.60	-7.19	-9.06
7	0.00	-4.08	-10.16	-8.89	-7.54	0.00	-4.33	-19.72	-14.96	-14.18	0.00	-4.84	-17.06	-11.35	-7.51
8	0.00	-6.38	-6.72	-8.34	-7.12	0.00	-6.89	-10.14	-9.13	-9.59	0.00	-3.48	-6.55	-7.21	-3.08
9	0.00	-10.04	-10.59	3.13	-7.08	0.00	-9.37	-12.52	-9.37	-8.17	0.00	-8.44	-10.17	-5.04	-6.85
10	0.00	-6.04	-10.35	-7.75	-6.04	0.00	-9.86	-9.31	-9.40	-12.03	0.00	-10.55	-15.22	-14.02	-10.92
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	0.00	-5.72	-10.94	-7.64	-7.54	0.00	-6.26	-10.20	-9.54	-9.27	0.00	-6.77	-11.13	-9.13	-8.23
SD	0.00	2.82	3.35	4.26	5.00	0.00	2.43	4.21	2.54	2.63	0.00	2.60	3.66	2.63	2.23

Table B2. 38 Study 2 Control subject arterialised venous sodium concentration ([Na^+], $mmol.L^{-1}$) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	135.05	140.90	140.20	139.00	135.85	139.00	144.50	144.50	143.00	141.00	139.45	145.95	144.80	143.70	141.90
2	138.05	140.70	142.40	140.40	139.15	137.40	138.35	141.35	140.35	138.90	136.65	137.75	140.10	139.55	138.30
3	139.3	141.10	144.05	143.10	141.20	138.35	140.55	143.25	142.90	141.10	137.00	139.15	142.70	141.20	139.45
4	139.5	144.05	146.95	144.80	143.50	138.20	140.40	145.00	143.50	141.30	137.75	143.15	145.15	144.50	142.70
5	140.55	142.70	146.60	146.20	144.50	139.75	144.10	147.50	145.90	144.45	138.65	141.40	144.00	145.00	143.80
6	138.85	143.65	144.85	143.85	142.30	138.65	141.60	145.30	144.60	143.20	138.80	141.20	144.30	143.75	142.70
7	136	138.05	140.90	141.95	140.40	137.55	138.55	142.50	140.85	139.75	137.40	140.20	145.00	144.30	142.35
8	140.45	145.65	147.70	145.50	143.85	138.15	142.40	144.85	142.80	141.60	140.50	145.65	147.10	145.55	144.30
9	139.05	144.40	143.70	143.15	142.00	138.45	142.60	144.65	143.25	141.85	137.60	141.80	142.80	141.35	140.15
10	136.8	140.10	143.65	142.70	140.85	137.50	142.25	144.45	143.35	142.15	137.10	140.50	143.80	142.70	140.85
11	137.75	140.20	142.50	141.05	140.40	139.75	141.90	144.20	142.90	142.30	138.80	142.55	143.75	141.55	141.40
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	138.3	141.95	143.95	142.88	141.27	138.43	141.56	144.32	143.04	141.60	138.15	141.75	143.95	143.01	141.63
SD	1.77	2.29	2.43	2.19	2.43	0.82	1.98	1.59	1.52	1.51	1.18	2.50	1.76	1.89	1.83

Table B2. 39 Study 2 LHTLc subject arterialised venous sodium concentration ([Na^+], $mmol.L^{-1}$) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	138.00	141.00	144.00	142.00	141.00	137.30	139.55	142.80	141.00	139.85	139.30	140.20	143.30	142.40	141.15
2	139.45	142.35	142.40	141.35	140.75	138.85	140.70	141.25	139.80	140.35	137.70	138.90	141.00	140.70	139.25
3	138.45	143.30	143.10	141.20	140.30	137.30	134.65	142.30	140.70	139.95	138.35	141.05	143.10	142.05	140.55
4	136.20	139.10	141.40	139.85	138.50	135.90	138.55	141.15	139.40	138.55	137.25	139.45	142.40	141.45	138.10
5	138.50	141.65	143.80	142.10	141.05	138.85	143.60	144.65	143.00	141.45	133.90	140.00	142.25	140.55	140.00
6	138.85	139.90	141.80	141.05	140.15	137.50	140.20	143.60	139.80	139.30	139.50	140.06	142.10	141.50	140.75
7	139.75	142.65	143.00	142.20	141.25	138.85	139.30	142.60	140.80	140.60	140.40	140.70	144.55	142.55	141.40
8	137.60	138.55	140.95	138.80	138.65	137.60	140.75	142.90	141.20	139.65	136.15	138.80	142.50	140.55	139.35
9	137.85	140.00	143.70	142.75	141.70	136.95	137.45	141.80	140.80	139.75	135.15	135.35	141.60	140.40	139.10
10	138.15	140.75	141.20	140.35	139.20	138.20	143.25	142.90	139.75	136.00	139.00	145.55	143.60	141.85	140.65
11	138.30	141.85	143.20	142.65	140.50	138.65	141.10	143.85	141.45	140.35	136.45	139.10	141.40	139.10	138.70
12	137.70	139.70	141.65	140.90	139.00	138.05	143.25	143.00	141.25	140.05	135.70	141.60	141.80	139.75	138.65
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	138.23	140.90	142.52	141.27	140.17	137.83	140.20	142.73	140.75	139.65	137.40	140.06	142.47	141.07	139.80
SD	0.92	1.49	1.09	1.18	1.08	0.91	2.59	1.03	0.99	1.35	1.98	2.34	1.02	1.07	1.09

Table B2. 40 Study 2 LHTLi subject arterialised venous sodium concentration ([Na^+], $mmol.L^{-1}$) (Chapter 5).

LHTLi	Pre					d5					Post				
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	137.50	141.00	144.00	143.00	141.00	137.50	141.00	141.60	141.80	140.25	140.15	142.65	145.65	144.55	143.00
2	136.10	140.15	139.80	138.00	140.19	137.30	139.80	141.20	140.65	140.30	136.05	139.45	141.40	140.60	139.65
3	138.80	140.60	141.15	141.70	140.10	138.40	140.60	142.30	141.10	139.95	137.90	140.25	141.80	141.75	140.50
4	137.65	140.90	143.05	141.35	139.75	137.85	141.85	144.40	141.95	140.80	137.90	140.60	143.90	142.45	141.15
5	136.30	138.80	139.70	138.60	137.80	137.10	139.80	141.20	140.20	138.95	136.20	138.45	140.60	139.05	137.90
6	137.95	139.50	144.15	141.80	140.30	137.30	139.10	141.30	141.80	140.20	n/a	n/a	n/a	n/a	n/a
7	137.55	139.55	142.40	139.90	138.75	136.30	139.35	144.35	141.85	140.70	n/a	n/a	n/a	n/a	n/a
8	137.45	140.10	142.30	141.10	139.80	138.45	140.50	142.20	142.05	141.10	138.60	140.15	141.10	141.00	140.40
9	140.65	143.90	145.10	145.15	142.55	138.50	142.15	144.05	142.10	141.60	139.15	141.50	143.15	141.05	140.85
10	139.80	141.85	144.30	142.95	141.65	139.10	142.00	143.90	142.75	140.85	137.90	140.70	143.40	141.65	140.60
n	10	10	10	10	10	10	10	10	10	10	8	8	8	8	8
mean	137.98	140.64	142.60	141.36	140.19	137.78	140.62	142.65	141.63	140.47	137.98	140.47	142.63	141.51	140.51
SD	1.43	1.44	1.89	2.13	1.35	0.84	1.11	1.37	0.76	0.72	1.38	1.26	1.70	1.58	1.43

Table B2. 41 Study 2 Control subject arterialised venous potassium concentration ($[K^+]$, $mmol.L^{-1}$) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	4.29	6.75	4.82	4.05	3.78	4.40	5.80	4.60	4.00	4.10	4.34	5.92	5.47	3.94	3.74
2	4.16	4.84	4.55	3.69	3.56	4.03	4.68	5.43	3.72	3.49	4.04	5.08	5.21	5.20	4.00
3	4.25	5.12	5.31	3.98	3.66	3.98	5.06	5.00	3.86	3.62	4.27	5.35	4.77	3.82	3.77
4	4.27	5.93	5.29	4.02	3.73	4.33	5.17	6.11	4.04	3.84	4.52	6.25	5.85	4.45	4.11
5	3.89	5.19	4.79	3.50	3.19	3.86	5.19	5.14	3.34	3.07	3.98	4.99	5.66	3.39	3.25
6	4.07	5.88	4.88	3.79	3.55	3.74	5.04	5.21	3.50	3.27	3.88	5.02	4.90	3.71	3.37
7	4.23	5.20	5.68	3.94	3.54	4.00	4.91	5.47	3.62	3.34	3.95	5.57	5.25	3.56	3.30
8	3.99	5.01	4.66	3.56	3.54	3.92	4.82	4.99	3.55	3.38	3.77	5.43	4.93	3.45	3.21
9	4.07	6.07	5.38	4.08	3.56	4.42	6.09	5.94	3.80	3.53	4.01	5.94	5.57	3.90	3.53
10	4.15	5.65	5.54	3.77	3.46	3.97	5.82	4.96	3.73	3.47	4.02	5.67	5.28	3.65	3.37
11	3.92	5.05	5.34	3.50	3.11	3.88	5.26	5.04	3.62	3.36	3.92	6.03	5.40	3.46	3.38
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	4.11	5.52	5.11	3.81	3.51	4.05	5.26	5.26	3.71	3.49	4.06	5.57	5.30	3.86	3.55
SD	0.14	0.59	0.38	0.22	0.20	0.23	0.45	0.45	0.21	0.28	0.22	0.43	0.34	0.54	0.31

Table B2. 42 Study 2 LHTLc subject arterialised venous potassium concentration ([K^+], mmol.L^{-1}) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	4.30	5.90	5.25	3.80	3.50	4.12	5.12	5.22	3.79	3.37	4.30	5.64	5.47	3.80	3.55
2	3.85	5.18	4.32	3.46	3.28	4.15	5.11	5.03	4.24	3.61	4.35	5.06	5.15	3.76	3.61
3	4.41	6.25	4.77	3.82	3.88	4.41	5.27	4.97	3.73	3.58	4.25	5.99	5.63	3.80	3.68
4	4.61	5.49	5.16	3.92	3.86	4.18	5.34	5.06	3.85	3.67	4.55	5.37	5.21	3.89	3.84
5	4.25	5.64	5.45	3.93	3.80	4.00	5.40	5.39	3.81	3.59	4.28	5.17	5.34	3.96	3.73
6	4.06	4.89	4.67	3.98	3.79	4.11	5.27	3.33	3.85	3.70	4.19	5.32	5.06	4.55	3.71
7	4.02	5.70	5.24	3.93	3.87	4.33	5.15	5.76	4.21	3.82	4.39	5.24	5.80	4.04	4.00
8	3.62	5.38	4.41	3.36	3.07	3.77	5.10	4.82	3.38	3.15	3.95	4.97	4.63	3.38	3.35
9	4.11	5.11	5.09	4.06	3.53	4.05	4.74	5.52	4.07	3.78	4.39	4.96	4.77	3.97	3.79
10	4.08	5.74	5.09	4.07	3.81	3.93	6.12	5.15	4.66	3.57	4.03	5.63	5.71	4.10	3.68
11	3.63	4.66	4.14	3.27	3.48	3.78	4.74	4.80	3.62	3.39	3.59	4.70	4.57	3.56	3.43
12	4.06	5.59	5.27	3.64	3.35	3.91	5.89	4.81	3.83	3.67	4.00	5.81	5.23	3.63	3.39
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	4.08	5.46	4.90	3.77	3.60	4.06	5.27	4.99	3.92	3.57	4.19	5.32	5.21	3.87	3.64
SD	0.29	0.44	0.43	0.27	0.27	0.20	0.40	0.60	0.33	0.19	0.26	0.38	0.41	0.30	0.19

Table B2. 43 Study 2 LHTLi subject arterialised venous potassium concentration ($[K^+]$, $mmol.L^{-1}$) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	4.29	6.75	4.82	4.05	3.78	4.40	5.80	4.60	4.00	4.10	4.34	5.92	5.47	3.94	3.74
2	4.16	4.84	4.55	3.69	3.56	4.03	4.68	5.43	3.72	3.49	4.04	5.08	5.21	5.20	4.00
3	4.25	5.12	5.31	3.98	3.66	3.98	5.06	5.00	3.86	3.62	4.27	5.35	4.77	3.82	3.77
4	4.27	5.93	5.29	4.02	3.73	4.33	5.17	6.11	4.04	3.84	4.52	6.25	5.85	4.45	4.11
5	3.89	5.19	4.79	3.50	3.19	3.86	5.19	5.14	3.34	3.07	3.98	4.99	5.66	3.39	3.25
6	4.07	5.88	4.88	3.79	3.55	3.74	5.04	5.21	3.50	3.27	3.88	5.02	4.90	3.71	3.37
7	4.23	5.20	5.68	3.94	3.54	4.00	4.91	5.47	3.62	3.34	3.95	4.92	5.25	3.56	3.30
8	3.99	5.01	4.66	3.56	3.54	3.92	4.82	4.99	3.55	3.38	3.77	5.43	4.93	3.45	3.21
9	4.07	6.07	5.38	4.08	3.56	4.42	6.09	5.94	3.80	3.53	4.01	5.94	5.57	3.90	3.53
10	4.15	5.65	5.54	3.77	3.46	3.97	5.82	4.96	3.73	3.47	4.02	5.67	5.28	3.65	3.37
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	4.13	5.56	5.09	3.84	3.56	4.06	5.26	5.28	3.71	3.51	4.08	5.46	5.29	3.91	3.56
SD	0.13	0.60	0.39	0.21	0.16	0.24	0.48	0.46	0.22	0.29	0.23	0.47	0.35	0.55	0.32

