Effects of high-intensity training on components of muscle pH regulation

Cian McGinley

August 2015

Submitted in fulfilment of the requirements of the degree of Doctor of Philosophy

Supervisor: Professor David J. Bishop

College of Sport and Exercise Science

Victoria University

Melbourne, Australia

Abstract

Regulation of pH in skeletal muscle comprises intracellular buffering of hydrogen ions (H^+) and acid/base (H^+/HCO_3^-) transport across the sarcolemma. During high-intensity exercise H^+ transport is primarily lactate-coupled through the monocarboxylate transporters (MCT)1/4, with non-lactate-coupled transport provided by the sodium/hydrogen exchanger (NHE) system. The chaperone protein basigin is essential for MCT functioning. Intracellular buffering comprises metabolic and physicochemical buffering (βm), the latter mainly from the histidine-based proteins and dipeptides, and inorganic phosphate. The sodium-coupled bicarbonate transport proteins (NCBT) enhance intracellular buffering and H^+ efflux, while the cytosolic and sarcolemmal carbonic anhydrase (CA) isozymes may enhance activity of each transport system by physical or functional interactions.

To better inform timing of post-training muscle biopsies, the first study profiled a timecourse of H^+ transport protein content and βm, measured by titration ($\beta m_{in\ vitro}$), following a single bout of high-intensity interval exercise. The data showed greater MCT1 protein content 24–72 h after exercise compared to the first 9 h post-exercise, whereas NHE1 abundance was lower 9 h post-exercise compared to every other timepoint. There was no change in MCT4 abundance over the same period. $\beta m_{in\ vitro}$ was found to be highly variable in resting samples taken over a 72 h period. These data have important implications for biopsy timing in studies investigating the response to training of the H^+ transport proteins. It is recommended post-training biopsies should be taken at least 24 h after the final training session.

The first major aim of the second study was to comprehensively measure the known isoforms of each of the acid/base transport families by western blotting of human muscle. The second aim was to investigate whether training intensity, either above the lactate threshold (LT) or close to peak aerobic power (W_{peak}), during work-matched high-intensity interval training (HIT), influenced adaptations in protein content, $\beta m_{in\ vitro}$, and exercise capacity. MCT4 and basigin demonstrated intensity-dependent upregulation, while abundance of MCT1, NHE1, NBCe1 (electrogenic sodium/bicarbonate cotransporter 1), CAII, and CAXIV increased after 4 weeks of training, but with no difference between the two training intensities. And there was no change or a decrease in CAIII and CAIV abundance, and no change in $\beta m_{in\ vitro}$. Improvements in repeated-sprint ability (RSA) were modest and there was little change in aerobic capacity. Detraining was evident from an almost complete loss of adaptations for all of the proteins, and for RSA, six weeks after stopping training. In conclusion, the intensity at which HIT is performed may not be a deterministic factor in muscle adaptations, suggesting training need not be maximal to achieve improvements.

In the third study, by manipulating the bioenergetic status of the muscle through altering the duration of the rest interval (1 min versus 5 min), the aim was to investigate potential

mechanisms for upregulation of acid/base transporters and $\beta m_{in\ vitro}$ following 4 weeks of work-matched sprint-interval training (SIT). The second aim was to see whether detraining could be mitigated by training one day per week over the subsequent 6 weeks. There was little difference between groups in transport protein changes, with increased abundance of MCT1, NHE1, and CAXIV seen after 4 weeks, but no change or a decrease for MCT4, NBCe1, basigin, CAII, and CAIII. $\beta m_{in\ vitro}$ did not change either. The 5-min rest group improved most in RSA, while modest improvements in $\dot{V}O_{2peak}$ were found for both groups. Training 1 d per week maintained adaptations in protein content and RSA. In conclusion, rest interval duration was not important for muscle adaptations but did result in different improvements in exercise performance.

This thesis has, for the first time, comprehensively measured the acid/base transport proteins in human muscle and their plasticity to high-intensity training. Protein content was shown to be upregulated following 4 weeks of HIT in active men but intensity of training did not discriminate between adaptations. Four weeks of SIT provoked lesser adaptations in active women, and protein content did not differ between rest intervals of 1 min or 5 min. Contrary to the hypotheses, it seems that manipulating the bioenergetic status of the muscle by altering exercise intensity, or duration of rest, did not influence adaptations in acid/base transport protein content, but did result in some distinct changes in exercise capacity. Variable individual adaptations in protein content and exercise performance suggest that genetic factors and unexplored random responses are more important than manipulating training variables. In addition, the two training studies have collectively shown that reversal of adaptations for acid/base transport proteins occur within 6 weeks of stopping high-intensity training, but most adaptations can be maintained by training 1 d per week at the same intensity. Finally, $\beta m_{in\ vitro}$ changed little with training, but was highly variable over a 72-h period, calling into question the ecological validity of the technique.

Declaration

Doctor of Philosophy Declaration

I, Cian McGinley, declare that the PhD thesis entitled "Effects of high-intensity training on components of muscle pH regulation" is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, 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.

Signature _____ Date _____

Acknowledgments

Thank you to my supervisor Professor David Bishop for providing me with the opportunity and resources to undertake a PhD in Australia.

I am extremely grateful to the participants who volunteered for each of the studies. They dedicatedly performed hours of exhausting, painful, and occasionally nauseating exercise, often long before the sun had risen. They underwent several muscle biopsies and multiple blood samples, and that this was all cheerfully undertaken and without any remuneration means I cannot thank them enough.

Without hundreds of hours of assistance from colleagues I would not have been able to conduct any research trials. I am privileged and grateful to have had the help of Chris Hedges, James Broatch, Rodrigo Oliveira, Jackson Fyfe, Ben Perry, Vicky Wyckelsma, Briar Rudsits, Rémi Delfour-Peyrethon, and Jess Dudley. I am also very grateful to the VU interns who assisted with training of participants and conducting of exercise trials – Cameron, Catherine, Evan, Francisco, Lewis, and Ned.

Thank you to the many colleagues and friends who helped with biochemical techniques, in particular troubleshooting the countless problems detecting novel proteins using stubborn antibodies – Roddy, Chris, Ben, Vicky, Amy, Jackson, Cesare, JJ, Xu, Dani, and Andrew.

I am hugely indebted to the lab technicians and lab managers, especially Brad Gatt and Jess Meilak, who, amongst hundreds of small tasks, set up and maintained equipment for each and every trial day, always with good humour despite the considerable demands of space and time. Thanks also to Sam and Collene for help setting up trials, and to Rhett and Rob, who finally helped solve the electrical noise problems with the pH microelectrode.

Thank you to each of the medical doctors who performed the muscle biopsies, in particular Dr. Mitch Anderson, who performed the vast majority of procedures, but also Dr. Andrew Garnham and Dr. Andrew Hodder, who provided their services when requested.

I received generous and invaluable advice by correspondence on disparate topics from several researchers, in particular Dr. Claire Thomas-Junius, Dr. Robyn Murphy, Prof. Andrew Halestrap, Prof. Will Hopkins, and Prof. Johannes Vogel.

I am grateful to have been the recipient of an International Postgraduate Research Scholarship from the Australian State, and to have won an Exercise and Sports Science Australia (ESSA) Applied Sports Science Research Grant.

Finally, thank you to my family for providing me with support and encouragement throughout.

List of Publications and Awards

Conference Presentations

- McGinley, C. & Bishop D. J. Influence of training intensity on adaptations in acid/base transport protein abundance and non-bicarbonate muscle buffer capacity in active men. AuPS Meeting, 29 November – 2 December 2015, Hobart, Australia. 164P.

- McGinley, C. & Bishop, D. J. Exercise intensity is an important determinant of changes in muscle monocarboxylate transporter abundance in active men. Proc Physiol Soc 31, 30 June – 2 July 2014 London, UK. C41.

Awards

- Victoria University International Postgraduate Research Scholarship 2011–2014 ($80,000)
- Exercise & Sports Science Australia (ESSA) Applied Sports Science Research Grant 2013 ($10,000)
- Physiological Society Travel Grant 2014 (£500)

Table of Contents

List of Figures

List of Tables

List of Abbreviations

[ ]	concentration
β_{CO_2}	buffering capacity of bicarbonate/carbon dioxide
βm	muscle buffer capacity
$\beta m_{in\ vitro}$	non-bicarbonate physicochemical muscle buffer capacity
$\beta m_{in\ vivo}$	*in vivo* muscle buffer capacity
Δ	change or difference
µg	microgram
µL	microlitre
µM	micromolar
^{31}P-MRS	phosphorous-31 magnetic resonance spectroscopy
A^-	conjugate base
[A] or A_{tot}	total weak acid concentration
acetyl-CoA	acetyl coenzyme A
ADP	adenosine 5'-diphosphate
AE1–3	anion exchanger (isoforms 1–3)
AMP	adenosine 5'-monophosphate
AMPK	adenosine 5'-monophosphate-activated protein kinase
ANOVA	analysis of variance
ATP	adenosine 5'-triphosphate
ATPase	adenosine 5'-triphosphatase
au	arbitrary unit
Bis-Tris	2,2-Bis(hydroxymethyl)-2,2',2''-nitrilotriethanol
BLAST	Basic Local Alignment Search Tool
BM	body mass
BSA	bovine serum albumin
CA (II/III/IV/XIV)	carbonic anhydrase (isoforms II/III/IV/XIV)
Ca^{2+}	calcium ion

cAMP	adenosine 3',5'-cyclic monophosphate
CCD (camera)	charge-coupled device (camera)
cCHX	cytosolic calcium/hydrogen ion exchange
CD147	cluster of differentiation 147 (alias of basigin)
$C_{24}H_{39}NaO_4$	sodium deoxycholate
CHO	carbohydrate
CI	confidence interval
CK	creatine kinase
Cl^-	chloride ion
Cl^-/HCO_3^-	chloride/bicarbonate exchanger (see also AE1–3)
cm	centimetre
CO_2	carbon dioxide
CO_3^{2-}	carbonate ion
-COOH	carboxyl group
Cr	creatine
CR-10 (scale)	category-ratio (scale)
cRNA	complementary ribonucleic acid
C-terminus	carboxyl (-COOH)-terminus
CV	coefficient of variation
d	Cohen's standardised effect size
d	day
DHPR	dihydropyridine receptor complexes
dm	dry mass
DMA	5-*N*,*N*-dimethylamiloride-hydrochloride
DTT	DL-dithiothreitol
E–C	excitation–contraction (coupling)
EDL	*extensor digitorum longus*
EDTA	ethylenediaminetetraacetic acid

EGTA	ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*,*N´*,*N´*-tetraacetic acid
*E*m	membrane potential
EMBOSS	European Molecular Biology Open Software Suite
EMMPRIN	extracellular matrix metalloproteinase inducer (alias of basigin)
ES	effect size
FADH	reduced flavine adenine dinucleotide
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GXT	graded-exercise test
h	hour
H^+	hydrogen ion, proton
HA	ionised weak acid
HCl	hydrochloric acid
HCO_3^-	bicarbonate ion
H_2CO_3	carbonic acid
HEPES	*N*-2-hydroxyethylpiperazine-*N´*-2-ethanesulphonic acid
HIF(-1α)	hypoxia-inducible factor(-1α)
HIE	high-intensity interval exercise
HIT	high-intensity interval training
H_2O	water
H_2O_2	hydrogen peroxide
H_3O^+	hydronium ion
HPO_4^{2-}	monoprotonated inorganic phosphate (hydrogen phosphate ion)
$H_2PO_4^-$	diprotonated inorganic phosphate (dihydrogen phosphate ion)
HR_{max}	maximum heart rate
HRP	horseradish peroxidase
iEMG	intergrated electromyography
IgG	immunoglobulin G
IMP	inosine 5'-monophosphate

IRBIT	Inositol 1,4,5 trisphosphate receptor binding protein released with inositol 1,4,5 trisphosphate
IV	intravenous
J	joule
K^+	potassium ion
K_a	acid dissociation constant
kDa	kilodalton
kg	kilogram
kJ	kilojoule
K_m	Michaelis–Menten constant
kPa	kilopascal
L	litre
La^-	lactate ion
LDH	lactate dehydrogenase
LT	lactate threshold
M	molar
m	metre
MCT(1/4)	monocarboxylate transporter (isoforms 1/4)
meq	milli-equivalent
Mg^{2+}	magnesium ion
min	minute
MJ	megajoule
mL	millilitre
mM	millimolar
mmHg	millimetre of mercury
mmol	millmole
mRNA	messenger ribonucleic acid
MRS	magnetic resonance spectroscopy
mV	millivolt

MVIC	maximal voluntary isometric contraction
N	newton
Na^+	sodium ion
NaCl	sodium chloride
$NaCO_3^-$	sodium carbonate ion
NAD^+	oxidised nicotine adenine dinucleotide
NADH	reduced nicotine adenine dinucleotide
NaF	sodium fluoride
$NaHCO_3^-$	sodium bicarbonate
Na^+-K^+-ATPase	sodium–potassium adenosine triphosphatase (sodium–potassium pump)
NaN_3	sodium azide
NaOH	sodium hydroxide
$Na_4P_2O_7$	sodium pyrophosphate tetrabasic
Na_3VO_4	sodium orthovanadate
NBCe(1/2)	electrogenic sodium bicarbonate cotransporter (isoforms 1/2)
NBCn(1/2)	electroneutral sodium bicarbonate cotransporter (isoforms 1/2)
NCBT	sodium-coupled bicarbonate transporter
NDCBE	sodium-driven chloride bicarbonate exchanger
NFDM	non-fat dry milk
$-NH_2$	amine group
NH_3	ammonia
NH_4^+	ammonium ion
NH_4Cl	ammonium chloride
NHE(1)	sodium/hydrogen exchanger (isoform 1)
N-terminus	amino ($-NH_2$)-terminus
O_2	oxygen
OH^-	hydroxyl ion
pCa	negative logarithm (base 10) calcium concentration

PCO_2	partial pressure of carbon dioxide
PCr/PCr^{2-}	phosphocreatine
PDC	pyruvate dehydrogenase complex
PDH	pyruvate dehydrogenase
PDH_a	pyruvate dehydrogenase, active form
PFK	phosphofructokinase
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator-1α
pH	negative logarithm (base 10) hydrogen ion activity/concentration
pH_i	intracellular pH
pH_o	extracellular pH
Phos	glycogen phosphorylase
Phos *a*	active (phosphorylated) glycogen phosphorylase
Phos *b*	less active (unphosphorylated) glycogen phosphorylase
P_i	inorganic phosphate (see also HPO_4^{2-})
pK_a	negative logarithm (base 10) acid dissociation constant
PMSF	phenylmethanesulfonyl fluoride
PO_2	partial pressure of oxygen
PRO	protein
RIPA (buffer)	radioimmunoprecipitation assay (buffer)
RNA	ribonucleic acid
RPE	rating of perceived exertion
rpm	revolutions per minute
RSA	repeated-sprint ability
RSE	repeated-sprint exercise
RyR	ryanodine receptors
s	second
s	solubility coefficient
SD	standard deviation

SDS	sodium dodecyl sulphate
SDS–PAGE	sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SE	standard error of the mean
SID	strong ion difference
SIT	sprint-interval training
SLC	solute carrier
SNP	single nucleotide polymorphism
SO_4^{2-}	sulphate ion
SR	sarcoplasmic reticulum
SR Ca^{2+}-ATPase (SERCA pump)	sarco(endo)plasmic reticulum calcium adenosine triphosphatase
t	time in seconds of the final stage of a graded-exercise test
TA	*tibialis anterior*
TBS	Tris-buffered saline
TBST	Tris-buffered saline with Tween-20
Tris	tris(hydroxymethyl)aminomethane
T-tubule	transverse-tubule
V	volt
V_{max}	maximum velocity
$\dot{V}O_2$	volume of oxygen uptake (per minute)
$\dot{V}O_{2max/peak}$	maximal/peak volume of oxygen uptake (per minute)
$v\dot{V}O_{2max}$	velocity at maximal oxygen utpake
W	watt
wk	week
W_{final}	power output of the last completed stage of a graded-exercise test
W_{peak}	peak aerobic power
y	year

Chapter 1 Introduction

For much of the last two centuries one of the axioms of exercise physiology was that lactic acid was a cause of fatigue (258). In recent decades it has become clear lactate oxidation to pyruvate is a crucial component of ATP resynthesis – for both slow and fast glycolysis. Subsequently, fatigue has been more specifically attributed to an accumulation of hydrogen ions (158). Yet, even the role of hydrogen ions (H^+) in fatigue is disputed (3), which is perhaps, in part, a reflection of the differing definitions of fatigue. The primary argument against acidosis being a factor in fatigue is based on the putative cause-effect relationship between muscle tension and H^+ concentration, although H^+ have also been implicated in the velocity component of fatigue (158). Given that all bodily systems operate within a limited physiological range of pH, if we make an *a priori* assumption that H^+ concentrations outside of this range adversely affects cellular biochemistry (220), then it follows that the pH-regulating capacity of the muscle fibres may play an important role in mitigating fatigue. And it is pH regulation that forms the central focus of the current thesis.

Intracellular skeletal muscle pH regulation entails buffering of H^+ within the muscle fibre by specific buffer compounds (physicochemical buffers) and through metabolic processes. Non-bicarbonate physicochemical buffer capacity of muscle, measured *in vitro* ($\beta m_{in\ vitro}$), has occasionally been shown to improve in response to high-intensity interval training (HIT), but there are conflicting data that indicate the mechanisms are not yet fully understood. A second key component of pH regulation involves the transport of acid (H^+) and base (bicarbonate, HCO_3^-) across the cell membrane by specialised proteins, with the direction of transport typically dependent on pH and concentration gradients. There are several families of acid/base transport proteins, and for some of these, there are multiple isoforms in human muscle. The primary regulators of muscle pH during high-intensity exercise are the lactate/H^+ (monocarboxylate, MCT) transporters, and the response of these to exercise has been much studied. Increased abundance of the MCTs has sometimes been found following high-intensity exercise, yet uncertainty remains about how best to stimulate their upregulation. Equivocation also remains regarding the sodium/hydrogen exchange (NHE) protein response to training, while no evidence currently exists in humans on how the sodium-coupled bicarbonate transport (NCBT) proteins adapt to a training stimulus. And a growing body of evidence has demonstrated in different models functional synergies between the acid/base transport proteins and the carbonic anhydrase (CA) enzymes, but no research exists on the plasticity of the four skeletal muscle CA isozymes to a training intervention.

Prior to conducting training interventions, understanding H^+ transport protein turnover in the short-term following a single bout of exercise was needed to ensure post-training samples were representative of the training response. Therefore, the aim of the first study was to profile

a timecourse of H^+ transport protein content, and *in vitro* muscle buffer capacity, over the 72 hours following a single bout of high-intensity interval exercise (Chapter 3).

The primary methodological aim of the second study was to try to identify the known isoforms of each of the acid/base transport families in human muscle. In Chapter 4, immunoblotting techniques were developed and validated for detection of these proteins. The second major aim of this study was, through manipulating the bioenergetic status of the muscle during HIT by altering the intensity of the work interval, to investigate the changes in acid/base transporters and $\beta m_{in\ vitro}$ after four weeks of HIT. How these adaptations translated to performance in tests of repeated-sprint ability and aerobic capacity was also studied. And six weeks after stopping HIT, any subsequent detraining was then investigated.

Following on from study 2, the third study (Chapter 5) explored potential stimuli for the upregulation of acid/base transport protein content and $\beta m_{in\ vitro}$ through manipulating rest interval duration during 10 weeks of sprint-interval training (SIT). Repeated-sprint ability and aerobic capacity were measured as performance indices once again. Anticipating from previous research that many adaptations would be almost completely lost six weeks after removing the stimulus of high-intensity training, this study also investigated whether training one day per week over a similar six-week period would be sufficient to maintain adaptations, or at least mitigate detraining.

Commencing with a literature review, this thesis further comprises three experimental chapters:

I. Chapter 3 (Study 1) – Changes in H^+ transport protein content and muscle buffer capacity following a single bout of high-intensity interval exercise
II. Chapter 4 (Study 2) – Influence of training intensity on adaptations in acid/base transport proteins, muscle buffer capacity, and repeated-sprint ability
III. Chapter 5 (Study 3) – Influence of rest interval duration on adaptations in acid/base transport proteins, muscle buffer capacity, and repeated-sprint ability

The main findings of this thesis are summarised with a general discussion and conclusions chapter (Chapter 6), including limitations and some suggested future research.

Chapter 2 Literature Review

2.1 Chapter Outline

This review of literature begins with an explanation of acidosis and measurement of pH in human skeletal muscle. The conflicting evidence for and against acidosis in the aetiology of fatigue is then presented. The next sections detail the different components of pH regulation within muscle, with a particular focus on physicochemical buffering and the acid/base transport proteins. There then follows a comprehensive review of studies that have investigated training-induced adaptations in muscle pH regulation, with some potential mechanisms for upregulation proposed. Finally, the review ends with an exploration of the limited evidence for reversal of adaptations in muscle buffering and acid/base transport proteins, placed in context of the wider body of detraining studies.

2.2 The Hydrogen Ion

According to the Brønsted–Lowry theory, an acid (HA) is a molecule or ion that is capable of donating a hydrogen cation (H^+; or simply a proton), whereas a base (or alkali) is capable of gaining a H^+ (434).

Eqn. 1 $HA \rightleftharpoons H^+ + A^-$ (A^- is the conjugate base)

In aqueous solutions, the proton readily bonds to a water molecule, so when reference is made to the H^+ concentration ($[H^+]$), it is actually the resultant hydronium ion concentration[1] ($[H_3O^+]$) that is of interest. In turn, the pH scale quantifies the concentration of H_3O^+ in a given solution, or more specifically the negative logarithm of same ($pH = -\log_{10} [H_3O^+]$; in neutral water $[H_3O^+]$ is 1.0×10^{-7} M at 25°C, i.e., pH = 7)[2]. Nevertheless, tradition holds that the water molecule to which the proton is attached is essentially ignored, and H^+ is the ion of interest when referring to pH and acid–base theory (291). To avoid unnecessary confusion this thesis shall hold with convention.

Lactic acid is almost completely dissociated[3] to its lactate anion (La^-) and a H^+ at physiological pH because of its high acid dissociation constant[4] ($K_a \cong 1 \times 10^{-3}$ equivalents•L^{-1}, or $pK_a = 3.86$ at 20°C) (75, 229, 452). Therefore, while lactate and H^+ are often linked, it is H^+ regulation that is of interest in traditional acid–base chemistry, rather than lactate *per se*.

[1] pH actually measures the activity of H^+, which is normally less than H^+ concentration (64). Activity varies with ionic strength, but in dilute solutions activity and concentration are assumed to be equivalent.

[2] At 25°C pH scale: acid < 7.0 (neutral) > base/alkali. Acid–base neutrality exists when the concentrations of hydrogen ions and hydroxyl ions (OH^-) are equal. Neutral pH is 7 at 25°C but varies with temperature, pH 6.8 being neutral at 37°C (381).

[3] A^-:HA = 1380:1 at pH = 7.

[4] $K_a = \frac{[H^+][A^-]}{[HA]}$ and $pK_a = -\log_{10}[K_a]$. The stronger the acid, the greater the dissociation, and the lower the pK_a.

Conversely, under the alternative Stewart strong ion difference (SID) model, lactate is an important independent variable as one of the strong ions, and $[H^+]$ is simply a dependent variable in determining pH (451). This separate approach is detailed in section 2.6.2.

During low-intensity exercise, H^+ efflux from working muscle can be mainly attributed to mechanisms other than lactate-coupled release (13, 16, 253). Indeed, lactate removal is the result of not only transport, but also of diffusion and metabolism (246). Nevertheless, in the context of muscle pH regulation it is sometimes useful to measure and report lactate concentration because of its near 1:1 stoichiometry with H^+ production (305, 482), albeit that this relationship is pH-dependent has been the focus of much debate in the literature (50, 62, 392, 412, 482).

2.3 pH Measurement

The resting range for venous blood pH at 37°C is around 7.35–7.45 (220, 299). Reported values for resting skeletal muscle intracellular pH (pH_i) typically range between 6.90 and 7.20 (see Appendix G). The variation is predominantly a function of the measurement technique used. For example, one limitation of measurements in muscle homogenate is that the sample will be a mixture of intracellular and extracellular fluids, increasing pH_i by an estimated 0.03 pH units (405). Indeed, measurement of pH in crude homogenate is arguably of limited epistemological value, given that different organelles and compartments in the myocyte will have distinct pH values. At 37°C the pH of the mitochondrial matrix is about 8.1, whereas the pH of the cytosol reflects the pH_i above (220). Nevertheless, until such techniques exist to discriminate pH between compartments *in vivo* then the averaging of pH in crude homogenate must suffice.

Because there are so many factors influencing pH_i measurement, caution must be used in comparing data between studies and between techniques. Theoretically, the loss of CO_2 during the freeze-drying process should return higher pH_i values in freeze-dried compared to wet muscle (198, 448), but in practice this difference disappears at pH_i lower than ~6.8 (198, 474). Delays in snap-freezing samples in liquid nitrogen may lead to increases in pH_i due to phosphagen hydrolysis (111). Conversely, failure to adequately blot wet muscle with filter paper to remove blood prior to freezing may artefactually increase the pH_i (198). This may be a particular source of error with more hyperaemic, end-exercise samples. If metabolite assays requiring instant freezing of muscle are to be performed on the same samples, then the potential error due to blood contamination increases.

Phosphagen hydrolysis initiated through the homogenisation process has been reported to produce only a modest acidification (0.01 pH units) of resting muscle samples, and a slight

alkanisation of electrically-stimulated muscle (0.03 pH units), when measured at 37°C (442). Evaporative water loss will result in progressively more acidic samples the longer the sample is maintained above ambient temperature (see Appendix J). This may, in part[5], explain the previously unidentified cause for acidic pH drift (375). Nevertheless, the temperature at which measurement is performed will always affect pH (381). Thus, to report pH_i at a physiologically valid temperature, these are currently unavoidable limitations of the homogenate measurement technique.

Finally, measurement of pH_i by phosphorous-31 magnetic resonance spectroscopy (^{31}P-MRS) is not affected by artefact arising from phosphagen hydrolysis, nor is measurement contaminated by extracellular proteins. Hence, theoretically and often practically, MRS delivers marginally more acidic values (108), though there is not always an appreciable difference between pH_i values in muscle at rest when comparing MRS and biochemical analyses (457). Using MRS, pH_i is calculated from the chemical shift of inorganic phosphate (P_i) relative to phosphocreatine (PCr) (14, 121, 262):

Eqn. 2 $$pH_i = 6.75 + \log_{10}\left(\frac{\sigma - 3.27}{5.69 - \sigma}\right)$$

where σ is the chemical shift of P_i relative to PCr in parts per million.

Yet, the use of MRS to calculate pH_i is also not without criticism. Because the estimation of pH_i is dependent on $[P_i]$, greater weight will be given to those fibres that show greater hydrolysis of PCr to P_i (411). This is likely only to produce significant error of estimation during the early seconds of exercise. A second criticism is that the coefficients in the equation, representing the chemical shifts of monoprotonated (3.27) and diprotonated (5.69) phosphate, are not constants, but may vary depending on the bioenergetic status of the muscle studied (108). One more fundamental limitation with the use of MRS during exercise studies is that the active muscle mass is limited by the bore of magnet and the type of exercise is restricted to comparatively immobile, single-limb modalities. For multi-joint dynamic exercise, biochemical analysis remains the only pragmatic option for muscle pH measurement.

2.4 H^+ Accumulation

Changes in pH_i can be caused by factors external to the muscle cell, for example during hypercapnia, or more importantly for the current thesis, by activity within the muscle cell itself. During periods of high energetic demand, the required rate of adenosine triphosphate (ATP) production cannot be met by the maximal rate of oxidative phosphorylation. An immediate but low yield of ATP is provided through hydrolysis of free ATP (ATPase) and phosphorylation of

[5] Applying a correction factor for volume loss does not adequately normalise pH, indicating that there are other methodological factors affecting acidic drift using a glass microelectrode.

adenosine diphosphate (ADP) by PCr. These are conceptually referred to as the coupled Lohmann reaction, albeit they are separate enzyme-catalysed reactions (392):

Eqn. 3 ATPase reaction: $ATP \rightleftharpoons ADP + P_i + \alpha H^+$

Eqn. 4 + Creatine Kinase (CK) reaction: $\underline{PCr + ADP + \beta H^+ \rightleftharpoons ATP + Cr}$

Eqn. 5 = Lohmann reaction: $PCr + \gamma H^+ \rightleftharpoons Cr + P_i$

The values of the proton stoichiometric coefficients α and β are a function of pH (305), and are in units of moles of H^+ per mole of either reactant or product (277). At constant pH, the ATPase reaction by definition does not produce H^+. Production of H^+ is represented by a positive coefficient (α), while H^+ consumption has a negative coefficient (β). In the physiological pH_i range $\beta > \alpha$, resulting in net H^+ consumption: $\gamma = \alpha + \beta$, i.e., γ is negative (105).

Almost immediately after exercise begins, ATP turnover requires an increasingly larger contribution from fast or 'anaerobic' glycolysis/glycogenolysis coupled to ATP hydrolysis (230), with the resultant formation of lactate – through the lactate dehydrogenase (LDH) reaction – and H^+.[6] This is essentially a reversal of the Lohmann reaction (Eqn. 8) (173, 305, 390):

Eqn. 6 ATP synthesis: *N*-Glycogen + 3ADP + $3P_i$ + $3\varepsilon H^+$ ⇌ 3ATP + $2La^-$ + (*N*-1)-Glycogen (where *N* is the number of glucose residues)

Eqn. 7 + CK reaction: $\underline{3ATP + 3Cr \rightleftharpoons 3PCr + 3ADP - 3\beta H^+}$

Eqn. 8 = PCr synthesis: *N*-Glycogen + $3P_i$ + 3Cr ⇌ 3PCr + $2La^-$ + (*N*-1)-Glycogen + $3\delta H^+$

Eqn. 9 LDH: $Pyr^- + NADH + H^+ \rightleftharpoons La^- + NAD^+$

(Pyr^-: pyruvate, $NADH/NAD^+$: reduced/oxidised nicotine adenine dinucleotide)

Here, the proton stoichiometric coefficient δ of the reverse Lohmann reaction (Eqn. 8) is the sum of the coefficients of Eqn. 6 and Eqn. 7: $\delta = \varepsilon + \beta$ (305). Eqn. 7 results in H^+ production; hence, the negative sign means the β coefficient is positive, i.e., a double negative. Ultimately, at resting pH_i the primary source of H^+ is ATP hydrolysis (260, 390), whereas during exercise resulting in decreased pH_i, the relative proportion of H^+ from ATP hydrolysis decreases as H^+ from glycolysis to lactate increases (260).

[6] The stoichiometry has traditionally been considered as: 1 Glucose/Glucosyl unit → $2La^- + 2H^+$. But this proton stoichiometry varies with pH, $[Mg^{2+}]$, and whether the substrate is glycogen or glucose (173, 219). With glycogen as the substrate, 3 mol of ATP are produced per mol of glucosyl unit; whereas 2 mol of ATP are produced per mol of glucose (220). Nevertheless, the net yield of H^+ remains the same at $2H^+$ per glucosyl unit regardless of starting substrate *if* glycolysis is coupled to ATP hydrolysis (61, 173, 219). And this stoichiometry is independent of pH and $[Mg^{2+}]$ (219). One caveat is that, in an open system, non-stoichiometric appearance of La^- and H^+ can be expected with differential transport of both across the cell membrane (305).

During low-intensity exercise, sufficient O_2 availability facilitates acceptance of H^+ at the end of the electron transport chain, as well as electrons from NADH (or FADH), with the resultant formation of water. However, during high-intensity exercise the rate of H^+ production through ATP hydrolysis coupled to glycolysis exceeds consumption by the pH_i-regulatory processes. This results in intracellular acidity, measured as a reduction in pH_i. Substantial drops in pH_i are typically only seen following dynamic high-intensity exercise of less than 10 minutes[7] (84, 241). A mean decline of 0.4 pH units is evident following both high-intensity continuous and intermittent exercise (*cf.* Appendix G), with reductions of up to 0.8 units occasionally reported (e.g., 319). The greatest reductions in pH_i are seen in type II fibres (158). Decreases below 6.0 have been documented (500), but this has been attributed to calibration issues with MRS measurement (247). Finally, the timecourse of pH_i recovery is initially slow[8], but returns to resting levels within 10 minutes depending on the extent of the initial H^+ load (13, 53, 231). Some large inter-individual variations in the kinetics of recovery have also been reported (212).

2.5 $[H^+]$ in the Aetiology of Fatigue

Dysregulation of pH is known to be involved in a wide range of pathologies across many organ systems (360). But what are the potential implications for a reduction in pH_i in skeletal muscle? Before assessing these, it is important to note that whilst muscle fatigue is associated with a negative inotropic effect, i.e., a reduction in the force-producing capability of the active muscle, it may also be a consequence of the failure of ATP regeneration to match the rate of ATP usage. Essentially, a limited rate of energy supply could be the cause of task failure rather than contractile activity being impaired. As described by Fitts (158), defining fatigue in terms of power output "recognizes the ability to sustain a given work capacity without decrement requires the maintenance of both force and velocity".

In this regard, it is of interest to note that accumulation of intracellular H^+ has been implicated in both the force and velocity components of fatigue. Increased acidity has been postulated as being an important mediator of peripheral fatigue by inhibition of excitation–contraction (E–C) coupling, impairment of contractile activity, and inactivation of key enzymes (158), but the evidence is by no means unequivocal (3, 494). Indeed, some research supporting a deleterious effect of $[H^+]$ on specific mechanisms of ATP turnover concluded that there was no direct effect on muscle contractility (408).

[7] Large drops in pH_i have also been reported with common experimental models such as finger flexion exercise (with or without occlusion) and sustained or intermittent maximal voluntary contractions (Appendix G).

[8] The recovery in blood pH is markedly slower, remaining depressed 30 and 39 min after intense exercise (111, 212). Another study reported approximately 60 min recovery time after 2–3 min high-intensity exercise, albeit with biphasic kinetics, wherein the half-time of recovery was ~10 min (389). Similar data are presented in Appendix F, showing venous blood pH kinetics following a repeated-sprint ability test, with a half-time of 7.3 min.

Part of the equivocation regarding the effects of H^+ on muscle force production can be explained by disparate causes for different stages of fatigue and recovery. For example, convincing evidence of a pH-dependent component of the fatigue-induced reduction in force has been documented in humans (83). But looking at force recovery upon cessation of exercise, though the second slow phase of force recovery has been reported to be highly correlated[9] (r = 0.98) with pH_i (469), the initial fast phase of force recovery strongly points to a distinct pH-independent component (3). Others have provided supporting data using ^{31}P-MRS of a pH-independent component of fatigue, showing a distinct dissociation between force and $[H^+]$ during the early stages of a maximal voluntary isometric contraction (MVIC), and during recovery (125). Ultimately, perhaps the pertinent question to ask is not whether acidity is a factor in the aetiology of fatigue, but rather, how do the physiological mechanisms necessary for sustained power output in specific tasks respond to variations in $[H^+]$?

2.5.1 $[H^+]$ and Excitation–Contraction Coupling

Inhibition of distinct mechanisms of E–C coupling has been attributed in part to greater $[H^+]$ (158), but increasingly research has challenged the importance of acidity in disrupting E–C coupling (3). Much of the earlier intact single muscle fibre research was performed at non-physiological temperatures of 5–25°C (346, 493). As Figure 2.1 shows, when temperatures closer to physiological temperatures are used in similar models, the effects of reduced pH on muscle tension are mitigated, although not eliminated (270, 279, 346, 364, 493). In addition, the Ca^{2+} concentrations used have often been the equivalent of maximal or supramaximal physiological $[Ca^{2+}]$, with less reduction of the effects of increasing temperature seen at submaximal $[Ca^{2+}]$ (346).

It is important to note that data from an isolated muscle fibre model can only be as valid as the model itself and caution should be used in extrapolating to muscle contracting *in vivo* (17, 292). Indeed, even the higher temperatures used in *in vitro* models are still lower than mammalian muscle temperature, in particular the elevated temperatures during high-intensity contractions. In addition, studying the action of one ion in isolation risks ignoring the interaction between ions (85). Conversely, it is difficult to isolate the effects of acidosis on contracting muscle in human models from the other peripheral and central factors potentially affecting fatigue (280). The following sections document sequentially the key stages of E–C coupling and present the evidence for and against any inhibitory effect of elevated $[H^+]$.

[9]The following augmented Cohen's convention for qualitatively describing correlation coefficients will be used throughout this thesis: trivial < 0.1, small < 0.3, moderate < 0.5, large < 0.7, very large < 0.9, extremely large ≥ 0.9 (225).

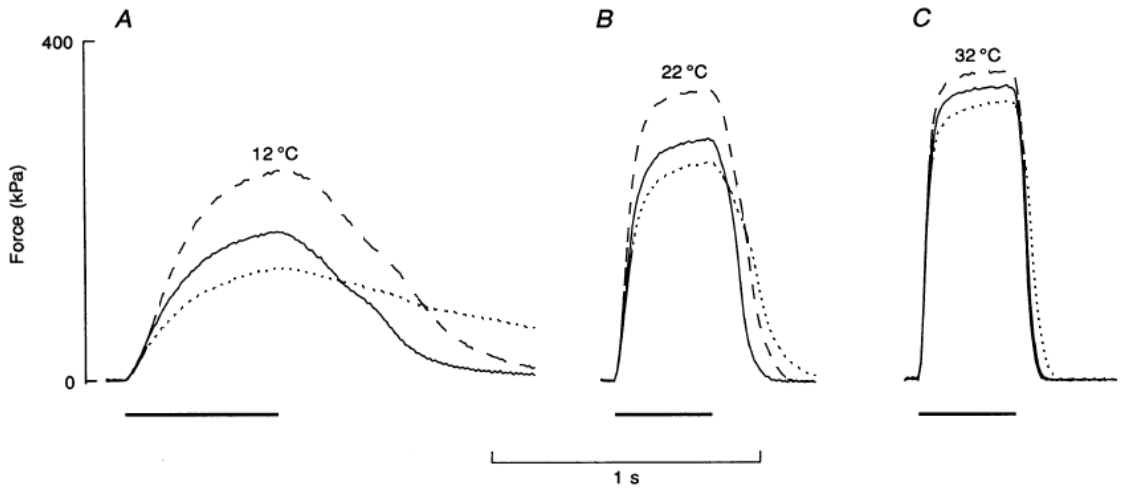


Figure 2.1 Mitigation of the pH-dependent reduction in force with increasing temperature. Electrically-induced tetani were performed on single intact mouse muscle fibres at three temperature, under three different pH conditions: acidic (30% CO_2, dotted lines), alkali (0% CO_2, dashed lines), and control (5% CO_2, solid lines). Modified from Westerblad *et al.* (493).

2.5.1.1 Action Potential Propagation

Release of acetylcholine at the neuromuscular junction facilitates propagation of an action potential by the motor neuron to the muscle, causing a wave of depolarisation to travel across the sarcolemma to the fibre interior via the T-tubules (449). Failure of the ability of the T-tubules to conduct an action potential has been implicated in fatigue, and may be due to an accumulation of interstitial potassium (K^+) (432). Some *in vitro* research has shown that acidosis enhances excitability. In frog sarcolemmal vesicles at room temperature, reduced pH maintained activity of ATP-sensitive K^+ channels (119). In resting isolated rat muscle at 30°C, acidity counteracted the reduction in excitability caused by increased extracellular [K^+] (353). Similarly, in the presence of chloride (Cl^-), a reduction in pH increased the excitability of T-tubules of single intact mouse muscle fibres at 25°C, enhancing propagation of an action potential (365). Others reported similar results in isolated rat muscles at rest, but when the muscles were stimulated *in vitro* there was no lessening of force production with lactate or lactic acid (272). And *in vivo* research in humans has shown indirect evidence of a link between reduced interstitial [K^+] and reduced interstitial [H^+] (15, 454).

2.5.1.2 Depolarisation Stimulates Ca^{2+} Release From the Sarcoplasmic Reticulum

Following propagation of an action potential, the change in membrane potential causes a conformational change in the voltage-sensitive Ca^{2+} channels (dihydropyridine receptor complexes, DHPRs) of the T-tubules, in turn initiating Ca^{2+} release into the cell from the close proximity ryanodine receptors (RyR) of the sarcoplasmic reticulum (SR) (3). Impaired Ca^{2+}

release from the SR is considered to be one of the primary causes of fatigue (4). Ca^{2+} release has been reported to be inhibited by elevated $[H^+]$ at 37°C in an isolated SR model (341), and in SR vesicles prepared from rabbit muscle (153). In contrast, in single intact mouse muscle fibres, Ca^{2+} release failure measured at 22°C was less inhibited at the lower pH of high-intensity stimulation (97). And using single intact skinned toad fibres at 23°C (278), or similarly prepared rat fibres at 37°C (279), voltage-sensor activated Ca^{2+}-release was only marginally inhibited at a pH of 6.2.

2.5.1.3 Ca^{2+} Binds to Troponin C, Freeing up the Actin Binding Site

Release of Ca^{2+} from the SR causes a transient increase in free myoplasmic $[Ca^{2+}]$, which binds to troponin C, detaching tropomyosin from the binding site on actin (3, 449). Early data in an isolated protein model at room temperature suggested that H^+ can inhibit the binding of Ca^{2+} to troponin (164). Competitive inhibition of H^+ with Ca^{2+} for binding to SR Ca^{2+}-ATPase (SERCA pumps) has been found to result in inhibition of the latter in rabbit SR vesicles at 25°C (234). Separately, lower pH was associated with reduced affinity of troponin C for Ca^{2+} in rabbit skeletal muscle and dog cardiac muscle, leading to deactivation of ATPase activity (51, 52).

The pCa-tension curve – pCa is $-\log[Ca^{2+}]$ – is an indicator of myofibrillar Ca^{2+} sensitivity, where shifts in the slope or shape of the curve represent a change in the free $[Ca^{2+}]$ required to elicit a given force. Elevated $[H^+]$ has been reported to directly reduce Ca^{2+}-activated force of rabbit muscle fibres at 23°C (133), and rat and rabbit fibres at 10 and 15°C (322, 323). A rightward shift in the pCa-tension curve, indicating reduced Ca^{2+} sensitivity, was found following higher-intensity contractions inducing lower pH in single intact mouse fibres (97). More recently, a rightward shift in the pCa-tension curve was found at pH 6.2 in rat single type I and type II muscle fibres, with the effect greater at 30°C than 15°C (346). Separately, though a shift in the pCa-tension curve in skinned muscle fibres was found at reduced pH, a direct competition between H^+ and Ca^{2+} for binding sites on troponin was disputed on the basis of increasing $[Ca^{2+}]$ failing to overcome these effects (152). The latter authors also reported that small reductions in pH had opposite effects on cardiac (rat) and skeletal (frog) muscle because of differing optimum pH for Ca^{2+} loading of the SR, i.e., lower pH for skeletal muscle. One explanation may be the presence of different troponin isoforms in the different tissues (489). Finally, it has been argued that given the SERCA pumps bind less Ca^{2+} at low pH, this negates any H^+-induced reduction in Ca^{2+} sensitivity by increasing the Ca^{2+} available to bind to troponin C (280).

2.5.2 $[H^+]$ and Crossbridge Cycling

The final stages of E–C coupling involve removal of tropomyosin from the actin binding site, with crossbridge cycling then initiated by binding of myosin to actin, powered by ATP hydrolysis (3, 449). Inhibition of specific phases of crossbridge cycling has been attributed to increased $[H^+]$ (158, 298). The evidence for these mechanisms is dealt with next.

2.5.2.1 Force Development

After the myosin head attaches to the binding site on actin, P_i is released from actomyosin and the crossbridge undergoes the powerstroke (159). The final stage involving release of ADP breaks the actomyosin crossbridge. Repetition of the phases of crossbridge cycling leads to the development of muscular force. $[H^+]$ has been postulated as directly affecting contractile activity by reducing the number of crossbridges and/or reducing the force per individual crossbridge (158, 231). Moreover, actin and myosin initially bind in a weak state, and following the release of P_i, bind in a strong state, which respectively form low- or high-force crossbridges (159). Some evidence suggests that a reduction in pH_i can inhibit the number of high-force crossbridges (159).

Doubt remains over whether there is any direct inhibitory effect of H^+ on contractility (408). As was noted in section 2.5.1, many of the studies demonstrating an effect of pH on reduced crossbridge interaction have been undertaken at non-physiological temperatures (92, 109, 320-323), and temperature has a marked effect on the contractile characteristics of skeletal muscle (383). A reduction in force after fatiguing contractions has been found to be either diminished at higher temperatures (30°C) in isolated rat muscle (398), or conversely increased at higher temperatures (37°C) in humans (123, 124). When temperatures closer to mammalian body temperature have been used (25 to 32°C), a reduction of the depressive effect of acidity on force production (and shortening velocity) is seen (364, 493, 504). In fact, twitch tension of rat *extensor digitorum longus* (EDL) muscle at 35°C has been reported to increase as pH decreases (range: 8.0–6.5), while at 15°C tension decreased across the same pH range (382). Criticisms of these data are that they do not report the fibre type of the single fibres tested – there being well-established contrasting fatiguing properties of type I and type II muscle fibres – and they typically study the effects of pH on peak tension or velocity of shortening, but not the effects on peak power, which occurs at submaximal tension (270). The latter authors have also reported a lessening of the $[H^+]$ effects on peak force in rat type I and type II fibres at 30°C versus 15°C (270). However, they found peak power to be reduced more by low pH at 30°C than 15°C in type I fibres, but reduced less at 30°C in type II fibres (Figure 2.2). A counter-criticism of these data is that pH 6.2 is non-physiological for type I fibres (3).

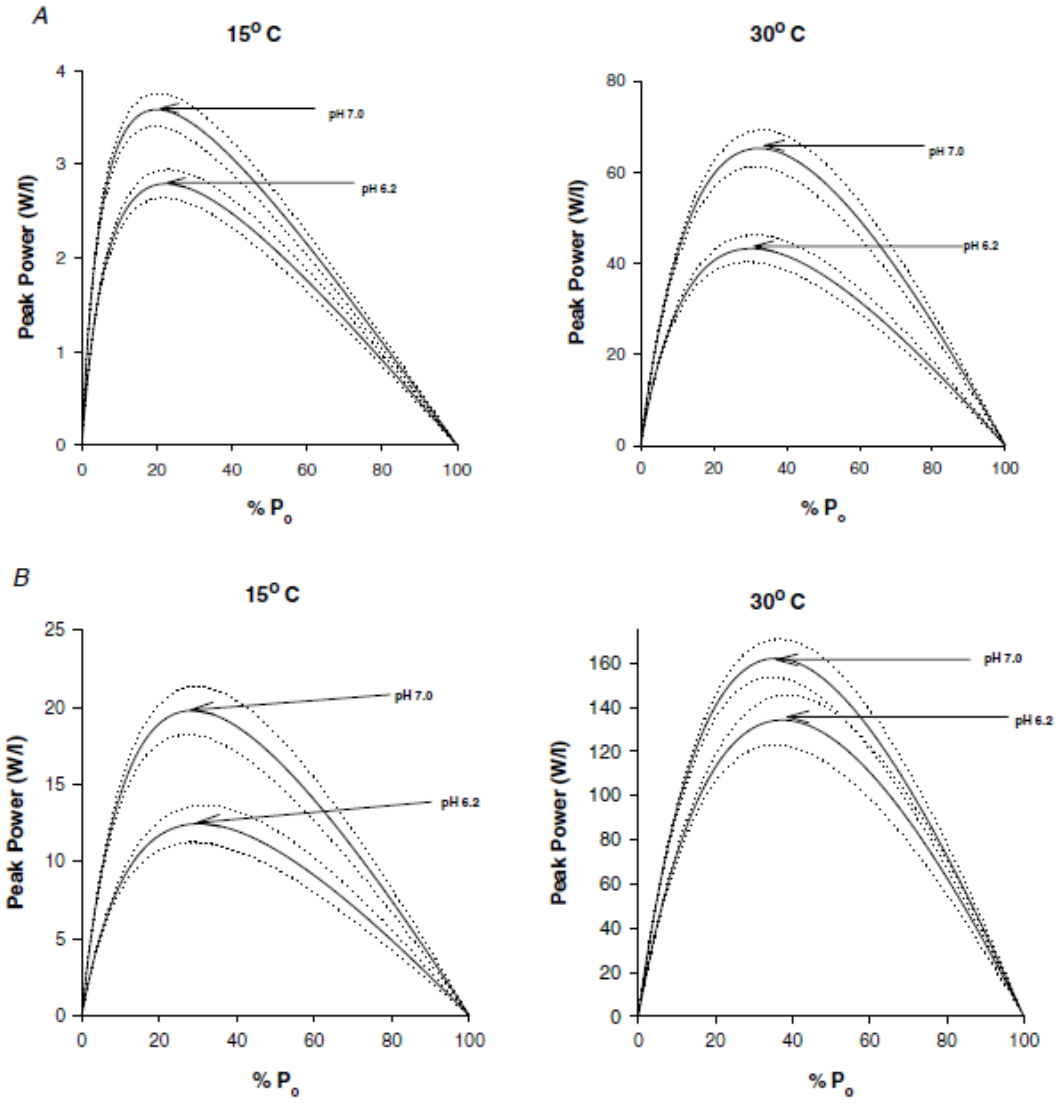


Figure 2.2 Effects of pH on force-power curves for *soleus* type I (A) and *gastrocnemius* type IIa (B) mouse fibres at 15 and 30°C. The curves are mean (solid) ± SE (dashed) values for pH 7.0 and 6.2. P_0 = maximal fibre force. Taken from Knuth *et al.* (270).

2.5.2.2 Relaxation

Muscle relaxes when the transient increase in myoplasmic [Ca^{2+}] is reversed by pumping of Ca^{2+} back into the SR by the ATP-dependent SERCA pumps (3, 449). The reduction in free [Ca^{2+}] results in dissociation of Ca^{2+} from troponin (3). A slowing of relaxation results in increased time between contractions, and fatigue has sometimes been associated with reduced relaxation time in both *in vitro* (492) and *in vivo* models (122, 240). There are some data to suggest that relaxation may also be H^+-sensitive. A non-linear relationship between [H^+] and relaxation rate in anaerobic frog muscle at 4°C was found using ^{31}P-MRS, though similarly strong relationships were reported for other metabolites (122). Cady *et al.* (82) reported evidence in humans that a prolonged relaxation time following fatiguing

contractions had two distinct components, one of which was caused by reduced pH_i. Slowed relaxation rate has similarly been attributed to acidosis in isolated rat muscle stimulated at 20°C (407), and single intact mouse muscle fibres contracted at 28°C (76). In contrast to muscle tension, in humans the increase in relaxation time of fatigued muscle relative to unfatigued muscle is greater at more physiological temperatures (123).

2.5.3 $[H^+]$ and ATP Turnover

In addition to a negative inotropic effect, fatigue has also been attributed to the failure of ATP regeneration to match the rate of ATP usage, i.e., the velocity component of fatigue. For some intracellular proteins, such as the histidyl-imidazole residues (*cf.* 2.7.2.1), the temperature dependence of pH is paralleled by a similar change in pK_a with temperature, which serves to maintain the protonated:deprotonated ratio of these proteins (220). However, a reduction in pH can provoke changes in the rates of some enzymatic reactions (436). The ionisation state of enzyme-binding sites may affect their substrate affinity or catalytic capacity (397). Of particular note is that the rate-limiting enzymes for glycogenolysis and glycolysis, namely glycogen phosphorylase (Phos) and phosphofructokinase (PFK), are inhibited *in vitro* at low pH (94, 229, 460, 473, 475). Conversion of the less active Phos *b* form to the more active (phosphorylated) Phos *a* form by phosphorylase kinase has been shown to be inhibited by reduced pH (93, 221, 363). Moreover, Phos conversion is regulated by increased concentrations of Ca^{2+}, epinephrine, and cyclic adenosine monophosphate (cAMP), and accumulation of the latter has also been reported to be inhibited following a reduction in pH (93, 446). Similarly, PFK has been reported in *in vitro* experiments to have a large reduction in activity with just a 0.1 pH unit decrease (473), and to be almost completely inactivated at a pH of 6.4 (405). ATP is the primary allosteric inhibitor of PFK, and increased $[H^+]$ enhances binding of ATP to PFK (132, 445).

Despite the evidence presented above, inhibition of glycolysis by H^+ accumulation may not occur *in vivo* (445, 446). The optimum pH for activity of specific enzymes shifts with changing temperature (436, 437). The inhibitory effects of increased $[H^+]$ may be counteracted by allosteric activation of the glycolytic enzymes, whereby specific substrates bind to a secondary (allosteric) site on the enzyme, keeping the active site open. For example, Hultman & Sjöholm (230) found no change in glycolytic rate at the end of exercise despite very high muscle $[La^-]$ and, though not measured, presumably low pH_i. There are several proposed allosteric activators, including ADP, AMP, IMP, and cAMP; the hexose phosphates: fructose 6-phosphate, fructose 1,6-diphosphate, fructose 2,6-diphosphate, glucose 1,6-diphosphate; and ammonium (NH_4^+) (12, 15, 132, 443, 445). Some of the potential allosteric modulators are pH-dependent too though, with, for example, [ADP] decreasing at lower pH because of a shift in the

equilibrium of the CK reaction (106). This shift in equilibrium will also reduce monoprotonated P_i, the substrate for Phos (93).

Dobson *et al.* (132) postulated that any modulator that increases the ratio of protonated to unprotonated forms of enzymes allows the muscle cell to mitigate pH-dependent ATP inhibition. For example, they showed 10 μM of fructose 2,6 bis-phosphate to counteract the pH-dependent inhibitory effects of 5 mM ATP on PFK in rabbit muscle. A caveat is that their experiments were conducted at 25°C and at a pH no lower than 6.8.

Around the same time, Spriet *et al.* (443) provided supporting data in humans following intermittent electrical stimulation with occluded blood flow. They found both the glycogenolytic and glycolytic rates to be quite stable, after an initial increase then decrease, despite a continued reduction in pH to 6.45. PFK activity was assumed to be equivalent to the glycolytic rate, and therefore changed little over a period of decreasing pH. Over the same period of contractions there was also little change in specific allosteric activators, the hexose monophosphates, fructose 1,6-diphosphate, and P_i; and similarly so with the allosteric inhibitors, ATP and PCr. Conversely, during the initial period of increased PFK activity, where pH reduced from 7.00 to 6.70, concentrations of these same proposed activators of PFK increased and concentrations of the inhibitors decreased. The authors further suggested that a measured increase in IMP formation would lead to a stoichiometric increase in NH_4^+ accumulation, the latter also acting as a positive modulator of PFK activity. Ultimately, PFK activity was considered to be maintained in the face of reducing pH, at least until the final few contractions, where pH reduced to 6.40 and was considered to be inhibiting contraction, possibly through inhibiting PFK.

Although the rate of glycolysis may be inhibited at low pH (473), this may actually serve as a protective mechanism, preventing further acidification of the cell, sparing glycogen, and facilitating greater relative oxidative phosphorylation (220). Evidence for the latter was shown by sustained maximal pyruvate dehydrogenase (PDH)[10] activity parallel to reduced Phos activity as pH_i dropped with repeated bouts of maximal cycling (363). Exercise capacity remained compromised though, and indeed the rate of oxidative phosphorylation has also been reported to be inhibited at lower pH (244).

Finally, it is important to note that most of the animal research cited in this section was conducted using a frog model, typically *Xenopus*. When extrapolating from data elicited using frog muscle to mammalian muscle, one must be cautious, given frog muscle is highly glycolytic and has poor oxidative capacity (158). This dependence on non-mitochondrial ATP turnover means acidosis tends to be more pronounced in frogs compared to mammals (84). Furthermore,

[10] PDH_a is the active form of the pyruvate dehydrogenase complex (PDC) and catalyses decarboxylation of pyruvate to acetyl-CoA. PDH_a is the rate-limiting enzyme for carbohydrate oxidation by the Krebs (tricarboxylic acid) cycle (263).

in comparing rodent and human models, it is important to note the distinct fibre type differences that will contribute to contrasting contractile characteristics and metabolic activity. For example, small mammals such as rodents have primarily type IIx and type IIb muscle fibre types, whereas the muscles of large mammals such as humans are primarily comprised of type I and IIa fibres (420). Moreover, small mammals have greater relative oxidative enzyme content than humans. These contrasting capacities for relative substrate level and oxidative phosphorylation will impose different perturbations in pH during exercise, in particular for metabolic pH regulation (see section 2.8). Therefore, a comparison between rodent and human muscle data should be undertaken with these important distinctions in mind.

2.5.4 Induced Alkalosis or Acidosis

A popular approach in seeking to clarify the role of acidity in fatigue *in vivo* is to induce alkalosis, typically through provision of an exogenous buffer such as sodium bicarbonate ($NaHCO_3^-$), or to induce acidosis through ammonium chloride (NH_4Cl) supplementation or hypercapnic ventilation. Inducing alkalosis will not only have local effects at the tissue of interest but also systemic and central effects throughout the body, notably by altering oxygen–haemoglobin desaturation (3). Moreover, because the sarcolemma is relatively impermeable to ions such as bicarbonate (HCO_3^-) (130), supplementation has no direct effect on pH_i (43, 68, 129), or at most a small alkalising effect (352, 448). Extracellular buffers such as HCO_3^- mediate pH_i through several mechanisms, each of which are detailed later on (sections 2.7.3, 2.9.2, and 2.10.2). For example, in buffering extracellular H^+, and thus creating a greater ionic gradient, extracellular buffers enhance efflux of H^+ from the intracellular space via specific transport proteins.

One explanation for the conflicting data between *in vitro* and *in vivo* models is that acidosis can affect systemic or central factors other than muscle (84). Therefore, potential improvements in fatigue-resistance after induced systemic alkalosis cannot be directly attributed to attenuating the effects of acidity in the working muscle. Nevertheless, there is a large body of evidence showing performance improvements with alkalosis in high-intensity events lasting less than 10 minutes [for meta-analysis see Carr *et al.* (88)]. For example, improved repeated-sprint ability (RSA) with $NaHCO_3^-$ supplementation, and the parallel increase in post-exercise muscle [La^-], was explained in part by a greater anaerobic energy contribution (43). This is supported by findings of enhanced glycolysis with $NaHCO_3^-$ supplementation after high-intensity exercise (222), resulting from elevated Phos *a* and PFK activity. And from the same group, induced acidosis through NH_4Cl supplementation resulted in lesser glycolytic contribution to high-intensity exercise because of inhibition of Phos *a* and PFK activity (221). These data support earlier evidence in humans showing less glycogen utilisation and inhibited

La^- efflux following exercise to fatigue under acidosis (460). Inhibition of glycogenolysis was also shown in perfused rat muscle undergoing exercise after both metabolic and respiratory acidosis (440), though a similar study with alkalosis found only greater La^- efflux but no altered glycogen utilisation (441). Contrary to the evidence documented for *in vitro* studies in section 2.5.3, these data provide support for a pH-dependence of enzyme activity.

Also in contrast to the *in vitro* data detailed above (section 2.5.1.1), where acidosis enhanced membrane excitability (353, 365), some *in vivo* research in humans has found indirect evidence that extracellular alkalosis enhanced excitability. Prior $NaHCO_3^-$ supplementation resulted in greater K^+ efflux during finger-flexion exercise (438). Similarly, inducing alkalosis through sodium citrate supplementation reduced interstitial $[K^+]$ following one-legged knee extension to fatigue (454). In both studies fatigue was delayed by supplementation. Again, however, that these data could not be replicated in isolated rat muscle incubated with high HCO_3^- (68), indicates that neither experimental model should be considered in isolation.

2.5.5 Summary of H^+ in Fatigue

There seems little doubt that the role of acidosis in fatigue was overestimated in the past. Considering a reduction in pH_i as detrimental to muscle functioning is too simplistic without a greater understanding of the whole organism. Nevertheless, when considering all of the evidence, there do seem to be pH-dependent components of muscle contractile function. However, these need to be considered additive to other factors, rather than in isolation. Acidosis has been found to maintain membrane excitability in resting frog and rodent muscle, but the data conflict with stimulated muscle *in vitro*, or with human research. *In vitro* studies have shown that peak force and velocity of shortening are less affected by pH at temperatures closer to physiological than earlier data indicated. However, power appears to be reduced more by low pH at higher than at lower temperatures, but in a fibre-type dependent manner. H^+ accumulation may affect Ca^{2+} sensitivity, but this may be negated by the effects of pH on the affinity of the SERCA pumps for Ca^{2+}. And similar to force production, muscle relaxation seems to have pH-dependent and pH-independent components, which indicate task or exercise-intensity specificity. Finally, the conflicting data between *in vitro* and *in vivo* models regarding the effects of pH on glycolytic enzyme activity highlight the limitations of relying on either model in isolation to interpret data.

Whether pH_i-regulatory capacity influences exercise capacity is not yet fully understood. Cognisant of the limitations of studying specific physiological mechanisms in humans, one of the main objectives of the present thesis was to investigate the parallel effects of exercise training on pH_i regulation and exercise performance. The following sections describe in detail the components of muscle pH_i regulation and how they respond to exercise.

2.6 Introduction to pH Regulation

2.6.1 Traditional Acid–Base Theory

Within the traditional Brønsted–Lowry acid–base theory, a pH buffer is any chemical or physiological mechanism that reduces the magnitude of change in pH. Subsequent to exhaustive exercise in humans, pH_i typically falls no lower than 6.2 in muscle, with further reduction mitigated by the buffering capacity of the muscle (158, 229). Regulation of pH in skeletal muscle involves balancing H^+ production, buffering, and removal, and, under traditional acid–base chemistry, can be categorised into the following systems:

1. Physicochemical buffering
2. Dynamic/metabolic buffering
3. Transmembrane acid (H^+) and base (HCO_3^-) transport

An important distinction to make is that transmembrane acid/base transport mechanisms are not considered buffers *per se*, in part to reflect the time dependence of ion transport. While physicochemical buffering reactions reach completion within fractions of a second, and metabolic reactions within a few seconds, ion transport across the cell membrane is much slower, taking several minutes (397). Moreover, buffers minimise reductions in pH_i, but have a limited capacity to do so, whereas transport mechanisms remove all of an acid load, but are rate-limited in doing so.

2.6.2 Strong Ion Difference

An alternative approach to understanding acid–base physiology is Stewart's physicochemical or strong ion difference ([SID]) model, which focuses on the three fundamental laws governing the behaviour of aqueous solutions: conservation of mass, electrical neutrality, and dissociation equilibrium (451). In a solution, the [SID] is defined as the difference between the concentrations of strong (i.e., fully dissociated) base cations and strong acid anions[11] (451). In mammalian intracellular compartments K^+ is the most important strong ion (452). Extracellularly[12], the most important strong ions are Na^+ and Cl^-, and to a lesser extent, because of their low concentrations, K^+, SO_4^{2-}, Ca^{2+}, and Mg^{2+}. Two important organic strong ions, because of their high K_a values, are phosphocreatine (PCr^{2-}) and La^-.

According to Stewart (451), the [SID] constitutes an independent variable for a given system, whilst $[H^+]$ is a dependent variable[13] (451). Therefore, changes in $[H^+]$ – or the other dependent variable HCO_3^- – are dependent on changes in the [SID], or the other independent

[11] Hence, [SID] is a negative value if strong anion concentration is greater than strong cation concentration.
[12] In plasma, $SID = Na^+ + K^+ - Cl^- - La^-$ (243).
[13] In biological systems the dependent variables are $[H^+]$, $[OH^-]$, $[HCO_3^-]$, $[CO_3^{2-}]$, [HA], and $[A^-]$.

variables, PCO_2 and total weak acid concentration (A_{tot} or [A], i.e., protein buffers). During exercise the most important contribution to muscle [H^+] comes from the [SID] (291). Crucial to this understanding is that changes in any of the independent variables such as the [SID] can cause changes in the dependent ones; however, while changes in dependent variables may be correlated because of this reason, a dependent variable cannot cause changes in any of the dependent variables. To put it in terms relevant to the current thesis, with this model, changes in [H^+] are not explained simply by proton movements, albeit they can contribute to the mechanism involved.

As a given solution must obey the principle of electrical neutrality, the [SID] must be balanced by the sum of the charge on all the weak ions:

$\sum$[strong base cations] – $\sum$[strong acid anions] + $\sum$[weak base cations] – $\sum$[weak acid anions] + [H^+] – [OH^-] = 0

where the [SID] = $\sum$[strong base cations] − $\sum$[strong acid anions].

The value and mechanistic validity of the SID approach has been criticised by some (254, 275, 351, 430). Kurtz *et al.* (275) have shown the SID approach to be a mathematical construct rather than an experimentally-derived theory. In a specific critique, these authors prove that a change in the [SID] cannot by itself determine [H^+]. Essentially, rather than being cause and effect relationships, there is an arbitrariness about the choice of dependent and independent variables. Interestingly, the authors also show the [SID] to be quantitatively equivalent to the Henderson–Hasselbalch equation (see section 2.7). Therefore, while the [SID] may be a useful quantitative tool, as a model it does not provide additional mechanistic explanation for the biochemistry underpinning pH regulation.

Finally, a corollary of the SID approach is that the physical transport of H^+ (detailed in section 2.10) across the plasma membrane is deemed by some as merely a construct (291). According to Lindinger *et al.* (291), "consideration of the physical chemistry of water reveals that it is highly improbable that H^+ is physically transported and that movement of $lactate^-$ alone from one side of a membrane to the other is sufficient to produce the measured H^+ responses". They later argued there was no evidence a proton is transported, in particular given that the H^+ (or H_3O^+) is in existence for only 10^{-6} s (293). Halestrap (193) countered that the transient existence of a proton is a moot point because free protons are not moving through the substrate-binding channel, rather their model shows that specific residues on, for example, the La^-/H^+ (monocarboxylate) transporter 1, such as lysine (K38), aspartate (D302), and arginine (R306), undergo protonation/deprotonation. Juel (254) more robustly stated "although the authors who advocate the Stewart approach question that co-transfer of H^+ takes place, they have never clearly stated what the nature of these systems are, whether they exist and whether they transport only Na^+, lac^- or HCO_3^- in an electrogenic manner".

2.7 Physicochemical Buffering

The buffering capacity of non-living systems is determined solely by physical and chemical processes (229). This physicochemical buffering also plays an important role in humans and other living organisms, and entails H^+ uptake by weak acids and bases. Because buffering powers are additive, the total (intrinsic) buffering capacity (β) of a cell is the sum of the individual buffering powers (64).