Table B2. 44 Study 2 Control subject change in plasma [K^+] from rest ($\Delta[K^+]$, $mmol.L^{-1}$) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	0	2.46	0.53	-0.24	-0.51	0.00	1.40	0.20	-0.40	-0.30	0.00	1.59	1.14	-0.40	-0.60
2	0	0.68	0.39	-0.47	-0.60	0.00	0.65	1.40	-0.31	-0.54	0.00	1.04	1.17	1.16	-0.04
3	0	0.87	1.06	-0.27	-0.59	0.00	1.08	1.03	-0.12	-0.36	0.00	1.08	0.50	-0.46	-0.50
4	0	1.66	1.02	-0.25	-0.55	0.00	0.84	1.78	-0.29	-0.50	0.00	1.73	1.33	-0.07	-0.41
5	0	1.31	0.91	-0.39	-0.70	0.00	1.33	1.28	-0.52	-0.80	0.00	1.02	1.69	-0.59	-0.73
6	0	1.81	0.81	-0.28	-0.52	0.00	1.30	1.47	-0.24	-0.47	0.00	1.14	1.02	-0.18	-0.51
7	0	0.98	1.45	-0.29	-0.69	0.00	0.91	1.47	-0.38	-0.66	0.00	1.62	1.31	-0.39	-0.65
8	0	1.03	0.68	-0.43	-0.45	0.00	0.91	1.07	-0.37	-0.54	0.00	1.66	1.16	-0.32	-0.56
9	0	2.01	1.31	0.01	-0.51	0.00	1.68	1.53	-0.62	-0.89	0.00	1.93	1.56	-0.12	-0.49
10	0	1.51	1.40	-0.38	-0.69	0.00	1.86	1.00	-0.24	-0.50	0.00	1.65	1.26	-0.37	-0.65
11	0	1.13	1.42	-0.42	-0.82	0.00	1.38	1.16	-0.27	-0.52	0.00	2.11	1.48	-0.47	-0.55
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	0	1.40	1.00	-0.31	-0.60	0.00	1.21	1.22	-0.34	-0.55	0.00	1.50	1.24	-0.20	-0.52
SD	0	0.54	0.37	0.13	0.11	0.00	0.37	0.41	0.14	0.17	0.00	0.38	0.31	0.48	0.18

Table B2. 45 Study 2 LHTLc subject change in plasma [K^+] from rest (Δ [K^+], mmol.L^{-1}) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	0.00	1.60	0.95	-0.50	-0.80	0.00	1.00	1.10	-0.33	-0.75	0.00	1.34	1.18	-0.50	-0.75
2	0.00	1.33	0.47	-0.40	-0.57	0.00	0.96	0.88	0.09	-0.54	0.00	0.72	0.80	-0.59	-0.74
3	0.00	1.84	0.36	-0.59	-0.53	0.00	0.86	0.56	-0.69	-0.84	0.00	1.74	1.38	-0.45	-0.58
4	0.00	0.89	0.56	-0.69	-0.75	0.00	1.16	0.89	-0.33	-0.51	0.00	0.83	0.66	-0.66	-0.71
5	0.00	1.39	1.21	-0.32	-0.45	0.00	1.40	1.39	-0.19	-0.41	0.00	0.89	1.06	-0.32	-0.56
6	0.00	0.83	0.61	-0.08	-0.27	0.00	1.16	-0.78	-0.26	-0.42	0.00	1.14	0.88	0.37	-0.48
7	0.00	1.68	1.22	-0.08	-0.15	0.00	0.83	1.44	-0.12	-0.51	0.00	0.85	1.41	-0.35	-0.39
8	0.00	1.76	0.79	-0.26	-0.55	0.00	1.34	1.06	-0.39	-0.62	0.00	1.02	0.68	-0.57	-0.60
9	0.00	1.00	0.98	-0.05	-0.58	0.00	0.69	1.48	0.02	-0.27	0.00	0.58	0.39	-0.42	-0.60
10	0.00	1.66	1.01	-0.01	-0.27	0.00	2.20	1.22	0.73	-0.35	0.00	1.60	1.68	0.07	-0.35
11	0.00	1.03	0.51	-0.37	-0.15	0.00	0.96	1.02	-0.16	-0.39	0.00	1.11	0.98	-0.03	-0.16
12	0.00	1.54	1.22	-0.42	-0.71	0.00	1.98	0.90	-0.08	-0.25	0.00	1.81	1.24	-0.37	-0.61
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	0.00	1.38	0.82	-0.31	-0.48	0.00	1.21	0.93	-0.14	-0.49	0.00	1.13	1.03	-0.32	-0.54
SD	0.00	0.36	0.32	0.22	0.23	0.00	0.46	0.60	0.34	0.18	0.00	0.41	0.37	0.30	0.17

Table B2. 46 Study 2 LHTLi subject change in plasma [K^+] from rest (Δ [K^+], $mmol.L^{-1}$) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	0.00	2.46	0.53	-0.24	-0.51	0.00	1.40	0.20	-0.40	-0.30	0.00	1.59	1.14	-0.40	-0.60
2	0.00	0.68	0.39	-0.47	-0.60	0.00	0.65	1.40	-0.31	-0.54	0.00	1.04	1.17	1.16	-0.04
3	0.00	0.87	1.06	-0.27	-0.59	0.00	1.08	1.03	-0.12	-0.36	0.00	1.08	0.50	-0.46	-0.50
4	0.00	1.66	1.02	-0.25	-0.55	0.00	0.84	1.78	-0.29	-0.50	0.00	1.73	1.33	-0.07	-0.41
5	0.00	1.31	0.91	-0.39	-0.70	0.00	1.33	1.28	-0.52	-0.80	0.00	1.02	1.69	-0.59	-0.73
6	0.00	1.81	0.81	-0.28	-0.52	0.00	1.30	1.47	-0.24	-0.47	0.00	1.14	1.02	-0.18	-0.51
7	0.00	0.98	1.45	-0.29	-0.69	0.00	0.91	1.47	-0.38	-0.66	0.00	0.97	1.31	-0.39	-0.65
8	0.00	1.03	0.68	-0.43	-0.45	0.00	0.91	1.07	-0.37	-0.54	0.00	1.66	1.16	-0.32	-0.56
9	0.00	2.01	1.31	0.01	-0.51	0.00	1.68	1.53	-0.62	-0.89	0.00	1.93	1.56	-0.12	-0.49
10	0.00	1.51	1.40	-0.38	-0.69	0.00	1.86	1.00	-0.24	-0.50	0.00	1.65	1.26	-0.37	-0.65
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	0.00	1.43	0.95	-0.30	-0.58	0.00	1.19	1.22	-0.35	-0.55	0.00	1.38	1.21	-0.17	-0.51
SD	0.00	0.56	0.36	0.13	0.09	0.00	0.39	0.44	0.14	0.18	0.00	0.36	0.32	0.49	0.19

Table B2. 47 Study 2 Control subject arterialised venous chloride concentration ([Cl^-], $mmol.L^{-1}$) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	104	108.00	107.00	106.50	105.00	108.00	113.50	113.00	110.50	110.00	106.50	111.50	112.00	110.00	109.00
2	102.5	106.50	107.00	104.50	103.50	102.00	104.00	107.00	104.00	103.50	106.50	108.00	109.00	109.50	106.00
3	107.5	110.00	112.00	111.00	109.00	106.00	109.00	112.50	108.50	108.50	105.00	106.50	109.00	107.50	107.00
4	106	111.00	111.00	110.00	108.00	105.50	109.00	110.00	108.00	108.00	106.00	111.00	112.00	110.50	110.00
5	107	111.00	114.00	112.00	111.00	106.00	110.00	111.50	109.00	108.50	106.00	109.00	111.50	110.00	109.00
6	106.5	109.50	111.00	109.00	108.00	104.50	108.00	110.50	109.00	107.00	105.00	109.00	110.50	109.00	108.50
7	103	105.50	107.50	108.50	108.50	104.00	106.50	108.00	106.50	106.00	103.50	106.50	109.50	108.00	106.50
8	105	110.50	111.00	109.50	107.00	104.00	109.00	109.00	107.00	107.50	105.00	109.00	110.00	108.00	107.00
9	102.5	105.00	107.00	106.50	106.50	102.00	105.00	107.00	105.50	105.00	101.00	104.50	105.50	104.00	105.00
10	103	107.00	110.50	108.50	107.00	104.00	106.50	108.50	107.00	106.00	102.00	104.50	107.50	106.00	105.50
11	102	104.50	105.00	104.50	106.00	101.00	103.00	105.00	104.00	103.00	103.00	107.00	107.50	105.50	105.00
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	104.45	108.05	109.36	108.23	107.23	104.27	107.59	109.27	107.18	106.64	104.50	107.86	109.45	108.00	107.14
SD	2.02	2.47	2.78	2.47	2.03	2.07	3.00	2.49	2.09	2.18	1.87	2.32	2.05	2.10	1.75

Table B2. 48 Study 2 LHTLc subject arterialised venous chloride concentration ([Cl^-], $mmol.L^{-1}$) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	106.50	107.50	111.00	111.00	110.00	108.00	111.00	112.50	111.50	110.50	109.00	112.00	112.00	111.00	110.50
2	104.00	105.50	107.00	106.00	105.00	106.00	107.00	107.00	105.00	106.50	104.00	106.00	107.00	106.00	106.00
3	104.00	110.00	108.00	106.50	105.50	106.00	111.50	109.50	108.00	108.00	105.00	108.00	109.00	108.00	106.50
4	106.00	108.50	110.00	108.00	107.00	105.00	109.00	109.50	107.00	107.00	107.00	110.00	112.00	110.00	109.00
5	105.50	109.00	109.50	108.00	106.50	105.50	109.50	109.50	106.50	107.00	105.50	109.50	109.50	108.00	107.00
6	104.50	106.00	108.50	106.00	105.00	104.50	108.68	119.50	106.00	106.00	107.00	107.95	111.00	109.50	109.50
7	105.50	109.00	109.00	108.00	107.50	107.00	108.00	110.50	110.00	109.50	106.00	106.50	111.50	109.00	108.50
8	102.50	103.00	104.50	103.00	103.00	105.50	106.50	107.50	108.50	108.50	106.00	106.50	109.00	108.50	108.00
9	103.00	104.00	107.00	105.00	105.50	104.50	106.50	109.50	108.00	107.00	103.00	104.50	108.00	106.00	106.50
10	105.50	109.00	108.50	107.50	107.00	108.50	112.00	111.00	110.50	110.00	107.00	113.00	110.00	108.50	109.50
11	103.00	106.50	107.50	106.50	104.50	104.00	108.00	109.50	108.00	107.50	102.00	106.00	108.50	105.50	104.00
12	100.50	105.00	105.50	104.00	103.00	100.00	106.50	106.50	105.00	103.50	101.00	105.50	106.50	104.00	103.00
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	104.21	106.92	108.00	106.63	105.79	105.38	108.68	110.17	107.83	107.58	105.21	107.95	109.50	107.83	107.33
SD	1.74	2.25	1.85	2.11	1.97	2.19	1.98	3.39	2.08	1.92	2.33	2.67	1.86	2.06	2.28

Table B2. 49 Study 2 LHTLi subject arterialised venous chloride concentration ([Cl^-], $mmol.L^{-1}$) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	107.00	110.00	111.00	110.50	108.50	107.50	111.00	113.50	112.00	111.00	109.00	112.00	114.00	112.00	112.00
2	103.50	108.00	108.00	105.50	105.61	105.50	108.50	109.50	108.50	107.50	105.00	108.00	108.50	107.00	106.00
3	104.50	107.00	109.00	106.50	106.00	105.00	109.00	108.50	108.50	106.50	107.00	109.50	110.50	109.50	108.00
4	104.00	106.00	110.00	107.00	106.00	105.00	110.50	111.00	109.00	107.50	105.00	108.00	110.00	109.50	107.50
5	102.00	104.50	105.00	104.00	103.00	103.50	106.00	107.00	105.00	105.00	104.00	108.00	108.00	107.00	106.50
6	102.00	103.50	104.00	106.00	103.50	105.50	106.50	110.50	110.00	108.00	104.00	110.00	109.00	107.50	105.00
7	103.00	106.50	108.00	105.00	104.00	107.00	109.00	112.50	110.50	109.00	106.50	113.00	113.00	111.00	110.00
8	102.00	104.50	107.50	105.50	104.50	103.00	105.50	107.50	107.50	107.00	103.00	106.00	106.50	106.00	107.50
9	106.50	108.00	110.00	113.00	108.50	107.00	110.00	112.00	110.00	110.50	106.00	109.50	110.50	109.50	107.00
10	105.00	106.50	109.00	107.00	106.50	106.00	108.50	111.50	109.50	108.50	105.50	108.00	110.00	107.50	107.50
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	103.95	106.45	108.15	107.00	105.61	105.50	108.45	110.35	109.05	108.05	105.50	109.20	110.00	108.65	107.70
SD	1.82	1.95	2.21	2.73	1.91	1.47	1.89	2.17	1.89	1.80	1.73	2.08	2.24	1.94	2.00

Table B2. 50 Study 2 Control subject arterialised venous lactate concentration ([Lac^-], $mmol.L^{-1}$) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	1.35	8.80	12.70	14.70	14.35	1.95	5.95	13.25	14.55	14.15	2.05	6.65	11.70	12.95	13.35
2	0.7	2.30	9.80	12.70	11.70	1.15	2.00	8.70	11.35	11.50	0.80	1.80	8.20	7.90	9.00
3	1.4	3.45	12.60	15.00	14.40	1.70	3.45	10.95	13.50	12.75	1.60	3.95	13.90	16.00	16.00
4	1.35	6.00	14.90	16.00	17.00	0.90	2.90	10.50	13.10	13.30	1.35	5.65	13.20	16.00	16.00
5	1.3	4.80	17.00	20.00	19.00	1.50	6.50	19.00	20.00	21.00	1.25	3.90	15.00	20.00	20.00
6	1.75	7.50	16.00	18.00	18.00	1.70	4.00	13.15	18.50	19.00	1.50	3.90	14.00	18.00	17.00
7	1.4	2.85	8.80	16.00	16.00	0.90	1.80	8.80	12.50	12.85	1.40	3.05	9.15	11.45	12.40
8	1	4.40	13.40	14.30	14.50	0.90	3.35	12.85	15.00	15.00	1.40	5.95	11.30	12.60	12.25
9	1.5	6.85	14.30	16.00	16.00	2.25	7.65	16.00	18.00	18.00	1.20	6.65	13.85	15.00	14.80
10	1.05	4.20	15.00	18.00	17.00	1.00	7.20	17.00	18.00	18.00	0.95	4.25	14.10	16.00	15.00
11	1.4	3.45	8.90	14.00	12.60	1.25	3.45	12.55	13.55	13.25	1.55	5.55	9.30	11.80	11.30
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	1.29	4.96	13.04	15.88	15.50	1.38	4.39	12.98	15.28	15.35	1.37	4.66	12.15	14.34	14.28
SD	0.28	2.07	2.82	2.11	2.23	0.47	2.07	3.28	2.87	3.12	0.33	1.55	2.37	3.40	3.03