A buffer consists of a weak acid (HA) and its (dissociated) conjugate base (A^-), or similarly a weak base and its conjugate acid. The total concentration of the weak acid and its conjugate base ([A]) is one determinant of buffering power (242). Weak acids[14] only partially ionise in solution, reaching dissociation equilibrium such that their behaviour can be described by the following equations:

Eqn. 10 Weak acid: $HA \rightleftharpoons H^+ + A^-$

Eqn. 11 Dissociation equilibrium: $[H^+] \times [A^-] = K_a \times [HA]$

Eqn. 12 $\therefore K_a = \frac{[H^+][A^-]}{[HA]}$

Eqn. 13 $\therefore \log K_a = \log \frac{[H^+][A^-]}{[HA]} = \log H^+ + \log \frac{[A^-]}{[HA]}$

Eqn. 14 $\therefore pH = pK_a + \log \frac{[A^-]}{[HA]}$, giving the Henderson–Hasselbalch equation

When the acid is 50% dissociated, the concentrations of the acid and conjugate base are equal, hence, the dissociation constant and $[H^+]$ are equal. A weak acid provides its maximum buffering power at this point, i.e., when $pK_a = pH$ (229). Using the Henderson–Hasselbalch equation, and knowing or estimating the muscle concentration of a buffer, it is possible to quantify the buffering power over a specific pH range (199, 302). For example, between an assumed physiological pH transit range of 7.1 to 6.5:

Eqn. 15 At pH 7.1: $[HA]_{7.1} = \frac{[A]}{1+10^{(7.1 - pKa)}}$ (where $[A] = [HA] + [A^-]$)

Eqn. 16 At pH 6.5: $[HA]_{6.5} = \frac{[A]}{1+10^{(6.5 - pKa)}}$

Eqn. 17 $\therefore [HA]_{7.1} - [HA]_{6.5} = H^+$ buffered over this range

If the ratio of $[HA]:[A^-]$ is beyond 1:50 or 50:1, the buffer is considered to have lost its buffering power[15], i.e., about 1.7 pH units either side of the pK_a (36). Clearly for a buffer to be physiologically relevant it must have a pK_a close to the physiological pH range. Crucial to the understanding of muscle buffer capacity (βm) is that as pH_i changes during exercise, the buffering power for a given compound will vary as pH_i approaches or deviates from its pK_a (see

[14] Note that total weak acids constitute one of the independent variables of the Stewart approach, *viz.* $[A]_{tot}$, whereas [HA] and $[A^-]$ are considered dependent variables.
[15] At this point buffer power is about 8% of maximum.

Figure 2.3). Therefore, total buffering is a dynamic function of pH_i and the pK_a of constituent buffers.

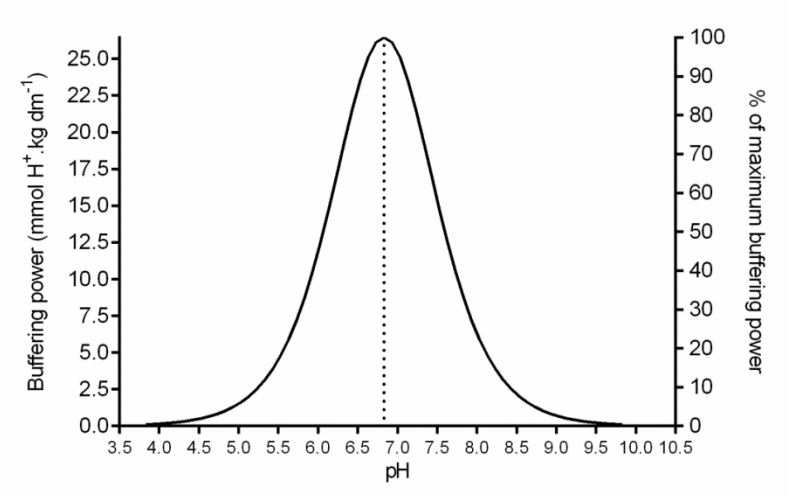


Figure 2.3 Buffering power of a single physicochemical buffer as a function of pH. Example for protein-bound histidine with an assumed pK_a of 6.83 and a concentration of 41 mmol H^+•kg dm^{-1}, calculated as per (64). Additional details are provided in section 2.7.2.1.

One additional factor not accounted for by the description up to now of buffers is their mobility. Buffers such as proteins can be categorised as fixed, and thus compartmentalised, whereas mobile buffers such as phosphate and carnosine are free to move throughout the cytoplasm (55, 255). Further information on the potential role of mobile buffers is covered in section 2.10.5. The following sections detail the significant physicochemical buffers in skeletal muscle.

2.7.1 Phosphates

Of the phosphate compounds, ATP and ADP contribute little to βm, despite having pK_a values (6.7–7.0) within the physiological pH transit range. This is because they exist in the cell primarily as Mg^{2+}-bound complexes – 92% and 53% respectively – with ADP also bound to proteins such as actin (81, 229, 405, 426). P_i, on the other hand, provides comparatively high buffering power. One group calculated the relative contribution to be 60–70% of total buffer capacity following exhaustive exercise in rainbow trout (362), while early buffering research reported that [ortho]phosphate accounted for 50% of non-protein buffering, albeit measured in rigour (19). Burton (81) previously stated that phosphate buffering in most cell types was less than the histidine imidazole groups (see 2.7.2.1), but acknowledged that there was uncertainty because of phosphate variability and the interaction with other compounds (81).

Recall from Eqn. 3 that hydrolysis of ATP to ADP releases P_i and a proton. Rewritten slightly to indicate the charge state of P_i (HPO_4^{2-}) gives:

Eqn. 18 ATPase: $ATP \rightleftharpoons ADP + HPO_4^{2-} + \alpha H^+$

One of the three single bond oxygen atoms has a pK_a in the physiological pH range of 6.82 (390), and is therefore readily protonated[16], converting P_i from its monoprotonated to diprotonated form:

Eqn. 19 $HPO_4^{2-} + \alpha H^+ \rightleftharpoons H_2PO_4^-$

As exercise intensity increases, greater phosphagen hydrolysis leads to an increased phosphate buffering contribution (Figure 2.4). Using ^{31}P-MRS, Marcinek *et al.* (305) calculated phosphate buffering to be 11% of total buffering at rest, but to increase to 44% of total buffering during ischaemia.

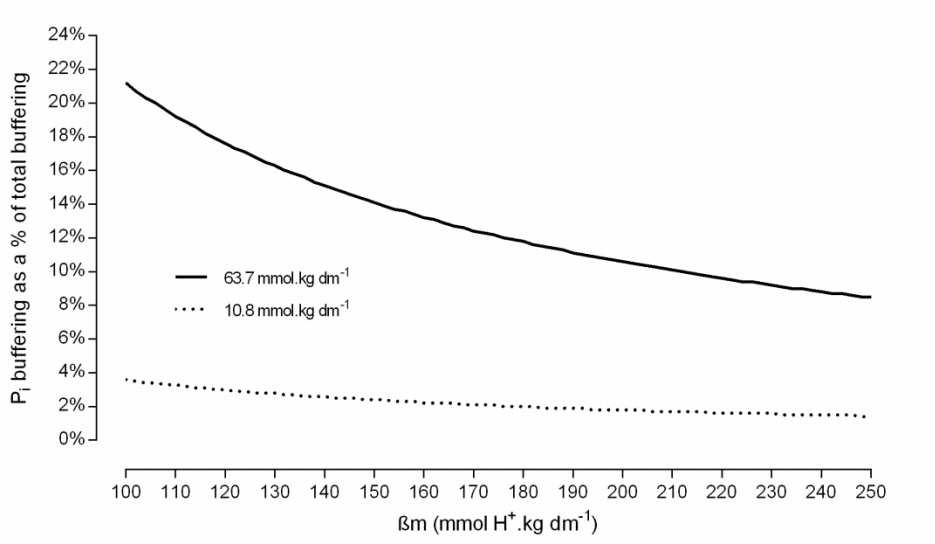


Figure 2.4 Total inorganic phosphate (P_i) buffering as a percentage of a broad range of total muscle buffer capacity (βm) values. The two curves represent an example resting (10.8 mmol•kg dm^{-1}) and end-exercise (63.7 mmol•kg dm^{-1}) content after 3 × 30-s maximal sprints (363). Buffering is calculated from the Henderson–Hasselbalch equation over an assumed physiological pH range of 7.1–6.5, and for a pK_a of 6.82 for P_i. For example calculations see Appendix M.

It is important to note that the free P_i is not stoichiometric to ATP turnover because HPO_4^{2-} serves as a substrate not only for glycolysis/glycogenolysis, but also for oxidative phosphorylation (390). As pH decreases and [HPO_4^{2-}] increases, $H_2PO_4^-$ becomes more important as a substrate for the glycolytic reactions Phos and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), increasing H^+ release from these reactions and thereby reducing net phosphate buffering (392). Moreover, much of the P_i released during phosphagen hydrolysis

[16] pK_a for the other two oxygen atoms are 2.15 and 12.38.

will form hexose monophosphates with glucose – glucose 1-phosphate, glucose 6-phosphate, and fructose 6-phosphate – and glycerol 3-phosphate[17] with glycerol (54, 229, 403, 443). Concentration of the hexose monophosphates at rest is low, but with the large glycogenolytic ATP turnover during high-intensity exercise, [glucose 6-phosphate] in particular increases (53, 54, 95). With pK_a values of 6.11–6.13 the hexose monophosphates are comparatively poor buffers. Glycerol 3-phosphate concentration has been found to increase ~20-fold after 3 × 30-s sprints (363), which, with a pK_a of 6.67, would contribute more to phosphate buffering as pH decreases (2). Clearly the relative contribution of total phosphate buffering will vary depending on the composition of the phosphate pool. And phosphate buffering calculated on the basis of resting phosphate concentrations is arguably of limited value, given that it is buffering during exercise that is of interest. Therefore, phosphate buffering becomes increasingly important as a result of PCr hydrolysis during exercise, although it likely still remains secondary to buffering by proteins and dipeptides.

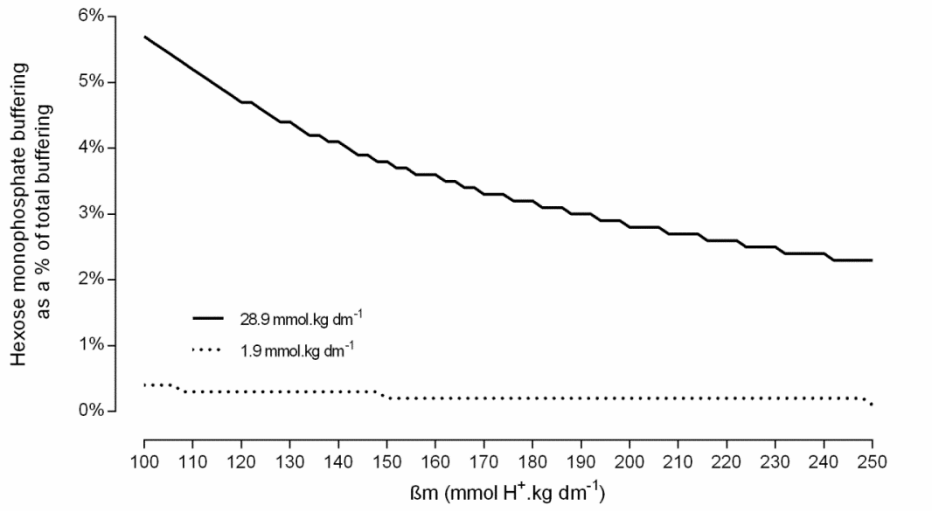


Figure 2.5 Hexose monophosphate buffering as a percentage of a broad range of total muscle buffer capacity (βm) values. The two curves represent an example resting (1.9 mmol•kg dm^{-1}) and end-exercise (28.9 mmol•kg dm^{-1}) content after 2 × 30-s maximal sprints (54). Buffering is calculated from the Henderson–Hasselbalch equation over an assumed physiological pH range of 7.1–6.5, and, having the highest concentration of the hexose monophosphates, a pK_a of 6.11 for glucose 6-phosphate. For example calculations see Appendix M.

2.7.2 Proteins and Dipeptides

Amino acids are fully dissociated in the cell because of pK_a values of < 2.5 and > 8.4 for the carboxyl (-COOH) and amine ($-NH_2$) groups, respectively (229). Hence, in free form these possess no physiological buffering power. The side chain (R-group) is the defining factor

[17] Referred to as glycerol 1-phosphate in some papers, these are enantiomers, or mirror images, of each other.

in the buffering power of amino acids, peptides, and proteins. Histidine is the only amino acid whose R-group, imidazole, has a pK_a (5.83) even close to the physiological pH range (200, 381). Somero (436) wrote that the imidazole group is the dominant intracellular buffer (436), but the combination of a comparatively low pK_a and reported concentrations of around 0.89 g•kg wet mass^{-1} in untrained human quadriceps indicate that the buffering power of free histidine is negligible (0.48 mmol H^+•kg dm^{-1} between pH 7.1 and 6.5) (200, 229). Crucially, the pK_a of imidazole changes when histidine forms peptides and proteins (55), thereby increasing its importance as a buffer[18]. And as noted earlier, because the pK_a and pH of histidyl residues change in parallel with temperature (436), the buffering power of imidazole compounds does not change with temperature. This is not the case for the pK_a of phosphates (436), though these vary by only ~0.001 pH unit per degree change in temperature (220).

2.7.2.1 Protein-Bound Histidine

The estimated protein content of muscle is 170–200 g•kg wet mass^{-1} (229, 258), with the buffering power of protein dependent on histidyl residues (229). Using data reported by Fürst *et al.* (165) for protein-bound histidine content in human muscle of 46 mmol•L^{-1} (153 mmol•kg dm^{-1})[19], Hultman & Sahlin (229) estimated that, with a pK_a of 6.83 for the imidazole group, the buffering power would be 42 mmol H^+•kg dm^{-1} between pH 7.08 and 6.6 (87 mmol H^+•kg dm^{-1}•pH^{-1}), or about 50% of what they estimated total physicochemical buffer capacity to be (see Figure 2.6). This assumes that all histidyl residues are available to buffer H^+, whereas *in vivo* they have additional roles in protein function (436).

More recently, the relative contribution of protein buffers[20] to non-bicarbonate physicochemical buffering ($\beta m_{in\ vitro}$) was estimated by titration of deproteinised homogenate to be between 30–35% of total buffer capacity, or on average 52 mmol H^+•kg dm^{-1}•pH^{-1} (48). Figure 2.6 shows how the relative contribution of protein buffering changes as a function of estimated histidine-containing muscle protein content and total buffer capacity.

[18] The charge profile of the microenvironment around any protonatable amino acid R-group can change the pK_a to a value nearer to physiological pH (220).

[19] Fürst *et al.* (165) reported histidine to be 2.7% of total amino acids. Hultman & Sahlin (229) assumed protein content of muscle to be 200 g•kg wet mass^{-1}, giving 5.4 g histidine•kg^{-1}, or 7.02 g•L^{-1}. Histidine has a molecular mass of 155.15 g•mol^{-1}, giving an estimated [histidine] in muscle of 45.2 mmol•L^{-1} (7.02 ÷ 155.15 × 1000).

[20] Assuming $\beta m_{protein} = \beta m_{total} - \beta m_{non\text{-}protein}$

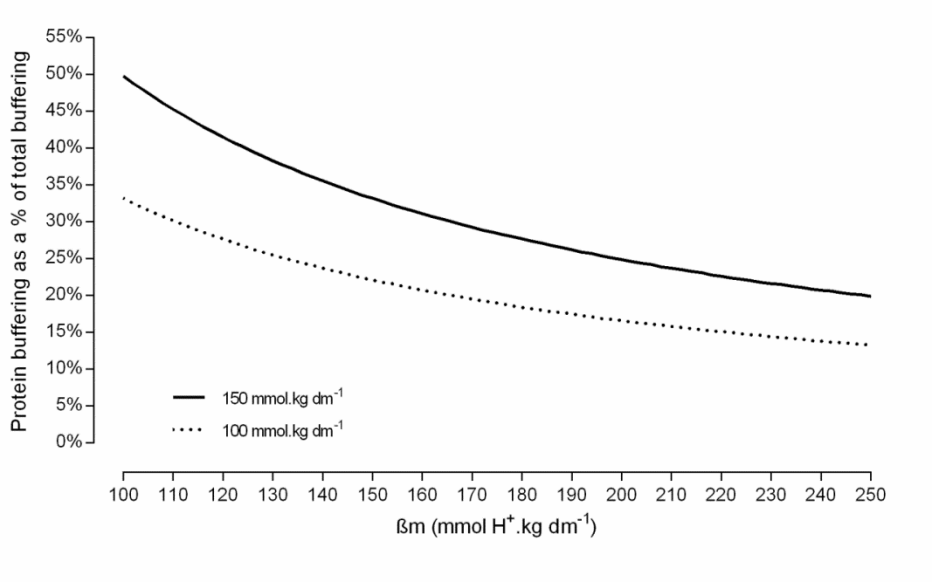


Figure 2.6 Protein buffering as a percentage of a broad range of total muscle buffer capacity (βm) values. The two curves represent an example low (100 mmol•kg dm^{-1}) and high (150 mmol•kg dm^{-1}) histidine-containing protein content. Both calculations use the histidine concentration measured by Fürst *et al.* (165) of 2.7% total amino acids. The former assumes total protein content of 170 g•kg wet mass^{-1} (258), the latter assumes 200 g•kg wet mass^{-1} (229). Buffering is calculated from the Henderson–Hasselbalch equation over an assumed physiological pH range of 7.1–6.5, and for a pK_a of 6.83 for protein-bound histidine. For example calculations see Appendix M.

2.7.2.2 Carnosine

Carnosine (β-alanyl-L-histidine) and anserine (methylated analogue of carnosine) are dipeptides of β-alanine and L-histidine (55, 284, 361). The potential importance of these compounds as muscle buffers was identified early last century (19, 145), with their dissociation constants – pK_a of 6.83 and 7.04 respectively – suited to buffering at physiological pH. Evidence suggested that together the dipeptides contribute as much as 40% of buffering capacity in different species of mammalian muscle (19), but anserine levels have since been reported to be negligible in humans (302, 361). Carnosine, on the other hand, can be synthesised in skeletal muscle from β-alanine and histidine by carnosine synthase (9, 55). β-alanine in turn can be synthesised in the liver, or provided by dietary sources or through supplementation (203). Meat is particularly high in carnosine and, not surprisingly, vegetarians have been reported to have comparatively low muscle carnosine content (202).

From a mean muscle carnosine content of 21.3 mmol•kg dm^{-1} measured in men, Mannion *et al.* (302) calculated buffering power of carnosine from pH 7.1 to 6.5 to be 7.0 mmol H^+•kg dm^{-1}(302). This equates to 7% of non-bicarbonate physicochemical buffer capacity using the same researchers' buffer capacity data. While intracellular bicarbonate buffering confers an additional small fraction to physicochemical buffering (see following

section), metabolic buffering constitutes perhaps 40% of total intracellular buffering (229, 303), thus the relative contribution of carnosine to total intracellular buffering becomes comparatively minor. Nevertheless, as a mobile buffer carnosine likely contributes relatively more buffering power than is estimated from the Henderson–Hasselbalch equation (55). Moreover, the predominance of carnosine in type II muscle fibres suggests that it may become of greater importance when those fibres are recruited during high-intensity exercise (217, 361). Overall, the evidence supports total buffering by histidyl-imidazole residues as being the largest contributor to physicochemical buffering.

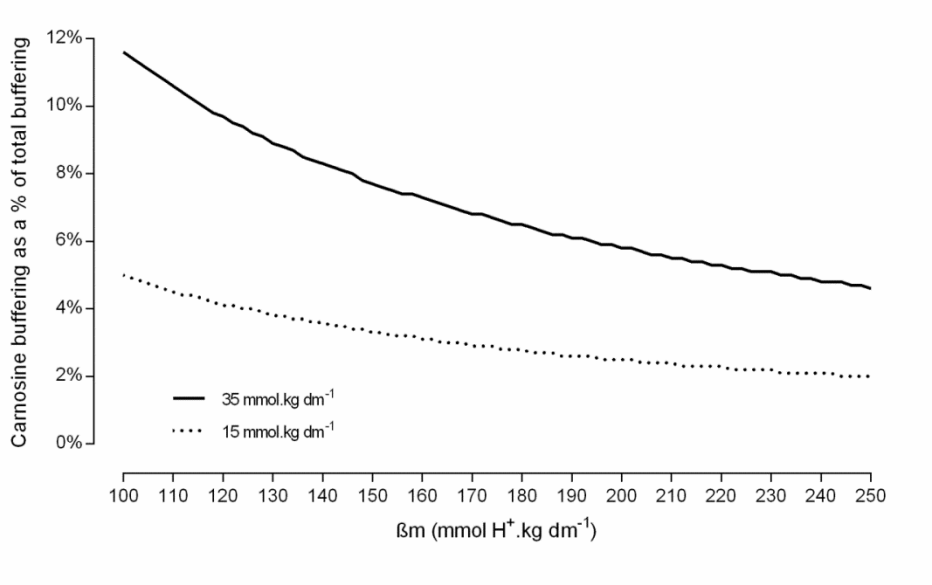


Figure 2.7 Carnosine buffering as a percentage of a broad range of total muscle buffer capacity (βm) values. The two curves represent an example low (15 mmol•kg dm^{-1}) and high (35 mmol•kg dm^{-1}) carnosine content, where the high carnosine content represents an 80% increase above baseline content following 10 weeks of β-alanine supplementation (217). Buffering is calculated from the Henderson–Hasselbalch equation over an assumed physiological pH range of 7.1–6.5, and for a pK_a of 6.83 for carnosine. For example calculations see Appendix M.

2.7.3 Bicarbonate

The reversible hydration of CO_2 in aqueous solutions describes how HCO_3^- combines with H^+, with the resultant carbonic acid (H_2CO_3) converted to CO_2, catalysed by carbonic anhydrases (CAs):

Eqn. 20 $HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$

Despite the CO_2/HCO_3^- system being regarded as the most important buffering system in mammals (110), some disagreement remains about the relative buffering power of HCO_3^- intracellularly, i.e., assuming a closed system (CO_2/HCO_3^- as an open-system buffer is discussed separately in section 2.9.2). For example, Beaver *et al.* (20) claimed that HCO_3^- is the primary muscle buffer, but Péronnet & Aguilaniu (366) subsequently presented an argument

that HCO_3^- contributes perhaps 16–18% of total buffer capacity but only 5–7% of intracellular buffering, supporting earlier[21] research (229, 406).

The Henderson–Hasselbalch equation (Eqn. 14) describes how, as CO_2 decreases, pH increases:

Eqn. 21 $$pH = 6.1 + \log\left(\frac{[HCO_3^-]}{[CO_2]}\right)$$

where $[CO_2]$ is calculated using Henry's law:

Eqn. 22 $$[CO_2] = s \times PCO_2$$

and where s is the solubility coefficient[22] of CO_2 in the intracellular water phase (64), 0.237 mM•kg H_2O^{-1}•kPa^{-1} at 37°C, or 0.03 mM•kg H_2O^{-1}•$mmHg^{-1}$ (406). The buffering power of CO_2/HCO_3^- (βCO_2) can be calculated from the Henderson–Hasselbalch equation as shown in Eqn. 17.

[For an open-system the calculation can be simplified to $\beta CO_2 = 2.3[HCO_3^-]$ (64), but this equation is not valid for contracting muscle, which effectively becomes a closed-system with restricted blood flow (211)]

As Péronnet & Aguilaniu (366) showed, on rearranging the Henderson–Hasselbalch equation it is clear that $[HCO_3^-]$ is dependent on both pH and PCO_2:

Eqn. 23 $$[HCO_3^-] = [0.03 \times PCO_2] \times 10^{(pH - 6.1)}$$

Thus, at a resting muscle pH of 7.1 and PCO_2 of 45 mmHg, $[HCO_3^-]$ is 13.5 mmol•L^{-1}. And despite the large increase in PCO_2 following high-intensity exercise to ~80–100 mmHg, with a parallel reduction in pH to 6.5, $[HCO_3^-]$ drops to 6.0–7.5 mmol•L^{-1}. Péronnet & Aguilaniu (366) calculated βCO_2 to range from 3 mmol•L^{-1}•pH^{-1} at rest, to 4.5 mmol•L^{-1}•pH^{-1} following exercise inducing a drop in pH to 6.4. In assuming a total intracellular βm[23] of 63 mmol•L^{-1}•pH^{-1}, this equated to just 5–7% contribution from βCO_2. These calculations use a version of the Henderson–Hasselbalch equation reported in Kemp *et al.* (262):

Eqn. 24 $$\beta CO_2 = 2.3[C] / [(1 + 10^{(pH - pK_a)}) \times (1 + 10^{(pK_a - pH)})]$$

where [C] is the total concentration of the buffer, in this case:

---

[21] Sahlin (405) calculated resting and end exercise $[HCO_3^-]$ to be 10.2 and 3.0 mmol•L^{-1}, respectively, equating to a buffering power of 12 mmol•L^{-1}•pH^{-1} (Slykes). Total buffering capacity was calculated to be 68 mmol•L^{-1}•pH^{-1} (or 224 mmol H^+•kg dm^{-1}•pH^{-1}), giving a 17.6% contribution from CO_2/HCO_3^-.

[22] A more comprehensive calculation takes into account that muscle is a multiphase system, with a second solubility coefficient for PCO_2 in the muscle of 0.273 mM•kg H_2O^{-1}•kPa^{-1} at 37°C (404, 406).

[23] 63 mmol•L^{-1}•pH^{-1} is the mean of $\beta m_{in\ vivo}$ and $\beta m_{in\ vitro}$ from several studies in this review. As detailed in section 2.11, the former is a measure of total intracellular buffering (physicochemical + metabolic), whereas the latter is solely physicochemical buffering. Both are normally measured in freeze-dried tissue and therefore exclude CO_2/HCO_3^- buffering. Péronnet & Aguilaniu (366) inappropriately treat both measures as being representative of total buffering. Given that the fraction of total βm that βCO_2 constitutes is their point of interest, the $\beta m_{in\ vivo}$ data are more representative. However, this does not greatly affect their calculations because their mean $\beta m_{in\ vivo}$ was 65 mmol•L^{-1}•pH^{-1}.

Eqn. 25 $[C] = [H_2CO_3] + [HCO_3^-] = (0.03 \times PCO_2) + (0.03 \times PCO_2 \times 10^{(pH - pK_a)})$

Although [C] is correct, Eqn. 24 does not account for the pH-dependence of buffering (262). Using Eqn. 17 to modify the calculations of Péronnet & Aguilaniu (366) gives an even lower contribution for β_{CO2} to total intracellular βm of 2.7–3.3% for a PCO_2 of 80–100 mmHg, assuming their pH transit range of 7.09–6.40. How the bicarbonate contribution to total physicochemical buffering changes as a function of pH and PCO_2 is illustrated below in Figure 2.8. The importance of the total (open-system) buffering of the CO_2/HCO_3^- system is also shown, i.e., physicochemical and volatile (diffusible) buffering, for a fixed PCO_2 of 45 mmHg and end-exercise pH of 6.5. The CO_2/HCO_3^- system as a volatile buffer is discussed further in section 2.9.2.

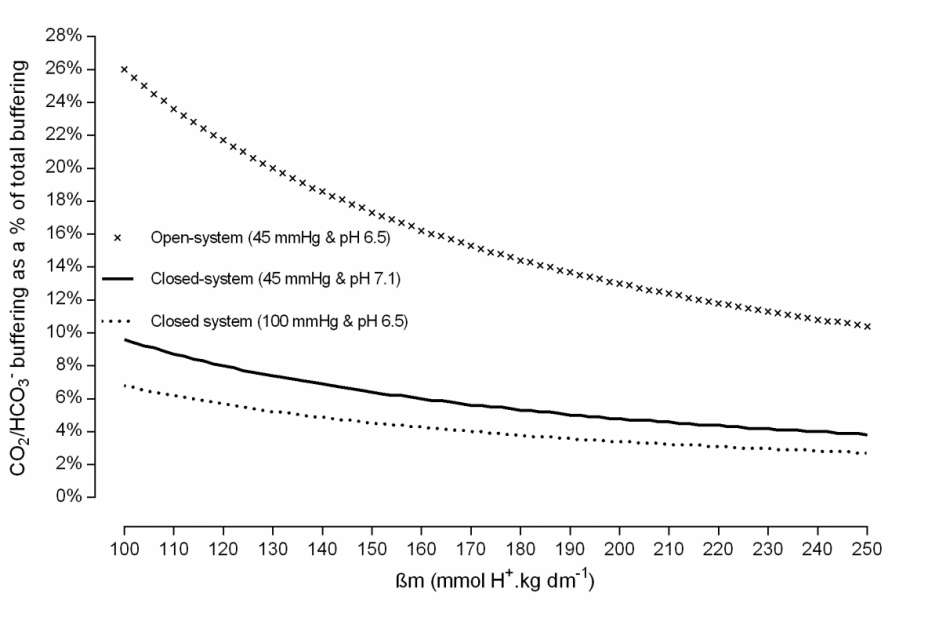


Figure 2.8 Physicochemical (closed-system) and total (open-system) CO_2/HCO_3^- buffering as a percentage of a broad range of total muscle buffer capacity (βm) values. Physicochemical buffering is shown at resting PCO_2 of 45 mmHg and pH 7.1, and at an example end-exercise PCO_2 of 100 mmHg and pH of 6.5. Buffering is calculated from the Henderson–Hasselbalch equation over an assumed physiological pH range of 7.1–6.5, and for a pK_a of 6.10 for HCO_3^-. Open-system buffering is calculated from $\beta_{CO_2} = 2.3[HCO_3^-]$ at fixed PCO_2 of 45 mmHg and pH of 6.5. For example calculations see Appendix M.

Although the significance of HCO_3^- as an intracellular physicochemical buffer has sometimes been overstated, as the primary form of CO_2 in the body, HCO_3^- has a vital role in respiratory regulation of acidosis (172, 243). HCO_3^- is further involved in pH regulation as a substrate for the family of bicarbonate transport proteins, and these are detailed later (section 2.10.2).

2.8 Metabolic Buffering

A number of metabolic changes play important roles in regulating H^+, whereby cellular metabolism causes changes in pH_i parallel to energy production (229). Total intracellular buffering comprises this metabolic, or dynamic buffering, and physicochemical buffering. Estimations of the contributions of metabolic processes to βm are calculated from biochemical changes within the muscle, rather than changes in pH_i or PCO_2. The most significant metabolic processes for H^+ consumption are the utilisation of phosphocreatine (PCr) and the formation of inosine 5'-monophosphate (IMP). The former essentially refers to the Lohmann reaction that was introduced earlier (*cf.* section 2.4), the sum of two conceptually coupled but separate enzyme-catalysed reactions (260):

Eqn. 26 ATPase reaction: $ATP \rightleftharpoons ADP + P_i + \alpha H^+$

Eqn. 27 CK reaction: $PCr + ADP + \beta H^+ \rightleftharpoons ATP + Cr$

Whilst IMP is formed as follows:

Eqn. 28 IMP formation: $ATP + H^+ \rightleftharpoons IMP + 2P_i + NH_4^+$

Buffering from metabolic reactions is dependent on the bioenergetic status of the cell, including being pH-dependent; thus, any calculation of metabolic buffering power is specific to a distinct metabolic state (406). Nevertheless, as Hultman & Sahlin (229) calculated following high-intensity exercise, it is clear that the relative buffering power of the Lohmann reaction[24] is high compared to buffering due to IMP formation[25].

2.9 Volatile Acids

Volatile acids are those that are completely dissociated in water, the concentration of which is dependent on the partial pressure of the substance in the gas phase (229). While physicochemical buffers are closed systems, such that the weak acid and its conjugate base are not removed from (or added to) the intracellular space, the volatile, or diffusible buffers, constitute open-systems, the components of which can be removed from (or added to) the cell (64). These open-system buffers are thus distinguished by their ability to exchange freely outside the cell and subsequently with the environment. The two main volatile buffer systems in mammals are:

Eqn. 29 Ammonia/ammonium: $NH_4^+ \rightleftharpoons H^+ + NH_3$, $pK_a = 9.25$

Eqn. 30 CO_2/HCO_3^- system: $HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2$, $pK_a = 6.10$

The following sections elaborate on both of these buffer systems.

[24] 11.6 meq.L^{-1}, assuming a decrease in PCr of 19 mmol•L^{-1} following high-intensity exercise.

[25] 0.41 moles H^+ buffered per mole IMP produced. Calculated under specific conditions: pH = 6.6, $[Mg^{2+}]$ = 1 mmol•L^{-1}, $[K^+]$ = 160 mmol•L^{-1}.

2.9.1 Ammonia

At rest ammonia (NH_3) is primarily produced from dietary sources (339). There is little change in muscle NH_3 at exercise intensities less than 50% $\dot{V}O_{2max}$, but with increasing intensity muscle becomes a greater source of NH_3 production (409). This has been reported to result from deamination of AMP to IMP by AMP-deaminase during the purine nucleotide cycle (229, 409), or alternatively through deamination of amino acids (229, 477). In addition, prolonged moderate-intensity exercise (~70% $\dot{V}O_{2max}$) resulting in glycogen depletion has been shown to lead to increased AMP deamination and a five-fold increase in NH_3 release from the active muscle (67). NH_3 combines with the amino acid glutamate forming glutamine, the accumulation of which contributes to buffering of protons (229). The toxicity of NH_3 ensures it is kept at a low concentration in the body and only contributes to systemic buffering in the kidneys. At physiological pH, ammonia exists mainly as NH_4^+, with < 5% existing as NH_3 (339). NH_4^+ has a pK_a of 9.25 (490), effectively negating its relevance as an intracellular buffer at physiological pH_i.