Table B2. 51 Study 2 LHTLc subject arterialised venous lactate concentration ([Lac^-], $mmol.L^{-1}$) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	1.40	4.25	9.65	14.45	14.35	0.85	3.20	11.50	13.00	12.45	1.65	3.20	10.10	12.60	12.40
2	0.80	3.25	9.25	10.10	10.00	0.95	2.75	8.80	9.20	9.35	1.00	1.85	9.55	11.00	10.05
3	2.20	7.25	13.50	14.50	13.40	1.10	8.65	12.30	12.80	11.85	0.90	4.10	12.40	14.90	14.65
4	1.05	3.35	11.10	12.30	11.60	0.70	2.90	10.70	11.25	10.50	1.05	2.90	12.20	13.90	13.15
5	1.20	4.65	12.40	14.85	14.10	1.10	5.30	13.70	16.00	15.00	1.00	3.60	13.65	14.60	14.65
6	1.00	1.80	10.35	11.95	11.00	0.80	4.23	2.75	11.30	10.90	1.10	3.41	10.65	11.45	11.05
7	2.45	4.75	11.20	11.95	11.50	0.90	1.35	10.55	12.10	12.30	1.50	1.75	7.90	9.60	10.45
8	1.60	2.75	10.70	12.50	12.00	2.10	3.30	6.40	12.95	12.50	1.50	1.95	8.75	10.00	9.75
9	2.05	2.95	10.65	14.55	15.00	2.90	1.90	8.75	15.00	15.50	1.40	2.25	8.80	11.10	11.65
10	1.00	5.50	11.25	12.80	12.80	1.10	4.25	8.40	10.40	10.40	1.35	4.60	7.95	9.95	10.05
11	1.90	4.45	12.50	12.90	13.35	1.25	4.20	12.20	14.00	13.50	1.50	4.50	9.75	11.55	11.65
12	2.40	6.25	12.60	17.00	17.00	1.75	8.70	12.70	14.10	15.00	1.40	6.80	11.30	12.65	12.55
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	1.59	4.27	11.26	13.32	13.01	1.29	4.23	9.90	12.68	12.44	1.28	3.41	10.25	11.94	11.84
SD	0.59	1.56	1.27	1.82	1.94	0.65	2.34	3.09	1.95	2.00	0.25	1.46	1.84	1.80	1.70

Table B2. 52 Study 2 LHTLi subject arterialised venous lactate concentration ([Lac^-], $mmol.L^{-1}$) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	1.30	4.50	11.80	14.35	15.00	0.80	5.05	11.70	13.80	13.85	0.80	3.95	11.25	14.25	13.65
2	1.25	4.25	11.60	12.05	13.56	0.90	3.15	8.15	12.95	13.55	1.00	3.45	9.75	13.20	13.50
3	0.80	2.70	9.65	12.15	11.40	1.30	3.15	11.25	12.25	11.55	1.35	2.35	8.05	12.75	13.15
4	1.30	4.25	12.65	16.00	14.90	1.15	4.15	14.70	16.00	16.00	4.10	4.70	16.00	17.00	17.00
5	1.00	2.55	10.40	11.00	10.55	1.00	2.05	10.50	11.20	10.60	0.60	5.00	9.60	11.10	11.10
6	1.30	2.25	8.10	14.55	15.50	1.40	2.05	4.80	14.40	14.80	1.55	5.05	10.80	11.55	11.60
7	1.40	4.30	13.00	16.00	15.50	0.80	3.10	14.50	17.00	16.00	1.70	6.60	10.15	12.90	12.60
8	1.30	2.05	11.85	14.20	13.05	1.70	3.40	13.80	14.35	14.20	1.95	3.30	12.40	13.90	12.85
9	1.25	4.60	10.90	11.40	13.05	0.85	3.00	11.40	13.00	13.15	0.80	2.65	9.90	11.05	12.40
10	1.35	2.80	12.55	13.85	13.05	0.80	2.80	11.30	12.80	12.55	2.10	2.45	10.00	13.10	12.65
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	1.23	3.43	11.25	13.56	13.56	1.07	3.19	11.21	13.78	13.63	1.60	3.95	10.79	13.08	13.05
SD	0.18	1.03	1.52	1.81	1.70	0.31	0.90	3.00	1.74	1.76	1.02	1.38	2.15	1.76	1.60

Table B2. 53 Study 2 Control subject arterialised venous strong ion difference ([SID], mmol.L^{-1}) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	39.7	34.10	30.50	27.80	26.50	39.05	35.05	28.25	27.95	26.85	40.90	37.80	31.10	30.75	29.55
2	38.98	37.46	30.69	27.04	27.51	38.31	37.61	30.93	28.71	27.41	33.43	33.41	28.19	26.06	26.86
3	30.53	28.25	19.84	17.31	17.96	30.89	28.58	20.26	21.12	20.14	30.63	29.17	20.15	18.25	16.77
4	32.19	27.24	21.17	18.87	18.55	31.87	28.65	24.65	22.47	20.09	30.47	26.65	20.06	18.17	16.80
5	36.54	33.65	20.42	18.25	18.28	36.65	33.40	21.60	20.90	19.05	35.74	34.42	22.97	18.94	18.54
6	34.76	31.49	22.40	20.54	19.86	36.48	34.28	27.08	20.82	20.69	36.34	33.38	25.01	21.95	21.20
7	35.85	34.82	29.91	21.43	19.56	36.63	35.31	30.70	25.71	24.52	36.77	36.00	31.12	28.67	27.22
8	38.72	36.68	28.59	25.72	26.08	37.58	35.22	29.11	24.84	22.94	38.62	36.95	31.65	29.40	29.16
9	38.94	37.74	27.19	24.15	22.69	38.06	35.14	26.79	23.09	21.92	39.38	35.64	29.11	25.74	23.60
10	36.82	34.78	23.03	19.99	20.40	36.24	33.59	24.16	21.85	21.42	38.03	36.77	27.10	24.41	23.72
11	38.58	37.45	34.28	26.49	25.34	41.50	40.36	32.12	28.97	29.39	38.20	35.57	32.20	27.81	28.40
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	36.51	33.97	26.18	22.51	22.06	36.66	34.29	26.88	24.22	23.13	36.23	34.16	27.15	24.56	23.80
SD	2.99	3.63	4.98	3.84	3.66	3.02	3.42	3.87	3.18	3.44	3.43	3.43	4.51	4.60	4.87

Table B2. 54 Study 2 LHTLc subject arterialised venous strong ion difference ([SID], mmol.L^{-1}) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	34.40	35.15	28.60	20.35	20.15	32.57	30.47	24.02	20.29	20.27	32.95	30.64	26.67	22.60	21.80
2	38.50	38.78	30.47	28.71	29.03	36.05	36.06	30.48	29.84	28.11	37.05	36.11	29.60	27.46	26.81
3	36.66	32.30	26.37	24.02	25.28	34.61	19.77	25.47	23.63	23.68	36.70	34.94	27.33	22.95	23.08
4	33.76	32.74	25.46	23.47	23.76	34.38	31.99	26.01	25.00	24.72	33.75	31.92	23.41	21.44	19.79
5	36.05	33.64	27.35	23.18	24.25	36.25	34.20	26.84	24.31	23.04	31.68	32.07	24.44	21.91	22.08
6	37.41	36.99	27.62	27.08	27.94	36.31	32.56	24.68	26.35	26.10	35.59	34.02	25.51	25.10	23.91
7	35.82	34.60	28.04	26.18	26.12	35.28	35.10	27.31	22.91	22.62	37.29	37.69	30.95	27.99	26.45
8	37.12	38.18	30.16	26.66	26.72	33.77	36.05	33.82	23.13	21.80	32.60	35.32	29.38	25.43	24.95
9	36.91	38.16	31.14	27.26	24.73	33.60	33.79	29.07	21.87	21.03	35.14	33.56	29.57	27.27	24.74
10	35.73	31.99	26.54	24.12	23.21	32.53	33.12	28.65	23.51	19.17	34.68	33.58	31.36	27.50	24.78
11	37.03	35.56	27.34	26.52	26.13	37.18	33.64	26.95	23.07	22.74	36.54	33.30	27.72	25.61	26.48
12	38.86	34.04	28.82	23.54	22.35	40.21	33.94	28.61	25.98	25.22	37.30	35.11	29.23	26.73	26.49
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	36.52	35.18	28.16	25.09	24.97	35.23	32.55	27.66	24.16	23.21	35.10	34.02	27.93	25.17	24.28
SD	1.49	2.39	1.75	2.35	2.46	2.17	4.34	2.71	2.45	2.54	1.97	1.96	2.53	2.37	2.23

Table B2. 55 Study 2 LHTLi subject arterialised venous strong ion difference ([SID], mmol.L^{-1}) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	29.49	26.94	21.63	18.42	17.77	29.40	25.35	17.00	16.33	15.59	30.61	27.08	20.81	18.60	17.54
2	31.43	28.03	20.32	20.53	21.10	30.96	28.27	23.72	19.30	19.30	30.12	28.11	23.27	20.49	20.21
3	37.79	37.65	27.32	27.10	26.48	36.50	34.25	27.15	24.35	26.00	33.89	34.32	28.72	23.44	23.09
4	36.51	35.49	24.95	22.04	22.41	35.73	31.88	24.13	20.67	20.79	32.84	32.98	23.11	21.15	20.65
5	37.55	36.87	29.61	27.58	27.91	36.58	36.81	28.70	27.86	26.97	35.87	30.80	27.77	24.77	24.07
6	38.92	39.68	37.34	25.27	25.03	34.73	35.72	32.11	21.44	21.24	n/a	n/a	n/a	n/a	n/a
7	37.04	33.94	26.19	22.40	22.44	32.36	32.44	22.49	17.69	18.77	n/a	n/a	n/a	n/a	n/a
8	38.22	39.43	27.83	25.19	25.80	37.49	36.64	26.11	23.70	23.17	37.53	35.87	27.10	24.81	23.42
9	37.13	36.50	29.88	24.69	24.54	34.65	34.06	26.12	22.72	21.29	36.30	34.27	28.00	24.06	24.75
10	37.44	37.56	27.41	25.66	25.64	36.22	35.52	26.09	24.00	23.18	34.07	35.68	28.33	24.50	23.66
n	10	10	10	10	10	10	10	10	10	10	8	8	8	8	8
mean	36.15	35.21	27.25	23.89	23.91	34.46	33.09	25.36	21.81	21.63	33.90	32.39	25.89	22.73	22.17
SD	3.10	4.41	4.72	2.95	3.00	2.68	3.75	4.00	3.45	3.39	2.65	3.37	3.02	2.34	2.46

Table B2.56 Study 2 Control subject pCO_2 (mm Hg) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	33.4	56.45	40.20	36.10	29.55	40.35	56.30	34.95	33.25	32.95	40.15	55.20	38.30	34.65	30.50
2	41.00	36.30	32.70	31.30	31.30	42.40	40.55	36.90	35.55	34.10	39.60	37.10	35.70	34.65	35.20
3	37.55	38.05	36.75	32.05	30.45	39.70	36.00	37.20	33.70	31.35	36.60	41.25	37.10	33.15	30.45
4	41.2	43.30	51.35	38.35	35.60	41.00	38.45	63.75	42.10	38.60	42.45	46.40	45.60	42.85	38.45
5	35.6	41.20	34.70	31.90	30.40	38.90	37.55	32.30	31.15	28.70	40.10	38.30	35.85	30.15	30.00
6	34.9	47.90	34.75	32.15	29.30	36.95	35.40	36.20	29.15	29.05	37.70	34.55	33.05	30.40	28.85
7	39.35	45.85	46.25	30.60	30.65	35.65	37.85	41.30	35.20	31.25	36.50	40.00	40.50	32.90	30.00
8	39.6	35.75	34.00	31.90	33.25	40.00	36.00	38.55	33.15	30.80	39.50	40.00	35.15	33.10	31.90
9	40.9	61.80	40.30	36.70	32.95	39.70	44.05	38.90	33.95	30.35	40.25	46.35	39.00	34.05	30.55
10	37.4	42.90	37.35	31.85	29.30	36.15	44.10	39.55	34.80	32.55	37.60	46.10	36.25	33.00	28.85
11	39.35	40.90	46.30	35.55	32.25	41.65	40.60	40.70	35.05	32.90	39.70	42.45	40.55	30.10	33.25
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	38.2	44.58	39.51	33.50	31.36	39.31	40.62	40.03	34.28	32.05	39.10	42.52	37.91	33.55	31.64
SD	2.65	8.18	6.06	2.64	1.97	2.21	6.01	8.29	3.22	2.73	1.81	5.73	3.43	3.52	2.94

Table B2.57 Study 2 LHTLc subject pCO_2 (mm Hg) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	37.10	47.10	48.20	29.60	27.75	34.00	31.15	32.40	26.70	26.20	36.10	33.25	36.50	30.40	28.05
2	38.70	44.40	33.80	32.90	31.65	35.05	37.25	34.30	31.30	32.40	34.65	33.50	36.35	29.70	29.20
3	41.80	39.20	35.15	33.55	33.20	36.00	37.30	33.30	29.15	27.85	38.90	39.45	37.25	30.45	29.45
4	38.50	37.15	35.50	31.70	31.80	37.00	34.80	33.05	30.75	30.00	39.20	34.70	33.70	29.35	29.15
5	36.90	39.95	42.15	33.00	35.10	38.65	35.55	33.60	32.85	31.85	35.90	33.25	33.50	29.20	29.00
6	37.30	39.40	33.50	33.00	33.05	34.65	38.21	23.60	28.75	28.65	37.20	38.20	29.20	30.40	30.25
7	37.40	37.30	35.35	31.40	31.75	33.65	31.10	32.05	26.00	27.40	39.95	40.75	39.95	33.75	31.55
8	40.25	46.00	37.85	32.20	31.25	38.20	48.80	56.10	31.55	30.00	37.10	41.20	38.80	33.00	32.10
9	38.50	44.50	41.80	38.65	32.95	37.20	34.80	35.30	28.80	27.50	40.30	35.85	39.85	35.15	30.00
10	40.60	40.60	37.55	32.60	29.75	38.15	57.00	48.85	33.40	29.55	37.65	59.00	46.05	32.90	29.75
11	38.05	37.40	33.00	29.90	31.20	36.30	33.40	35.40	27.90	27.55	37.30	32.85	29.65	28.10	28.05
12	44.25	40.40	43.25	35.45	31.05	38.70	39.20	38.90	36.95	32.10	38.00	36.45	37.35	33.05	29.85
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	39.11	41.12	38.09	32.83	31.71	36.46	38.21	36.40	30.34	29.25	37.69	38.20	36.51	31.29	29.70
SD	2.22	3.50	4.75	2.41	1.85	1.81	7.52	8.44	3.11	2.07	1.69	7.20	4.65	2.19	1.21

Table B2.58 Study 2 LHTLi subject pCO_2 (mm Hg) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	34.75	36.50	45.65	35.00	34.15	35.20	39.75	30.00	27.60	26.00	31.75	38.40	40.35	30.25	26.00
2	36.65	34.85	34.65	29.95	33.07	38.75	35.85	38.40	30.40	29.90	35.35	35.90	37.85	31.95	30.90
3	36.55	36.75	37.60	33.55	33.10	37.55	37.00	38.40	33.45	31.40	34.90	34.90	36.50	31.90	28.95
4	37.30	38.80	36.50	31.25	29.60	37.20	34.80	32.70	27.25	28.20	34.35	34.40	30.50	29.50	27.70
5	39.70	39.95	39.25	35.10	33.60	38.70	39.25	38.30	34.20	32.00	38.65	43.10	41.60	31.45	30.65
6	38.75	45.90	68.15	38.30	36.00	36.90	40.05	42.70	29.40	26.95	36.40	52.60	31.85	28.35	28.60
7	37.75	41.75	37.55	31.80	31.20	32.80	36.20	38.90	28.50	28.15	35.80	35.70	37.40	28.95	25.45
8	39.50	39.50	39.50	32.00	39.50	37.70	38.55	30.70	32.15	30.20	37.20	37.60	32.45	32.25	30.20
9	31.40	31.40	31.40	21.20	31.40	32.20	34.05	30.70	26.85	24.35	33.85	31.55	29.25	27.30	25.70
10	29.10	29.10	29.10	35.40	29.10	28.15	37.30	36.35	30.55	31.80	31.50	37.05	39.60	30.90	28.50
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	36.15	37.45	39.94	32.36	33.07	35.52	37.28	35.72	30.04	28.90	34.98	38.12	35.74	30.28	28.27
SD	3.48	4.91	10.91	4.62	3.08	3.45	2.09	4.38	2.59	2.60	2.25	5.91	4.39	1.70	2.03

Table B2.59 Study 2 Control subject [HCO_3^-] ($mmol.L^{-1}$) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	26.20	19.25	15.90	14.10	13.30	25.90	20.50	15.90	14.45	14.15	25.35	20.75	16.60	14.15	13.30
2	25.80	24.95	18.80	16.60	16.75	26.80	26.45	21.05	17.50	17.00	23.90	24.05	18.65	17.65	17.00
3	23.60	22.20	15.55	12.85	12.50	24.10	23.40	16.20	13.55	13.60	24.60	22.70	14.80	12.80	12.45
4	25.70	22.25	15.40	13.55	12.40	25.70	24.35	17.25	13.85	13.40	24.45	21.45	14.50	12.05	11.30
5	25.05	21.90	13.25	11.55	10.90	26.05	21.95	14.00	12.10	11.50	26.15	23.50	15.50	11.60	11.20
6	24.4	20.90	15.00	12.60	12.10	24.95	23.85	16.70	12.55	11.95	25.25	23.45	15.55	12.90	12.15
7	25.15	23.20	19.15	13.50	12.65	25.85	24.30	19.05	15.15	14.65	24.70	23.65	17.80	14.25	13.30
8	26.00	22.25	15.30	13.70	13.70	26.30	23.45	16.80	13.70	13.35	25.80	22.80	16.25	13.65	13.20
9	26.05	21.15	15.95	13.70	12.70	26.30	20.95	15.30	13.00	12.25	27.95	23.10	16.60	14.00	13.15
10	24.30	21.30	13.95	11.20	10.60	25.75	20.70	13.55	11.50	10.80	25.35	22.40	15.00	12.35	11.60
11	24.50	23.20	19.20	14.85	14.30	22.70	21.30	15.40	13.95	13.70	24.30	21.50	17.75	14.20	14.55
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	25.16	22.05	16.13	13.47	12.90	25.49	22.84	16.47	13.75	13.30	25.25	22.67	16.27	13.60	13.02
SD	0.86	1.47	2.04	1.49	1.67	1.17	1.90	2.14	1.62	1.70	1.12	1.05	1.36	1.64	1.67

Table B2.60 Study 2 LHTLc subject [HCO_3^-] ($mmol.L^{-1}$) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	23.80	22.10	16.65	11.90	11.40	23.50	21.60	15.15	12.65	12.20	22.95	21.95	16.25	13.10	12.80
2	25.10	23.35	18.45	17.05	16.80	25.20	24.15	19.90	18.50	17.35	25.30	25.15	18.60	16.80	17.10
3	25.20	21.80	16.15	14.65	15.00	23.90	17.70	15.10	13.75	13.55	25.45	23.45	16.65	13.50	13.30
4	23.00	21.30	15.35	13.80	13.90	23.65	22.25	15.55	14.40	14.40	23.25	21.60	14.60	12.65	12.60
5	24.95	22.25	16.20	13.60	14.05	25.50	23.20	17.20	15.10	14.75	23.75	22.10	15.00	13.30	12.75
6	25.15	24.50	17.45	16.30	16.55	25.10	22.23	18.05	15.60	15.45	24.05	22.72	16.75	15.40	15.15
7	22.85	21.10	15.75	14.50	14.50	22.25	23.15	15.80	13.50	12.65	23.20	23.70	17.60	14.15	13.55
8	25.10	24.15	17.50	15.65	15.20	23.90	23.15	19.95	14.65	14.15	23.55	24.00	16.85	14.50	14.50
9	25.10	24.30	18.00	14.80	13.60	23.85	24.55	19.00	13.75	12.95	25.00	24.60	17.00	14.40	13.35
10	25.35	20.95	16.90	15.00	14.60	24.05	20.65	17.25	15.00	14.45	24.50	19.30	18.05	14.40	13.70
11	24.05	21.75	16.55	15.05	15.35	25.15	22.90	17.00	14.80	14.50	24.95	22.10	17.40	15.65	15.05
12	24.25	21.25	16.25	12.90	12.35	24.90	21.20	16.90	14.65	14.00	25.55	21.95	16.50	14.40	13.80
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	24.49	22.40	16.77	14.60	14.44	24.25	22.23	17.24	14.70	14.20	24.29	22.72	16.77	14.35	13.97
SD	0.89	1.32	0.93	1.42	1.55	0.94	1.82	1.71	1.45	1.36	0.95	1.58	1.14	1.17	1.30

Table B2.61 Study 2 LHTLi subject [HCO_3^-] ($mmol.L^{-1}$) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	24.10	21.10	16.20	12.70	11.60	22.55	19.10	13.55	11.45	10.85	24.55	21.75	14.95	11.35	10.55
2	25.40	23.55	17.50	15.20	14.88	25.30	23.55	19.00	15.05	14.25	25.00	23.35	18.10	14.95	14.10
3	25.35	24.30	18.65	16.25	16.55	25.45	23.90	17.75	15.95	16.35	25.05	23.85	19.20	15.50	15.20
4	25.55	23.45	16.85	14.15	15.45	25.05	22.35	15.10	13.15	13.10	23.55	23.05	15.25	13.25	13.25
5	27.00	26.00	19.35	18.05	18.20	25.55	25.00	18.40	17.20	17.35	25.05	21.70	17.95	16.40	16.15
6	25.35	24.80	18.50	14.30	14.10	23.60	22.90	19.20	12.15	11.55	24.20	19.90	14.85	13.15	13.40
7	26.30	22.45	16.25	13.50	13.50	24.55	22.45	14.80	11.40	11.15	23.95	18.80	16.30	12.10	11.55
8	24.90	24.90	16.85	14.80	15.30	25.15	23.90	15.50	14.00	13.90	26.05	25.35	16.60	15.45	14.85
9	23.20	20.80	16.20	12.40	13.45	23.15	21.65	15.55	13.05	12.75	23.60	22.35	16.75	14.35	13.90
10	26.85	24.40	17.30	15.45	15.75	26.55	23.90	16.75	15.30	15.20	25.35	24.70	18.45	15.30	14.90
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	25.40	23.58	17.37	14.68	14.88	24.69	22.87	16.56	13.87	13.65	24.64	22.48	16.84	14.18	13.79
SD	1.17	1.68	1.12	1.69	1.84	1.23	1.64	1.95	1.97	2.20	0.81	2.04	1.54	1.65	1.70

Table B2.62 Study 2 Control subject pH (arbitrary units) (Chapter 5).

Con			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	7.48	7.23	7.23	7.20	7.22	7.42	7.26	7.26	7.23	7.22	7.41	7.26	7.25	7.21	7.21
2	7.42	7.44	7.34	7.30	7.30	7.42	7.43	7.36	7.29	7.29	7.40	7.42	7.32	7.32	7.28
3	7.40	7.38	7.23	7.18	7.18	7.40	7.41	7.25	7.19	7.21	7.43	7.37	7.21	7.17	7.17
4	7.41	7.35	7.16	7.17	7.15	7.42	7.41	7.16	7.17	7.16	7.39	7.31	7.12	7.10	7.10
5	7.44	7.35	7.18	7.14	7.12	7.44	7.38	7.22	7.16	7.15	7.43	7.40	7.23	7.15	7.13
6	7.43	7.28	7.23	7.18	7.18	7.43	7.43	7.27	7.19	7.17	7.43	7.43	7.26	7.20	7.18
7	7.42	7.34	7.27	7.21	7.18	7.46	7.42	7.29	7.24	7.24	7.43	7.39	7.27	7.22	7.21
8	7.43	7.40	7.25	7.21	7.20	7.43	7.42	7.26	7.20	7.21	7.43	7.37	7.26	7.20	7.19
9	7.42	7.25	7.23	7.18	7.17	7.43	7.31	7.21	7.17	7.17	7.46	7.35	7.25	7.20	7.20
10	7.42	7.35	7.18	7.12	7.12	7.45	7.31	7.16	7.12	7.11	7.43	7.34	7.22	7.16	7.16
11	7.41	7.38	7.28	7.22	7.23	7.36	7.34	7.21	7.20	7.20	7.40	7.34	7.27	7.24	7.23
n	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
mean	7.43	7.34	7.23	7.19	7.18	7.42	7.37	7.24	7.20	7.19	7.42	7.36	7.24	7.20	7.19
SD	0.02	0.06	0.05	0.05	0.05	0.03	0.06	0.06	0.05	0.05	0.02	0.05	0.05	0.06	0.05

Table B2.63 Study 2 LHTLc subject pH (arbitrary units) (Chapter 5).

LHTLc			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	7.41	7.33	7.21	7.17	7.16	7.43	7.42	7.25	7.21	7.20	7.40	7.41	7.26	7.20	7.21
2	7.42	7.38	7.33	7.30	7.30	7.45	7.42	7.36	7.32	7.31	7.46	7.46	7.31	7.32	7.33
3	7.40	7.36	7.26	7.23	7.24	7.42	7.29	7.24	7.23	7.23	7.43	7.39	7.26	7.21	7.21
4	7.39	7.37	7.24	7.22	7.22	7.41	7.40	7.26	7.24	7.25	7.39	7.39	7.23	7.19	7.19
5	7.43	7.37	7.22	7.20	7.20	7.43	7.42	7.30	7.25	7.24	7.42	7.40	7.24	7.21	7.20
6	7.43	7.41	7.30	7.28	7.28	7.45	7.38	7.41	7.29	7.28	7.41	7.40	7.31	7.27	7.26
7	7.39	7.36	7.25	7.24	7.24	7.41	7.46	7.27	7.25	7.21	7.39	7.39	7.27	7.22	7.21
8	7.41	7.36	7.28	7.27	7.26	7.41	7.34	7.26	7.24	7.24	7.41	7.40	7.26	7.23	7.24
9	7.42	7.38	7.27	7.21	7.20	7.41	7.45	7.33	7.23	7.22	7.41	7.42	7.26	7.22	7.21
10	7.41	7.33	7.26	7.25	7.26	7.41	7.25	7.22	7.24	7.25	7.42	7.34	7.25	7.23	7.23
11	7.41	7.38	7.29	7.29	7.27	7.44	7.42	7.28	7.27	7.26	7.44	7.41	7.33	7.29	7.28
12	7.38	7.34	7.22	7.16	7.17	7.42	7.35	7.26	7.21	7.22	7.43	7.38	7.26	7.22	7.23
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	7.41	7.36	7.26	7.23	7.23	7.42	7.38	7.29	7.25	7.24	7.42	7.40	7.27	7.23	7.23
SD	0.02	0.02	0.04	0.04	0.04	0.02	0.06	0.06	0.03	0.03	0.02	0.03	0.03	0.04	0.04

Table B2.64 Study 2 LHTLi subject pH (arbitrary units) (Chapter 5).

LHTLi			Pre					d5					Post		
subject	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min	Rest	End Ex	1-min	3-min	5-min
1	7.43	7.36	7.21	7.16	7.13	7.40	7.31	7.22	7.16	7.15	7.47	7.37	7.20	7.14	7.14
2	7.44	7.44	7.30	7.27	7.24	7.42	7.42	7.31	7.26	7.24	7.44	7.41	7.29	7.24	7.23
3	7.44	7.42	7.31	7.27	7.28	7.43	7.42	7.28	7.27	7.29	7.46	7.43	7.33	7.26	7.28
4	7.44	7.39	7.27	7.23	7.25	7.43	7.40	7.25	7.22	7.22	7.43	7.42	7.26	7.21	7.22
5	7.44	7.43	7.31	7.31	7.32	7.43	7.42	7.30	7.29	7.31	7.42	7.34	7.27	7.29	7.29
6	7.42	7.38	7.18	7.20	7.20	7.41	7.38	7.30	7.18	7.17	7.42	7.26	7.25	7.22	7.23
7	7.45	7.37	7.25	7.21	7.21	7.46	7.40	7.21	7.15	7.15	7.42	7.34	7.26	7.17	7.18
8	7.41	7.43	7.28	7.24	7.25	7.43	7.41	7.27	7.22	7.23	7.45	7.44	7.32	7.26	7.26
9	7.45	7.34	7.27	7.23	7.22	7.44	7.40	7.27	7.23	7.23	7.43	7.43	7.32	7.27	7.26
10	7.53	7.40	7.27	7.24	7.27	7.54	7.42	7.27	7.27	7.26	7.48	7.43	7.29	7.26	7.27
n	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mean	7.45	7.40	7.27	7.24	7.24	7.44	7.40	7.27	7.22	7.22	7.44	7.39	7.28	7.23	7.23
SD	0.03	0.03	0.04	0.04	0.05	0.04	0.03	0.03	0.05	0.06	0.02	0.06	0.04	0.05	0.05

APPENDIX B3 SUBJECT PERFORMANCE AND $\dot{V}O_2$ DATA

Table B3.1 Study 1 Control subject submaximal and peak $\dot{V}O_2$ ($L.min^{-1}$) (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
CON	1.5	2.5	3.5	4.5	peak
1	1.68	2.41	3.08	3.89	5.32
2	2.07	2.90	3.75	4.58	5.37
3	2.05	2.65	3.62	4.35	4.74
4	1.50	2.19	2.95	3.75	4.33
5	1.83	2.52	3.36	4.16	4.66
6	1.86	2.72	3.48	4.40	5.53
7	1.45	2.31	3.35	4.14	4.72
n	7	7	7	7	7
mean	1.78	2.53	3.37	4.18	4.95
sd	0.25	0.25	0.28	0.29	0.45
sem	0.09	0.09	0.11	0.11	0.17
Mid					
1	1.83	2.55	3.40	4.33	5.27
2	2.08	2.89	3.77	4.51	5.22
3	2.00	2.75	3.50	4.24	4.79
4	1.58	2.16	2.88	3.56	4.16
5	1.79	2.39	3.22	3.89	4.85
6	1.99	2.75	3.55	4.53	5.62
7	1.48	2.26	3.23	4.08	4.72
n	7	7	7	7	7
mean	1.82	2.54	3.36	4.16	4.95
sd	0.22	0.28	0.29	0.35	0.47
sem	0.08	0.10	0.11	0.13	0.18
Post					
1	1.70	2.48	3.42	4.12	4.95
2	2.00	2.84	3.57	4.46	5.35
3	1.96	2.76	3.51	4.20	4.73
4	1.71	2.25	2.96	3.63	4.10
5	1.65	2.38	3.17	3.88	4.81
6	1.88	2.66	3.58	4.46	5.41
7	1.57	2.33	3.22	4.13	4.72
n	7	7	7	7	7
mean	1.78	2.53	3.35	4.13	4.87
sd	0.16	0.23	0.24	0.30	0.44
sem	0.06	0.09	0.09	0.11	0.17