2.9.2 Ventilation and CO_2

Unlike the limited buffering power provided by CO_2/HCO_3^- as a closed-system physicochemical buffer, the capacity to eliminate CO_2 from the blood through ventilation effectively provides an open system with respect to the cell (397). Because CO_2 rapidly equilibrates across the membrane, in an open system intracellular [CO_2] is effectively considered constant (64, 229). Thus, at constant [CO_2], the Henderson–Hasselbalch equation gives (64):

Eqn. 31 $\beta_{CO_2} = 2.3[HCO_3^-]$

At fixed PCO_2, [HCO_3^-] increases with increasing pH. Therefore, β_{CO_2} reduces as pH decreases during exercise. In addition, as intracellular water increases during exercise, [HCO_3^-] decreases, but there is no change in [$H_2CO_3 + CO_2$] (229). The effect of this will be to shift the equilibrium hydration reaction to the left, further increasing [H^+]:

Eqn. 32 $HCO_3^- + H^+ \rightleftharpoons H_2CO_3$

Ultimately, by regulating arterial PCO_2, ventilation serves to regulate pH_i, but this capacity reduces during exercise (Figure 2.9).

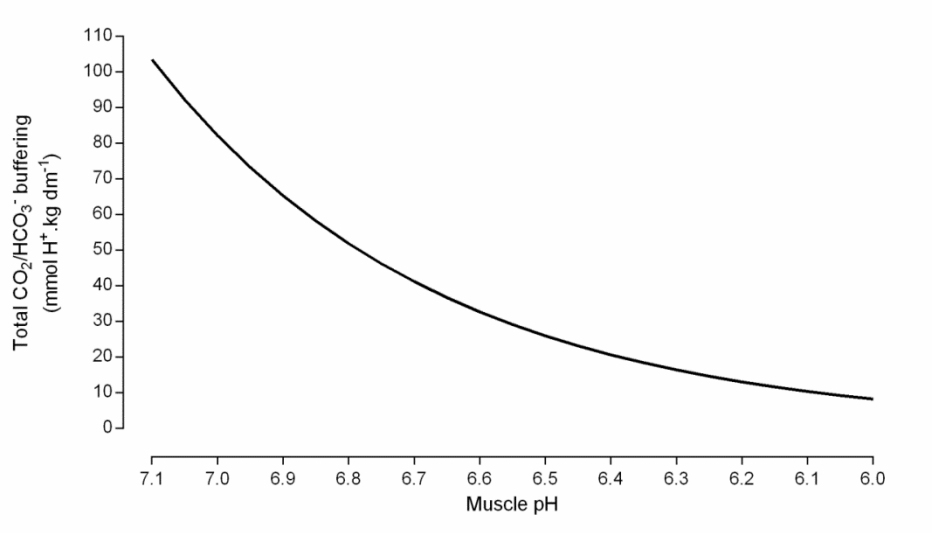


Figure 2.9 Total buffering of the CO_2/HCO_3^- system as a function of pH at fixed PCO_2 of 45 mmHg. Buffering is calculated as β_{CO_2} = 2.3[HCO_3^-] for an open-system and comprises total physicochemical and volatile buffering of CO_2/HCO_3^-.

2.10 Transmembrane Acid/Base Transport

At rest there is a greater H^+ concentration inside the muscle cell than outside it. And if H^+ distribution was defined simply by the principle of electroneutrality, a resting membrane potential[26] of −88 mV would, with an extracellular pH of 7.4, result in an intracellular pH of 6.0 (405). That pH_i is more alkaline than predicted indicates H^+ efflux requires active transport rather than simply passive distribution (63, 246, 254, 486). To counteract the electrochemically-driven influx of protons, H^+ efflux is mediated by a number of active transporters that are located primarily in the sarcolemma (250), as well as the T-tubules and sarcoplasmic reticulum (57). Categorisation of these transporters as either lactate-dependent or lactate-independent H^+ transport allows for estimation of their relative activity (254). Importantly, the specific transport systems work synergistically (254), even if a complete understanding of the interactions remains to be established.

2.10.1 Lactate/Proton Cotransport

One of the most important groups of H^+ transporters in skeletal muscle are the solute carrier (SLC)16 gene family, known as monocarboxylate (MCT) transporters because of the function of the first identified isoforms. While 14 isoforms of MCT have been identified, only

[26] Sources vary on the exact resting membrane potential (*E*m) of healthy skeletal muscle cells. Precise values of −88 ± 3.8 mV calculated from the Nernst equation have been measured in human muscle (115), and similarly reported elsewhere (e.g., 35, 229). Others have suggested a range of between −60 and −80 mV (254). Regardless, the fundamental point made here remains.

four have been found experimentally to transport monocarboxylates[27], all of which are also proton-linked (193). These MCTs enable transport of monocarboxylates – such as lactate and pyruvate – and H^+ across the plasma membrane with a 1:1 stoichiometry (246). The direction of transport is dictated by H^+ and ionic concentrations (193, 254), meaning that MCT activity does not require ATP (318). In skeletal muscle the two most important mediators of lactate/H^+ cotransport are MCT1 (SLC16A1) and MCT4 (SLC16A3). Some transcriptional and protein evidence[28] for MCT2 (SLC16A7) has also been reported in skeletal muscle (60, 157, 290), albeit with relatively low expression and high variability depending on the muscle and species analysed (157, 192). MCT2 has high affinity for monocarboxylates, meaning maximal transport activity is reached at very low concentrations of these substrates – Michaelis–Menten constants (K_m)[29] of 0.74 mM and 0.08 mM for L-lactate and pyruvate, respectively (193). Hence, MCT2 expression in human skeletal muscle is likely low compared to MCT1 and MCT4, and is of comparatively little functional importance.

Collectively, MCT1 and MCT4 contribute the largest portion of muscle H^+ regulation during high-intensity exercise (13, 246, 250), whilst during low-intensity exercise non-lactate-coupled H^+ transport predominates (16, 253). Evidence suggests that MCT1 and MCT4 have independent regulatory mechanisms (58, 135, 206) and that because of both this, and their different cellular locations (57), the two transporters perform different functions in muscle. MCT1 protein content in rat muscle has been reported to be very highly correlated with MCT1 mRNA (r = 0.94), whereas no such relationship was seen between MCT4 protein and MCT4 mRNA (no specific data given) (57). But later research from the same group found the relationship between MCT protein and mRNA expression post-exercise was actually muscle specific (104). It seems from this and other research that MCT1 and MCT4 are regulated by both transcriptional and post-transcriptional mechanisms following exercise (58, 208), though they do not appear to be glycosylated (87, 188). As with many other genes, there may be distinct fibre-type specific responses (216).

It has been postulated that MCT1 acts to remove lactate from the circulation for oxidation in muscle fibres (59, 310, 311), whereas the main role of MCT4 may be in enabling lactate efflux from fibres. Lactate would then either undergo 'direct oxidation', i.e., oxidised to

[27] MCTs: -5, -6, -7, -9, -12, and -13 have, as yet, unidentified transport properties (193).

[28] A caveat in interpreting evidence for expression of a particular MCT is that different antibodies for the same MCT have been found to produce contrasting results (60). This is perhaps of most significance in that it leaves open the possibility that other MCTs are also expressed in skeletal muscle (MCT5, MCT6, and MCT7) (57). To further complicate matters, evidence for the latter MCTs in skeletal muscle was criticised for being determined solely by western blots using commercial antibodies, with no correlation with MCT mRNA and no validation of antibody specificity (318).

[29] The Michaelis–Menten constant is the substrate concentration at which a given reaction reaches half of its maximum reaction rate. Essentially it is a measure of the kinetics of an enzymatic reaction. A low K_m indicates a high binding affinity between enzyme and substrate. The kinetics studies for the MCTs have typically been performed in *Xenopus* oocytes (e.g., 301). These cells have little inherent MCT activity and are injected with the cRNA of the requisite MCT isoform (192).

pyruvate for use as a substrate[30], or 'indirect oxidation', whereby lactate enters the Cori cycle for gluconeogenesis and subsequent oxidation of the glucose residue (148). This evidence is a central component of the intercellular (or cell–cell) lactate shuttle hypothesis promulgated by Brooks (71, 73), wherein the MCTs are part of a symporter system involving cotransport of lactate anions and protons into, or out of the cell, relative to concentration and pH, respectively. Whether this extends to a putative *intra*cellular lactate shuttle involving lactate oxidation in the mitochondria is the matter of much debate (e.g., 73, 191, 478), and is expanded upon below. Yet, a dichotomy of influx for MCT1 and efflux for MCT4 may not paint the whole picture, with MCT1 also found to be associated with increased extrusion of lactate from the muscle (10, 56). In support, MCT1 has been found to correlate highly (r = 0.70) with the velocity constant of lactate removal after 1 min of all-out exercise (465).

The function of both MCTs requires their targeting to the plasma membrane by glycosylated basigin[31], a member of the immunoglobulin superfamily (189, 318, 502, 503). This widely-expressed protein (463) acts as a chaperone for MCT1 and MCT4 (205, 264), in the absence of which, the MCTs remain in the Golgi apparatus (503). The MCTs are predicted to have 12 transmembrane domains (193), while basigin has a single transmembrane domain and remains associated with the MCTs at the membrane (325). Several co-immunoprecipitation studies using cell models have co-located basigin and MCT1 or MCT4 at the cell membrane (205, 264, 507). One study has also co-precipitated basigin and MCT1 in mitochondrial fractions of L6 cells (205), though this evidence is questioned by others, and discussed in the next section. Finally, further evidence of the importance of basigin for MCT functioning has been shown in a cell model, whereby basigin was inferred to act not simply as a chaperone protein for both MCTs, but also to be essential in maintaining their activity (503).

2.10.1.1 MCT1

In cell and rodent studies, MCT1 has been found primarily in type I muscle fibres (57, 60, 169, 204, 311, 501). Similar data have been reported in one human study, with MCT1 located in the sarcolemma, although the total content varied depending on the specific muscle examined (374). There are also some convincing data showing MCT1 in the mitochondrial membranes (70, 135, 205-207), but the very existence of a mitochondrial MCT remains experimentally (101), and theoretically, controversial (191, 192). Halestrap's group have found no evidence of MCT1 in purified mitochondria (101). Moreover, they, and others (384, 410), argue that the different NADH/NAD^+ redox ratios in the cytosol and mitochondria would not be

[30] Whether this occurs in the cytosol, or in the mitochondria through a mitochondrial lactate dehydrogenase (LDH) enzyme, remains controversial (176, 410).

[31] Basigin is the HUGO Gene Nomenclature Committee recommended name (178), but there are several aliases for the human protein, including CD147, EMMPRIN, M6, and OX-47 (30, 472), reflecting naming of the protein in different species before homology was ascertained.

possible if oxidation of lactate by LDH was occurring in both compartments, along with transport of lactate and/or pyruvate by MCT1 (191). The conflicting findings between theirs and Brooks' work cannot simply be explained by the crude nature of fractionation by centrifugation, because confocal microscopy has also been used to locate MCT1 to the mitochondrial inner membrane (205). And proteomic analysis of the mitochondria of several mouse tissues have identified LDH and MCT1 (331).

Using permeabilised muscle fibres and isolated mitochondria, subsarcolemmal but not intermyofibrillar mitochondria have been found to oxidise lactate (147). These authors suggest that mitochondrial LDH is present in the outer mitochondrial membrane but not within the matrix. Brooks (72) previously argued that the failure to find evidence for mitochondrial LDH may have been a result of protease or detergent disruption of mitochondrial membranes during 'purification' and subsequent loss of constituents (72). Therefore, the controversy of whether mitochondria have an MCT could possibly be explained by the compartmentalisation of MCTs to subsarcolemmal but not intermyofibrillar mitochondria (510), and to oxidation of lactate occurring outside of the inner mitochondrial membrane. Owing to greater permeability, the outer membrane of the mitochondrion is in closer equilibrium with the cytoplasm than is the matrix (318). Pyruvate could then be transported into the matrix for entry into the Krebs Cycle by the known (H^+-linked) mitochondrial pyruvate carrier (192).

MCT1 catalyses exchange of one monocarboxylate for another, or cotransport of one monocarboxylate anion and one proton (189, 192). A low K_m of ~3–5 mM for MCT1 indicates a high affinity for L-lactate (193, 301), theoretically allowing influx of lactate from the interstitial space, or from type IIx fibres following efflux, for usage as a substrate in more oxidative type I and type IIa fibres (318). A simple fibre-type dichotomy may not completely encompass MCT function though. Given the compartmentalisation of glycogen in muscle (358), there may be concurrent activity of MCT1 and MCT4 in myocytes regardless of fibre type. No evidence exists yet to support this idea, although MCT1 and MCT4 have been detected by western blot in sarcolemmal and mitochondrial fractions of human skeletal muscle (135). Separately, both MCTs were found in subsarcolemmal but not intermyofibrillar mitochondria of rat muscle (32). As already stated, uncertainty remains about the purity of such mitochondrial fractions.

Overexpression of the important transcriptional coactivator PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α) has been found to upregulate expression of both MCT1 (but not MCT4) and basigin, resulting in an increased rate of lactate uptake (33). Regulation of MCT1 by PGC-1α would sit well with the latter's now well-recognised role[32] in inducing the expression of genes involved in oxidative phosphorylation (377). And PGC-1α has

[32] PGC-1α has been suggested to be non-essential for most adaptive responses to endurance training (144). This argument, and the implied biological redundancy (74), is beyond the remit of the current thesis.

subsequently been identified as playing a crucial role in lactate homeostasis, including enhancing MCT1, but not MCT4 or basigin transcription (458). MCT1 also appears to be upregulated at the protein and mRNA level in cell and rodent models by AMP-activated protein kinase (AMPK) (191, 268, 462), but there are conflicting data for a similar upregulation of MCT4 by AMPK (166, 191, 268, 462).

2.10.1.2 MCT4

There is evidence from different species of MCT4 expression[33] in both type I and type II fibres (501), but it appears to predominate in type II fibres (131, 204, 374). Another tentative difference with MCT1 is the potential existence of an intracellular pool of MCT4 in skeletal muscle (57); although, as has been noted elsewhere (46), this remains to be substantiated by others[34]. Moreover, in a further hint at their disparate roles, MCT4 has been found in the sarcolemma like MCT1 (57, 135), but also in significant amounts in the T-tubules and sarcoplasmic reticulum (57). Notwithstanding the apparent ubiquitous nature of MCT4 across muscle fibre types, its high K_m (low affinity) for L-lactate of ~30 mM (131, 193, 301) has been suggested to be ideally suited to facilitating the efflux from type II fibres of the high concentrations of lactate produced during intense exercise (318) – potentially up to 40–50 mM (132–155 mmol•kg dm^{-1}) in skeletal muscle at exhaustion (246, 366). In support, the transport activity of MCT4 in oocytes has been found to increase with decreasing extracellular pH (131). Therefore, while MCT1 is saturated at low concentrations of L-lactate because of its low K_m, MCT4 transport rate continues to increase with increasing L-lactate concentration. This partly explains the greater efflux rate compared to influx measured in rat isolated EDL (though not *soleus*) fibre bundles (498), in rat and guinea pig isolated cardiac myocytes (487), and also presumed in human skeletal muscle during high-intensity exercise (247). However, absolute transport rates are more complex and variable, being dependent on the total content of the specific MCT isoforms (301). Finally, L-lactate, rather than other monocarboxylates, is the preferred substrate for MCT4 (131), whereas a high K_m of ~150 mM for pyruvate suggests a protective mechanism that prevents loss of pyruvate and subsequent failure in NAD^+ regeneration from NADH, which would effectively stop glycolysis (191, 467).

As was noted above, MCT4, unlike MCT1, is not regulated by PGC-1α. And MCT4 may also be upregulated by AMPK, but the limited data to date in cell and rodent models are equivocal (166, 191). The mechanisms regulating MCT4 expression require elucidation, in particular the probable post-transcriptional factors involved. Nevertheless, hypoxia is

[33] Wilson *et al.* (501) originally referred to MCT3; however, as an addendum they stated that, in light of further evidence, the transporter would be more accurately named MCT4.
[34] Actually, some evidence for an intracellular pool of MCT1 has been reported in cardiac myocytes of congestive heart failure rats (239).

considered to be the primary regulator of MCT4 expression (191); although in human studies, MCT4 protein content has not changed following 3–8 weeks of hypoxia (100, 252, 355), or hypoxic high-intensity training (324, 380). MCT4 has been found *in vitro* to be upregulated via the hypoxia-inducible factor (HIF)-1α pathway (343, 476). HIF-1 is a heterodimer consisting of an oxygen-labile α subunit and a constitutively expressed β subunit (456). Thus, a considerable difficulty in ascertaining any role for HIF-1 at the protein level is that the α subunit degrades rapidly in normoxia, with a half-life of only 4-8 minutes (37, 237). In reality, measurement of HIF-1α protein abundance *in vitro* can provide little quantitative information of use, other than identifying the presence of the protein. Finally, activity of both MCT1 and MCT4 appears to be increased by interaction with other acid/base transporters and the CAs. Evidence for this is presented in the following sections.

2.10.2 Sodium/Hydrogen Exchanger

The sodium/hydrogen exchanger (NHE; SLC9 gene family) proteins are a form of cation–proton antiporter. Of the 13 known mammalian isoforms, nine of these comprise the SCL9A subgroup: the plasmalemmal isoforms NHE1–5 and the intracellular isoforms NHE6–9 (167). The NHE proteins are reported to form the most important system in regulating pH in many cell types (252, 254), and along with the bicarbonate transport proteins (detailed in section 2.10.3) are considered the main regulators of skeletal muscle pH_i homeostasis at rest (172, 248, 251, 357). During submaximal exercise non-lactate-coupled H^+ transport predominates (16, 253), remaining high, but lower relative to total transport, during high-intensity exercise (13), and this has been attributed primarily to the NHE system (16).

NHE1 is the primary isoform thus far identified in human skeletal muscle and is almost ubiquitously expressed across cell types (134, 167, 486). Transcriptional evidence exists for NHE-2, -4, -5, -7, and -8 in skeletal muscle (134, 167, 342); however, protein evidence and functional relevance remains to be identified for most of these, in particular the intracellular isoforms (167). It appears in rats, at least, that NHE1 is found to a greater extent in glycolytic muscle – approximately four times more in type IIb than type I fibres (251). In humans, NHE1 is expressed in both type I and type II fibres, albeit with some suggestion of elevated expression in more glycolytic muscle (252).

The NHE proteins are electroneutral and, therefore, do not require ATP hydrolysis to function. The direction of the 1:1 exchange stoichiometry is dependent on the transmembrane Na^+ and H^+ chemical gradients (486). That said, NHE activity is reduced under conditions of cellular ATP depletion, but this does not appear to directly relate to reduced NHE phosphorylation (486). NHE1 seems to be phosphorylated by several different kinases (134), and is activated by endothelin-elevated Ca^{2+} increasing binding of NHE1 with the Ca^{2+}-binding

protein calmodulin (288). Intracellular pH has been identified in rat sarcolemmal giant vesicles (249), and in a cell model (66), as being an important factor in stimulating activity of NHE. In addition, the constant Na^+ influx by the sodium-potassium pump (Na^+-K^+-ATPase) will, by changing the Na^+ gradient, cause an efflux of H^+ from the cell by NHE.

Using cell or rodent models, NHE1 has also been found to be important for pH recovery post-exercise (245, 508). Indeed, in Slc9a1 knock-out mice, not only was basal pH lower in specific brain cells, but pH recovery was attenuated following acidification (134, 167). Similarly, inhibition of NHE1 with DMA[35], a specific NHE inhibitor, completely prevented recovery of pH in rabbit and mouse cell lines following acidification caused by anoxia (505). Moreover, inhibition of CA by acetazolamide prevented pH recovery, which the authors attributed to some interaction between NHE1 and CA. More detail is provided on this interaction in section 2.10.4.

2.10.3 Sodium-Coupled Bicarbonate Transport

While the sarcolemma is comparatively impermeable to HCO_3^- (393, 394, 448), pH_i can be regulated by transport or exchange of HCO_3^- or bicarbonate equivalents (CO_3^{2-} or $NaCO_3^-$), through proteins encoded by the SLC4 gene family. The function of eight out of ten of these proteins has been established, allowing for subsequent categorisation as the three Cl^-/HCO_3^- exchangers (anion exchangers: AE1–3), and the five Na^+-coupled HCO_3^- cotransporters/exchangers – two electrogenic (NBCe1–2) and three electroneutral (NBCn1–2 and NDCBE[36]) (396). The latter five transporters are collectively known as the sodium-coupled bicarbonate transporters (NCBT) (360).

The two NCBT isoforms that have been tentatively identified in human skeletal muscle at the protein level are the electrogenic cotransporters NBCe1 and NBCe2 (271), encoded by SLC4A4 and SLC4A5, respectively (396, 435, 483). Transcriptional evidence for all three electroneutral transporters has also been reported in human skeletal muscle (110, 296, 359, 360, 378), although Romero *et al.* (396) asserted that NBCn1 is not a skeletal muscle isoform. NBCe1 and NBCe2 are alternatively known as NBC1 and NBC4, respectively; however, the terminology for all of the NCBT isoforms has been confusingly varied and conflicted by separate investigators assigning names before ascertaining protein function (271, 360). This confusion is muddied further by the multiple splice variants of each isoform, with NBCe1 and NBCe2 reportedly having as many as five and six splice variants[37], respectively (396).

[35] 5-*N*,*N*-dimethylamiloride-hydrochloride
[36] Sodium-driven chloride bicarbonate exchanger.
[37] Different sources list different numbers of splice variants, although it seems some of these are cloning artefacts. For example, NBCe1-B (the canonical variant) is present in many tissues and is the primary skeletal muscle variant,

One difficulty in confidently claiming evidence for a specific NCBT isoform is that these proteins have considerable sequence similarity[38]. Table 2.1 shows the high percentage identity in the amino acid sequences of the five putative skeletal muscle isoforms.

Table 2.1 Percentage identity between the putative human skeletal muscle NCBT isoforms. Sequences were aligned using Clustal Omega 2.1 (429). See Appendix L-1 for an example alignment.

Gene Name	SLC4A4	SLC4A5	SLC4A7	SLC4A8	SLC4A10
Protein Name	NBCe1-B	NBCe2-C	NBCn1-D	NDCBE-A	NBCn2-B
NBCe1-B		62.8%	53.8%	53.0%	53.6%
NBCe2-C			47.8%	47.9%	49.1%
NBCn1-D				72.7%	72.4%
NDCBE-A					76.5%
NBCn2-B					

Antibodies to detect specific SLC4 proteins that are raised against epitopes in the amino (N)-terminus in particular may also cross-react with other SLC4 species. For example, the protein evidence for NBCe1 in human skeletal muscle comes from western blotting using a single polyclonal antibody raised against a 54 amino acid sequence of the N-terminus of the NBCe1-A isoform (this epitope is shared 100% among the five NBCe1-(A–E) splice variants) (252, 271). The earlier of these papers referred to a non-specific NBC isoform, whereas the latter refined their interpretation to an isoform of NBC1/NBCe1 that the antibody detected, partially on the basis that a separate antibody detected a 200 kDa band, which was assumed to be NBC4/NBCe2. While others have more recently concluded that it is likely to be NBCe1 that was detected (360), the epitope of this antibody may bind to more than one NCBT isoform. On performing a BLAST alignment of this specific sequence between NBCe1-A and four other putative skeletal muscle NCBTs, the sequence identities range from 49% to 57% (Table 2.2).

NBCe1-A is found predominantly in the kidney, and NBCe1-C is found in the brain (276, 396). NBCe1-D and NBCe1-E have not been detected on the protein level (276). And for NBCe2, Romero *et al.* (396) stated that of the six reported splice variants only one is a functional clone, NBCe2-C; though NBCe2-A is also predicted to encode a functional transporter (360).

[38] Percentage identity is a quantitative score calculated from the percentage of amino acids identical between the sequences of two proteins, divided by the shorter sequence length (256). Depending on the algorithm, gaps in the sequences may or may not be taken in to account. Whereas percentage similarity quantifies not only the identity between sequences, but also matches non-identical amino acids with similar properties. For example, a pairwise sequence alignment of NBCe1-B and NBCe2-C returns a percentage identity of 59% and a percentage similarity of 72% using EMBOSS Needle software (385). See Appendix L-1 for details.

Table 2.2 Percentage identity using pairwise alignments of a 54 amino acid sequence of the N-terminus of NBCe1-A with each of the putative human skeletal muscle NCBT isoforms. To date, reported NBCe1 protein from human muscle has come from immunoblotting with a polyclonal antibody raised against this sequence. Sequences were aligned using Clustal Omega 2.1 (429). See Appendix L-2 for full sequence alignments.

Gene Name	SLC4A4	SLC4A5	SLC4A7	SLC4A8	SLC4A10
Protein Name	NBCe1-A	NBCe2-C	NBCn1-D	NDCBE-A	NBCn2-B
NBCe1-A		57.4%	49.0%	54.2%	47.9%

For western blotting, relying solely on the molecular mass at which a band appears is insufficient confirmation of the specific NCBT detected, or indeed any protein (336). Not only do the NCBTs have relatively similar predicted molecular masses of between 116 and 140 kDa (360), they are all potentially glycosylated, thereby increasing the apparent molecular mass upon immunoblotting (98, 257, 271). A further complication in relying on apparent molecular mass is that NBCe1, for example, readily forms dimers/oligomers (257).

While little is known about the specific role of the NCBTs in muscle pH_i regulation, other than that they function on a Na^+ gradient (254), their function does follow sequence similarity (360). The electrogenic NBCs normally transport two, but sometimes three HCO_3^- anions or bicarbonate equivalents, and one Na^+ cation across the membrane, causing a negative shift in membrane potential (396, 483). The exact anion transported is currently unknown (396). Thermodynamics predict that NBCe1 and NBCe2 should move Na^+ and HCO_3^- into the myocyte[39], with subsequent buffering of intracellular H^+ (274). In other tissues at least, an NHE contributes to the accumulation of HCO_3^- through efflux of H^+ (396). And all of the NCBTs, apart from NBCe2, may be activated by IRBIT[40] (283, 360, 428).

Keeping in mind the uncertainty around the identity of specific NBC isoforms detected by different antibodies, there are some fibre-type specific data. In human muscle, what were nominally identified as NBCe1 and NBCe2 have been found to be moderately negatively correlated with the percentage of type I fibres, r = 0.41 and r = 0.42 respectively, although considerable inter-individual variation was also observed (271). In rats, NBC(e1) has been identified in all fibre types (271, 466). The latter study reported non-isoform-specific NBC (466), despite using the same antibody as the former study (271). Additionally, both isoforms have been identified in sarcolemmal fractions, with some evidence of interior cellular location too (271, 360). In rats, a larger correlation has been reported between NBC/NBCe1 and MCT1 content in type I *soleus* (r = 0.50) than in type II EDL (r = 0.31) muscle fibres (466). A caveat with all of these western blot data is that they may be prone to spurious correlation owing to

[39] This assumes a 1:2 Na^+:HCO_3^- stoichiometry. With a 1:3 stoichiometry NBCe1 would facilitate net efflux of HCO_3^- (360), thereby reducing intracellular pH. The stoichiometry has been reported to be specific to the cell type in which the protein is expressed (183).

[40] Inositol 1,4,5 trisphosphate receptor binding protein released with inositol 1,4,5 trisphosphate

mathematical coupling if the same housekeeping protein was used as a loading control for two target comparison proteins, and simple bivariate correlations were performed. None of these papers provide the requisite information to determine this. Furthermore, if the precision of the correlation coefficients are assessed there is low certainty of fibre-type specificity from these data. For example, taking the correlation between NBCe1 and percentage of type I fibres in Kristensen *et al.* (271), the 90% confidence interval for $r = 0.41$ and a sample size of 30, is 0.06 to 0.67 (*cf.* Figure 2.10) (224).

Functional synergy between the various transport proteins has been shown, albeit to a limited extent to date. Using a *Xenopus* oocyte model, coexpression of MCT1 with NBCe1-B was found to enhance lactate/H^+ transport approximately 2-fold (21). And NCBT function is intrinsically linked with that of the carbonic anhydrases, specifically CAII and CAIV (6, 23). CAs influence not only NCBT activity, but also that of MCT1/4 and NHE1 (27), being hypothesised to form transport metabolons (126). These are essentially direct functional and structural interactions between enzymes and transporters, allowing channelling of protons from one active residue to another. The evidence for their importance warrants elaboration here.

2.10.4 Carbonic Anhydrases

Of the four classes of CA (α, β, γ, δ), mammals possess only the 16 α-CA isozymes (459), 12 of which are known to be active in humans (337). The cytosolic CA isozymes identified to date in human skeletal muscle are CAII and CAIII and the membrane-bound isozymes are CAIV (sarcolemma, capillary endothelium, and SR) and CAXIV (specific location uncertain) (172, 252). One study has reported CAIV in both type I and type II fibres, but CAXIV solely in type I fibres (252). CAIV has also been shown to be expressed both intra- and extra-cellularly (338).

CAs catalyse the reversible hydration of CO_2 (see Eqn. 20, section 2.7.3) – at a rate 13 to 25,000 times greater than uncatalysed hydration (172) – thereby either consuming intracellular CO_2 (increasing $[H^+]$), or producing extracellular CO_2 (H^+ buffering) (6, 337, 338). Their activity varies greatly between isozymes, with CAIV and the ubiquitous CAII being highly active, whereas the most abundant skeletal muscle isozyme CAIII has the least catalytic activity of the 16 α-CAs, at about 0.2–0.5% of CAII activity *in vitro* (25, 422, 459). Of note for pH regulation, is that increasing evidence supports both a catalytic and non-catalytic function for specific CAs in H^+/HCO_3^- transport.

The importance of the catalytic function of the CA isozymes is readily apparent in regards to the NCBT systems, given that HCO_3^- is a shared substrate. CAII has been reported by some to directly bind to and enhance the transport activity of NBCe1 (23, 184), i.e., forming transport metabolons, thereby facilitating transport of HCO_3^- into the cell to buffer H^+. The

same group provided evidence supporting a similar role for CAIII (422); while others have argued that a direct binding between CAIV and NBCe1 was essential for restoring pH_i following an acid load (6). Although a functional interaction between CA isozymes and NBCs seems likely, direct binding of enzymes and transporters remains in dispute (65, 369). Nevertheless, even where conflicting data have shown no effect of extracellular CA on NBC activity in ventricular myocytes, inhibition of intracellular CA did reduce NBC activity (481). In the latter study, the authors suggested that intracellular CA catalyses the delivery of H^+ to NBCs.

In catalytically enhancing the buffer capacity of extracellular HCO_3^-, sarcolemmal CAs help maintain a pH gradient relative to the intracellular space that enhances MCT transport activity (172). In addition, of the cytosolic CAs, CAII, but not CAIII, has been shown to enhance activity of both MCT1 and MCT4, independent of its catalytic activity (22, 24, 25, 455). The increase in transport activity is proposed to involve a direct physical interaction between MCTs and CAII, whereby H^+ are exchanged between close proximity protonatable residues on the transporters and enzymes (25, 455). CAIII lacks the cluster of protonatable histidine residues that are crucial for this interaction (25). The same group have since demonstrated sarcolemmal CAIV to increase MCT transport independent of catalytic function, and importantly, the enhanced activity is additive to that of CAII (26). The authors have referred to the CAs as "H^+-collecting antennae" for the MCTs (24), describing the process as an "intramolecular H^+ shuttle" (25). An acknowledged limitation is that evidence to date, in CAIV at least, has been provided solely from a *Xenopus* oocyte model that overexpresses CAs (269). Whether this model translates to wild-type tissue, and *in vivo* functioning, remains to be determined. Finally, earlier supporting data using different techniques have shown that by knocking out individual CAs (IV, IX, and XIV) in mice (194), or by inhibiting extra- but not intra-cellular CAs in rat muscle (498), lactic acid transport was reduced. Similarly, in humans, following infusion of the CA inhibitor acetazolamide prior to exercise, the lower plasma [La^-] but similar muscle [La^-], compared to control, provided evidence of inhibited La^- efflux from the muscle (418, 419).

To complete the picture of the synergy between the pH-regulatory proteins and CA isozymes, some evidence has also been provided of a functional interaction between NHE1 and both cytosolic and sarcolemmal CAs. Inhibition of CAIV in a cell model seemed to reduce NHE1 activity (506). And as was mentioned above (2.10.2), acetazolamide prevented pH recovery following a period of anoxia in different muscle cell models, which was inferred to support an interaction between NHE1 and CA (505). Separately, CAII was shown to bind to a specific carboxyl (C)-terminal sequence of NHE1, a process the authors suggested required phosphorylation of NHE1 (286, 287). Using a cell-culture model they also reported that NHE1 activity decreased when CAII was inhibited both by acetazolamide and when they transfected

the cells with an inactive form of CAII (286). Subsequently, others have produced contradictory data showing no catalytic effect of intra- or extra-cellular CA on NHE activity in ventricular myocytes (481).

The role of the CAs in pH_i regulation seemingly involves more than simply catalysing hydration of CO_2/HCO_3^-. In catalysing H^+ buffering in the interstitium by HCO_3^- (228), the sarcolemmal CAs maintain a pH gradient relative to the intracellular space necessary for high MCT activity. And despite some doubt about the transport metabolon concept (65, 369), the CAs enhance MCT activity independent of their catalytic activity. If the cytosolic CAs do bind directly to NCBe1 forming transport metabolons, this would facilitate transport of HCO_3^- into the intracellular space to buffer H^+. Clearly these data show that the synergy between acid/base transporters and the CAs is important in maintaining pH_i regulation, but how this translates to *in vivo* function in skeletal muscle is unknown. Despite some evidence of changes in CA protein or mRNA expression in muscle following hypoxia (252, 511), there are currently no data on the CA response to exercise training.

2.10.5 Calcium/Hydrogen Exchange

A distinct transport mechanism worthy of brief mention is cytosolic Ca^{2+}/H^+ exchange (cCHX). As the name suggests, a unique feature of this exchange mechanism is that it is cytosolic rather than membrane-based (461). Briefly, Ca^{2+} and H^+ bind to small mobile buffers, in particular the histidyl dipeptides. The Ca^{2+}-bound buffer and protonated buffer are exchanged along their respective ionic gradients. Effectively, this compartmentalisation of Ca^{2+} to acidic regions of the cytoplasm can couple with NHE1 and compensate for the possible negative inotropic effects of acidity (461). Evidence remains to be shown for the existence of such a cytosolic transport mechanism in skeletal muscle, with the data to date coming from cardiac myocytes. Nevertheless, the reliance on mobile buffers such as carnosine to facilitate Ca^{2+}/H^+ exchange points towards a potentially important role in skeletal muscle too.

2.11 Techniques for Measuring Buffer Capacity

Before looking at the research to date into exercise and βm, now is an appropriate time to explain some of the different methodologies used to measure βm. The two most commonly-used techniques are the titration method for measuring non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) in homogenate, and what has been called the self-titration method (303), $\beta m_{in\ vivo}$, also measured in homogenate. A third non-invasive and dynamic measurement of βm can be obtained by ^{31}P-MRS. None of these techniques are without criticism though, and the strengths and weaknesses of each are briefly outlined here.

The $\beta m_{in\ vitro}$ technique entails the relatively simple titration of a weak base with a strong acid, the weak base(s) being the collection of physicochemical buffers in the homogenised tissue, and the strong acid used typically being HCl. Buffer capacity is defined as the number of moles of H^+ required to change the pH by one unit per mass of muscle. This is the only absolute measurement of βm currently used, but it is important to note the limitations. As the name suggests, measurement is performed on a small sample *in vitro*, having biopsied muscle from a participant. To enable dissection of the sample free from blood and connective tissue it is necessary to freeze-dry the muscle prior to homogenising, resulting in the loss of the bicarbonate buffering capacity of the sample[41]. Marlin & Harris (306) reported freeze-drying to reduce βm by about 24–30%. They found by adding HCO_3^- (60 mmol•kg dm^{-1}) to the sample βm increased by 18%. As noted earlier in section 2.7.3, although some equivocation remains regarding the relative contribution of HCO_3^- to intracellular buffering (20, 366), the physicochemical buffering of HCO_3^- is less than these data suggest (*cf.* Figure 2.8). In fact, that much HCO_3^- should, according to the Henderson–Hasselbalch equation (Eqn. 17), add a further 13.9 mmol H^+•kg dm^{-1} of physicochemical buffering power[42], increasing the titrated βm measured by Marlin & Harris (306) in dry muscle by 12.7%. This suggests that the difference in buffering between dry and wet muscle cannot simply be explained by loss of CO_2 during freeze-drying.

It is clear that $\beta m_{in\ vitro}$ cannot be an exact representative measurement of physiological physicochemical buffering. By breaking cell walls and membranes, extracellular proteins will be included in the calculation of βm after the homogenisation process (376); while disruption of organelles may expose protonatable residues that are unavailable to bind H^+ in the native state, artefactually inflating βm (277). Homogenisation has also been reported to cause almost complete phosphagen hydrolysis following Ca^{2+} release, in particular when combined with incubation at 37°C, with the P_i released increasing the total buffering capacity (1, 306, 442). As Harris *et al.* (200) noted, any subsequent variability in the phosphate contents[43] after homogenisation will contribute to the intra-assay variability (*cf.* section 2.7.1). Nevertheless, coefficients of variation (CV) between 1.7% and 7% have been reported in the literature (45, 303, 326, 349, 496). Notably, the lower CVs are typically calculated on repeat titrations from the same homogenate, rather than the entire process from dissection of muscle (e.g., 306). This will negate the primary sources of error, namely variable phosphagen hydrolysis during homogenisation, and indeed weighing of freeze-dried muscle, which can be greatly affected by

[41] CO_2 is also lost during the homogenisation process (111).

[42] Assuming $[HCO_3^-]$ of 60 mmol•kg dm^{-1} equates to a total concentration of buffer ($[H_2CO_3]$ + $[HCO_3^-]$) of 71.5 mmol•kg dm^{-1}, and for a pK_a of 6.1.

[43] PCr is a poor buffer with a pK_a of 4.58, whereas P_i and the hexose monophosphates have pK_a values of 6.82 and 6.11–6.13, respectively (200). Additionally, lipid-bound phosphates, such as glycerol 3-phosphate, with a pK_a of ~6.67 (2), may be released by homogenisation (414).

a static charge in the absence of a microbalance fitted with an ionising blower (see Appendix K for cumulative βm error with weighing imprecision).

As was described in section 2.7, by knowing the pK_a of a buffer and its concentration in muscle it is possible to estimate its buffering power using the Henderson–Hasselbalch equation. Sahlin (405) presented a thorough breakdown of the contribution of specific buffers based on assumptions of pK_a values and concentrations, but the relative contribution of the each of the physicochemical buffers to total buffering has received little direct investigation. In a review paper, Parkhouse *et al.* (361) reported the contribution of protein buffering in different studies[44] to range from 22% to 50% of total $\beta m_{in\ vitro}$. More recently, Bishop *et al.* (48) estimated protein $\beta m_{in\ vitro}$ to be 35–40% of the total $\beta m_{in\ vitro}$[45]. There seems to be little certainty and much variability regarding the importance of individual buffers.

The $\beta m_{in\ vivo}$ technique is based on the principle that by measuring the change in metabolic acids *in vivo* in response to exercise, and the concurrent change in pH_i, one can calculate the total intracellular buffer capacity. The difference between $\beta m_{in\ vivo}$ and $\beta m_{in\ vitro}$ should equal the contribution from metabolic buffering, notwithstanding the limitations of CO_2 loss in freeze-dried muscle. Theoretically, $\beta m_{in\ vivo}$ may therefore be 30–40% higher than $\beta m_{in\ vitro}$ (303). In practice, where both have been measured the data are highly variable, $\beta m_{in\ vivo}$ being on average 19% greater (see Appendix I), but ranging from 118% greater (349) to 48% lower than $\beta m_{in\ vitro}$ (232). Originally[46], $\beta m_{in\ vivo}$ was calculated from $\Delta[H^+]/\Delta pH$ (479), but latterly the calculation used has been $\Delta[La^-]/\Delta pH$ (e.g., 405). This technique assumes a 1:1 stoichiometry between La^- and H^+, a controversial topic and subject of much spirited debate (61, 62, 260, 291, 294, 305, 390-392, 412, 482). The details of the argument would be somewhat tangential here, suffice to say that anything affecting the assumed 1:1 stoichiometry will introduce error into the calculation, in particular the non-lactate-coupled transport of H^+, which will result in underestimation of actual βm. Upregulation of non-lactate-coupled H^+ transport, primarily through increased NHE1 protein abundance, will significantly affect $\beta m_{in\ vivo}$ (248). Compounded by the elevated pH artefact from phosphagen hydrolysis during the homogenisation process, the greater changes in pH relative to $[La^-]$ will also result in an underestimation of the true βm (303). And in excluding pyruvate, some error is introduced in considering lactate as the totality of metabolic acid in the muscle (303). Furthermore, $\beta m_{in\ vivo}$ does not take into account buffering by PCr hydrolysis (261).

[44] Owing to an apparent omission error in the editing process, the cited studies are not listed in the references; therefore it is not known which studies the authors referred to.

[45] The measured $\beta m_{in\ vitro}$ of the supernatant after deproteinsing with 5-sulfosalicylic acid was 60–65% of the total $\beta m_{in\ vitro}$, with an assumption made that the balance consisted of protein buffers.

[46] The paper by Van Slyke led to the later terming of the unit of buffer capacity as Slyke ($mmol \cdot L^{-1} \cdot pH^{-1}$) in his honour, but a paper eight years earlier by Koppel and Spiro provided a similar formula to calculate buffer capacity [cited in Deitmer & Thomas (127)].

$\beta m_{in\ vivo}$ is also very much context specific, i.e., the values obtained are dependent on the nature of the exercise performed. Indeed, Roos & Boron (397) argued that such time dependent βm estimates that are "contaminated" by ion fluxes are meaningless unless the experimental conditions are specified. In contrast, transmembrane ion flux is not an issue in a closed system, such as measured by the $\beta m_{in\ vitro}$ technique (361). For moderate-intensity exercise involving no change in muscle [La^-], $\beta m_{in\ vivo}$ will be zero, which has been argued to render it a conceptually obscure technique (260). One additional criticism that has been made is that, with $\beta m_{in\ vivo}$ being calculated from just two pairs of values, it is highly susceptible to measurement error (304), magnified by the potential blood contamination of hyperaemic post-exercise samples. In one study for example, though not reported, owing to a seemingly artefactually low resting pH for one of the groups, calculating mean $\beta m_{in\ vivo}$ for two randomised groups of similar participants pre-intervention would return values of 355 and 173 mmol•kg dm^{-1} (327). And others have reported $\beta m_{in\ vivo}$ values lower than their $\beta m_{in\ vitro}$ measured by titration (43, 177, 232), despite $\beta m_{in\ vivo}$ theoretically being a measure of physicochemical *and* metabolic buffering. To put the margin of error into context, mean resting pH_i values that were just 0.05 units lower than measured in Gore *et al.* (177) would have increased their mean $\beta m_{in\ vivo}$ by 16–20%.

βm can also be calculated in ischaemic exercise models using ^{31}P-MRS by estimating the rate of change in pH and [PCr] (261, 305). During the first few seconds of exercise pH_i becomes slightly basic due to consumption of H^+ by the CK reaction (1, 509). On this basis βm can be calculated from the change in PCr hydrolysis relative to the change in pH:

$$\text{Eqn. 33}\quad \beta = \alpha_p \times \frac{\Delta[PCr]}{\Delta pH}$$

where α_p is a stoichiometric coefficient for protons per mole of PCr hydrolysis, equal to 0.36–0.85 between pH 7 and pH 6 (1, 261). Using this calculation, Adams *et al.* (1) reported similar values as a titration assay of homogenate in some, but not all animal tissues assayed. Others have observed that the lower values for βm estimated from MRS are because of the absence of the artefactual PCr hydrolysis found with the biochemical techniques noted above (261). A fundamental criticism of estimating βm using MRS is that an assumption is made of negligible La^- accumulation during the initial stages of exercise (411), an assumption known to be false (230). It has been acknowledged that the technique would not be valid in high-intensity exercise models with a high early glycolytic ATP turnover (107).

2.12 pH_i Regulation as a Predictor of Performance

Working off the tenuous assumption that pH_i regulation is a limiting factor to exercise performance at high intensities, the evidence should support pH_i regulation in part predicting performance. In this regard, there are a number of studies that have sought to correlate βm or

transport protein abundance with exercise performance, showing contrasting results to date. Parkhouse *et al.* (361) speculated that sprint-trained athletes' greater ability to regulate pH_i compared to endurance-trained or sedentary participants could be a contributory factor to their enhanced anaerobic performance. Subsequently, relationships between the different measures of βm and exercise performance have been investigated. One study has reported large correlations between $\beta m_{in\ vitro}$ and both 200-m run time (r = −0.57) and peak power output during a 30-s sprint (r = 0.69) in male and female recreational runners[47] (349). Using a less well-trained female cohort[48], another study found a greater correlation between work decrement following repeated-sprint exercise and $\beta m_{in\ vivo}$ (r = −0.72), than with $\beta m_{in\ vitro}$ (r = −0.24) (44). The same investigators calculated a large correlation between $\beta m_{in\ vitro}$ and total work performed (r = 0.67) during repeated-sprint exercise in a female population of mixed training status[49], but again reported a lesser correlation with work decrement (r = 0.41) (141). It is difficult to make any clear inference from these data, given the imprecision inherent in correlation coefficients with small sample sizes. For example, as shown in Figure 2.10, with n = 13 in the study of Nevill *et al.* (349), the 90% confidence interval (CI) for the correlation coefficient, r = 0.69, between $\beta m_{in\ vitro}$ and peak power, is 0.32 to 0.88. Similarly, the correlation coefficient (90% CI) between $\beta m_{in\ vitro}$ and total work in Edge *et al.* (141) is r = 0.67 (0.40 to 0.83), for n = 21. While βm does appear to contribute to performance in some high-intensity exercise tests, its relative importance remains unclear from these data.

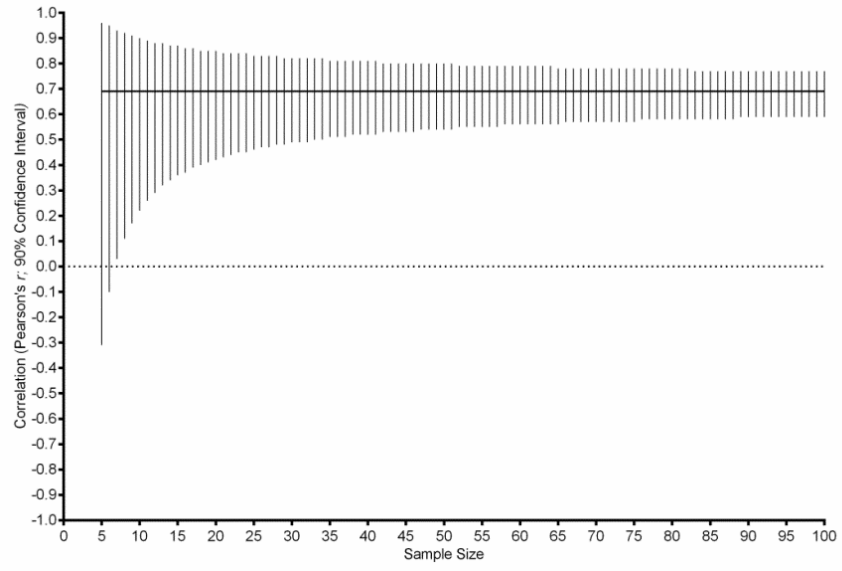


Figure 2.10 Relationship between Pearson's product-moment correlation coefficient (*r*) and sample size. Data are shown for an example correlation coefficient of *r* = 0.69 (horizontal line) between $\beta m_{in\ vitro}$ and peak power taken from Nevill *et al.* (349), and the corresponding 90% confidence interval (vertical lines). Calculations were performed using a custom spreadsheet (224), which corrects for small sample size bias.

[47] Mean $\dot{V}O_{2max}$ of 4 men and 4 women: 52.6 mL•kg^{-1}•min^{-1}
[48] Mean $\dot{V}O_{2peak}$: 42.3 mL•kg^{-1}•min^{-1}
[49] Mean $\dot{V}O_{2peak}$: team-sport athletes 46.6 mL•kg^{-1}•min^{-1}; endurance-trained athletes 53.6 mL•kg^{-1}•min^{-1}; active participants 38.5 mL•kg^{-1}•min^{-1}

Performance improvements have been reported without any substantial change in muscle pH_i regulation. Mannion *et al.* (304) saw no change in $\beta m_{in\ vitro}$ after 16 weeks of isokinetic knee-extensor training, despite improvements in total work performed during a modified all-out cycling test[50]. It seems likely that βm is not a determining factor in such single-bout sprint performance. Separately, improved sprint performance after 8 weeks of repeated-sprint training was not mirrored by any change in $\beta m_{in\ vitro}$ (349). As mentioned above, the latter study did find that individuals with the highest $\beta m_{in\ vitro}$ also had the best sprint performance. One possible inference is the well-trained participants already had elevated $\beta m_{in\ vitro}$ and were less likely to improve further during a comparatively short-term training intervention (139). In support of this, no relationship (data not given) was reported between repeated-sprint performance and $\beta m_{in\ vivo}$ in endurance-trained cyclists[51] (187).

Others have suggested that, distinct from untrained populations, improving exercise capacity in well-trained individuals is achieved through physiological adaptations other than muscle pH_i regulation (233). This is supported by their own research, which has found enhanced exercise performance after various high-intensity training interventions despite failing to see any changes in MCT1 or MCT4 abundance (18, 186, 187, 232, 468). A number of these studies have found increases in NHE1 abundance (186, 187, 232), suggesting disparate signalling factors are involved in upregulation of the MCT and NHE transport systems.

The importance of pH_i regulation for performance in specific exercise tests cannot be confidently ascertained from cross-sectional data studies without larger sample sizes. And the lower the correlation, the greater the imprecision in the estimate of a relationship, and consequently the larger the sample size needed (332). Nevertheless, individuals undertaking high-intensity training do appear to have greater βm than untrained individuals, or those who perform moderate-intensity training. Though there may be a dissociation between pH_i regulation and performance in the more highly-trained, it seems that high-intensity training may increase βm, and possibly also H^+ transport capacity, in comparatively untrained individuals. This brings us on to the key research question of the current thesis.

2.13 Training-Induced Adaptations in pH_i Regulation

If, as the balance of evidence suggests, H^+ accumulation contributes to the aetiology of fatigue, what potential is there for enhancing muscle pH_i regulation? Many studies have sought to identify whether different modalities of training can upregulate an individual's capacity to buffer and/or transport H^+. The wide variety of training interventions used, with participant

[50] All-out cycling until cadence dropped below 60 rpm. Tests were performed at six different external loads, equivalent to 9–14% of maximal voluntary isometric contraction of the quadriceps.
[51] Mean $\dot{V}O_{2max}$: 59.1 $mL \bullet kg^{-1} \bullet min^{-1}$

populations of mixed training statuses, as well as the heterogeneity of muscle samples, make comparative analysis of the research difficult, and reflects the incomplete understanding of the area. Nevertheless, there are some convincing data to indicate the plasticity of muscle pH_i regulation.

As was noted earlier, it is typically only following high- to supramaximal-intensity intermittent or continuous exercise that pH_i decreases appreciably (see Appendix G). Clearly, pH_i regulation is adequately equipped for buffering or transport of H^+ during lower-intensity exercise. Intuitively, it may then follow that only exercise resulting in H^+ accumulation in the muscle – where sufficient stress is exerted on pH-regulating capacity to overwhelm it – would be effective in eliciting adaptations in pH_i regulation (495).

Many of the training interventions used to date have involved various modalities of high-intensity interval training (HIT), which are repeated bouts of short duration exercise (typically < 5 min) at an intensity typically greater than the lactate threshold (LT), but usually not greater than maximal aerobic power (77, 282). Supramaximal forms of HIT that are often used include sprint-interval training (SIT), which entails shorter duration (< 30 s) bouts greater than maximal aerobic power, and repeated-sprint training (RST), which consists of all-out sprints (< 10 s in duration), interspersed with short recovery periods (77). HIT and/or SIT have been found to induce improvements in oxidative metabolism similar to endurance training, but with considerably less training load (78, 80, 174, 281). And such modes of high-intensity training have displayed the greatest efficacy in eliciting adaptations in most pH_i-regulatory mechanisms.

The next section details the response to training of $\beta m_{in\ vitro}$. Changes in $\beta m_{in\ vivo}$ following training have also been reported several times, but these data are not included below on the basis that training-induced changes in $\beta m_{in\ vivo}$ are of questionable epistemological value, given the limitations of the technique detailed in section 2.11 (304). In particular, changes in $\beta m_{in\ vivo}$ do not just reflect changes in intracellular buffering capacity, but may indicate changes in lactate metabolism, or increased activity of non-lactate-coupled H^+ transporters such as NHE1. Training adaptations in individual acid/base transport proteins are covered in the subsequent sections.

2.13.1 Muscle Buffer Capacity

On pooling the existing research, it is apparent that most training interventions have failed to elicit clear improvements in $\beta m_{in\ vitro}$ (Figure 2.11). And though there are some studies that have reported a clear positive effect of training, there are also others that have found a clear negative effect. While methodological limitations with the assay cannot be discounted from explaining some of the variance, training intensity in human studies may also, in part, explain

the equivocation around changes in $\beta m_{in\ vitro}$ following training. Figure 2.11 displays data from individual human studies, separated by training intensity: moderate intensity (< LT), high-intensity (> LT and < $\dot{V}O_{2max}$), maximal intensity (≈ $\dot{V}O_{2max}$), and supramaximal intensity (> $\dot{V}O_{2max}$). At exercise intensities above the LT there are greater improvements in $\beta m_{in\ vitro}$, with no improvements evident for moderate-intensity continuous training, i.e., less than the LT. When training is conducted at the supramaximal intensities of SIT, the changes in $\beta m_{in\ vitro}$ have typically been small and unclear. Given that there are also some conflicting data in HIT studies, it is likely that other programme variables such as the volume of exercise and the amount of recovery between and within training sessions need to be optimised for a given exercise intensity to promote adaptations. On the basis of the evidence to date, some potentially influential factors are discussed below.

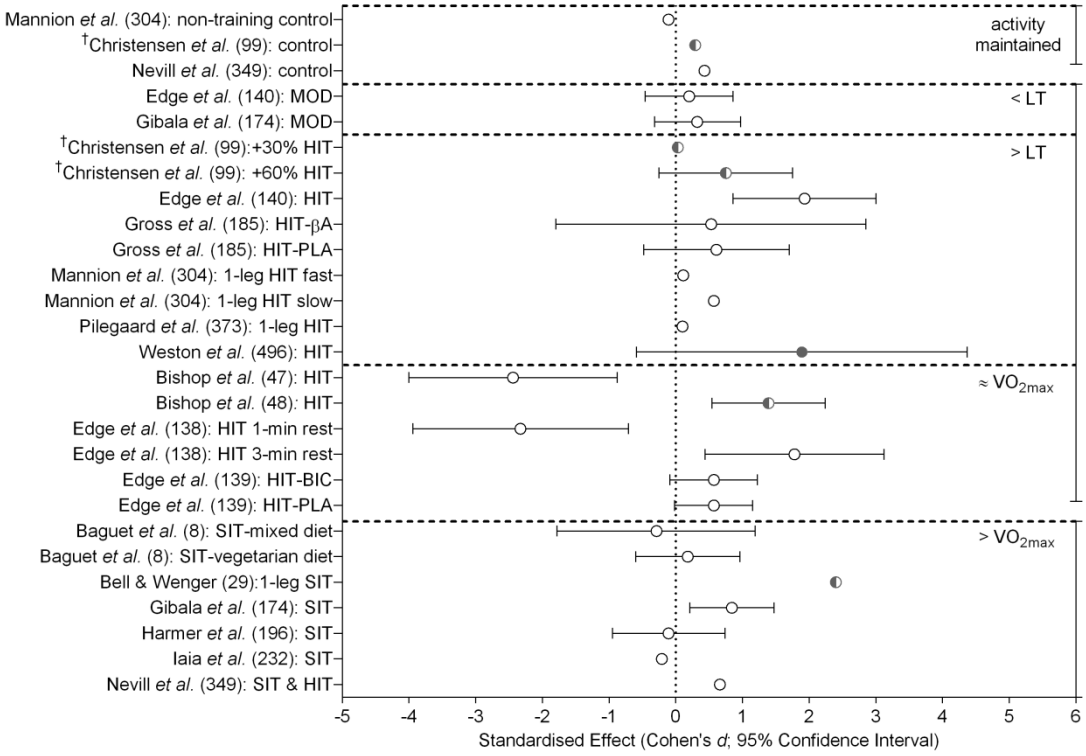


Figure 2.11 Forest plot of Cohen's *d* effect size and 95% confidence intervals (CI) for non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) following different training interventions, categorised by four zones of training intensity: control (maintained normal activity), moderate intensity continuous (< LT), high intensity (> LT and < $\dot{V}O_{2max}$), maximal intensity (≈ $\dot{V}O_{2max}$), supramaximal intensity (> $\dot{V}O_{2max}$). For studies that reported error of change scores or individual data, CIs were calculated by multiplying the standard error (SE) of the change scores by the t-statistic for a 95% CI with *n*-1 degrees of freedom. If raw data were not reported but there was an exact *P* value, 95% CIs were calculated from *P* values (5). Abbreviations: βA (β-alanine supplementation), BIC (sodium bicarbonate supplementation), HIT (high-intensity interval training), MOD (moderate-intensity continuous), PLA (placebo), SIT (sprint-interval training). †No details given on modality of HIT. Training status: ● highly-trained, ◐ well-trained, ○ recreationally active. See text for details of individual studies.

Cross-sectional research with different cohorts illustrates the potential trainability of βm. Female recreational team-sport athletes[52] have been reported to possess greater $\beta m_{in\ vitro}$ than either untrained or endurance-trained groups (141), but no differences were observed between the endurance-trained and untrained groups. The same authors subsequently reported $\beta m_{in\ vitro}$ to increase after high-intensity training, but not after work-matched moderate-intensity continuous training (140). Active females[53] who performed 5 weeks of continuous training for 3 d•wk^{-1}at 80–95% of the LT showed no change in $\beta m_{in\ vitro}$, whereas a group who underwent work-matched HIT[54] at 120–140% of the LT had a 25% improvement (140). This was despite similar improvements for both groups in both the LT and $\dot{V}O_{2peak}$. The authors postulated that training needs to be greater than the LT to provoke an adaptation in βm, which is supported by the meta-analysis in Figure 2.11.

Some tentative evidence that training intensity influences adaptations to $\beta m_{in\ vitro}$ was provided in an earlier unpublished study[55] (99). Without changing the distance each group ran, two groups of male endurance runners[56] increased their intensity of training by either 30% or 60%, through the addition of HIT for 4 weeks, while a third group maintained their normal training. The group who increased their intensity by 60% showed a large improvement in $\beta m_{in\ vitro}$ of 24%, compared to no change for the +30% intensity group, and a 5% increase for the control group. Similarly, when male endurance-trained[57] cyclists substituted part of their regular continuous training with six HIT sessions over 4 weeks (6–9 × 5 min at 80% peak power output, 1 min of active recovery), there was a 16% improvement in $\beta m_{in\ vitro}$ (496). Having previously found no improvement in $\beta m_{in\ vitro}$ in type I or type II muscles[58] of rats that underwent spontaneous running for 4 weeks (495), these findings supported their hypothesis that the exercise undertaken by the rats was of insufficient intensity to induce changes in βm.

It seems that exercise performed above the LT is necessary to induce improvements in $\beta m_{in\ vitro}$. Nevertheless, progressively higher training intensities are not necessarily paralleled with increasingly greater adaptations in βm. At the supramaximal intensities at which SIT is performed, improvements in $\beta m_{in\ vitro}$ have typically not been observed. Two training interventions using 30-s sprints with rest intervals of 3–4 min reported no improvement in $\beta m_{in\ vitro}$ in either active males[59] (196), or male endurance-trained runners[60] (232). Though increases in $\beta m_{in\ vitro}$ have been reported in active men[61] undergoing 30-s SIT with rest intervals

[52] Mean $\dot{V}O_{2peak}$: team-sport 42.1 mL•kg^{-1}•min^{-1}; untrained 38.5 mL•kg^{-1}•min^{-1}; endurance 53.6 mL•kg^{-1}•min^{-1}
[53] Mean $\dot{V}O_{2peak}$: continuous group 42.1 mL•kg^{-1}•min^{-1}; HIT group 43.7 mL•kg^{-1}•min^{-1}
[54] Periodised model: 3 d•wk^{-1}, 2–10 × 2-min intervals, 2:1 work:rest
[55] Owing to this being a conference abstract, there was limited information on the type of high-intensity training.
[56] Mean $\dot{V}O_{2max}$: control 4.55 L•min^{-1}; +30% intensity group 4.17 L•min^{-1}; +60% intensity group 4.55 L•min^{-1}
[57] Mean $\dot{V}O_{2max}$: 66.2 mL•kg^{-1}•min^{-1}
[58] Superficial *vastus* was found to contain 100% type II fibres; *soleus* was a minimum of 73% type I fibres.
[59] Mean $\dot{V}O_{2peak}$: 3.8 L•min^{-1}. Performed 4–10 sprints for 7 weeks.
[60] Mean $\dot{V}O_{2max}$: 55.8 mL•kg^{-1}•min^{-1}. Performed 8–12 sprints for 4 weeks.
[61] Mean $\dot{V}O_{2peak}$: 4.1 L•min^{-1}

of 4 min (174), the magnitude of the effect is considerably diminished if a comparison with the moderate-intensity continuous training[62] group is made (7.6% versus 4.2% respectively). In reality, the magnitude of change relative to control is less than the coefficient of variation (1.7% to 7%) commonly reported for the βm titration assay (*cf.* section 2.11). It seems likely that if progressively higher exercise intensities do result in better improvements in $\beta m_{in\ vitro}$, then the absolute accumulation of muscle La^- and H^+ are not important stimuli.

Indirect evidence in support of this hypothesis that greater intracellular [La^-] and [H^+] do not lead to greater increases in $\beta m_{in\ vitro}$ has been provided by two related studies. Rats that performed 5 weeks of HIT (6–12 × 2 min, 2:1 work:rest) showed large increases in $\beta m_{in\ vitro}$ for both the *soleus* (34–40%) and EDL (22–26%) muscles, regardless of whether they received $NaHCO_3^-$ supplementation or a placebo prior to each training session (49, 466). An earlier human study found no clear difference in the increase in $\beta m_{in\ vitro}$ for moderately-trained female participants[63] who received chronic $NaHCO_3^-$ supplementation (19%) throughout a 5-week HIT programme, when compared to a group who received a placebo (9%) (139). While providing $NaHCO_3^-$ does not improve intracellular buffer capacity (43), supplementation increases extracellular pH (pH_o), and the resultant greater ionic gradient would enhance activity of acid/base transport proteins (297, 397, 441), notably NBCe1 and MCT1 (21). HCO_3^- therefore indirectly acts to regulate pH_i by enhancing H^+ transport during exercise. Furthermore, $NaHCO_3^-$ supplementation has been shown to result in a greater muscle glycogen depletion, and higher muscle [La^-] and [H^+], following exercise at 75% $\dot{V}O_{2max}$, but not at lower intensities (222). Therefore, while exercise-induced increases in La^-/H^+ accumulation may provide some stimulus for upregulation of $\beta m_{in\ vitro}$ (495), further increases appear to have little effect. Moreover, given that there was no additional benefit of prior $NaHCO_3^-$ supplementation on $\beta m_{in\ vitro}$, despite the probable enhancement of transport activity, these data also suggest greater H^+ production, and subsequent efflux, are not important stimuli.

It seems from the majority of studies to date that longer duration intervals performed above the LT are favourable for improved $\beta m_{in\ vitro}$, rather than shorter supramaximal intervals. Indeed, the first study to investigate training-induced changes in $\beta m_{in\ vitro}$ is the only study that has shown clear improvements following exercise with a short-duration, 20-s work cycle (60-s rest periods) (29). Using single-leg cycling at 150% of the power at $\dot{V}O_{2max}$, there was an increase of 16% after 7 weeks in a group of both sexes[64], compared to 4% for the non-exercising leg. In contrast to this type of single-leg dynamic exercise with a large muscle mass, 30 to 60-s

[62] 90–120 min at 65% $\dot{V}O_{2peak}$. Although the LT is not reported, this intensity is likely to be close to, or above the LT, for this active but untrained population.
[63] Mean $\dot{V}O_{2peak}$ for placebo and supplementation groups: 2.3 and 2.4 $L \cdot min^{-1}$.
[64] Mean $\dot{V}O_{2max}$ for 8 men and 1 woman: 52.9 $mL \cdot kg^{-1} \cdot min^{-1}$

single-leg knee extensions with 2 min of rest, performed 3–5 d•wk^{-1} for 8 weeks[65], failed to induce improvements in $\beta m_{in\ vitro}$ in active men[66] compared to their untrained leg (373). Others have similarly failed to observe any clear change in $\beta m_{in\ vitro}$ following a single-joint, single-leg exercise intervention for 16 wk at fast or slow angular velocities[67] (304). While the size of the exercising muscle mass may explain the difference between single-leg modalities, it is not clear why there was such a large increase in the study of Bell & Wenger (29) compared to other SIT interventions. It is possible that this study is simply an outlier of random sampling, or an incidence of the Proteus phenomenon, whereby the first published effect can be larger and contradictory to subsequent findings (236). A greater sample of SIT studies is required to confirm or reject this speculation.