Table B3. 2 Study 1 LHTL subject submaximal and peak VO_2 ($L.min^{-1}$) (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
LHTL	1.5	2.5	3.5	4.5	peak
1	1.55	2.37	3.16	4.03	4.88
2	2.10	2.90	3.74	4.53	5.53
3	2.24	3.18	4.13	4.97	5.46
4	1.74	2.52	3.22	4.03	5.03
5	1.82	2.57	3.31	4.01	4.88
6	1.87	2.59	3.36	3.91	4.71
n	6	6	6	6	6
mean	1.89	2.69	3.49	4.25	5.08
sd	0.25	0.30	0.38	0.42	0.34
sem	0.10	0.12	0.15	0.17	0.14
Mid					
1	1.48	2.22	3.02	3.74	4.61
2	2.09	2.90	3.68	4.57	5.44
3	2.12	3.05	3.84	4.75	5.16
4	1.72	2.54	3.22	4.10	4.86
5	1.74	2.40	3.13	3.77	4.68
6	1.88	2.50	3.20	3.87	4.64
n	6	6	6	6	6
mean	1.84	2.60	3.35	4.13	4.90
sd	0.24	0.31	0.33	0.43	0.33
sem	0.10	0.13	0.13	0.18	0.14
Post					
1	1.38	2.1	2.96	3.72	4.45
2	2.10	2.87	3.69	4.58	5.34
3	2.07	3.02	3.62	4.01	4.65
4	1.6	2.44	3.17	3.89	4.74
5	1.72	2.43	3.25	3.91	4.64
6	1.84	2.46	3.21	3.89	4.45
n	6	6	6	6	6
mean	1.79	2.55	3.32	4.00	4.71
sd	0.28	0.33	0.28	0.30	0.33
sem	0.11	0.14	0.11	0.12	0.13

Table B3. 3 Study 1 Control subject submaximal and peak $\dot{V}_E$ ($L.min^{-1}$) (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
CON	1.5	2.5	3.5	4.5	peak
1	40.82	59.04	76.07	106.71	179.09
2	54.42	74.09	92.98	124.92	187.42
3	54.70	65.40	97.87	136.42	160.99
4	33.91	50.03	71.60	103.89	140.77
5	40.69	56.90	85.80	123.21	174.98
6	47.53	69.54	82.67	116.17	195.35
7	39.91	56.85	87.52	122.32	189.88
N	7	7	7	7	7
Mean	44.57	61.69	84.93	119.09	175.50
sd	7.88	8.35	9.14	11.21	19.03
sem	2.98	3.16	3.45	4.24	7.19
Mid					
1	40.22	57.40	77.01	106.91	186.50
2	53.86	76.70	103.04	124.01	187.20
3	47.98	73.48	95.61	150.22	195.52
4	37.50	54.30	75.20	99.82	142.58
5	43.44	57.79	87.12	116.37	194.81
6	50.39	69.91	88.49	123.49	206.63
7	41.41	58.66	89.16	129.39	195.98
n	7	7	7	7	7
mean	44.97	64.03	87.95	121.46	187.03
sd	5.93	9.04	9.76	16.36	20.71
sem	2.24	3.42	3.69	6.18	7.83
Post					
1	39.00	56.93	82.85	111.91	176.00
2	52.59	74.44	93.66	126.47	198.75
3	46.27	66.04	95.13	136.74	188.92
4	38.78	56.07	74.94	102.12	145.95
5	39.15	58.99	79.98	105.74	185.11
6	48.94	65.15	93.11	128.09	193.85
7	40.17	60.66	85.06	140.80	194.72
n	7	7	7	7	7
mean	43.56	62.61	86.39	121.70	183.33
sd	5.66	6.46	7.76	15.21	18.09
sem	2.14	2.44	2.93	5.75	6.84

Table B3. 4 Study 1 LHTL subject submaximal and peak V_E ($L.min^{-1}$) (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
LHTL	1.5	2.5	3.5	4.5	peak
1	40.10	56.01	77.22	132.50	193.57
2	42.15	62.89	84.88	116.42	209.08
3	46.41	68.17	101.41	134.42	167.21
4	39.78	56.82	77.20	109.42	189.80
5	38.33	58.89	81.81	107.49	199.26
6	43.47	55.89	78.39	97.00	176.98
n	6	6	6	6	6
mean	41.71	59.78	83.49	116.21	189.32
sd	2.94	4.88	9.28	14.75	15.16
sem	1.20	1.99	3.79	6.02	6.19
Mid					
1	41.85	57.30	85.65	129.92	206.84
2	50.06	72.71	95.68	127.38	220.89
3	49.99	71.69	95.53	134.26	171.65
4	39.26	59.76	86.02	124.41	193.34
5	41.36	58.94	76.40	110.73	201.87
6	52.92	67.20	91.73	115.78	192.16
n	6	6	6	6	6
mean	45.91	64.60	88.50	123.75	197.79
sd	5.73	6.81	7.38	8.89	16.53
sem	2.34	2.78	3.01	3.63	6.75
Post					
1	36.64	55.06	89.17	140.21	205.6
2	42.77	65.17	86.35	126.57	221.82
3	50.76	74.46	95.34	131.64	150.79
4	37.87	57.39	84.74	118.28	191.42
5	39.58	57.08	77.53	110.21	196.93
6	48.17	68.11	90.83	122.54	190.11
n	6	6	6	6	6
mean	42.63	62.88	87.33	124.91	192.78
sd	5.74	7.64	6.06	10.47	23.65
sem	2.34	3.12	2.47	4.27	9.66

Table B3. 5 Study 1 CON subject submaximal and peak RER (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
CON	1.5	2.5	3.5	4.5	peak
1	0.87	0.92	0.93	0.98	1.11
2	0.91	0.93	0.98	1.04	1.14
3	0.94	0.90	0.97	1.03	1.04
4	0.81	0.88	0.92	1.02	1.10
5	0.78	0.83	0.89	0.97	1.13
6	0.91	0.94	0.93	1.00	1.14
7	0.92	0.93	0.99	1.02	1.15
n	7	7	7	7	7
mean	0.88	0.90	0.94	1.01	1.11
sd	0.06	0.04	0.04	0.02	0.04
sem	0.02	0.02	0.01	0.01	0.01
Mid					
1	0.83	0.88	0.89	0.94	1.10
2	0.87	0.91	0.96	1.01	1.18
3	0.87	0.94	0.98	1.08	1.16
4	0.88	0.93	0.98	1.06	1.12
5	0.88	0.91	0.97	1.03	1.18
6	0.91	0.92	0.93	0.99	1.17
7	0.95	0.95	1.01	1.07	1.14
n	7	7	7	7	7
mean	0.88	0.92	0.96	1.02	1.15
sd	0.04	0.02	0.04	0.05	0.03
sem	0.01	0.01	0.01	0.02	0.01
Post					
1	0.90	0.94	0.97	1.04	1.14
2	0.86	0.91	0.96	1.03	1.16
3	0.85	0.89	0.94	1.03	1.16
4	0.81	0.90	0.95	1.06	1.13
5	0.87	0.92	0.95	1.02	1.19
6	0.94	0.96	0.97	1.03	1.18
7	0.83	0.90	0.96	1.05	1.09
n	7	7	7	7	7
mean	0.86	0.92	0.95	1.03	1.15
sd	0.04	0.02	0.01	0.01	0.03
sem	0.02	0.01	0.00	0.01	0.01

Table B3. 6 Study 1 LHTL subject submaximal and peak RER (Chapters 3 and 4).

Pre	Exercise Workrate ($W.kg^{-1}$)				
LHTL	1.5	2.5	3.5	4.5	peak
1	0.81	0.81	0.84	0.94	1.03
2	0.79	0.89	0.91	0.99	1.19
3	0.78	0.84	0.92	0.98	0.99
4	0.79	0.86	0.93	1.02	1.16
5	0.84	0.91	0.97	1.02	1.18
6	0.81	0.82	0.89	0.96	1.12
n	6	6	6	6	6
mean	0.80	0.85	0.91	0.98	1.11
sd	0.02	0.04	0.04	0.03	0.08
sem	0.01	0.02	0.02	0.01	0.03
Mid					
1	0.89	0.86	0.92	1.00	1.15
2	0.86	0.92	0.93	0.98	1.16
3	0.86	0.91	0.95	1.02	1.06
4	0.78	0.84	0.92	1.00	1.18
5	0.86	0.93	0.96	1.05	1.20
6	0.86	0.88	0.93	0.97	1.12
n	6	6	6	6	6
mean	0.85	0.89	0.94	1.00	1.14
sd	0.04	0.03	0.02	0.03	0.05
sem	0.02	0.01	0.01	0.01	0.02
Post					
1	0.84	0.86	0.925	1.005	1.12
2	0.80	0.87	0.895	0.97	1.17
3	0.9	0.94	0.99	1.07	1.055
4	0.835	0.86	0.935	1.015	1.19
5	0.845	0.885	0.915	1	1.19
6	0.805	0.875	0.9	0.98	1.1
n	6	6	6	6	6
mean	0.84	0.88	0.93	1.01	1.14
sd	0.04	0.03	0.03	0.04	0.05
sem	0.01	0.01	0.01	0.01	0.02

Table B3. 7 Study 1 CON subject submaximal and peak HR (beats.min^{-1}) (Chapters 3 and 4).

Pre	Exercise Workrate (W.kg^{-1})				
CON	1.5	2.5	3.5	4.5	peak
1	109	129	148	163	181
2	105	125	144	166	184
3	132	146	174	185	191
4	102	126	148	180	204
5	106	120	135	155	181
6	108	122	140	161	186
7	106	122	147	170	195
n	7	7	7	7	7
mean	110	127	148	168	189
sd	10	9	12	10	8
sem	4	3	5	4	3
Mid					
1	111	132	145	162	183
2	102	120	143	162	183
3	116	147	167	182	192
4	102	124	156	186	205
5	101	119	134	152	185
6	109	125	140	154	179
7	108	127	153	172	195
n	7	7	7	7	7
mean	107	128	148	167	189
sd	6	10	11	13	9
sem	2	4	4	5	3
Post					
1	115	134	153	164	182
2	108	127	150	166	184
3	125	144	167	181	192
4	116	137	166	192	210
5	103	116	135	154	184
6	117	131	149	165	187
7	113	137	156	172	190
n	7	7	7	7	7
mean	114	132	154	170	190
sd	7	9	11	12	9
sem	3	3	4	5	4

Table B3. 8 Study 1 LHTL subject submaximal and peak HR (beats.min^{-1}) (Chapters 3 and 4).

Pre	Exercise Workrate (W.kg^{-1})				
LHTL	1.5	2.5	3.5	4.5	peak
1	100	119	135	155	173
2	110	130	150	166	184
3	115	134	157	178	184
4	95	117	139	158	180
5	101	116	135	153	180
6	124	144	163	181	200
n	6	6	6	6	6
mean	108	127	146	165	183
sd	11	11	12	12	9
sem	4	5	5	5	4
Mid					
1	103	119	140	157	178
2	109	128	148	166	180
3	106	129	157	179	190
4	88	108	135	160	182
5	91	106	129	155	183
6	103	124	136	163	195
n	6	6	6	6	6
mean	100	119	141	163	185
sd	9	10	10	9	6
sem	3	4	4	3	3
Post					
1	93	120	138	159	177
2	105	124	144	161	176
3	101	130	159	179	189
4	104	119	140	163	186
5	102	116	139	161	181
6	104	126	156	173	190
n	6	6	6	6	6
mean	101	122	146	166	183
sd	4	5	9	8	6
sem	2	2	4	3	2

Table B3. 9 Study 1 CON subject submaximal and peak work (kJ) (Chapters 3 and 4).

CON	Pre	Mid	Post
1	55.0	55.0	52.4
2	53.6	53.6	55.4
3	45.2	45.2	48.8
4	42.1	42.1	40.9
5	48.6	48.6	51.6
6	59.4	59.4	57.9
7	49.4	49.4	45.4
n	7	7	7
mean	50.5	50.5	50.3
sd	6.0	6.0	5.8
sem	2.2	2.2	2.2

Table B3. 10 Study 1 LHTL subject submaximal and peak work (kJ) (Chapters 3 and 4).

LHTL	Pre	Mid	Post
1	47.3	50.9	48.3
2	56.7	55.4	54.0
3	49.2	50.8	49.2
4	52.8	54.4	53.1
5	49.3	49.8	48.6
6	44.7	44.4	42.1
n	6	6	6
mean	50.0	51.0	49.2
sd	4.2	3.9	4.2
sem	1.7	1.6	1.7

Table B3. 11 Study 3 subject and $\dot{V}O_{2peak}$ (ml.kg^{-1}.min-1) (Chapter 6).

subject	Baseline	Pre	Post
1			
2	66.5	64.2	
3	64.0	67.0	64.6
4	61.8	60.9	66.4
5			
6	73.4	69.4	73.7
7	58.3		
8		64.9	64.5
9	66.3	66.3	66.4
10	62.6		
11	61.9	59.5	59.0
12		60.7	62.5
n	8	7	7
mean	64.4	64.1	65.3
SD	4.5	3.8	4.5

Table B3. 12 Study 3 subject peak power output (W) (Chapter 6).

subject	Baseline	Pre	Post
1	359.0		
2	393.0		
3	385.0	385.0	377.0
4	353.0	353.0	352.0
5			
6	422.0	422.0	438.0
7	325.0	325.0	337.0
8	355.0	355.0	380.0
9		415.0	435.0
10			
11	335.0	335.0	343.0
12	365.0	365.0	370.0
n	9	8	8
mean	365.8	369.4	379.0
SD	30.0	35.3	38.7

APPENDIX B4 MUSCLE DATA.

Table B4.1 Study 1 Control subject metabolites (Chapters 3 and 4).