Despite HIT performed above the LT appearing to provide the greatest improvements in $\beta m_{in\ vitro}$, there are some conflicting data between studies that have employed similar training intensities and work interval durations. One potential explanation for these disparate findings may be the duration of rest intervals. Five weeks of HIT at 100% of $\dot{V}O_{2peak}$ for 3 d•wk^{-1} (6–12 × 2 min, 2:1 work:rest) resulted in a mean decrease of 11% in $\beta m_{in\ vitro}$ in female team-sport[68] participants (47). A second study from the same group reported a 9% increase in $\beta m_{in\ vitro}$ following 5 weeks of running HIT for 3 d•wk^{-1} (5–8 × 2 min, 1:1 work:rest), performed at the velocity associated with $\dot{V}O_{2max}$, in well-trained[69] male team-sport athletes (48). They measured the greater physicochemical buffering to be due to increases in both protein and non-protein buffering components. Other than the obvious sex difference in populations used, an important distinction between the studies was that despite also performing 2-min work intervals, the latter study utilised 2-min rest intervals. Though it is unlikely that there would be much difference in pH_i recovery following 1 min versus 2 min of rest (54), 2 min of high-intensity exercise causes a large depletion of PCr (142, 349), which would be less replenished after 1-min rest intervals, ensuring increasing oxidative ATP turnover for subsequent work intervals (54, 168, 308, 379, 444). If La^-/H^+ production is an important stimulus for upregulation of $\beta m_{in\ vitro}$, then training at or near $\dot{V}O_{2max}$ combined with short rest intervals would not maximise non-mitochondrial ATP turnover. Support for this premise was shown in a similar study by the same group. Active females[70] undertook five weeks of HIT (6–10 × 2 min) at 92–111% of $\dot{V}O_{2peak}$ for 3 d•wk^{-1}, but participants were split into 1-min rest and 3-min rest groups (138, 201). Despite being work-matched, there was a mean decrease in $\beta m_{in\ vitro}$ of 11% (95% CI: 4 to 18%) for the 1-min group, but an increase of 6% (1 to 10%) for the 3-min group.

[65] 3–5 sets of 2 × 30-s and 3 × 60-s bouts at a resistance /kick frequency of 100–120 N /80 rpm and 50–100 N /60 rpm for the 30-s and 60-s bouts, respectively.
[66] No measure of training status was given in this study.
[67] Isokinetic knee extensions at 4.19 rad•s^{-1} or 1.05 rad•s^{-1}
[68] Mean $\dot{V}O_{2max}$: 43.2 mL•kg^{-1}•min^{-1}
[69] Mean $\dot{V}O_{2max}$: 55.6 mL•kg^{-1}•min^{-1}
[70] Mean $\dot{V}O_{2peak}$: 1-min rest group 45.6 mL•kg^{-1}•min^{-1}; 3-min rest group 45.6 mL•kg^{-1}•min^{-1}

The rest component of the duty cycle may be important at both ends of the spectrum. There are some data to suggest the duration of the relief interval may have been too long to sufficiently stress pH_i-regulatory mechanisms and stimulate upregulation. An intervention incorporating varied HIT and SIT in a mixed population[71], and using 5–10 min rest between intervals, found no change in $\beta m_{in\ vitro}$ (349). More recently, there was little change in $\beta m_{in\ vitro}$ following 5 weeks of cycling and running SIT[72] that incorporated rest intervals of 20 min (8). If H^+ accumulation is a stimulus for βm improvement (495), the length of rest periods needs to be sufficiently short, given pH_i returns to baseline within about 10 min, depending on the intensity of the exercise (13, 231). Lower pH_i and higher muscle $[La^-]$ are seen following 1-min versus 3-min rest intervals between similar work intervals (142). Therefore, with 20 min rest it is almost certain that the second of two sprints was performed at normal resting pH_i (8).

A confounding factor in assessing changes in $\beta m_{in\ vitro}$ is methodological artefact with the technique. Factors contributing to within-sample variation were detailed in section 2.11, but between-sample variation may add additional error. In one example, an increase in $\beta m_{in\ vitro}$ of 8% was reported in active men[73] who undertook two weeks of 4 × 4-min cycling intervals at 90–95% of maximum heart rate, interspersed with 3 min of rest (185). However, if the change in $\beta m_{in\ vitro}$ following training was measured relative to a pre-supplementation phase (β-alanine or placebo), there were group increases of only 1–2%. The difference from pre-supplementation to pre-training is evidence of a potentially large variation in $\beta m_{in\ vitro}$ measured in separate samples taken from the same muscle. Therefore, to reliably assess the effect of training on $\beta m_{in\ vitro}$, the magnitude of the effect needs to be greater than the known within-sample, and unknown between-sample variations. Moreover, given there was a measured decrease in $\beta m_{in\ vitro}$ of 3% despite a 32% increase in muscle carnosine content after 38 days of β-alanine supplementation, it is clear that the effect of increased carnosine content on $\beta m_{in\ vitro}$ is not meaningful relative to the typical error of measurement for the titration assay.

Furthermore, the timing of the biopsy relative to an exercise bout has the potential to influence interpretation of potential training effects. As was stated in section 2.11, muscle samples taken at the end of high-intensity exercise resulting in substantial PCr depletion, and subsequent phosphate accumulation, will have elevated phosphate buffer capacity compared to the theoretical resting intracellular buffer capacity (375). Variation in phosphate buffering will depend on the specific phosphate pool, with the hexose monophosphates formed as intermediates of glycogenolysis being comparatively poor buffers over most of the pH_i transit range – pK_a values of ~6.1 (*cf.* section 2.7.1) – whereas with a pK_a of 6.87, P_i provides greater

[71] Training was performed on a non-motorised treadmill 3–4 $d \bullet wk^{-1}$, broken down as follows: 2 d of 2 × 30-s maximal sprints separated by 10 min; 1 d of 6–10 × 6-s sprints, 1:9 work:rest; and 1 d of 2–5 × 2-min runs at 110% $\dot{V}O_{2max}$, 2:5 work:rest. Mean $\dot{V}O_{2max}$ of 4 men and 4 women: 52.6 $mL \bullet kg^{-1} \bullet min^{-1}$.

[72] Training consisted of alternating sessions of 1–2 × 30-s all-out cycling, or 1–2 × 200-m sprints. Participants were described as active males and females.

[73] Mean $\dot{V}O_{2max}$ for placebo and supplementation groups: 61 and 59 $mL \bullet kg^{-1} \bullet min^{-1}$

buffering power. However, the homogenisation process causes almost complete phosphagen hydrolysis (306, 442), and without prior glycogenolysis the phosphates will be as free P_i, rather than hexose monophosphates or glycerol-3-phosphate, potentially increasing $\beta m_{in\ vitro}$ at rest compared to post-exercise. A post-exercise sample is, therefore, probably more representative of actual intracellular muscle buffering than a resting sample.

One group have reported decreases in $\beta m_{in\ vitro}$ in muscle samples taken immediately after different modes of high-intensity exercise (46, 48). An 11% reduction in $\beta m_{in\ vitro}$ was found following 45 s of maximal cycling (46), and 5–7% reductions were seen following cycling to fatigue at 120% $\dot{V}O_{2max}$ or 6 × 4-s maximal sprints (48). These reductions were attributed in the latter study to an equimolar reduction in protein buffering, having measured stable non-protein $\beta m_{in\ vitro}$. However, the differences were of a similar magnitude to their reported CV of 5.4% for the titration technique (45, 140). Though a reduction in $\beta m_{in\ vitro}$ has been shown in rat muscle (372), this was two days after lengthening contractions expected to damage membrane integrity, causing intracellular proteins to leak into the extracellular space. Sufficient reduction in protein content to reduce $\beta m_{in\ vitro}$ after such short-duration exercise is thought unlikely (254). For example, to produce a decrease in protein buffering from 52 to 45 mmol H^+•kg dm^{-1}•pH^{-1} after the 6 × 4-s sprints would require a reduction in the content of protein-bound histidine from 156 to 136 mmol•kg dm^{-1} (*cf.* section 2.7.2.1). Not all histidyl residues are free to buffer H^+ because of the different functions they have *in vivo* (436); hence, there may be less protonatable histidyl residues at lower pH. Nevertheless, it is possible that hyperaemic post-exercise samples were snap-frozen without adequately blotting away blood, in particular given that rapid freezing is essential to measure metabolites, as was done in the first study (46). Thoroughly separating blood from freeze-dried muscle fibres is difficult, increasing the possibility that the titrated samples were contaminated with blood of little buffer capacity, having lost CO_2/HCO_3^-. This would underestimate $\beta m_{in\ vitro}$ because the value is expressed per dry mass of sample.

Clearly, mixed results have been found to date where studies have sought to improve $\beta m_{in\ vitro}$ through training, reflecting the failure to identify mechanisms for upregulation and the inherent limitations of the titration technique. With due consideration to the signal-to-noise ratio of measurement, balancing the volume and intensity of training with appropriate relief durations could prove to be important factors in provoking changes in βm. Training at an intensity greater than the LT to necessitate high non-mitochondrial ATP turnover, but not at maximal or supramaximal intensities that can induce a rapid shift to oxidative phosphorylation (54, 168, 308, 363), may prove favourable for adaptations. And rest intervals less than 5 min that prevent recovery in pH_i (53, 142), maintaining the potential stimulus of La^-/H^+ accumulation, yet are sufficiently long to allow substantial PCr resynthesis (53, 142), may further optimise non-mitochondrial ATP turnover (413). Finally, some studies that failed to

measure changes in $\beta m_{in\ vitro}$ did report increased La^- transport and greater MCT protein abundance (373), or greater NHE1 content (232). It is reasonable to conclude from this that there are distinct stimuli for upregulation of intracellular buffering and the acid/base transport proteins. These transporters are the topic of the next section.

2.13.2 H^+ Transport Proteins

From the body of literature investigating acid/base transport protein response to training, it is clear the MCT proteins have been the most studied (Figure 2.13), perhaps befitting their progressively greater importance as pH_i regulators as exercise intensity increases (13, 16). During moderate-intensity exercise most H^+ transport is non-lactate-coupled, provided primarily by the NHE proteins (16, 253). As noted earlier, the NHE and NCBT systems are also the main regulators of basal pH_i, while NHE1 may be important during recovery following exercise. Despite some data on human skeletal muscle NCBT proteins, tentatively identified as NBCe1 and NBCe2, to date no study in humans has investigated training-induced changes in any of the NCBT isoforms. There are also currently no data on the CA protein response to training. On the other hand, several training interventions have investigated the plasticity to training of NHE1 (Figure 2.15), and these are documented next, after the MCT studies.

2.13.2.1 MCT1 and MCT4

Initial evidence for the potential trainability of the MCTs was provided by cross-sectional research using sarcolemmal giant vesicles[74], which showed that sprint and endurance athletes had greater lactate-transport capacity than either untrained or active participants (371). On comparing all of the trained participants, though the similarity in muscle fibre capillarisation suggested participants were equally aerobically-trained, lactate-transport capacity was greatest in the group of elite cyclists who undertook regular high-intensity training. The authors speculated that intensity of training, rather than volume of training, was a more important determinant of lactate-transport capacity.

Subsequent to this research, the first studies measuring MCT content in rodents suggested there may be a minimum threshold of training intensity required to elicit adaptations in MCT1. Rats that trained 5 $d \cdot wk^{-1}$ for 3 weeks at a moderate intensity (1 h × 21 $m \cdot min^{-1}$, 8% incline)[75] showed little change (< 10%) in MCT1 protein content, whereas in a parallel group of rats that trained at a high-intensity (1 h × 31 $m \cdot min^{-1}$, 15%

[74] The sarcolemmal giant vesicle model does not contain T-tubule membranes (372), one location for MCT4 (57).
[75] Predicted $\dot{V}O_2$ at 21 $m \cdot min^{-1}$ and 10% incline = 64 $mL \cdot kg^{-1} \cdot min^{-1}$ (39), which is 74% of the $\dot{V}O_{2max}$ reported for similar age Sprague-Dawley rats in Bedford *et al.* (28).

incline)[76], MCT1 was 30–32% greater after just 1 week (10). After 3 weeks of high-intensity training, MCT1 content was 70% and 94% above baseline in type I *soleus* and red *gastrocnemius* muscles respectively, but there were no changes in MCT1 when measured in more glycolytic muscles (EDL and white *gastrocnemius*). Baker *et al.* (10) suggested that, given they had previously (and have subsequently) found MCT1 to increase in the EDL, and red and white *tibialis anterior* (TA) muscles, after 7 d of continuous electrical stimulation (33, 310), the contractile stimulus in the low-intensity group was insufficient to provoke upregulation. Support for this idea came in a separate study that deliberately chose a training intensity (25 min at 10% incline)[77] between the two intensities used by Baker *et al.* (10), which found 5 weeks of training to increase MCT1 content by 28% in rat *soleus* muscle (151).

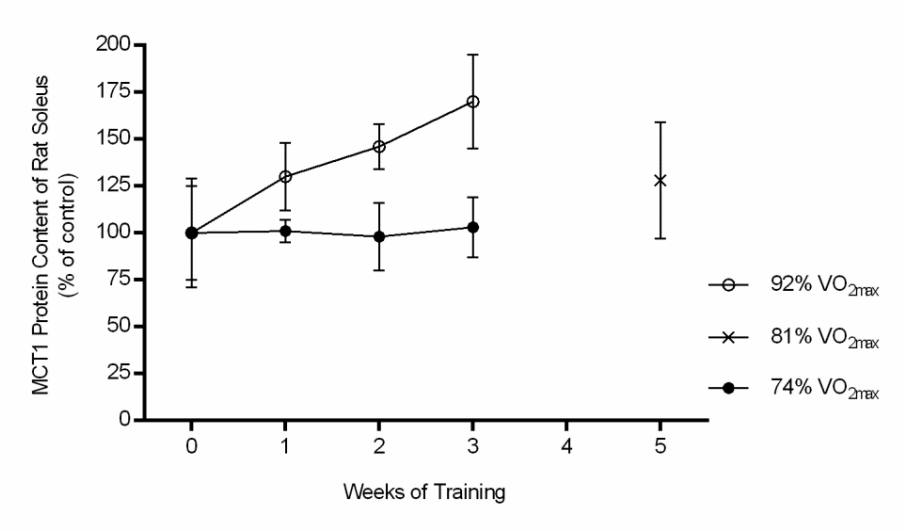


Figure 2.12 MCT1 protein content of rat *soleus* following training at three different intensities. Modified from (10), with the addition of data from (151). Training intensities as a percentage of $\dot{V}O_{2max}$ are estimated from the speed and incline data reported in the respective studies, extrapolating from data published elsewhere (28, 39). Both studies used the same MCT1 antibody but different breed and sex of rats. Data are mean (SD). See text for additional details.

While there are no comparison studies that have trained participants at low or moderate intensities, upregulation of MCT1 in humans has been shown with continuous training just above the LT (Figure 2.13). In contrast, increased MCT4 content is less readily achieved, seemingly only responding to higher training intensities, and typically of a lower magnitude than for MCT1. In the first training study published using humans, 7–8 days of cycling for 2 h per day at 60% $\dot{V}O_{2max}$ was sufficient to increase muscle MCT1 content by 18% in men[78], albeit with large inter-individual variations (range 0–62%) (56). From their arterial [La^-] data, it can

[76] As a comparison, 27 m•min^{-1} at 17% incline = 92% $\dot{V}O_{2max}$ in similar age Sprague-Dawley rats (28).
[77] 27 m•min^{-1} at 10% incline = 81% $\dot{V}O_{2max}$ in similar age, mixed-species rats (28)
[78] Mean $\dot{V}O_{2peak}$: 3.7 L•min^{-1}

be interpolated[79] that training was just above the LT (> 3 mM [La^-]). There were similar findings in moderately-trained men[80] exercising 6 d•wk^{-1} for 9 wk, at 75% $\dot{V}O_{2peak}$ for 1 h, again above the LT (34, 135). Large increases of 93%, 61%, and 80% were reported for MCT1 content in total muscle, sarcolemmal fractions, and mitochondrial fractions, respectively. There was no statistically significant change reported for MCT4 in total muscle fractions, and it can be seen from Figure 2.13 there was a only moderate but uncertain effect, indicating highly variable responses in a small heterogeneous sample (n = 7). There was also no change reported for MCT4 in the mitochondrial fractions, but in the sarcolemmal fractions content increased by 48%. Again, it is important to re-emphasise that the existence of any mitochondrial MCT remains controversial (191), with others failing to show the presence of MCT1 in purified mitochondrial fractions (101).

Training intensity has been proposed as being an important determinant of upregulation of the MCTs (467), but whether there is progressively greater upregulation of MCT1 and MCT4 at higher exercise intensities is unclear. Six weeks of 4-min HIT intervals[81] at 90% $\dot{V}O_{2peak}$, interspersed by 2 min of rest, produced increases in MCT1 (~18%) and MCT4 (~15%) abundance in active men and women (367). In contrast, 5 weeks of 2-min HIT intervals[82] at 100% $\dot{V}O_{2max}$, with 1 min of rest, resulted in no change (−4%) in MCT1 protein content in active women, and a similar magnitude, but less clear increase (19%) in MCT4 protein content (47). That these participants were all regularly participating in intermittent team-sport training may indicate already high MCT content. Alternatively, the very high intensity of training combined with short rest periods may not have sufficiently balanced stress with recovery to optimise adaptations. Some support for this premise comes from a parallel reduction of 11% in $\beta m_{in\ vitro}$.

[79] Mean arterial [La^-] at 65% $\dot{V}O_{2peak}$ was ~4 mM pre-training, while muscle [La^-] was ~6 mM.

[80] Participants were inaccurately described as sedentary. There were no subgroup analyses but mean $\dot{V}O_{2peak}$ was categorised into untrained and trained participants as 43.5 and 50.1 mL•kg^{-1}•min^{-1} respectively, where untrained was considered < 2 h activity per week and $\dot{V}O_{2peak}$ < 45 mL•kg^{-1}•min^{-1}. During the final 2 weeks of training, participants performed interval training for the last 10 min of each session. No specific details were given.

[81] 10 intervals 3 d•wk^{-1}. Mean $\dot{V}O_{2peak}$: 3.3 L•min^{-1}.

[82] 6–12 intervals 3 d•wk^{-1}. Mean $\dot{V}O_{2max}$: 43.2 mL•kg^{-1}•min^{-1}.

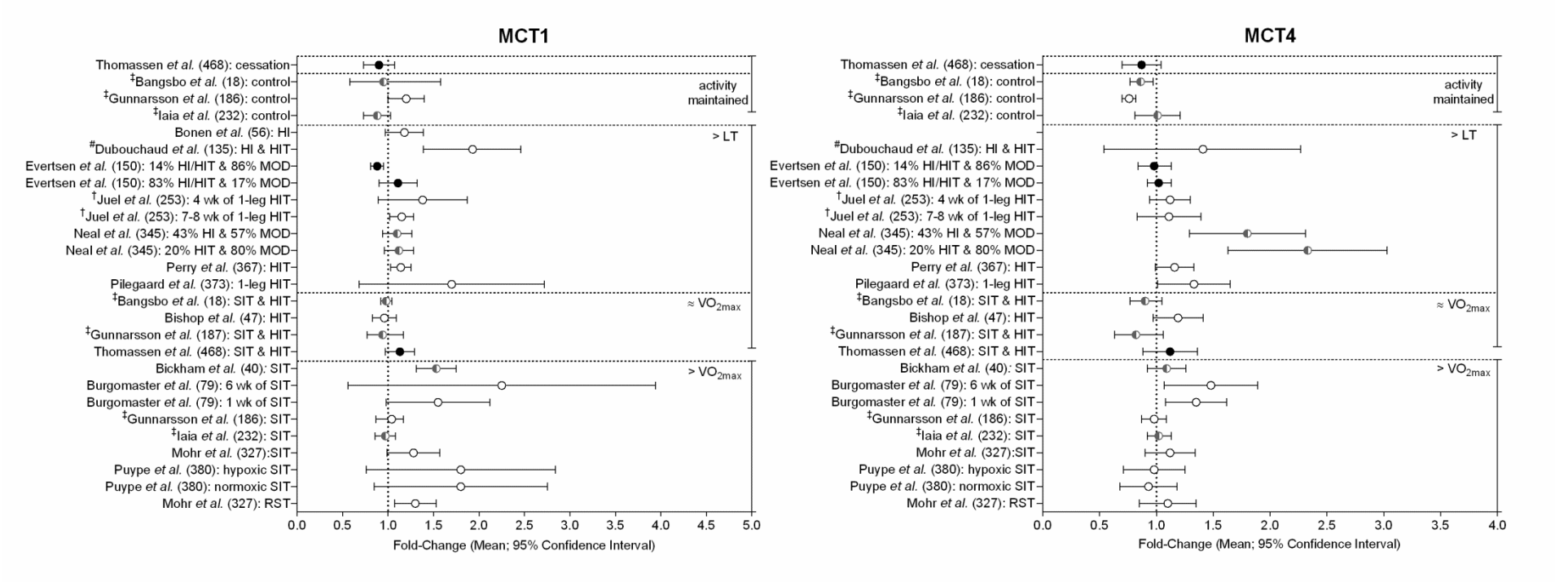


Figure 2.13 Forest plot of mean fold-change and 95% confidence intervals (CI) for MCT1 and MCT4 protein content following different training interventions, categorised by five zones of training intensity: cessation (stopped regular training), control (maintained regular training), high intensity (> LT and < $\dot{V}O_{2max}$), maximal intensity (≈ $\dot{V}O_{2max}$), supramaximal intensity (> $\dot{V}O_{2max}$). Data are arithmetic mean (95% CI) except where indicated as ‡geometric mean (95% CI). †Reported data assumed to be arithmetic mean (SD) on the basis of a similar study (252). #95% CI was calculated from the exact *P* value for MCT4, or for MCT1, from an estimated *P* value that was determined using a two-tailed t-distribution of the mean difference scores divided by the mean predicted standard error of the difference for *n*−1 degrees of freedom (5). Abbreviations: MOD (moderate-intensity continuous training), HI (high-intensity continuous training), HICT (high-intensity concurrent training), HIT (high-intensity interval training), RST (repeated-sprint training), SIT (sprint-interval training). Some studies that failed to report data (103, 433), or an estimate of the uncertainty of the effect (324), could not be included. Training status: ● elite, ◐ well-trained, ○ recreationally active. See text for details of individual studies.

Although higher-intensity HIT intervals do not necessarily lead to greater increases in MCT abundance, training at the even higher intensities of SIT has sometimes proven effective. Using a 6-week all-out SIT intervention (4–6 × 30 s, 1:8 work:rest), a large effect of training for both proteins was found in active men[83], with adaptations evident after just 1 week of training (79). The intermittent nature of the training appears to have been important, given that 6 weeks of a work-matched[84], single continuous bout of exercise provoked no adaptations in either MCT (no data given) in a separate study of similarly active[85] men and women (103). Nevertheless, the magnitudes of the increases in the former study were highly variable: ~30–530% and ~15–200% for MCT1 and MCT4 respectively. The authors attributed this to physiological variability rather than experimental error, with a CV of ≤ 10% reported for their within-gel western blots of MCTs, although no between-gel CV was given. The variability can in part be attributed to the mixed training backgrounds of these participants, but may also be an indication of fibre-type specific changes, as has been inferred in a rat study[86] (466). Given that MCT1 is primarily found in type I fibres and MCT4 primarily in type II fibres (374), it could be that changes in MCT content are hidden in the noise of mixed homogenate analysis. Direct measurement of the MCTs in separated fibres remains to be done.

While a between-study comparison has proven inconclusive as to the factors influencing MCT upregulation, by comparing two different training modalities with distinct bioenergetic costs, it is possible to not only assess whether training intensity is important in provoking adaptations in MCT abundance, but also to judge possible mechanisms for upregulation. In a direct comparison of different intensities of training, active men[87] undergoing 8 × 30-s sprints at 130% $\dot{V}O_{2max}$ (SIT) had similar improvement (28%) in MCT1 abundance as a non-work-matched group (30%) who performed 15 × 6-s RST at 95% maximal running speed (327). There were small and unclear increases in MCT4 abundance (10–12%). Others have also reported that 6 weeks of RST (4 × 5–15 s, work:rest 1:5–1:3) induced an increase in MCT1 content (53%), but no clear change in MCT4 (+9%) in endurance-trained runners[88] (40). In the former study, Mohr *et al.* (327) found post-exercise muscle pH_i was slightly decreased (6.98) after the first and final sessions of SIT compared to no reduction in pH_i following RST (7.06−7.07), providing some evidence that H^+ accumulation does not influence upregulation of MCT1, contrary to previous speculation (128, 249). In support of this conclusion, a separate

[83] Mean $\dot{V}O_{2peak}$: 50 mL•kg^{-1}•min^{-1}

[84] 1.0–1.25 kJ•kg bm^{-1} performed as quickly as possible during a single continuous bout of cycling for 3 d•wk^{-1}.

[85] Mean $\dot{V}O_{2peak}$ of 5 men and 4 women: 47 mL•kg^{-1}•min^{-1}

[86] MCT1 content increased by 30% in type I *soleus* muscle relative to non-training control, but there was a decrease of ~25% in the predominantly type II EDL muscle. There were similar changes in MCT4 for both muscles but much larger variance. It may simply be that the EDL was not sufficiently recruited throughout the training protocol.

[87] Mean $\dot{V}O_{2max}$: SIT 49.0 mL•kg^{-1}•min^{-1}, RST 50.2 mL•kg^{-1}•min^{-1}. Training comprised 3–6 d•wk^{-1} of SIT (1:3 work:rest) or RST (1:10 work:rest). SIT trained for longer each session (4 min versus 90 s) and covered a greater distance (1,264–1,360 m versus 600–630 m).

[88] Mean $\dot{V}O_{2max}$: 58.1 mL•kg^{-1}•min^{-1}

study found no improvements in MCT abundance after 5 weeks of HIT at 100% of $\dot{V}O_{2peak}$ for 3 d•wk^{-1} (6–12 × 2 min, 2:1 work:rest), despite reductions in post-exercise pH_i of ~0.3 units (47).

In addition to H^+ accumulation, La^- has been proposed as being a potential MCT signalling molecule. Data in L6 cells have shown MCT1, but not MCT4, to be similarly upregulated after incubation with 10 mM and 20 mM [La^-] (206). In the study of Mohr *et al.* (327), muscle [La^-] increased after SIT (14 mM) and RST (9 mM)[89]. Thus, La^- accumulation may have been sufficient following both training modalities to have acted as a signal for upregulation of MCT1. MCT1 reaches half of its maximum activity at 3–5 mM [La^-], whereas MCT4, with a K_m for L-lactate of ~30 mM, reaches high activity only following high-intensity exercise producing a large [La^-]. It could be that maximal activity of the transporters is a prerequisite to upregulation, in which case MCT4 requires high muscle [La^-], but MCT1 responds to lower concentrations. This could help explain why continuous exercise performed just above the LT sometimes results in increased MCT1, but not MCT4 content (56, 135). As noted above, in the first of these studies, muscle [La^-] was ~6 mM (20 mmol•kg dm^{-1}) following a single 15-min bout of exercise at 65% $\dot{V}O_{2max}$, with training conducted at 60% $\dot{V}O_{2max}$; while in the second study, muscle [La^-] after 1 h at 65% $\dot{V}O_{2max}$ was ~12 mM (40 mmol•kg dm^{-1}), with training performed at 75% $\dot{V}O_{2max}$. Following RST, [La^-] is normally no greater than 20 mM (43-45, 48, 137, 327, 439), while [La^-] can range from 20 to 40 mM after single or multiple 30-s sprints (53, 54, 95, 195, 349, 379) (*cf.* Appendix G). Therefore, if muscle [La^-] greater than 30–40 mM is a signal for MCT4, it becomes apparent why many high-intensity training studies have found no change in MCT4 content. Direct confirmation is required to confirm whether this is actually the case.

Another potential stimulus for upregulation of different transport proteins is flux of their substrates or ions. Greater La^-/H^+ flux requires high non-mitochondrial ATP turnover, leading to high La^-/H^+ production, with the elevated intracellular muscle [La^-] and [H^+] increasing MCT activity through an increased ionic gradient relative to the interstitium. Evidence that higher La^-/H^+ production and efflux may be important stimuli for upregulation of MCT4 has been provided in a rodent study (466). Chronic $NaHCO_3^-$ supplementation combined with 5 weeks of HIT (6–12 × 2 min, 2:1 work:rest)[90] resulted in a 115% increase in *soleus* MCT4 content compared to a non-training control group, which was more than three times the increase in a placebo training group. Recall that $NaHCO_3^-$ supplementation may result in a greater glycolytic

[89] [La^-] are reported in this study per dry mass as 47.1 mmol•kg dm^{-1} and 30.0 mmoL•kg dm^{-1} after the first training sessions for SIT and RST, respectively. These have been converted to mmol•L muscle water^{-1} (i.e., mM) to allow easy comparison with the cell data of Hashimoto *et al.* (206), using a conversion factor of 0.3 (229).

[90] The age of the male Wistar rats was not reported. Assuming an approximate $\dot{V}O_{2max}$ of 81 mL•kg^{-1}•min^{-1}, as has been reported for similar species 80–90 d old rats (28), the mean training intensity is estimated from data on Wistar rats in Brooks & White (69) as 85–93% $\dot{V}O_{2max}$ during weeks 1–4.

contribution to exercise depending on the intensity (222), which results in higher La^-/H^+ production. In addition, $NaHCO_3^-$ enhances transport activity through buffering extracellular H^+, thereby further increasing the ionic gradient relative to the intracellular space (297, 397). Thomas *et al.* (466) speculated that the stimulus for MCT4 upregulation may have been H^+ and/or La^- efflux from the muscle. A subsequent functional overload model in mice suggests this may not be the case regarding La^- efflux, with increased *plantaris* MCT1 and MCT4 content found 12 d after surgical removal of the *gastrocnemius* and *soleus* muscles, despite no change in tail vein [La^-] (265). However, it is possible that there was high La^-/H^+ production in the *plantaris*, but because of the increased energetic demand in the overloaded muscle, La^- was shuttled to more oxidative fibres in the same muscle. Ultimately, if La^-/H^+ flux is a signal for MCT adaptations, then exercise above the LT creates a demand for high non-mitochondrial ATP turnover, which in combination with long rest intervals, facilitates greater efflux.

As was detailed in section 2.10.1.2, the primary regulator of MCT4 is thought to be hypoxia (191), and *in vitro* data have shown HIF-1α to upregulate MCT4 (343, 476). Yet, there has been a failure to replicate these findings in human studies that have provided a hypoxic stimulus (100, 252, 324, 355). And when training and hypoxia have been combined, increases were found for MCT1 (~70%) but not MCT4 (~7% decrease) after 6 weeks of 30-s SIT in moderately-trained men[91], irrespective of whether training was performed in normoxia or hypoxia (380). In the *in vitro* study of Ullah *et al.* (476), PO_2 of 8 mmHg (1% O_2) was maintained for 48 h in different cells, prior to measuring increased MCT4 protein and mRNA expression. In normoxia, human muscle intracellular PO_2 is ~29 to 34 mmHg (89, 387), decreasing to ~23 mmHg under hypoxia of 10% O_2 (387). Therefore, while hypoxia has been shown to be an important regulator of MCT4 *in vitro*, the environmental hypoxia provided in these studies may not have induced sufficient cellular hypoxia to replicate this signal. Exercise training performed under occlusion may provide the requisite cellular hypoxia to stimulate upregulation of MCT4, and future research in this area may prove informative.

The timing of muscle biopsies may be a confounding factor for the failure to measure changes in MCT4 in some studies. In the hypoxic training study above, post-training biopsies were taken 12–13 days after the final SIT session (380). Just 1 wk after stopping SIT, Burgomaster *et al.* (79) had already observed an ~20% reduction in mean MCT4 content. Given that they saw a comparable reduction in MCT1, it is possible that neither MCT1 nor MCT4 content measured post-training in Puype *et al.* (380) were representative of peak content at the end of the training intervention. With the magnitude of the training effect for MCT4 being typically smaller than for MCT1, a small effect of training may have been missed due to rapid detraining (section 2.15 covers detraining in more detail).

[91] Mean $\dot{V}O_{2max}$ for normoxic and hypoxic groups: 53.3 and 55.1 $mL \bullet kg^{-1} \bullet min^{-1}$. Participants trained $3d \bullet wk^{-1}$, performing 4–9 intervals separated by 4.5 min rest.

Post-training biopsies can also be performed too close to the final training session. Following a single bout of exercise, an acute reduction in MCT protein content in membrane fractions has been documented in both humans (46, 48) and rodents (372, 471). Combining these data and others, Thomas *et al.* (467) proposed a potential timecourse of changes in MCT protein membrane content following a single exercise bout (Figure 2.14). According to this model, after a brief initial decrease (46, 471), MCT content increases over the hours following exercise (40, 104, 179, 181, 267). Though there seem to be disparate responses for MCT1 and MCT4 depending on the exercise modality (40, 181), these results indicate the importance of taking into account the short-term changes in MCT protein content when assessing training adaptations. A potential limitation with these studies is that the analyses were performed on either crude cytosolic or membrane fractions. Inconsistencies in separating the membrane fractions could introduce variation into target protein content. And although the MCTs are membrane-bound proteins, they are translated in the Golgi apparatus and subsequently targeted to the membrane by basigin. Moreover, one study has reported evidence of an intracellular pool of MCT1 in cardiac myocytes of congestive heart failure rats (239). Therefore, there is no certainty of measuring a consistent portion of the MCTs in fractionated muscle. A decrease in MCT membrane content following exercise may simply be an artefact of discarding much of the protein. Alternatively, it could represent a failure to break the disulphide bonds of the higher molecular weight (95 kDa) basigin-bound MCT heterodimer (507).