Pre	ATP		PCr		Cr		Glycogen		Lactate	
	mmol kg dm^{-1}		mmol kg dm^{-1}		mmol kg dm^{-1}		mmol glucosyl units kg dm^{-1}		mmol kg dm^{-1}	
CON	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex
1	27.2	17.9	82.4	62.4	46.7	73.4	630.7	229.1	5.7	47.4
2	27.4	17.6	84.2	62.7	44.5	73.5	601.4	259.7	5.2	45.0
3	26.5	17.6	85.0	62.0	46.5	74.0	595.2	245.7	4.4	47.1
4	25.9	18.2	85.7	63.4	43.7	74.1	612.8	286.6	4.5	45.3
5	26.8	17.8	83.1	61.9	46.1	72.9	623.3	229.7	5.5	52.0
6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
7	28.4	18.1	83.1	63.0	42.1	72.9	603.2	209.5	4.3	48.1
n	6	6	6	6	6	6	6	6	6	6
mean	27.0	17.9	83.9	62.6	44.9	73.5	611.1	243.4	4.9	47.5
sd	0.9	0.3	1.3	0.6	1.8	0.5	13.7	27.1	0.6	2.5
sem	0.3	0.1	0.5	0.2	0.7	0.2	5.6	11.1	0.3	1.0
Post										
1	27.2	18.0	83.4	62.4	46.9	72.7	613.8	236.3	5.1	46.1
2	27.6	17.7	85.4	62.9	46.0	72.4	596.5	259.9	5.2	52.3
3	26.4	17.3	84.7	61.5	46.2	75.1	601.1	242.7	4.4	45.9
4	26.7	18.4	86.4	64.8	43.9	74.3	n/a	301.3	5.1	43.9
5	26.9	17.9	82.1	62.7	45.7	74.0	600.2	197.6	5.2	50.6
6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
7	28.6	18.0	84.1	62.4	42.5	72.1	585.4	200.9	4.1	50.6
n	6	6	6	6	6	6	5	6	6	6
mean	27.2	17.9	84.4	62.8	45.2	73.4	599.4	239.8	4.9	48.2
sd	0.8	0.4	1.5	1.1	1.7	1.2	10.2	38.7	0.5	3.4
sem	0.3	0.1	0.6	0.4	0.7	0.5	4.6	15.8	0.2	1.4

Table B4.2 Study 1 LHTL subject metabolites (Chapters 3 and 4).

Pre	ATP		PCr		Cr		Glycogen		Lactate	
	mmol kg dm^{-1}		mmol kg dm^{-1}		mmol kg dm^{-1}		mmol glucosyl units kg dm^{-1}		mmol kg dm^{-1}	
LHTL	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex
1	28.3	18.5	84.5	62.4	46.1	73.4	645.4	339.3	4.2	33.9
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	26.1	17.5	82.3	61.5	42.4	71.2	615.0	190.6	3.7	48.7
4	26.3	18.1	82.9	63.7	44.9	72.7	612.4	249.8	5.4	47.2
5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	27.2	17.7	81.9	61.9	45.7	73.5	430.1	200.6	10.1	48.2
n	4	4	4	4	4	4	4	4	4	4
mean	27.0	18.0	82.9	62.4	44.8	72.7	575.7	245.1	5.9	44.5
sd	1.0	0.4	1.1	1.0	1.7	1.1	98.2	67.9	2.9	7.1
sem	0.5	0.2	0.6	0.5	0.8	0.5	49.1	34.0	1.5	3.6
Post										
1	27.8	18.4	84.0	62.8	45.8	73.6	634.8	317.4	6.9	22.1
2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	26.2	17.4	82.4	61.9	43.1	71.5	600.3	181.9	3.8	46.4
4	26.5	18.4	81.8	63.0	44.7	73.5	595.2	258.3	4.2	43.4
5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	27.0	17.9	82.0	60.7	45.2	72.9	455.4	232.4	9.2	48.4
n	4	4	4	4	4	4	4	4	4	4
mean	26.9	18.0	82.6	62.1	44.7	72.9	571.4	247.5	6.0	40.1
sd	0.7	0.5	1.0	1.0	1.2	1.0	79.3	56.4	2.5	12.1
sem	0.3	0.2	0.5	0.5	0.6	0.5	39.7	28.2	1.3	6.1

Table B4.3 Study 1 Control subject protein and acid-base (Chapters 3 and 4).

Pre	Protein mg (mg muscle^{-1})		$[H^+]$ nmol.L^{-1}		β in-vitro μmol H^+ g dm^{-1} pH^{-1}		β in-vivo ($\Delta[H^+]/\Delta[Lac^-]$)
CON	Rest	Ex	Rest	Ex	Rest	Ex	Rest
1	0.17	0.17	66.1	163.0	144.3	139.4	106.5
2	0.17	0.17	73.0	183.3	134.7	137.5	99.4
3	0.14	0.15	62.4	132.2	175.8	163.6	131.1
4	0.18	0.18	74.3	190.7	130.9	123.0	99.7
5	0.18	0.18	71.6	151.4	139.2	145.9	142.8
6	0.17	0.17	65.3	141.0	150.8	151.7	n/a
7	0.18	0.17	70.2	174.7	143.8	141.0	112.9
n	7	7	7	7	7	7	6
mean	0.17	0.17	69.0	162.3	145.6	143.2	115.4
sd	0.01	0.01	4.4	21.9	14.8	12.6	17.8
sem	0.00	0.00	1.7	8.3	5.6	4.8	7.3
Post							
1	0.18	0.18	67.3	144.3	138.2	140.1	123.6
2	0.17	0.17	64.7	148.3	147.1	145.8	130.8
3	0.14	0.14	60.4	180.0	161.9	131.3	87.4
4	0.18	0.18	75.9	140.4	133.8	137.5	145.5
5	0.17	0.18	63.4	122.5	146.6	150.9	158.7
6	0.17	0.17	65.5	120.6	147.1	152.7	n/a
7	0.17	0.17	67.5	127.4	145.2	146.8	172.8
n	6	6	7	7	7	7	6
mean	0.17	0.17	66.4	140.5	145.7	143.6	136.5
sd	0.01	0.01	4.8	20.5	8.8	7.7	30.0
sem	0.01	0.01	1.8	7.8	3.3	2.9	12.2

Table B4.4 Study 1 LHTL subject protein and acid-base (Chapters 3 and 4).

Pre	Protein mg (mg muscle^{-1})		$[H^+]$ nmol.L^{-1}		β in-vitro μmol H^+ g dm^{-1} pH^{-1}		β in-vivo ($\Delta[H^+]/\Delta[Lac^-]$)
LHTL	Rest	Ex	Rest	Ex	Rest	Ex	Rest
1	0.18	0.18	70.8	134.7	154.4	165.8	84.4
2	0.18	0.18	67.2	143.6	150.4	157.3	n/a
3	0.18	0.18	72.3	191.1	132.5	130.1	101.3
4	0.17	0.16	75.7	144.3	119.5	122.5	149.1
5	0.18	0.18	70.8	134.7	143.7	147.9	n/a
6	0.17	0.17	74.3	179.2	130.1	139.7	87.4
n	5	6	6	6	6	6	4
mean	0.18	0.18	71.9	154.6	138.4	143.9	105.6
sd	0.01	0.01	3.0	24.3	13.3	16.4	30.0
Post							
1	0.18	0.18	61.0	126.3	183.4	183.2	55.8
2	0.18	0.18	67.2	128.0	163.3	176.8	n/a
3	0.17	0.18	72.3	151.8	154.0	166.8	136.6
4	0.16	0.16	75.2	144.3	144.8	137.1	138.4
5	0.18	0.18	61.0	126.3	174.5	172.0	n/a
6	0.16	0.16	64.7	172.3	156.3	149.1	88.0
n	4	6	6	6	6	6	4
mean	0.17	0.17	66.9	141.5	162.7	164.2	104.7
sd	0.01	0.01	5.9	18.5	14.2	17.6	40.1

Table B4.5 Study 1 Control subject Na^+,K^+ATPase activity (nmol (g wt weight)$^{-1}$ and nmol.min^{-1} (g protein)$^{-1}$) and content (pmol (g wt weight)$^{-1}$ and pmol.min^{-1} (g protein)$^{-1}$) (Chapters 3 and 4).

Pre	Na^+,K^+ATPase Activity		Na^+,K^+ATPase Activity		Na^+,K^+ATPase Content		Na^+,K^+ATPase Content	
CON	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Rest
1	283	243	1634	1397	330.7	n/a	1911.6	n/a
2	248	201	1459	1198	281.1	n/a	1653.5	n/a
3	280	246	1955	1674	n/a	n/a	n/a	n/a
4	300	262	1713	1470	268.9	n/a	1536.6	n/a
5	309	274	1744	1550	n/a	378.4	n/a	2137.9
6	285	248	1678	1436	284.4	310.7	1672.9	1796.2
7	286	252	1614	1446	367.8	n/a	2143.3	n/a
n	7	7	7	7	5	2	5	2
mean	284	247	1685	1453	307	345	1784	1967
sd	19	23	151	146	42	48	243	242
Post								
1	281	238	1607	1369	287.9	288.7	1645.1	1659.2
2	253	201	1505	1158	278.5	n/a	1657.7	n/a
3	280	247	2002	1755	406.9	519.5	2906.2	3684.3
4	299	266	1683	1509	n/a	n/a	n/a	n/a
5	305	273	1753	1544	359.4	303.7	2065.5	1715.8
6	281	252	1626	1438	361.0	285.2	2086.9	1626.8
7	281	254	1633	1496	n/a	n/a	n/a	n/a
n	7	7	7	7	5	4	5	4
mean	283	247	1687	1467	339	349	2072	2172
sd	17	23	158	181	54	114	512	1009

Table B4.6 Study 1 LHTL subject Na^+,K^+ATPase activity (nmol (g wt weight)$^{-1}$ and nmol.min^{-1} (g protein)$^{-1}$) and content (pmol (g wt weight)$^{-1}$ and pmol.min^{-1} (g protein)$^{-1}$) (Chapters 3 and 4).

Pre	Na^+,K^+ATPase Activity		Na^+,K^+ATPase Activity		Na^+,K^+ATPase Content		Na^+,K^+ATPase Content	
LHTL	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Rest
1	291.0	236.0	1580.0	1299.0	315.0	n/a	1711.0	n/a
2	268.0	228.0	1480.0	1331.0	322.0	312.0	1777.0	1820.0
3	254.0	238.0	1458.0	1361.0	246.0	n/a	1409.0	n/a
4	312.0	268.0	1888.0	1673.0	307.0	n/a	1861.0	n/a
5	300.0	265.0	1649.0	1455.0	353.0	n/a	1942.0	n/a
6	268.0	251.0	1577.0	1523.0	401.0	396.0	2357.0	2397.0
n	6	6	6	6	6	2	6	2
mean	282.0	248.0	1606.0	1440.0	324.0	354.0	1843.0	2109.0
sd	22.0	16.0	155.0	141.0	51.0	59.0	311.0	408.0
Post								
1	276.0	224.0	1503.0	1238.0	263.0	328.0	1433.0	1813.0
2	259.0	220.0	1445.0	1227.0	308.0	339.0	1718.0	1894.0
3	251.0	235.0	1441.0	1325.0	249.0	234.0	1431.0	1321.0
4	293.0	263.0	1840.0	1644.0	318.0	n/a	2002.0	n/a
5	302.0	259.0	1664.0	1421.0	351.0	432.0	1935.0	2375.0
6	264.0	253.0	1594.0	1545.0	329.0	370.0	1987.0	2254.0
n	6	6	6	6	6	5	6	5
mean	274.0	242.0	1581.0	1400.0	303.0	341.0	1751.0	1931.0
sd	20.0	19.0	154.0	169.0	39.0	72.0	267.0	415.0

Table B4.7 Study 2 Na^+,K^+ATPase content (pmol.(g wet wt.)$^{-1}$) (Chapter 5).

	Pre		d5		Post		**LHTLc**	Pre		d5		Post		**LHTLi**	Pre		d5		Post	
Con	Rest	End Ex	Rest	End Ex	Rest	End Ex	subject	Rest	End Ex	Rest	End Ex	Rest	End Ex	subject	Rest	End Ex	Rest	End Ex	Rest	End Ex
1	265.35	365.8	323.21	n/a	N/a	n/a	1	344	327.2	353.5	n/a	356.9	n/a	1	303.3	308.2	288.3	289.6	311.6	315.5
2	n/a	n/a	n/a	n/a	N/a	n/a	2	n/a	n/a	n/a	n/a	n/a	n/a	2	n/a	n/a	n/a	n/a	n/a	n/a
3	n/a	n/a	n/a	n/a	N/a	n/a	3	n/a	n/a	n/a	n/a	n/a	n/a	3	n/a	n/a	n/a	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	N/a	n/a	4	n/a	n/a	n/a	n/a	n/a	n/a	4	279.7	293.7	293.1	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	N/a	n/a	5	278.3	304.9	326.3	n/a	n/a	n/a	5	n/a	n/a	n/a	n/a	n/a	n/a
6	301.46	272.87	297.99	303.34	309.35	296.05	6	326.7	320.7	n/a	n/a	293.9	n/a	6	300.8	307.7	298.2	293.1	324.5	327.1
7	295.67	254.99	279.18	292.19	290.96	282.28	7	353.1	371	374	372.5	375.1	360	7	331.7	298.8	305.3	294.8	268.9	289.1
8	299.66	309.32	323.05	313.73	312.72	309.76	8	271.5	n/a	309.2	n/a	n/a	n/a	8	332.4	336.1	n/a	n/a	334.0	n/a
9	n/a	n/a	n/a	n/a	N/a	n/a	9	296.7	359.4	n/a	382.8	311.8	318.4	9	359.2	n/a	n/a	n/a	358.3	n/a
10	301.38	319.05	n/a	n/a	292.07	n/a	10	278.5	275.4	n/a	n/a	292.1	n/a	10	374.9	381.3	n/a	350.5	382.1	357.7
11	377.13	386.27	359.53	362.82	408.72	382.6	11	398.3	388.1	378	n/a	395.3	n/a							
							12	335.5	377	368.8	n/a	n/a	n/a							
n	6	6	5	4	5	4	n	9	8	6	2	6	2	n	7.0	6.0	4.0	4.0	6.0	4.0
mean	306.78	318.05	316.59	318.02	322.76	317.67	mean	320.3	340.5	351.6	377.6	337.5	339.2	mean	326.0	321.0	296.2	307.0	329.9	322.3
SD	37.14	51.05	30.31	31.13	49.05	44.71	SD	42.5	39.6	28.1	7.3	44.2	29.4	SD	33.8	33.0	7.3	29.1	39.1	28.4

Table B4.8 Study 2 maximal Na^+,K^+ATPase activity ($nmol.min^{-1}.(g\ wt\ weight)^{-1}$) (Chapter 5).

Con	Pre		d5		Post		**LHTLc**	Pre		d5		Post		**LHTLi**	Pre		d5		Post	
	Rest	End Ex	Rest	End Ex	Rest	End Ex	subject	Rest	End Ex	Rest	End Ex	Rest	End Ex	subject	Rest	End Ex	Rest	End Ex	Rest	End Ex
1	291.1	271.6	288.5	273.9	293.8	272.4	1	317.0	272.5	311.0	266.4	306.0	265.1	1	260.0	227.7	254.0	213.7	263.0	230.8
2	n/a	n/a	n/a	n/a	n/a	n/a	2	n/a	n/a	n/a	n/a	n/a	n/a	2	277.0	247.9	272.0	241.4	267.0	234.2
3	n/a	n/a	n/a	n/a	n/a	n/a	3	n/a	n/a	n/a	n/a	n/a	n/a	3	250.0	221.8	244.0	216.1	254.0	222.0
4	n/a	n/a	n/a	n/a	n/a	n/a	4	n/a	n/a	n/a	n/a	n/a	n/a	4	270.0	238.8	264.0	239.7	288.0	262.8
5	270.8	235.2	271.8	235.4	270.0	246.1	5	249.7	226.3	243.5	222.9	238.9	217.4	5	272.0	241.8	263.0	232.1	275.0	236.9
6	278.3	228.4	280.3	235.2	274.3	227.6	6	306.0	272.2	295.4	263.1	288.1	256.1	6	253.0	227.6	249.0	225.2	254.0	221.5
7	279.6	246.0	274.4	241.2	280.4	239.2	7	258.8	238.4	252.0	228.1	257.9	219.6	7	283.0	243.2	275.0	235.3	282.0	245.1
8	261.6	229.9	271.8	230.6	276.3	236.7	8	250.6	201.4	245.7	195.3	242.3	184.9	8	289.0	258.2	n/a	n/a	289.0	251.2
9	263.8	234.6	271.6	224.6	270.3	227.5	9	240.8	204.9	235.3	193.2	237.8	186.4	9	282.0	247.3	n/a	n/a	275.0	251.6
10	293.4	271.9	283.9	264.0	287.9	264.5	10	242.5	230.3	242.8	219.2	238.8	216.5	10	277.0	238.5	n/a	n/a	279.0	236.2
11	302.6	277.3	n/a	n/a	297.0	274.2	11	297.2	240.1	n/a	n/a	295.9	247.8							
							12	295.2	258.2	n/a	n/a	288.9	251.2							
n	8	8	7	7	8	8	n	9	9	7	7	9	9	n	10	10	7	7	10	10
mean	280.2	249.4	277.5	243.6	281.2	248.5	mean	273.1	238.2	260.8	226.9	266.1	227.2	mean	271.0	239.3	260.0	229.1	273.0	239.2
SD	14.6	20.8	6.8	18.3	10.5	19.3	SD	30.2	26.0	29.7	29.1	28.3	29.6	SD	13.2	11.0	11.6	11.0	12.7	13.4

Table B4.9 Study 2 total protein content (mg (mg muscle^{-1})) (Chapter 5).

Con	Pre		d5		Post		**LHTLc**	Pre		d5		Post		**LHTLi**	Pre		d5		Post	
subject	Rest	End Ex	Rest	End Ex	Rest	End Ex	subject	Rest	End Ex	Rest	End Ex	Rest	End Ex	subject	Rest	End Ex	Rest	End Ex	Rest	End Ex
1	0.178	0.179	0.177	0.177	0.179	0.177	1	0.182	0.182	0.182	0.181	0.181	0.182	1	0.175	0.175	0.175	0.174	0.175	0.175
2	n/a	n/a	n/a	n/a	n/a	n/a	2	n/a	n/a	n/a	n/a	n/a	n/a	2	0.18	0.18	0.179	0.181	0.179	0.181
3	n/a	n/a	n/a	n/a	n/a	n/a	3	n/a	n/a	n/a	n/a	n/a	n/a	3	0.172	0.173	0.172	0.172	0.172	0.174
4	n/a	n/a	n/a	n/a	n/a	n/a	4	n/a	n/a	n/a	n/a	n/a	n/a	4	0.175	0.174	0.174	0.174	0.175	0.173
5	0.173	0.174	0.174	0.172	0.174	0.174	5	0.171	0.171	0.172	0.171	0.172	0.171	5	0.177	0.177	0.178	0.173	0.179	0.178
6	0.174	0.172	0.176	0.175	0.175	0.176	6	0.176	0.175	0.176	0.176	0.177	0.175	6	0.173	0.174	0.175	0.175	0.175	0.174
7	0.175	0.173	0.175	0.176	0.177	0.175	7	0.176	0.177	0.177	0.176	0.175	0.177	7	0.177	0.175	0.175	0.177	0.177	0.175
8	0.177	0.178	0.231	0.176	0.175	0.177	8	0.177	0.178	0.175	0.177	0.177	0.176	8	0.171	0.173	n/a	n/a	0.173	0.172
9	0.175	0.173	0.177	0.175	0.176	0.176	9	0.171	0.17	0.17	0.17	0.172	0.169	9	0.17	0.169	n/a	n/a	0.171	0.171
10	0.179	0.177	0.18	0.181	0.18	0.179	10	0.172	0.172	0.173	0.173	0.173	0.172	10	0.175	0.175	n/a	n/a	0.175	0.175
11	0.17	0.17	n/a	n/a	0.171	0.17	11	0.176	0.175	n/a	n/a	0.175	0.177							
							12	0.17	0.171	n/a	n/a	0.171	0.17							
n	8	8	7	7	8	8	n	9	9	7	7	9	9	n	10	10	7	7	10	10
mean	0.175	0.174	0.184	0.176	0.176	0.175	mean	0.175	0.175	0.175	0.175	0.175	0.174	mean	0.174	0.175	0.175	0.175	0.175	0.175
SD	0.003	0.003	0.021	0.003	0.003	0.003	SD	0.004	0.004	0.004	0.004	0.003	0.004	SD	0.003	0.003	0.002	0.003	0.003	0.003

Table B4. 10 Study 3 Na^+,K^+ATPase content (pmol.(g wt weight)$^{-1}$)(Chapter 6)

subject	Baseline	Pre	Post
1	264.3	300.4	245.9
2	447.4	398.7	344.3
3	375.9	n/a	351.3
4	n/a	n/a	n/a
5	400.1	325.5	n/a
6	267.8	351.2	n/a
7	n/a	n/a	n/a
8	172.6	235.0	132.2
9	434.0	446.8	n/a
10	n/a	264.1	326.3
11	404.9	455.4	473.5
12	405.6	423.6	487.1
n	9	9	7
mean	352.5	355.6	337.2
SD	94.4	80.4	123.7

Table B4. 11 Study 3 Maximal Na^+,K^+ATPase activity (nmol (g wt weight)$^{-1}$) (Chapter 6).

	Baseline	Pre		Post	
Subject	Rest	Rest	Ex	Rest	Ex
1	273.9	271.9	238.3	281.2	243.5
2	n/a	n/a	n/a	n/a	n/a
3	n/a	n/a	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	n/a
6	311.0	306.0	280.4	310.5	286.4
7	n/a	n/a	n/a	n/a	n/a
8	299.8	294.8	240.4	305.4	259.9
9	285.4	284.8	253.2	304.9	275.5
10	284.4	274.6	249.3	297.3	274.0
11	255.6	254.0	206.2	271.5	210.7
12	280.7	285.6	253.7	305.1	268.7
n	7	7	7	7	7
mean	284.4	281.7	245.9	296.6	259.8
SD	17.8	16.8	22.3	14.6	25.5

Table B4. 12 Study 3 Na^+,K^+ATPase isoform mRNA expression (Arbitrary units) (Chapter 6)

	alpha 1				alpha 2				alpha 3				Beta 1				Beta 2				Beta 3			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post		Pre		Post		Pre		Post	
Subject	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex
1	1.00	0.10	1.00	6.96	1.00	2.13	1.00	1.79	1.00	2.34	1.00	3.75	1.00	0.85	1.00	9.76	1.00	0.43	1.00	3.70	1.00	0.52	1.00	5.29
2	1.00	3.99	1.00	2.41	1.00	4.64	1.00	1.32	1.00	4.17	1.00	1.43	1.00	1.39	1.00	2.52	1.00	1.66	1.00	7.34	1.00	9.80	1.00	4.86
3	1.00	7.57	1.00	4.26	1.00	7.28	1.00	10.41	1.00	3.47	1.00	7.59	1.00	3.08	1.00	4.38	1.00	3.69	1.00	7.75	1.00	0.70	1.00	1.73
4	1.00	1.63	1.00	1.83	1.00	2.41	1.00	1.09	1.00	1.25	1.00	4.30	1.00	0.67	1.00	1.53	1.00	0.06	1.00	0.12	1.00	0.18	1.00	0.27
5	1.00	2.11	1.00	3.96	1.00	4.27	1.00	1.85	1.00	0.54	1.00	13.36	1.00	1.01	1.00	1.42	1.00	0.98	1.00	1.18	1.00	1.58	1.00	1.61
6	1.00	3.04	1.00	3.80	1.00	0.27	1.00	1.30	1.00	5.02	1.00	4.30	1.00	1.13	1.00	0.72	1.00	2.30	1.00	12.20	1.00	1.82	1.00	1.50
7	1.00	2.59	1.00	0.43	1.00	3.61	1.00	0.25	1.00	11.56	1.00	0.42	1.00	1.70	1.00	0.09	1.00	0.43	1.00	2.30	1.00	2.64	1.00	0.27
8	1.00	0.46	1.00	12.33	1.00	3.66	1.00	2.80	1.00	4.61	1.00	4.30	1.00	2.09	1.00	9.24	1.00	2.30	1.00	1.71	1.00	2.19	1.00	0.88
9	1.00	9.64	1.00	0.58	1.00	3.72	1.00	8.75	1.00	22.35	1.00	4.30	1.00	0.98	1.00	1.06	1.00	8.88	1.00	3.70	1.00	0.40	1.00	0.68
10	1.00	1.89	1.00	0.94	1.00	5.60	1.00	0.22	1.00	1.13	1.00	0.11	1.00	1.12	1.00	0.59	1.00	1.16	1.00	1.54	1.00	4.42	1.00	0.60
11	1.00	1.18	1.00	4.14	1.00	2.77	1.00	2.11	1.00	1.96	1.00	3.78	1.00	1.86	1.00	2.21	1.00	3.85	1.00	1.60	1.00	1.62	1.00	0.51
12	1.00	2.27	1.00	4.14	1.00	0.68	1.00	2.11	1.00	1.87	1.00	3.78	1.00	0.50	1.00	2.21	1.00	1.87	1.00	1.60	1.00	0.99	1.00	0.51
n	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
mean	1.00	3.04	1.00	3.82	1.00	3.42	1.00	2.83	1.00	5.02	1.00	4.28	1.00	1.37	1.00	2.98	1.00	2.30	1.00	3.73	1.00	2.24	1.00	1.56
SD	0.00	2.84	0.00	3.29	0.00	1.97	0.00	3.26	0.00	6.20	0.00	3.50	0.00	0.72	0.00	3.24	0.00	2.40	0.00	3.57	0.00	2.66	0.00	1.72

Table B4. 13 Study 3 Na^+,K^+ATPase isoform mRNA expression (Arbitrary units)

	alpha 1				alpha 2				alpha 3				Beta 1				Beta 2				Beta 3			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post		Pre		Post		Pre		Post	
Subject	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex
1	1.00	0.27	1.00	0.75	1.00	0.64	1.00	0.46	1.00	2.77	1.00	9.42	1.00	1.03	1.00	0.81	1.00	0.59	1.00	0.80	1.00	1.26	1.00	0.95
2	1.00				1.00				1.00				1.00				1.00				1.00			
3	1.00	0.53	1.00	1.07	1.00	0.88	1.00	0.35	1.00	1.25	1.00	1.12	1.00	0.69	1.00	1.13	1.00	2.39	1.00	0.80	1.00	1.83	1.00	0.29
4	1.00	0.33			1.00	1.52			1.00	1.22			1.00	1.33			1.00	1.14			1.00	0.13		
5	1.00	2.46			1.00	0.80			1.00	0.87			1.00	0.83			1.00	1.07			1.00	0.87		
6	1.00	0.85			1.00	2.67			1.00	2.80			1.00	0.37			1.00	0.36			1.00	0.68		
7	1.00	5.31			1.00	0.67			1.00	0.73			1.00	0.28			1.00	1.06			1.00	1.84		
8	1.00	1.02	1.00	7.99	1.00	2.24	1.00	8.96	1.00	1.06	1.00	3.07	1.00	0.90	1.00	9.98	1.00		1.00	0.97	1.00	1.38	1.00	0.75
9	1.00	2.67	1.00	0.60	1.00	0.24	1.00	0.67	1.00	0.83	1.00	0.48	1.00	0.65	1.00	2.30	1.00	4.02	1.00	0.85	1.00	1.25	1.00	0.52
10	1.00	3.30	1.00	1.25	1.00	0.99	1.00	0.30	1.00	1.88	1.00	1.51	1.00	2.29	1.00	1.35	1.00	1.44	1.00	0.15	1.00	1.07	1.00	1.63
11	1.00	1.22			1.00	0.77			1.00	0.74			1.00	0.82			1.00	2.74			1.00	0.76		
12	1.00	0.51			1.00	0.34			1.00	1.46			1.00	1.13			1.00	0.53			1.00	1.19		
n	12	11	5	5	12	11	5	5	12	11	5	5	12	11	5	5	12	10	5	5	12	11	5	5
mean	1.0	1.7	1.0	2.3	1.0	1.1	1.0	2.1	1.0	1.4	1.0	3.1	1.0	0.9	1.0	3.1	1.0	1.5	1.0	0.7	1.0	1.1	1.0	0.8
SD	0.0	1.6	0.0	3.2	0.0	0.8	0.0	3.8	0.0	0.8	0.0	3.6	0.0	0.5	0.0	3.9	0.0	1.2	0.0	0.3	0.0	0.5	0.0	0.5

APPENDIX C1 THE EFFECTS OF HIGH INTENSITY INTERVAL EXERCISE AND TRAINING ON Na^+,K^+ATPase ACTIVITY AND CONTENT IN WELL-TRAINED ATHLETES.

METHODS

Muscle Biopsy Sampling and Analyses.