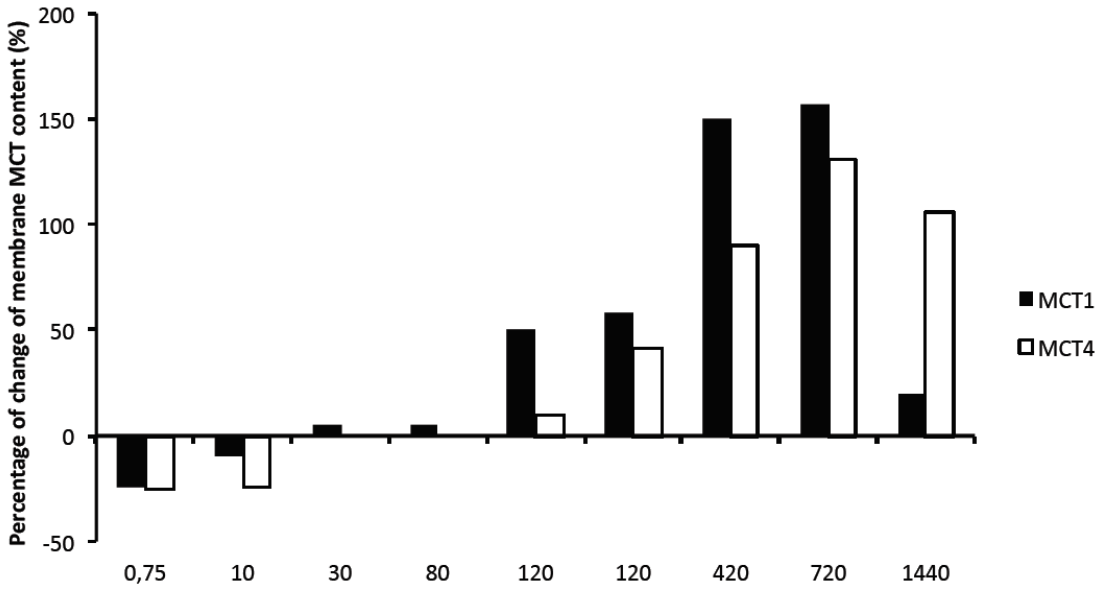


Figure 2.14 Modelled timecourse of the percentage changes in MCT1 and MCT4 protein membrane content following a single bout of exercise. The x-axis is in minutes from the *beginning* of exercise. The model is based on data from both human and rodent studies. Taken from Thomas *et al.* (467).

Another potential explanation for the failure of some studies to find any change in MCT content is the initial training status of participants. Following the end of a racing season, elite

male and female cross-country skiers[92] were randomised into a moderate-intensity training group and a high-intensity training group (150). For 5 months the moderate-intensity group performed 86% of their training at 60–70% $\dot{V}O_{2max}$, with the remainder comprising high-intensity continuous or intermittent training at 80–90% $\dot{V}O_{2max}$. The high-intensity group performed 83% high-intensity continuous or intermittent training, with the remainder at moderate intensity. Despite the contrastingly polarised nature of training, neither group had improved MCT1 or MCT4 abundance, but there was a small reduction (12%) in MCT1 for the moderate-intensity group. These data suggest that, not only are further adaptations in the MCTs for highly-trained individuals unlikely, but if a high-intensity stimulus is removed from the training volume then some detraining may occur.

Further evidence that upregulation of MCT content is atypical in more highly-trained individuals was provided by a series of studies from Copenhagen. Moderately-trained endurance runners[93] displayed no improvement in MCT1 or MCT4 protein abundance after 4 weeks of SIT (8–12 × 30-s sprints) (232), while elite footballers[94] undergoing 2 weeks of HIT/SIT at the end of their season had no change in MCT4 content, and a small but unclear increase in MCT1 (468). Two further studies found that, when endurance-trained runners[95] and cyclists[96] reduced their training volume by 25% and 70% respectively, but added HIT/SIT for 6–9 weeks, there were no changes in MCT1 or MCT4 content (18, 187). The authors ascribed the failure to elicit a change in MCT1 content to potentially high levels pre-intervention for these comparatively well-trained cohorts (18). The absence of an effect of training on MCT4 protein content was reasoned to be consistent with similar studies, and the data in Figure 2.13 suggest HIT and/or SIT interventions have seldom produced a training effect. The same group also failed to elicit changes in the abundance of either protein after 7 weeks of SIT in moderately-trained[97] men and women (186). Nor did they find any change in MCT4 after an 8-week period of concurrent[98] training in moderately-trained male endurance runners (433). One common factor with each of their studies was the use of relatively homogenous participant

[92] Mean $\dot{V}O_{2max}$: men 73.4 mL•kg^{-1}•min^{-1}, women 58.3 mL•kg^{-1}•min^{-1}

[93] Mean $\dot{V}O_{2max}$: 55.8 mL•kg^{-1}•min^{-1}

[94] Mean $\dot{V}O_{2max}$: 55.0 mL•kg^{-1}•min^{-1}. There were 10 training sessions in total: 5 sessions of small-sided games consisting of 8 × 2 min with 1 min rest at 88% mean HR_{max}, 4 sessions of 10–12 × 25–30-s all-out running at peak 88% HR, and 1 session of 16 × 40–60 s running at 84% mean HR_{max}.

[95] Mean $\dot{V}O_{2max}$: 63.0 mL•kg^{-1}•min^{-1}. Four participants trained for 6 weeks and 8 trained for 9 weeks. Training comprised 2–3 d•wk^{-1} of 8–12 × 30-s sprints at 95% max running speed, 1:9 work:rest; 1 d•wk^{-1} of 4 × 4 min > 85% HR_{max}, 2:1 work:rest; 1–2 d•wk^{-1} of low-intensity (< 75% HR_{max}) or moderate-intensity training (75–85% HR_{max}).

[96] Mean $\dot{V}O_{2max}$: 59.1 mL•kg^{-1}•min^{-1}. Training comprised 7 weeks × 2–3 d•wk^{-1} of 10–12 × ~30-s sprints at 85–95% peak power output (test not specified), 1:9 work:rest; 1–2 d•wk^{-1} of 4–5 × 3–4 min at 90–95% peak aerobic power, ~2:1 work:rest.

[97] Mean $\dot{V}O_{2max}$ for men and women: 52.2 mL•kg^{-1}•min^{-1}. Training comprised 2 d•wk^{-1} × 3–4 sets of 5 × 1-min bouts of running for 30 s, 20 s, and 10 s at 30%, 60%, and 90–100% of maximal intensity respectively, 5:2 work:rest.

[98] Mean $\dot{V}O_{2max}$: mL•kg^{-1}•min^{-1}. Four training sessions per week consisting of: 2 d•wk^{-1} of 4–12 × 30-s all-out SIT, followed 15 min later by lower body resistance training progressing from low load high repetitions to high load low repetitions; 1 d•wk^{-1} 4 × 4 min HIT at > 85% HR_{max}; 1 d•wk^{-1} of 40–70 min continuous running at 75–85% HR_{max}.

groups in terms of training background, the implication being that they may already have been in similarly adapted states.

Initial training status may also partially explain the wide dispersion of training responses in studies where an effect of training was measured, indicating high- and low-responders. As the width of the confidence intervals relative to the magnitude of the effects shows in Figure 2.13, it appears that many of the studies to date were underpowered to detect a clear change in MCT abundance. This is particularly the case for MCT4, given the smaller magnitude of any training response compared to MCT1, but may also explain some outliers such as Pilegaard *et al.* (373), where the sample size was just four participants.

One prominent outlier in Figure 2.13 is the only study to report large increases for MCT4 protein content but none for MCT1 (345). In a crossover design using moderately-trained cyclists[99], comparing 80% moderate-intensity and 20% high/supramaximal-intensity (polarised) training, versus 57% moderate-intensity and 43% high-intensity (threshold) training, there were small but unclear increases for MCT1 (10–12%), whereas MCT4 increased by ~130% and ~80%, for the polarised and threshold groups, respectively. Despite the use of a crossover design, a comparison of the two types of training is limited by an inadequate washout period of 4 weeks, and potential confounding factors such as the stage of their cycling season that the interventions commenced. Why this study found such large increases in MCT4 compared to all other studies can potentially be explained by limitations in their immunoblotting techniques. Their MCT antibodies detected multiple bands and the published blots for MCT4 are of poor fidelity, suggesting low quality antibodies with questionable antigen binding affinity were used. No validation of antibody specificity was performed and reliance solely on expected molecular weight for the band of choice is not sufficient validation of a novel antibody (336). Another major limitation was the use of GAPDH as a loading control. This is a highly abundant protein with a linear dynamic range lower than most target proteins, and may well have saturated at even the 7.5 µg protein loaded (328, 480), giving non-linear and non-proportional data (328).

The functional importance of changes in MCT content has not been directly measured in humans. Nevertheless, in both rodents (10, 58, 310, 312, 370) and humans (34) training has been found to result in greater La^- transport/uptake, and although increased La^-/H^+ transport activity cannot be assumed to directly ensue from increased MCT protein abundance, there are some data to suggest that greater La^- transport can, in part, be explained by enhanced MCT content. In one study, a strong correlation between muscle [La^-] and femoral [La^-] was maintained in response to one week of moderate-intensity training (r = 0.85), but the steeper slope of the relationship post-training was attributed in part to greater La^- extrusion by MCT1

[99] Mean peak aerobic power = 4.7 W•kg^{-1}

(56). Separately, increased La^- transport, as measured in sarcolemmal giant vesicles, was evident following single-leg knee-extension in active men[100], exercising 3 to 5 d•wk^{-1} for 8 weeks (373). There were also increases in both MCT1 (70%) and MCT4 (33%) protein content when compared to the untrained leg. Despite there being lower intracellular–to–interstitial gradients of La^- and H^+ in the trained compared to the untrained leg following exhausting exercise, there were similar release rates of La^- and H^+ measured for both legs. The authors concluded that upregulation of La^-/H^+ transport capacity, including increased MCT protein content, served to better regulate muscle pH_i and intracellular [La^-].

Another study out of the same lab found 7–8 weeks of similar training with active men[101] to induce small increases in MCT1 (15%) and MCT4 (11%) protein abundance (253). During an incremental test to exhaustion, training resulted in increased La^- removal from the muscle, increased H^+ release, and higher pH_i at exhaustion in the trained leg (6.82) versus untrained leg (6.69). However, the majority of H^+ release during low-intensity exercise was found not to be coupled with La^-; training increased non-lactate-coupled H^+ release by 83% and lactate-coupled H^+ release by 53%. In quantifying the functional importance of these changes, the authors were able to show that improvements in H^+ and La^- efflux could not be explained solely by greater transport protein content, but were also due to greater leg blood flow and increased muscle fibre capillarisation (254).

In summary, the determinants of upregulation of MCT1 and MCT4 remain uncertain. This may be a consequence of the failure to identify the optimum training intensity and volume for upregulation of these proteins. One important confounding factor is that there may be a ceiling of adaptation already attained by even moderately-trained individuals. Notwithstanding equivocal data for both proteins, evidence to date indicates the magnitude of increase for MCT1 is typically greater than for MCT4, which with small sample size studies makes it easier to distinguish between training interventions. MCT1 has been found to increase with different exercise modalities performed above the LT, whereas greater MCT4 abundance has seldom been reported, and possibly requires a higher training stimulus. It is unlikely that H^+ accumulation is an important stimulus for MCT1 upregulation, but the data are less clear for MCT4. Similarly, muscle La^- accumulation seems to be a signal for MCT1, but not MCT4, at least not at concentrations less than 20 mM (~67 mmol•kg dm^{-1}). Sustained La^-/H^+ production, and subsequent flux, may also be factors in provoking adaptations in both MCTs and studies can be designed to test this hypothesis. Training interventions that incorporate high-intensity training with long rest periods will, by maximising non-mitochondrial ATP turnover for sequential intervals, increase La^-/H^+ production. Longer rest intervals will facilitate greater PCr

[100] All measures of exercise capacity reported were for single-leg tests. Training comprised 3–5 sets of 2 × 30-s and 3 × 60-s bouts, 2 min rest, at a resistance / kick frequency of 100–120 N / 80 rpm and 50–100 N / 60 rpm for the 30-s and 60-s bouts respectively.

[101] Mean $\dot{V}O_{2peak}$: 50.2 mL•kg^{-1}•min^{-1}

resynthesis and thus higher capacity for PCr hydrolysis during subsequent intervals (120, 142). Nevertheless, it is important to remain cognisant of confounding factors such as the initial training status of participants. Finally, studies that take their post-training muscle biopsy too soon after the final training session risk short-term changes in protein content masking any longer term adaptation. Specific data on both MCTs is required to confirm this.

2.13.2.2 NHE1

While the MCTs provide the majority of H^+ transport during high-intensity exercise (13, 246, 250), one-third of H^+ transport has been reported to be non-lactate-coupled (13). These data were calculated from arteriovenous La^- and H^+ differences, and their respective gradients relative to the muscle, rather than directly measuring transporter activity. Nevertheless, it is likely that most of non-lactate-coupled H^+ transport is provided by the NHE proteins (16). And once the functional importance of NHE activity during exercise became clear, studies measuring the plasticity of the known skeletal muscle isoform, NHE1, soon followed (Figure 2.15).

The first measurement of NHE1 protein content in response to training in humans[56] proved inconclusive because of the high variability (99), but not long after more robust evidence was shown. Seven to eight weeks of one-legged intermittent knee-extension training of active men[102] provided sufficient stimulus to provoke an increase in NHE1 protein content by 16% compared to the untrained leg. Interestingly, elevated NHE1 (~31%) in biopsies taken at 2 and 4 weeks suggested adaptations might be quite rapid. That NHE1 content at the earlier timepoints was greater than at 8 weeks, though not statistically significant at 2 weeks, indicates either the temporal variability of protein turnover, or the variability of the immunoblotting technique.

As with the MCTs, there may be a minimum threshold of exercise intensity required to upregulate NHE1. Rats undergoing 6 weeks of moderate-intensity training 5 d•wk^{-1} (18–40 min at 15–20 m•min^{-1} and 8% incline) demonstrated no change in NHE1 activity, measured in sarcolemmal giant vesicles, compared to a non-exercising control group (249). In contrast, a parallel HIT group (3 × 3 min at 25–40 m•min^{-1} and 8–18% incline, 3:1 work:rest)[103] had a 52% increase in NHE1 activity. And three weeks of an almost identical HIT protocol[104] elicited increases in NHE1 protein content – using western blotting to measure skeletal muscle content for the first time – of 29% and 36% in predominantly type I and type II muscle, respectively (251).

---

[102] Mean $\dot{V}O_{2max}$: 50.2 mL•kg^{-1}•min^{-1}

[103] These were male Wistar rats with age not stated. 40 m•min^{-1} at 18% incline was assumed from data in (69) to be equivalent to 100% $\dot{V}O_{2max}$, or 70–80 mL•kg^{-1}•min^{-1}, although that study was in 2 month old female Wistar rats. From the latter study, 25 m•min^{-1} at 5% incline ≈ 60 mL•kg^{-1}•min^{-1} and 15 m•min^{-1} at 10% incline ≈ 55 mL•kg^{-1}•min^{-1}.

[104] Other than the duration of training, the only variation was a starting incline of 9%.

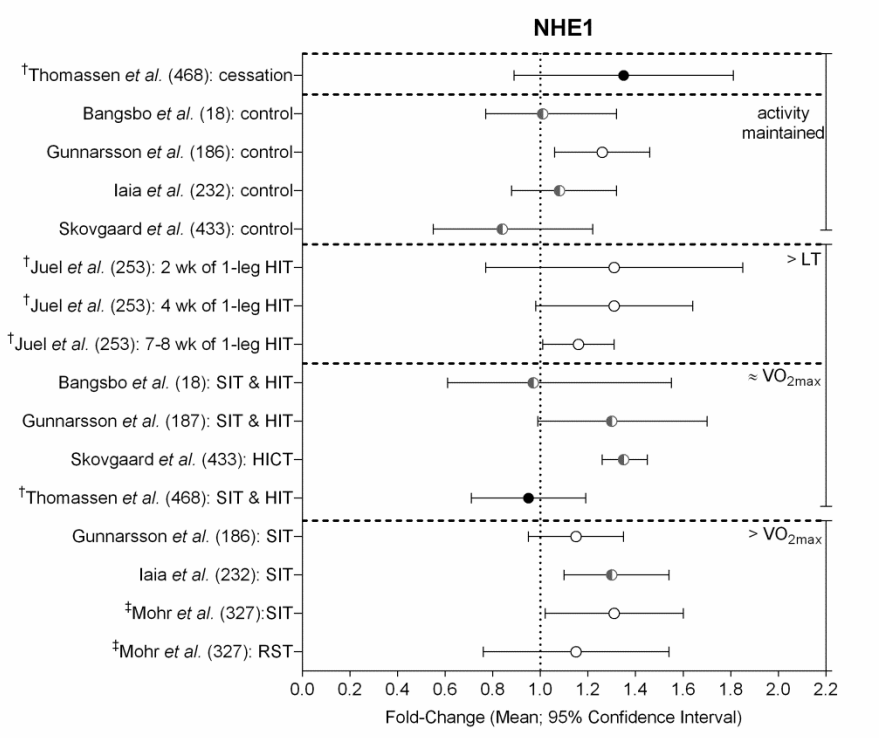


Figure 2.15 Forest plot of mean fold-change and 95% confidence intervals (CI) for NHE1 protein content following different training interventions, categorised by five zones of training intensity: cessation (stopped regular training), control (maintained regular training), high intensity (> LT and < $\dot{V}O_{2max}$), maximal intensity (≈ $\dot{V}O_{2max}$), supramaximal intensity (> $\dot{V}O_{2max}$). Data are geometric mean (95% CI) unless otherwise indicated. Where not reported, CIs were calculated by multiplying standard error by the t-statistic for a 95% CI with *n*-1 degrees of freedom. ‡Arithmetic mean (95% CI). †Reported data assumed to be arithmetic mean (SD) on the basis of a similar study (252). Abbreviations: HICT (high-intensity concurrent training), HIT (high-intensity interval training), RST (repeated-sprint training), SIT (sprint-interval training). Training status: ● elite, ◐ well-trained, ○ recreationally active. See text for details of individual studies.

Having identified the potential importance of training intensity for NHE1 in rats, follow-on studies out of the same lab investigated whether similar responses could be shown with more applied training interventions in humans. NHE1 protein changes have not been directly compared between moderate-intensity training and high-intensity training. However, several studies have indirectly assessed the effects of training intensity by using endurance-trained populations, and comparing groups who augmented their regular moderate-intensity training with HIT/SIT, to control groups who maintained their normal training programmes. One study showed NHE1 abundance to change little (+7%) for male endurance runners[105] who maintained their normal training, compared to a 30% increase for a cohort who performed 4 weeks of SIT (8–12 × 30-s sprints) (232). Similarly, cyclists[106] who reduced training volume by 70% for 7 weeks, but performed a mixture of HIT and SIT[96], demonstrated 30% greater

[105] Mean $\dot{V}O_{2max}$: 55.8 mL•kg^{-1}•min^{-1}
[106] Mean $\dot{V}O_{2max}$: 59.1 mL•kg^{-1}•min^{-1}, sex not stated.

NHE1 abundance (187), albeit reported as not statistically significant (P = 0.09). And NHE1 content increased by 35% in male endurance runners[107] who reduced running distance by 42%, while incorporating 8 weeks of concurrent training, including SIT and HIT[98], into their training load (433). It is possible that the combination of increased training intensity and reduced training volume provided the requisite stimulus for upregulation of NHE1. However, when 8 weeks of 15 × 6-s RST was compared with a greater volume of 8 × 30-s SIT[87], NHE1 abundance increased by 15% for the former group but by 31% in the latter (327). Therefore, while NHE1 abundance appears to increase in already trained participants with the addition of high-intensity training to their training programmes, the balance between intensity and volume is important.

One potential explanation for the greater improvement in NHE1 for the SIT group than the RST group (327) may be related to the lower post-training muscle pH_i in the former (6.98), than the latter (7.06) group. It is possible that a high work:rest ratio (1:10) for the RST group provided insufficient anaerobic training stimulus, with no change in pH_i and a higher reduction in glycogen, but lower muscle [La^-] (~30 mmol•kg dm^{-1}) compared to the SIT group. A single bout of 5 × 6-s RST with shorter (24 s) rest periods has often been shown to elicit reductions in muscle pH_i of 0.2–0.4 pH units and increases in [La^-] to 40–70 mmol•kg dm^{-1} (43-45, 137) (*cf.* Appendix G). High non-mitochondrial ATP turnover coupled with high [H^+] may provide the necessary cellular stress for upregulation of NHE1. Some support for the importance of H^+ accumulation for NHE1 function is seen with an increase in transport activity as pH decreases (66, 249). Although H^+ accumulation seems to be unimportant for MCT upregulation it may be a stimulus for changes in NHE1, potentially explaining the disparate findings between NHE1 and MCT upregulation.

While it does appear that the addition of high-intensity training can upregulate NHE1 in trained individuals, this does not seem to be the case when more highly-trained participants are studied. Using similar experimental designs to their studies above, when highly-trained male endurance[95] runners (18) and elite male footballers[94] (468) reduced their usual training volume, but augmented their training with mixed HIT/SIT, there were no changes in NHE1 abundance. In a comparison of two of the studies using endurance runners (18, 433), there was no change in NHE1 with the more highly-trained athletes (greater mean $\dot{V}O_{2max}$ and undertaking a greater weekly training load). They may have already had high NHE1 protein levels due to the performance of more high-intensity training, although control groups in both studies were documented as undertaking weekly high-intensity training (18).

As an alternative explanation, the contrasting results in Bangsbo *et al.* (18) and Iaia *et al.* (232) were argued to be due to the greater volume of training in the first study (6–9 wk

[107] Mean $\dot{V}O_{2max}$: 59.4 mL•kg^{-1}•min^{-1}.

versus 4 wk) inhibiting adaptations (18). Juel *et al.* (253) also found NHE1 abundance was greater after 2 and 4 weeks training compared to 8 weeks. And in the study of elite footballers who reduced training load but added HIT/SIT (468), a control group of footballers, who simply stopped their training, displayed an increase in NHE1 abundance of ~1.35-fold, albeit highly variable (95% CI: 0.89 to 1.81). While this may simply have been methodological variance, it is possible that NHE1 responded to the reduced training load after consecutive high load macrocycles. More evidence is needed to confirm this.

It should also be considered that the apparent differences between studies may be one of statistical artefact. In using null hypothesis significance testing to interpret changes in NHE1 abundance, similar effect sizes but greater variance affect the likelihood of detecting a true change using a similar alpha probability of 0.05 (*cf.* data in Figure 2.15). For example, Iaia *et al.* (232) reported[108] a mean change of 1.30-fold (95% CI: 1.10 to 1.54) as a significant change ($P < 0.05$); whereas a similar effect, but greater variance, from the same sample size ($n = 8$), was reported by Gunnarsson *et al.* (187) as not being significantly different (1.30-fold; 0.99 to 1.70). And Skovgaard *et al.* (433) reported $P < 0.0001$ ($n = 9$) for a change of 1.35-fold (1.26 to 1.45), but performed a within-group comparison rather than a between-group comparison against their control group. The control group had greater variance (0.85-fold; 0.55 to 1.22), which in a between-group analysis would have resulted in a higher *P* value. Therefore, a clearer inference can be drawn from these studies through categorising studies by effect sizes, while acknowledging the uncertainty of the effect from the width of the confidence intervals. In using this analysis (Figure 2.15), a 1.15–1.35-fold increase in NHE1 abundance has been elicited with different modalities of high-intensity training (186, 187, 232, 253, 327, 433). That there were also changes in some control groups can potentially be explained by a number of factors, including physiological fluctuations in NHE1 protein turnover, variability of the immunoblotting technique, or possibly changes in the activity profile of some of the control participants.

In summary, training intensity appears to be an important determinant of adaptations in NHE1. Improvements in humans have only been found when training incorporated HIT and/or SIT. Those studies have often reduced the training volume of participants while adding HIT/SIT, suggesting that the combination of lower volume and higher intensity of training influence changes in NHE1. However, in common with the MCTs, initial training status seems to be a confounding factor. It is likely that individuals who regularly perform high-intensity training as part of their programme already have elevated levels of these proteins and do not readily respond to a short-term intervention. In contrast, NHE1 is upregulated after two weeks in some individuals, showing gains can be rapidly achieved in the lesser-trained. Finally, although there are often parallel improvements in high-intensity exercise performance in these

[108] Means and confidence intervals are estimated from graphs.

populations (233, 253, 327, 433), there is a dissociation between changes in NHE1 and sprint performance in more highly-trained individuals (18). Given that NHE1 may be important for pH_i recovery, it could be that an association between upregulation of NHE1 and improved performance is only found with intermittent high-intensity exercise. This remains to be determined.

2.13.2.3 NBC(e1)

Only one study to date has investigated the potential plasticity of the NCBT proteins in response to training (466). Although the authors refer to a non-isoform-specific NBC, others have since written that it was probably NBCe1 that this antibody recognised (360). The uncertainty surrounding the specific isoform has been discussed in detail in section 2.10.3; suffice to say there remains insufficient evidence to confidently call the protein detected NBCe1. Rats that underwent 5 weeks of HIT (6–12 × 2 min, 2:1 work:rest)[90], with or without $NaHCO_3^-$ supplementation prior to each training suggestion, displayed fibre-type specific responses in NBC content. There were large increases in *soleus* NBC content of both training groups (60–85%), with no change apparent in the EDL muscle. Despite $NaHCO_3^-$ likely enhancing H^+ efflux transport activity by creating a greater ionic gradient relative to the intracellular space, in common with their similar findings with MCT1 abundance, a mechanism other than H^+ efflux appears to be responsible for upregulation of NBC. Given that there was a moderate correlation reported between MCT1 and NBC content[109], it is possible there is shared signal for upregulation of the two proteins. As noted earlier, a coupling in activity occurs between NBCe1 and MCT1 through increased influx of bicarbonate (or equivalents) buffering intracellular H^+ (21). One possibility is increased abundance and/or activity of sarcolemmal carbonic anhydrases (CAIV and CAXIV) enhances H^+ extrusion via both of these transport proteins (319, 498). Further evidence is required to confirm this.

2.14 Genetic Factors Influencing pH_i Regulation

A common feature of most studies investigating the response to training of the H^+ transport proteins is the high within-group variability. While there may be methodological limitations with western blot techniques contributing to variability, such as varied efficiency of protein extraction and fractionation, it is also probable that unidentified genetic factors influence the training response (300). The nascent stage of genomic studies into sporting or exercise performance means that there is not a great deal known about the genetic factors influencing pH_i

[109] As noted in section 2.10.3, protein abundance measured by western blotting may be prone to spurious correlation owing to mathematical coupling if the same housekeeping protein was used as a loading control for the two target comparison proteins. Information on loading controls is not stated in the paper.

regulation. It was only in the latter stages of the previous millennium that the first genetic polymorphism influencing human performance was identified (329). Nevertheless, this is an area that is rapidly advancing and some relevant recent data have been published.

In a pilot study, a role for the previously identified (317) A1470T polymorphism (SNP ID: rs1049434) of the SLC16A1 gene that codes for MCT1 was sought (116). Carriers of the mutant (T) allele have been reported to have impaired erythrocyte La^- transport (317). In a pilot study, carriers (AT and TT) were found to have greater capillary [La^-] at the end of a bout of high-intensity circuit training (116). A similar study from the same group found lower venous [La^-] in carriers of the T allele (117). There are little additional data to draw further conclusions, but the mixed blood La^- data were suggested to be a result of impaired La^- influx into type I fibres. In a separate study, male but not female rowers carrying the T allele, were reported to have higher capillary [La^-] following an incremental test to exhaustion (154). Blood [La^-] alone informs little about the kinetics of La^- appearance and clearance in the muscle. Greater blood [La^-] could be evidence of higher efflux from the muscle or increased glycogenolysis, rather than lower muscle MCT1 activity. In addition, given that the mutant allele is known to affect erythrocyte La^- transport (317), these data in whole blood more likely reflect impairment in erythrocytes than evidence of reduced muscle La^- transport.

Greater incidence of the A allele in athletes from a variety of endurance sports compared to non-athlete controls has been reported, while the T allele was no more prevalent in power-based sports compared to controls (154). Conversely, other researchers have reported no greater incidence of the A allele in endurance athletes compared to controls, but did report the T allele to be associated with sprint/power athletes (416). As if to balance the conflicting data, yet another study reported no difference in T or A allele frequency on comparing sprint and endurance athletes (31). Given that the first two studies used athletes of similar ethnic background and from similar sports, the contrasting results highlight one of the weaknesses of relying on candidate gene-association studies to infer phenotype–genotype relationships, especially when failing to adjust for multiple comparisons as in the study of Sawczuk *et al.* (416). Conflicting findings are inevitable from genetic association studies which, among other weaknesses such as low sample size, set an inappropriately low evidence threshold (235). In reality, researchers have often investigated multiple polymorphisms in the same populations, in addition to a *P* value of 0.05 providing increasingly weak evidence against the null hypothesis as sample size increases[110] (163, 450). Until more robust research is reported, there is little inference that can be drawn from these studies.

[110] Using the example in (163), for a fixed effect size of 0.5, if α of 0.05 is accepted as evidence against the null hypothesis for $n = 10$, then for $n = 100$ the same threshold of evidence requires α of 0.003.

2.15 Training Cessation and Training Maintenance

A corollary of the plasticity of muscle pH_i regulation evident from the training studies reported above is that any upregulation is potentially reversible. The reversibility of training-induced adaptations (outcome) following a period of training cessation or reduced training (process) is referred to as detraining (333). The rate at which training adaptations are lost typically varies between weeks and months, depending on the mechanism investigated, with endurance adaptations seemingly more rapidly reversed (295, 347, 447).

Reductions in oxidative enzyme activity have been found 3 or 7 weeks after stopping training (11, 96, 330), whereas glycolytic enzyme activity has been shown to take between 7 weeks and 6 months to return to baseline (400, 431). Performance adaptations similarly vary in their transience, with maximal sprint power changing little 7 weeks after stopping training (295), but large reductions in maximal aerobic power and 90-s cycling performance evident over the same period (431).

Limited research has been conducted into the timecourse of the reversibility of adaptations in pH_i regulation, but the evidence suggests such loss of adaptations occurs quickly. In perhaps the first study to demonstrate the rapidity of this, 4 weeks after swimmers stopped a 5-month intensive training programme, indirect evidence of a reduction in pH_i-regulatory capacity was shown by greater blood $[La^-]$ and $[H^+]$ at the end of a performance swim (112). It is only lately that direct follow-up of the transience of adaptations in pH_i regulation has been undertaken, primarily in horses.

Apparent improvements in $\beta m_{in\ vitro}$ of 19% after 34 weeks of training in horses were reported to be reversed following 12 weeks of no training (315). There was little change in $\beta m_{in\ vitro}$ after ~30 weeks of multiple phases of training in control and overload training groups of horses[111], but after overtraining had been identified in the overload training group at week 31, there were large increases for the subsequent data (Figure 2.16). Following 10 weeks confinement to their yards, complete loss of adaptations in $\beta m_{in\ vitro}$ was said to have occurred. However, it is more likely that the large increase at 32 weeks was methodological variation, given that the control group had a similar, if not greater increase than the overload group. As such, without a clear training effect there was no clear evidence of detraining in this study.

[111] The two groups of horses performed continuous training for 4 km at ~60% $\dot{V}O_{2max}$, for 7 weeks × 5 $d \bullet wk^{-1}$, followed by 9 weeks of 3 $d \bullet wk^{-1}$ moderate intensity training (3 km at ~80% $\dot{V}O_{2max}$) and 2 $d \bullet wk^{-1}$ of HIT (3 × 2 min at 100% $\dot{V}O_{2max}$). Their training then differed for the next 16 weeks, with the overload group performing HIT 3 $d \bullet wk^{-1}$ (11 wk of up to 8 × 2 min at 100% $\dot{V}O_{2max}$, followed by 5 wk of 10–15 × 1 min at 110% $\dot{V}O_{2max}$) and moderate-intensity training 3 $d \bullet wk^{-1}$ (6 km at ~80% $\dot{V}O_{2max}$), whereas the control group performed HIT 2 $d \bullet wk^{-1}$ (3–4 × 2 min at 100% $\dot{V}O_{2max}$) and moderate-intensity training 3 $d \bullet wk^{-1}$ (3.0–4.5 km at ~80% $\dot{V}O_{2max}$).