An initial resting muscle biopsy was taken at baseline with further biopsies taken at rest (Rest) and immediately post-exercise (Exercise), during the Pre-Train and Post-Train testing sessions. The needle biopsy sample was taken from the vastus lateralis muscle under local anaesthesia (Xylocaine, 1 %), with suction applied to the needle (Evans *et al.*, 1982). The post-exercise sample was taken immediately after cessation of the final exercise bout, with the subject lying supported on the cycle ergometer. Serial biopsies were taken from separate incisions in the same leg, with the exercise sample taken from an incision ~1.5 cm proximal to the rest sample. Muscle samples were removed, and rapidly frozen and stored in liquid N_2 for subsequent analysis of Na^+,K^+ATPase isoform mRNA and crude homogenate protein expression, content and maximal activity. Muscle was also analysed for AMPK response to exercise, as reported elsewhere (Clark *et al.*, 2004b), which restricted our ability to complete all analyses for each subject at each timepoint. Consequently, the sample size for each Na^+,K^+ATPase analysis was as follows, isoform mRNA, n=12; isoform protein expression, n=5; Na^+,K^+ATPase content, n=7; and maximal activity, n=7.

Real-Time RT-PCR measurement of mRNA

Na^+,K^+ATPase isoform mRNA expression was measured as previously described (Murphy *et al.*, 2004). Total RNA was extracted from 5-10 mg of muscle using the FastRNA reagents (BIO 101, Vista, CA, USA) (Murphy *et al.*, 2003). The resulting

RNA pellet was dissolved in EDTA-treated water and total RNA concentration was determined spectrophotometrically at 260 nm. RNA (1 μg) was transcribed into cDNA using the Promega AMV Reverse Transcription Kit (Promega, Madison, Wisconsin, USA), and the resulting cDNA was stored at –20°C for subsequent analysis.

Real-Time PCR (GeneAmp 5700 Sequence Detection System) was run for 1 cycle (50°C for 2 min, 95°C for 10 min) and 50 cycles (95°C for 15 s, 60°C for 60s). Primer sequences were designed for the Na^+,K^+ATPase α_1, α_2, α_3, β_1, β_2 and β_3 genes from published sequences (Murphy *et al.*, 2004). The Na^+,K^+ATPase α_4 gene was also probed for, but was undetected by RT-PCR. All samples were run in triplicate and measurements included a no-template control as well as a human skeletal muscle sample endogenous control. Cyclophilin (CYC) mRNA expression was unchanged with exercise ($P < 0.77$) and training ($P < 0.77$, data not shown), and was therefore used as a control (housekeeping gene) to account for any variations in the amount of input RNA and the efficiency of reverse transcription. Gene expression was quantified using a cycle threshold (C_T) method, whereby the relative expression of the genes compared with Pre HIT resting sample was made using the expression, $2^{-\Delta\Delta CT}$, in which the expression of each gene was normalised for input cDNA using the housekeeping gene, CYC (Murphy *et al.*, 2004).

Immunoblotting

Immunoblotting methods were as previously described (Murphy *et al.*, 2004). Muscle samples (20-30 mg) were diluted 1:40 with extraction buffer (25 mM Tris-HCl, pH 6.8, 1% sodium dodecyl sulfate (SDS), 5 mM EGTA, 50 mM NaF, 1 mM sodium vanadate, 10% glycerol, 17.4 $\mu g.ml^{-1}$ phenylmethylsulphonyl fluoride (PMSF), 10 $\mu g.ml^{-1}$ leupeptin and 1 $\mu g.ml^{-1}$ aprotinin) and homogenised on ice for 15 s at a speed rating of 4 (Polytron PT1200, Kinematica, Luzern, Switzerland). A portion of each homogenate

was heated for 10 min at 90 °C, and analyzed for total protein content (BCA Assay Kit, Pierce, Rockford, IL, USA), with bovine serum albumin (BSA) as the standard. Stabilising buffer (0.75 M Tris HCl, pH 6.8, 25% glycerol, 25 mM dithiothreitol (DTT), 5% SDS and 5% 2-mercaptoethanol) was then added to the remaining homogenate in a 1:5 dilution, and each sample was subsequently frozen at –80 °C for immunoblotting.

SDS-PAGE (10% separating gel, 5% stacking gel) was performed and gels were loaded with 20 (β_1) or 70 (α_1, α_2, α_3, β_2, β_3) µg protein. Following electrophoresis (20 min, 100 V and 90 min, 150 V), the protein was transferred (90 min, 100 V) to 0.45 µm nitrocellulose membrane, and blocked for 2 h with blocking buffer (5% non-fat milk in Tris-buffered saline Tween (TBST)). Membranes were incubated overnight at 4 ºC in primary antibodies diluted in blocking buffer containing 0.1% NaN_3. Membranes were washed in 0.05% TBST, and incubated for 1 h in horseradish peroxidase (HRP) conjugated secondary antibodies (goat anti-mouse immunoglobulins or goat anti-rabbit immunoglobulins) diluted 1:10,000 in TBST buffer. Following three washes in 0.05% TBST, membranes were dried and treated with chemiluminescent substrate (Pierce SuperSignal West Pico, Illinois, USA). The signal was captured and imaged (Kodak Digital Science Image Station 400_{CF}, Eastman Kodak Company, CT, USA). Positive control samples included rat brain and kidney homogenates and these were run on each gel to assess the reactivity of the probe.

Antibodies

Blots were probed with antibodies specific to each isoform. These were for α_1: monoclonal α6F (developed by D. Fambrough and obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA, USA); α_2: polyclonal anti-HERED (kindly donated by T. Pressley, Texas Tech University); α_3:

monoclonal MA3-915 (Affinity Bioreagents, Golden, CO, USA); β_1: monoclonal MA3-930 (Affinity Bioreagents); β_2: polyclonal 610915 (Transduction Laboratories, Lexington, KY, USA); and β_3: polyclonal 610993 (Transduction Laboratories).

RESULTS

Stability from Baseline to Pre-Train

None of Na^+,K^+ATPase isoform mRNA (α_1, 1.25±0.27; α_2, 0.65±0.40; α_3, 1.01±0.22; β_1, 1.20±0.13; β_2, 2.16±0.66; and β_3 1.01±0.26 arbitrary units, expressed relative to Baseline mRNA, n=12, CV 15.7, 1.3, 15.7, 7.9, 30.2 and 35.1% respectively, *NS*) or crude homogenate protein expression (α_1, 0.86±0.13; α_2, 0.80±0.10; α_3, 1.38±0.29; β_1, 1.17±0.24; β_2, 1.03±0.19; and β_3 1.11±0.25 arbitrary units, n=11, CV 10.8, 15.4, 22.4, 11.1, 2.4 and 7.4 % respectively, *NS*) were significantly different at Pre Train when expressed relative to Baseline. However, there was a tendency towards increased β_2 mRNA expression ($P = 0.06$) and reduced α_2 protein ($P = 0.08$) at Baseline.

Na^+,K^+ATPase isoform mRNA expression

A single bout of high-intensity interval exercise immediately elevated Na^+,K^+ATPase α_1, α_2, and α_3 mRNA expression by 2.0-, 2.4-, and 4.0-fold, respectively (Fig. 6.1A, $P>0.05$). Exercise also induced a tendency toward elevated β_2 mRNA expression ($P < 0.09$, β_2 mRNA elevated in 10 of 12 subjects), but had no significant effect on β_1 ($P < 0.11$) or β_3 mRNA expression (Fig. C.1A).

Na^+,K^+ATPase isoform protein abundance

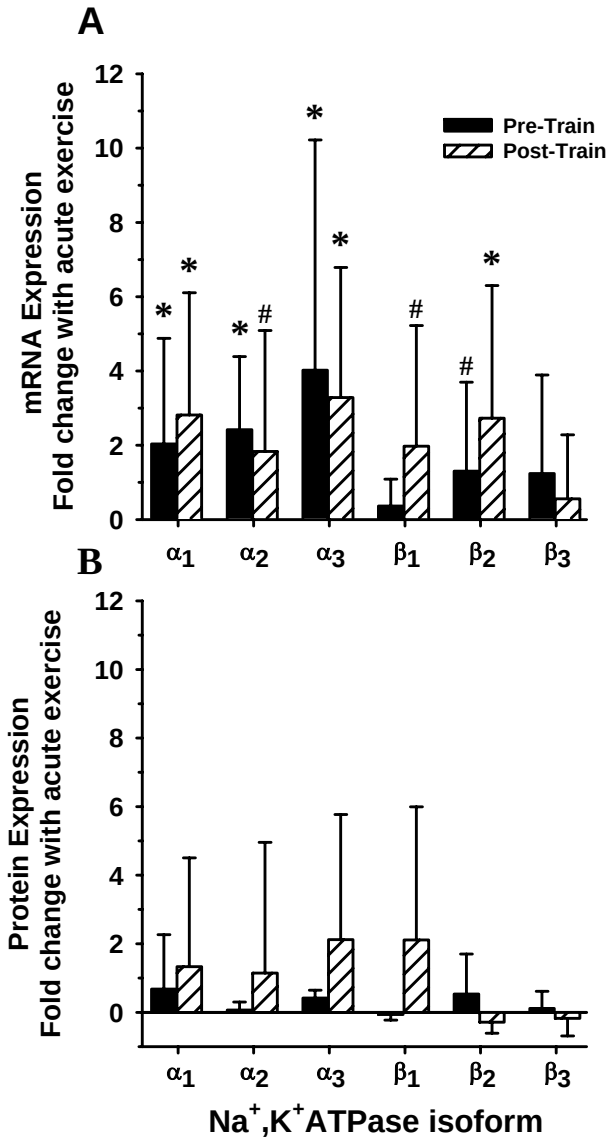
In contrast to the upregulatory effect on α-isoform mRNA, high-intensity interval exercise had no significant effect on the protein abundance of any of the Na^+,K^+-ATPase α_1, α_2, α_3, β_1, β_2, or β_3 isoforms (Fig. C.1B).

Na^+,K^+ATPase mRNA and protein expression

Resting muscle

HIT elevated resting muscle Na^+,K^+ATPase α_3 and β_3 mRNA by 4.6- and 2.5-fold, respectively but had no significant effect on mRNA expression of any other isoform (Fig. C.2A, $P<0.05$). HIT did not significantly alter muscle protein abundance for any Na^+,K^+ATPase isoform (Fig. C.2B).

Figure C.1 Acute exercise-induced change in Na^+,K^+ATPase α_1, α_2, α_3, β_1, β_2 and β_3 isoform mRNA (A) and protein expression (B) with an acute high-intensity interval exercise bout conducted before (Pre-Train, filled bars) and after (Post-Train, hatched bars) 3 wks HIT. Mean ± SD, n=12 for mRNA expression, n=11 for isoform protein expression Pre-Train and n=5 for Post-Train. * *E*xercise > Rest, $P < 0.05$, # $P < 0.09$ (Exercise main effect). Data expressed relative to resting levels for Pre-Train and for Post-Train and expressed as fold change with exercise for that training state.

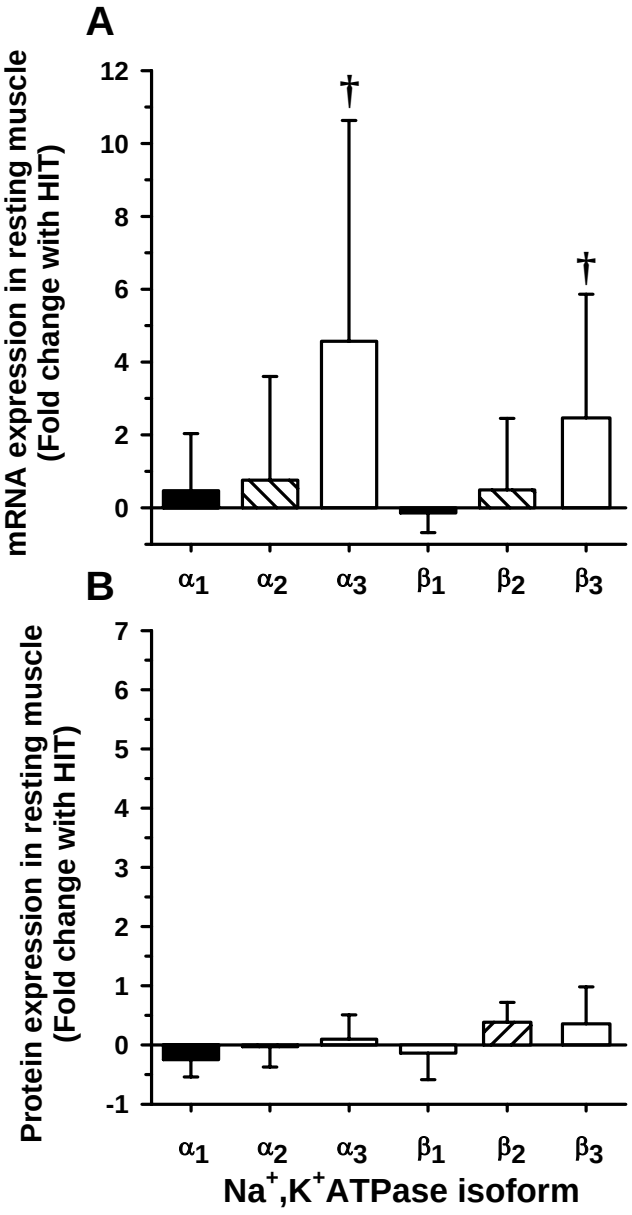


Changes with exercise

Following 3 wks of HIT, the up-regulatory effects of high-intensity, intermittent exercise on α_1 ($P < 0.02$) and α_3 mRNA expression ($P < 0.01$) were still present (Fig. C.1A), whilst exercise tended to again increase α_2 mRNA ($P < 0.08$, α_2 mRNA elevated in 10 of 12 subjects). Exercise also increased β_2 mRNA expression by 2.7-fold ($P < 0.03$), and tended to increase β_1 mRNA expression ($P < 0.06$, β_1 mRNA increased in 10 of 12 subjects), with no significant change in β_3 mRNA expression ($P < 0.29$, Fig. C.1A).

There was no significant difference between Pre-HIT and Post-HIT in the exercise effect on the mRNA expression of any of the α_1 ($P < 0.62$), α_2 ($P < 0.51$), α_3 ($P < 0.75$), β_1 ($P < 0.11$), β_2 ($P < 0.24$) or β_3 isoforms ($P > 0.36$, Fig. C.1B).

Figure C.2 High intensity interval training (HIT) effects on resting muscle Na^+,K^+ATPase α_1, α_2, α_3, β_1, β_2 and β_3 isoform mRNA (A) and protein (B) expression. All results were normalised against Pre-Train resting values and expressed as fold change compared to Pre-Train for that isoform (zero means no change). Mean ± SD; n=12 for mRNA, n=5 protein † Post > Pre, $P < 0.05$ (Training main effect).



Conclusions

1. Acute high-intensity interval exercise immediately increased mRNA expression of the three Na^+,K^+ATPase α-isoforms.
2. HIT did not modulate this acute exercise-induced change
3. HIT increased Na^+,K^+ATPase α_3 and β_3 isoform mRNA expression in resting muscle
4. Neither acute exercise nor HIT altered muscle Na^+,K^+ATPase isoform protein expression.