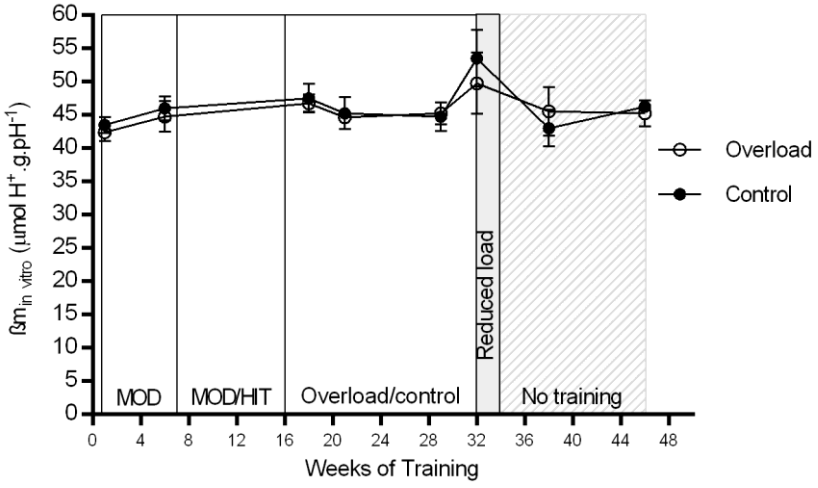


Figure 2.16 $\beta m_{in\ vitro}$ in horses after multiple stages of training and detraining. There were 7 weeks of moderate-intensity training (MOD), 9 weeks of moderate and high-intensity training (MOD/HIT), 15 weeks of overload training or control, 2 weeks of reduced training, and 10 weeks of no training. Data are mean (SD). ● control group and ○ overload training group. Modified from McGowan *et al.* (315). See text for additional details.

In the first published study investigating reversal of adaptations in any of the acid/base transporters in humans, following a 6-week period of repeated 30-s all-out sprints (1:8 work:rest), MCT1 content remained elevated one week after stopping training, but had reduced to the pre-training level after 6 weeks (79). The post-training increase in MCT4 content was reduced by 20% one week after stopping training and had fallen to the mean pre-training value after 6 weeks. An important caveat here in interpreting a potential physiological detraining response is that the individual increases in MCT1 and MCT4 content after training were highly variable, 30–530% and 15–200%, respectively (*cf.* Figure 2.13). Although not reported, presumably the individual detraining responses were equally varied.

Others subsequently reported no statistically significant change in MCT1 or MCT4 after two weeks of training cessation in football players following the end of their season (468). From their data it is apparent there was a progressive reduction in abundance of both proteins 3 d and 2 wk after stopping training. A detraining response probably commenced quite rapidly, but the variance meant an uncertain effect. Interestingly, the same participants demonstrated a rapid upregulation in NHE1 protein abundance upon stopping training, ~25% and ~35% increase at 3 d and 2 wk respectively, a response that was absent in a group who performed HIT over the same two-week period. It is unclear why NHE1 responded to such a reduction in training load, and whether this was simply an artefact of the high variance, but it certainly merits further investigation.

In another study with horses, a 6-week period of stall-rest following 18 weeks of high-intensity training[112] saw a reversal of increases in MCT1 and MCT4 protein content, and in the important mitochondrial enzyme citrate synthase (266). MCT1 but not MCT4 content remained elevated in a parallel group that maintained a moderate level of activity for 6 weeks[113]. It may be that maintenance of MCT4 upregulation requires inclusion of high-intensity training to prevent rapid detraining, while improvements in MCT1 content are more readily maintained. There is some evidence suggesting that reversal of adaptations in specific physiological variables such as maximal oxygen uptake and relative substrate usage can be avoided with a reduction in both intensity and volume of training (388), but the prevailing view in maintaining training adaptations favours reducing volume, while maintaining training intensity, to prevent or mitigate physiological detraining (334, 335, 347). And there is much research to support this view (214, 215, 226, 227, 309, 427). Given that the data presented earlier suggest upregulation of the MCTs and NHE1 is typically only achieved following high-intensity training, it is likely that the removal of a high-intensity stimulus can result in a reduction of protein content. Conversely, reducing training volume but maintaining a high-intensity stimulus may be sufficient to maintain protein content. Therefore, specific data on the acid/base transport proteins and βm should help inform how best to maintain training adaptations despite a reduction in training volume.

2.16 Summary of Adaptations in pH_i Regulation

Muscle pH_i regulation probably contributes to performance in high-intensity exercise tests, but it is doubtful whether this capacity is a limiting factor to performance in more highly-trained individuals. Notwithstanding limitations in measurement techniques, it is clear that upregulation of βm and H^+ transport protein content has sometimes been achieved with short-term training interventions in comparatively untrained individuals. Yet, the conflicting data indicate the physiological stresses that provoke adaptations are not firmly established. It does seem that when pH_i regulation has improved, it is typical that some high-intensity training was performed. However, progressively increasing intensity is not paralleled with greater adaptations, suggesting other factors such as duration of recovery may also be important. By manipulating individual programme variables such as these it should be possible to gain a better understanding of the factors determining pH_i-regulatory capacity. Furthermore, following removal of the physiological stress of high-intensity training, the limited data to date indicate that adaptations are rapidly lost. However, the minimum volume of high-intensity training required to maintain adaptations in pH_i regulation is currently unknown.

[112] 5 d•wk^{-1} at 90–110% $\dot{V}O_{2max}$ for 3 min. Described as HIT and defined as high-intensity training, but there is no mention of repeated bouts.
[113] 5 d•wk^{-1} at 70% $\dot{V}O_{2max}$ for 3 min

2.17 Study Aims

The general aim of this thesis was to investigate components of skeletal muscle pH_i regulation in response to high-intensity intermittent exercise, performed as either a single bout, or following training interventions. Specific aims of the three distinct research studies were as follows.

2.17.1 Study 1 (Chapter 3)

To ensure that measured training adaptations are representative, timing of the post-training muscle biopsy requires evidence of the response of the proteins of interest to the final training session. The aim of the first study was to profile the timecourse of protein content for specific H^+ transport proteins, and for $\beta m_{in\ vitro}$, following a single bout of high-intensity interval exercise.

2.17.2 Study 2 (Chapter 4)

While MCT1, MCT4, and NHE1 protein abundance have been measured in human muscle following exercise training, there are currently no such data for basigin, the NCBT proteins, or any of the CA isozymes. The initial aim of study 2 was to detect by immunoblotting a comprehensive selection of the acid/base transport proteins for the first time in human muscle, and to subsequently measure their plasticity to training. Second, equivocation between seemingly similar studies suggests that the intensity at which a programme of HIT is performed may be important in eliciting adaptations in acid/base transport protein abundance and βm. The next major aim of this study was to compare potential adaptations in muscle pH_i regulation and repeated-sprint ability following two different intensities of work-matched HIT. The third aim was to ascertain how rapidly potential adaptations were achieved by taking a muscle biopsy halfway through training. And the final aim was to investigate if 6 weeks after stopping training reversal of adaptations in acid/base transport proteins and βm would occur.

2.17.3 Study 3 (Chapter 5)

Following on from study 2, the duration of rest intervals may be a key factor in discriminating between equivocal findings in the existing research. The primary aim of this study was to compare adaptations in muscle pH_i regulation and repeated-sprint ability following work-matched SIT, differing only in rest interval duration. The second aim was to investigate if a six-week period of reduced training volume, but maintained intensity, was sufficient to mitigate the loss of adaptations in acid/base transport protein abundance and βm.

Chapter 3 Changes in H^+ transport protein content and muscle buffer capacity following a single bout of high-intensity interval exercise

3.1 Introduction

In response to a bout of high-intensity exercise, the increased proton (H^+) load in skeletal muscle requires buffering and removal from the intracellular space. Physicochemical and metabolic buffers mitigate reductions in intracellular pH (pH_i), but have a limited capacity for doing so (397). Removal of H^+ ultimately requires their active transport across the sarcolemma by transmembrane transport proteins (248, 467). During high-intensity exercise, the monocarboxylate transporters (MCT1 and MCT4) provide the bulk of the H^+ removal capacity through a 1:1 symport system, with L-lactate (La^-) as the preferred substrate (247). Non-lactate-coupled H^+ transport removes an estimated one third of the H^+ load (13), primarily through the sodium/hydrogen exchanger (NHE) system (16). The NHE proteins may also become increasingly important in removing the H^+ load during the recovery from exercise (248).

Although much research suggests MCT1, and to a lesser extent MCT4, may readily adapt to training (40, 79, 135, 327, 373, 380), there are several contrasting studies showing no training response (18, 47, 150, 186, 187, 232). Similar equivocation can be found with the known skeletal muscle NHE isoform, NHE1 (18, 232, 327, 433, 468). One possible explanation for the conflicting findings relates to the timing of the post-training muscle biopsy relative to the final training session (46, 467), typically 1 to 3 days later, where stated. Therefore, a better understanding of the timecourse of the transport protein response to a single exercise bout would help distinguish the short-term responses from any potential longer-term adaptations.

Several studies have investigated the MCT response to a single bout of exercise. On the basis of these studies, Thomas *et al.* (467) modelled a potential timecourse of MCT protein membrane content in different species after exercise (Figure 2.14). Their model indicates that, following a brief initial decrease from the onset of exercise (46, 471), MCT1 and MCT4 protein content progressively increases in the minutes and hours following exercise (40, 104, 179, 181, 267). As Thomas *et al.* (467) also noted, some of these studies found disparate responses for the two MCTs (40, 181). Additional data have shown that, following increases in MCT1 and MCT4 in response to 3 consecutive days of ~2 h of moderate-intensity exercise, MCT4 progressively decreased 48–72 h later, whereas MCT1 remained elevated over the same measurement time (180). In contrast, both MCTs progressively increased 2 and 4 days after 5 h of moderate-intensity continuous cycling, but were reduced 2 d later (179). A similar 2 h bout from the same group failed to elicit any changes in MCT1 or MCT4 protein content immediately post-exercise, or over the subsequent three days (182). It seems likely both the

volume and intensity of exercise – thus substrate usage and accumulation of metabolites – dictate the magnitude and direction of the protein response to a single bout of exercise.

The model proposed by Thomas *et al.* (467) remains to be validated, in particular given that the data come not only from different species and muscle fibre types, but also different fractions of muscle (i.e., membrane or crude cytosolic). Conflicting data may reflect varying efficacy of MCT protein extraction from spun muscle fractions (328). The same group reported MCT1 and MCT4 protein cytosolic content to change little over the 24-h period following a 3 × 30-s maximal cycling bout, interspersed with 20 min of rest (129). According to their model, MCT protein membrane content may have decreased from the onset of exercise, recovering rapidly thereafter. Nevertheless, the failure to observe any change in MCT abundance over the 24 h post-exercise indicates, first, that the model is currently incomplete, and second, the exercise protocol may have provided an insufficient stimulus to provoke changes in MCT expression. The study of Delfour-Peyrethon *et al.* (129) is also the first to report the NHE1 protein response to a single bout of exercise in humans. They showed NHE1 content to change little in response to 3 × 30-s sprints, but abundance was greater 24 h post-exercise with prior sodium bicarbonate supplementation, compared to a placebo, suggesting that alkalosis attenuated a reduction in NHE1 content following exercise.

In addition to changes in H^+ transport protein content, there may also be changes in intracellular buffering capacity (βm) following a single bout of exercise. The two most common measures of βm are the titration assay for measuring non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) and the ratio of the change in La^- to the change in pH_i in response to exercise ($\beta m_{in\ vivo}$). Timing of biopsies in the latter is moot given that the validity of this technique requires immediate post-exercise [La^-] and pH_i, giving a task-specific measure of βm. $\beta m_{in\ vitro}$, on the other hand, is a measure primarily of buffering by inorganic phosphate (P_i) and histidine-containing proteins and dipeptides, with P_i in particular varying in response to exercise. It might be expected that with increased phosphagen hydrolysis during high-intensity exercise, the resultant accumulation of P_i and glycolytic intermediates would elevate $\beta m_{in\ vitro}$ in biopsies taken soon after the end of exercise. However, homogenisation causes almost complete phosphagen hydrolysis (306, 442), and without prior glycogenolysis the phosphates will exist as free P_i in resting samples, rather than as hexose monophosphates or glycerol-3-phosphate, potentially increasing $\beta m_{in\ vitro}$ slightly in resting samples compared to post-exercise samples. Furthermore, Bishop *et al.* (48) calculated a decrease in $\beta m_{in\ vitro}$ immediately after 45 s of cycling at $\dot{V}O_{2peak}$ to be primarily due to reduced protein buffering. It is possible the reduction in protein buffering was due to methodological artefact in hyperaemic post-exercise samples, rather than a rapid depletion of protein-bound histidine content in the muscle or a change in the pK_a of the imidazole side chain of histidine. Regardless of the cause, it is clear that the timing

of a post-training biopsy relative to the final exercise session has the potential to confound interpretation of training-induced changes in $\beta m_{in\ vitro}$.

A failure to be cognisant of the short-term response of target proteins to a bout of exercise risks inappropriate timing of the post-training muscle biopsy following a training intervention (46). This may subsequently lead to misinterpretation of training-induced changes in protein abundance. Similarly, $\beta m_{in\ vitro}$ can vary following exercise due to both methodological and physiological reasons. Therefore, timing of the post-training biopsy becomes crucial in determining whether long-term upregulation has occurred, and the magnitude of any such change (104, 467). The current study profiled the 72-h timecourse of MCT1, MCT4, and NHE1 protein content following a single bout of accustomed high-intensity interval exercise (HIE). It was postulated that protein content would be lower during the first 24–48 h post-exercise, and the appropriate timing of a representative post-training biopsy would therefore be 2–3 days after the final training session. $\beta m_{in\ vitro}$ was also measured over the same period to similarly ascertain the point at which any confounding effects of the last exercise bout were negated.

3.2 Methods

3.2.1 Participants

Sixteen recreationally-active men gave written informed consent to participate in this study [age: 23 (5) y; height: 179.8 (8.6) cm; mass: 80.2 (11.9) kg; $\dot{V}O_{2peak}$: 48.5 (6.2) $mL \bullet min^{-1} \bullet kg^{-1}$; mean (SD)]. All procedures were approved by the Victoria University Human Research Ethics Committee.

3.2.2 Experimental Design

Participants underwent a resting muscle biopsy (Week 0) before undertaking four weeks of high-intensity interval training (HIT) as part of a separate study (Chapter 4). The final session of the HIT intervention was used for the present study as a single bout of HIE, following which a series of six muscle biopsies were performed over four days (see Figure 3.1 for experimental design). The exercise intensity was power at the lactate threshold (LT), plus 40% of the difference between power at the LT and peak aerobic power (W_{peak}) [(LT) + (40%)(W_{peak} − LT)], as measured during a graded-exercise test (GXT) before the HIT intervention. A familiarisation trial of the GXT was performed prior to baseline testing.

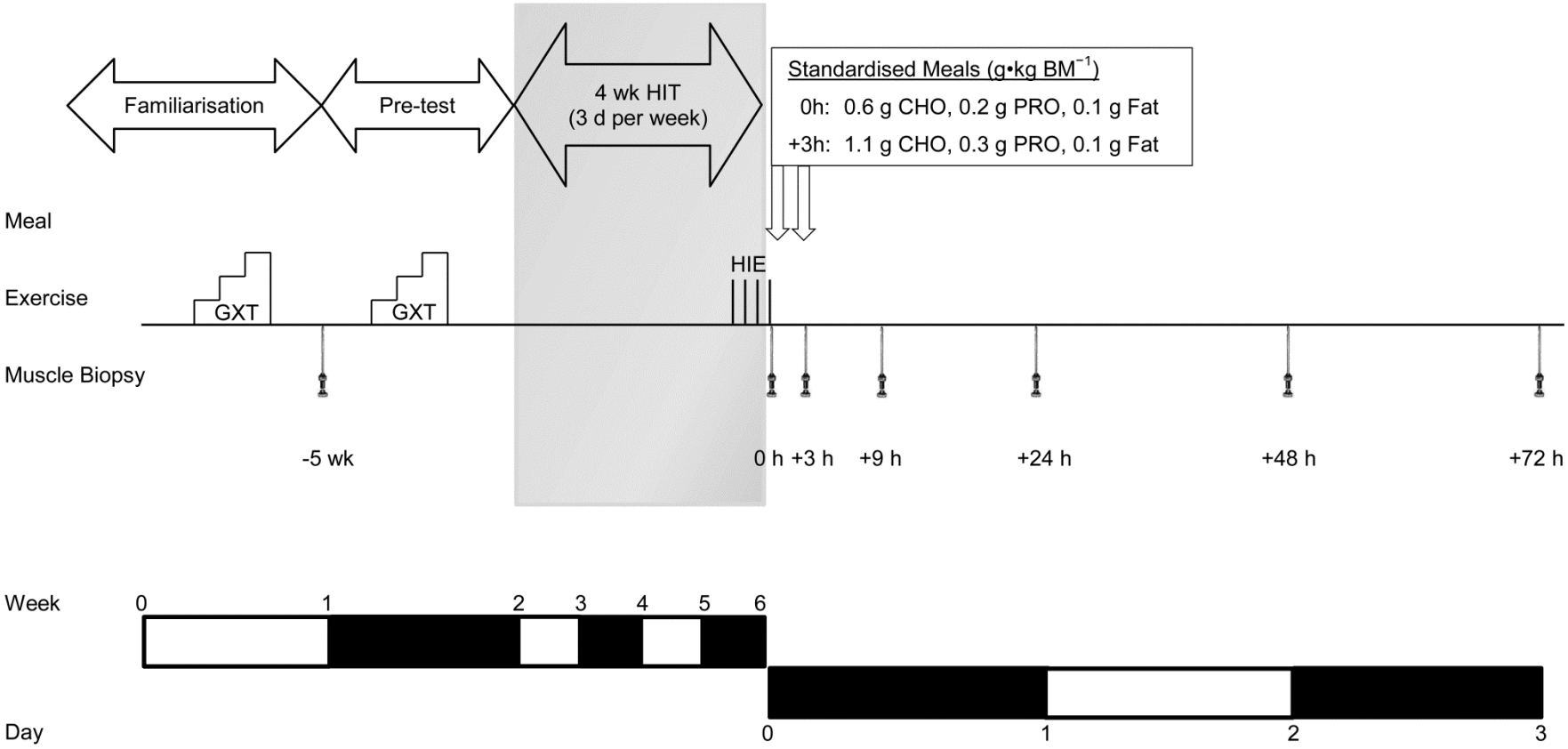


Figure 3.1 Experimental design. Abbreviations: BM (body mass); CHO (carbohydrate); GXT (graded-exercise test); HIE (high-intensity interval exercise); HIT (high-intensity interval training); PRO (protein).

3.2.3 Graded Exercise Test

A GXT was performed pre-training to determine the LT, from venous blood samples taken via an IV cannula, and W_{peak}. The tests were performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands), using an intermittent protocol, with 4-min exercise stages and 30-s rest stages. The starting power (90–150 W) was ascertained from the familiarisation GXT, with the goal of minimising the number of stages to a maximum of ten, and the power was subsequently increased by 30 W every 4.5 min. Participants were required to maintain a set cadence of 70 rpm and consistent verbal encouragement was provided for the latter stages. The test was terminated either volitionally by the participant, or by the assessors when the participant could no longer maintain the required cadence (± 10 rpm) despite strong verbal encouragement. The LT was identified as the power at which venous blood lactate increased 1 mM above baseline (114), and was calculated using Lactate-E software (350). W_{peak} was calculated as previously reported (209, 273):

$$W_{peak} = W_{final} + \left(\frac{t}{240} \bullet 30\right)$$

where W_{final} was the power output of the last completed stage and *t* was the time in seconds of any final uncompleted stage.

3.2.4 Peak Oxygen Uptake Test

After the GXT, the participants performed 5 min of active recovery at 20 W on the cycle ergometer, followed by a square-wave $\dot{V}O_{2peak}$ test. This comprised a steady-state cycle to volitional fatigue at a supramaximal power output, equating to 105% of W_{peak} achieved during the GXT. A similar protocol has previously been reported to elicit $\dot{V}O_{2peak}$ values no different to those determined during either a ramp incremental test performed 5 min previously (401), or a GXT performed 3 min previously (423). Participants were advised to accelerate to 90–100 rpm at the commencement of a 5-s countdown, and to maintain a high but not fixed cadence until volitional fatigue. Consistent verbal encouragement was provided throughout. Expired gases were analysed every 15 s using a custom-made metabolic cart. A two-point calibration of the gas analysers (S-3lA/II and CD-3A analysers, Ametek, PA, USA) was performed before each test using one certified gravimetric gas (16.1% O_2, 4.17% CO_2; BOC Gases, Chatswood, Australia) and ambient air. Ventilation was recorded every 15 s. The ventilometer (KL Engineering, Sunnyvale, CA, USA) was calibrated at the start of each day using a 3-L syringe (MedGraphics, St. Paul, MN). Peak $\dot{V}O_2$ was calculated as the mean of the two highest consecutive 15-s values.

3.2.5 High-Intensity Interval Exercise

A single bout of HIE was performed between 06.30 and 08.00 following an overnight fast. The exercise consisted of seven 2-min intervals performed on an electromagnetically-braked cycle ergometer (Velotron, Racer-Mate, Seattle, WA), separated by 1 min of passive recovery (2:1 work:rest). The exercise intensity was set to: [(LT) + (40%)(W_{peak} − LT)]. A standardised 5-min steady-state warm-up at 75 W was completed prior.

3.2.6 Muscle Sampling

Muscle biopsies were taken from the belly of the *vastus lateralis*, approximately halfway between the knee and hip, using the needle biopsy technique modified with suction (149). Subsequent samples were taken approximately 1 cm from a previous biopsy site. An incision was made under local anaesthesia (1% Xylocaine) and a muscle sample taken using a Bergström needle. Samples were blotted on filter paper to remove blood, before being immediately snap-frozen in liquid nitrogen, and then stored at −80°C until subsequent analyses. Muscle samples were taken from the non-dominant leg at rest pre-HIT (Week 0), and after a HIE bout performed four weeks later – immediately post-exercise (0 h), 3 h, 9 h, 24 h, 48 h, and 72 h post-exercise.

3.2.7 Dietary Control

The HIE bout was performed following an overnight fast, with participants instructed not to drink caffeine on the day of exercise, and to abstain from drinking alcohol or exercising in the preceding 24 h. Whilst in the laboratory, participants were provided with two meals totalling one third of their daily energy requirement, based on their predicted basal metabolism (197), and allowing for a 1.4 activity correction factor (136). Following the 0 h biopsy, they received a standardised 1,392 (189) kJ breakfast consisting of [per kg body mass (BM)]: 0.6 g carbohydrate, 0.2 g protein, and 0.1 g fat. Following the 3 h biopsy, participants received a standardised 2,416 (328) kJ meal consisting of (per kg BM): 1.1 g carbohydrate, 0.3 g protein, 0.2 g fat. The total relative macronutrient intake was therefore 64% carbohydrate, 16% protein, and 20% fat. Except for the meals provided, participants were instructed to ingest only water *ad libitum* until after the 9 h biopsy. Only light activities were permitted between the 0 h and 9 h biopsies. All other biopsies (Week 0, 0 h, 24 h, 48 h, 72 h) were performed following an overnight fast, with participants refraining from exercising or drinking alcohol until after the final muscle biopsy.

3.2.8 Muscle Buffer Capacity

Non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) was measured on 2–3 mg dry mass (dm) of freeze-dried muscle samples using the titration technique (303). Samples were dissected free of visible blood and connective tissue (198), and homogenised on ice for 3 × 30 s in a 10 mM solution of the glycolytic inhibitor NaF (0.1 mL of NaF per 3 mg dm). pH measurements were performed at 37°C with a microelectrode (MI-410, Microelectrodes, Bedford, NH) connected to a pH meter (Lab 850, Schott Instruments GmbH, Mainz, Germany). After the initial pH measurement, homogenates were adjusted to pH 7.1–7.2 with 0.02 M NaOH and then titrated to pH 6.1–6.2 with the serial addition of 2 µL aliquots of 0.01 M HCl. A linear regression was plotted and the number of moles of H^+ per kg dm required to change pH from 7.1–6.5 interpolated. $\beta m_{in\ vitro}$ was then expressed as mmol $H^+ \bullet kg\ dm^{-1} \bullet pH^{-1}$. The typical error for repeat titrations was 10.3 mmol $H^+ \bullet kg\ dm^{-1} \bullet pH^{-1}$, equivalent to a CV of 7%.

3.2.9 Quantitative Western Blotting

3.2.9.1 Muscle homogenate preparation

Approximately 30 mg of frozen muscle tissue was homogenised (Kontes Pellet Pestle, Kimble Chase, NJ, USA) in a 1:20 dilution of ice-cold buffer (pH 7.4) containing: 0.15 M NaCl, 1% Triton-X100, 0.5% sodium deoxycholate, 0.05 M Tris, 0.1% SDS, 0.1 M EDTA, 1 $\mu g \bullet mL^{-1}$ aprotonin, 1 $\mu g \bullet mL^{-1}$ leupeptin, 1 mM benzamidine, 1 mM Na_3VO_4, 1 mM NaF, 5 mM $Na_4P_2O_7$, 1 mM DL-dithiothreitol, 1 mM phenylmethanesulfonyl fluoride (PMSF). Homogenates were rotated end-over-end for 60 min at 4°C and centrifuged twice at 15,000*g* for 10 min at 4°C. The supernatant (i.e., crude cytosolic) was then collected and the pellet discarded.

3.2.9.2 Protein Assay

Protein content of muscle homogenate was measured in triplicate using a Bradford assay (Bio-Rad protein assay dye reagent concentrate, Bio-Rad Laboratories, Hercules, CA) against bovine serum albumin standards (BSA, A9647, Sigma-Aldrich).

3.2.9.3 Immunoblotting

Muscle homogenate was diluted in 2X Laemmli buffer (0.125 M Tris, 4% SDS, 20% glycerol, 0.015% bromophenol blue, 10% 2-mercaptoethanol) and equal amounts of total protein (15 or 20 µg) were loaded in different wells on 10% Tris-Glycine-HCl SDS-PAGE gels. All samples for an individual were loaded in adjacent lanes on the same gel. Four different concentrations of a mixed-homogenate internal standard were also loaded on each gel and a calibration curve plotted of density against protein amount. From the subsequent linear regression equation, protein abundance was calculated from the measured band intensity for

each sample on the gel (336). Coomassie blue (Phastgel Blue R-350, GE Healthcare, Rydalmere, Australia) staining of total protein was used as a loading control (491).

Gel electrophoresis ran for 60–80 min at 140 V. Proteins were wet-transferred to a 0.2 µm polyvinylidene fluoride membrane at 100 V for 80 or 100 min. Membranes were blocked for 60 min at room temperature in 5% non-fat dry milk (NFDM) diluted in Tris-buffered saline with 0.1% Tween-20 (TBST). Membranes were washed in TBST and incubated overnight with the appropriate primary antibody diluted in TBST containing 5% BSA and 0.02% NaN_3 (Table 3.1). Following an additional 1 h incubation at room temperature, membranes were washed in TBST and incubated in the relevant secondary antibody diluted in 5% NFDM in TBST for 90 min at room temperature.

After further washes, membranes were incubated in chemiluminescent solution (1.25 mM luminol, 0.2 mM ρ-coumaric acid, 100 mM Tris pH 8.5, 0.009% H_2O_2) for 2 min and images were taken with a VersaDoc Imaging System (Bio-Rad) fitted with a CCD camera (Bio-Rad). Densitometry was performed with Image Lab 5.0 software (Bio-Rad) using the volume calculation (sum of all band intensities). Background correction was applied individually to each lane using a rolling-ball algorithm, with contrast and brightness adjustments applied homogenously to the entire image (171). Images are displayed with at least five bandwidths above and below the band of interest (344).

3.2.9.4 Antibodies

Table 3.1 Details of primary and secondary antibodies used for western blotting

Primary Antibody	**Supplier/ Catalogue no./ Lot no.**	**Primary Dilution**	**Secondary Dilution**
Rabbit polyclonal anti-MCT1	Merck Millipore/ AB3540P/ 2136555	1:1,000	1:15,000
Rabbit polyclonal anti-MCT4	Merck Millipore/ AB3316P/ 2397059	1:1,000	1:20,000
Mouse monoclonal anti-NHE1	Merck Millipore/ MAB3140/ 2283852	1:500	1:7,500
Secondary Antibody	**Supplier/ Catalogue no.**		
Goat anti-mouse IgG	Perkin Elmer/ NEF822001EA		
Goat anti-rabbit IgG	Perkin Elmer/ NEF812001EA		

3.2.10 Statistical Analyses

To reduce bias from non-uniformity of error, western blot data were log-transformed (348), and back-transformed mean and standard deviation (geometric mean ×/÷ SD) are

reported. Otherwise, means and standard deviations [mean (SD)] are reported for centrality and dispersion. Data were analysed using linear mixed models with, for example, 'time' (repeated-measure) as a fixed factor, and 'subject' and 'intercept' as random factors. Model fit was assessed by −2 log-likelihood (156). Uncertainty of effects are expressed as both 90% confidence intervals (90% CI) and *P* values where relevant. The latter are presented as precise values unless $P < 0.001$ (118), and without arbitrarily defined significance thresholds (450). Effect sizes (ES) were assessed using Cohen's *d*, where ES thresholds were qualified as trivial < 0.2, small < 0.6, moderate < 1.2, large < 2.0, very large < 4.0, and extremely large ≥ 4.0 (225), and are reported as (ES; 90% CI) of the difference (post − pre) scores. Effects were not considered meaningful if there was < 75% probability of being either substantially positive or substantially negative relative to the smallest worthwhile change (ES = 0.2), and were deemed unclear if there was a greater than 5% probability of being both substantially positive and substantially negative (223, 225). Linear mixed models were analysed using IBM SPSS Statistics V21 (IBM Corporation, Somers, NY, USA) and effect sizes and confidence intervals were calculated using custom Excel spreadsheets (223).

3.3 Results

3.3.1 Protein Abundance

3.3.1.1 MCT1

The MCT1 antibody recognised a single band at 50 kDa (Figure 3.2). MCT1 protein abundance was greater at all timepoints post-HIE compared to Week 0 (time main effect: $F_{6,54.7} = 8.05$, $P < 0.001$). As Table 3.2 shows, the effect sizes comparing 0 h to all subsequent timepoints indicate no difference at 3 h or 9 h, but mean MCT1 abundance was 1.26-fold to 1.32-fold greater at 24 h, 48 h, and 72 h. There were no further changes in protein abundance between 24 h and 72 h.

Table 3.2 Magnitude-based inferences for MCT1 protein abundance. Effect sizes and 90% confidence intervals comparing specific timepoints following a single bout of high-intensity interval exercise (HIE).

Timepoint comparison	Effect Size	90% CI	Likelihood of a meaningful difference between timepoints	
			Probability	Inference
0 h – Week 0	1.68	1.04 to 2.33	100%	0 h greater than Week 0
3 h – 0 h	0.06	−0.69 to 0.80	37%	no meaningful difference
9 h – 0 h	0.00	−0.68 to 0.68	31%	no meaningful difference
24 h – 0 h	0.67	0.05 to 1.28	90%	24 h greater than 0 h
48 h – 0 h	0.75	−0.02 to 1.52	88%	48 h greater than 0 h
72 h – 0 h	0.80	0.11 to 1.48	93%	72 h greater than 0 h

3.3.1.2 MCT4

The MCT4 antibody recognised a strong band at ~50 kDa, and a weaker band at ~75 kDa in some samples (Figure 3.2). Only the 50 kDa band was quantified. Overall, mean MCT4 protein content was greater at all timepoints compared to Week 0 (time main effect: $F_{6,54.3} = 2.49$, $P = 0.03$). Following HIE, mean MCT4 content was unchanged at all timepoints from 0 h to 72 h (see Table 3.3)

Table 3.3 Magnitude-based inferences for MCT4 protein abundance. Effect sizes and 90% confidence intervals comparing specific timepoints following a single bout of high-intensity interval exercise (HIE).

Timepoint comparison	Effect Size	90% CI	Likelihood of a meaningful difference between timepoints	
			Probability	Inference
0 h – Week 0	0.47	0.08 to 0.85	88%	0 h greater than Week 0
3 h – 0 h	0.12	−0.37 to 0.60	39%	no meaningful difference
9 h – 0 h	−0.04	−0.34 to 0.27	18%	no meaningful difference
24 h – 0 h	0.14	−0.31 to 0.58	40%	no meaningful difference
48 h – 0 h	0.13	−0.33 to 0.58	39%	no meaningful difference
72 h – 0 h	0.27	−0.27 to 0.82	59%	no meaningful difference

3.3.1.3 NHE1

The predicted molecular mass of NHE1 is 91 kDa. The NHE1 antibody recognised a single or a double band below 100 kDa (Figure 3.2), likely the non- or partially-glycosylated forms (113, 210). Both bands were quantified where present (252). Overall there was a change in mean NHE1 abundance over time (main effect: $F_{6,60.2} = 6.36$, $P < 0.001$). Protein abundance was greater at all timepoints post-HIE compared to Week 0. As Figure 3.2 shows, NHE1 decreased by ~0.8-fold 9 h post-HIE compared to 0 h and 3 h, but had increased again by 24 h. The magnitudes of the effects are reported in Table 3.4.

Table 3.4 Magnitude-based inferences for NHE1 protein abundance. Effect sizes and 90% confidence intervals comparing specific timepoints following a single bout of high-intensity interval exercise (HIE).

Timepoint comparison	Effect Size	90% CI	Likelihood of a meaningful difference between timepoints	
			Probability	Inference
0 h – Week 0	1.09	0.72 to 1.47	100%	0 h greater than Week 0
3 h – 0 h	0.08	−0.39 to 0.54	33%	no meaningful difference
9 h – 0 h	−0.54	−0.88 to −0.20	95%	9 h lower than 0 h
24 h – 0 h	0.05	−0.30 to 0.41	24%	no meaningful difference
48 h – 0 h	−0.09	−0.64 to 0.47	36%	no meaningful difference
72 h – 0 h	0.24	−0.21 to 0.70	57%	no meaningful difference

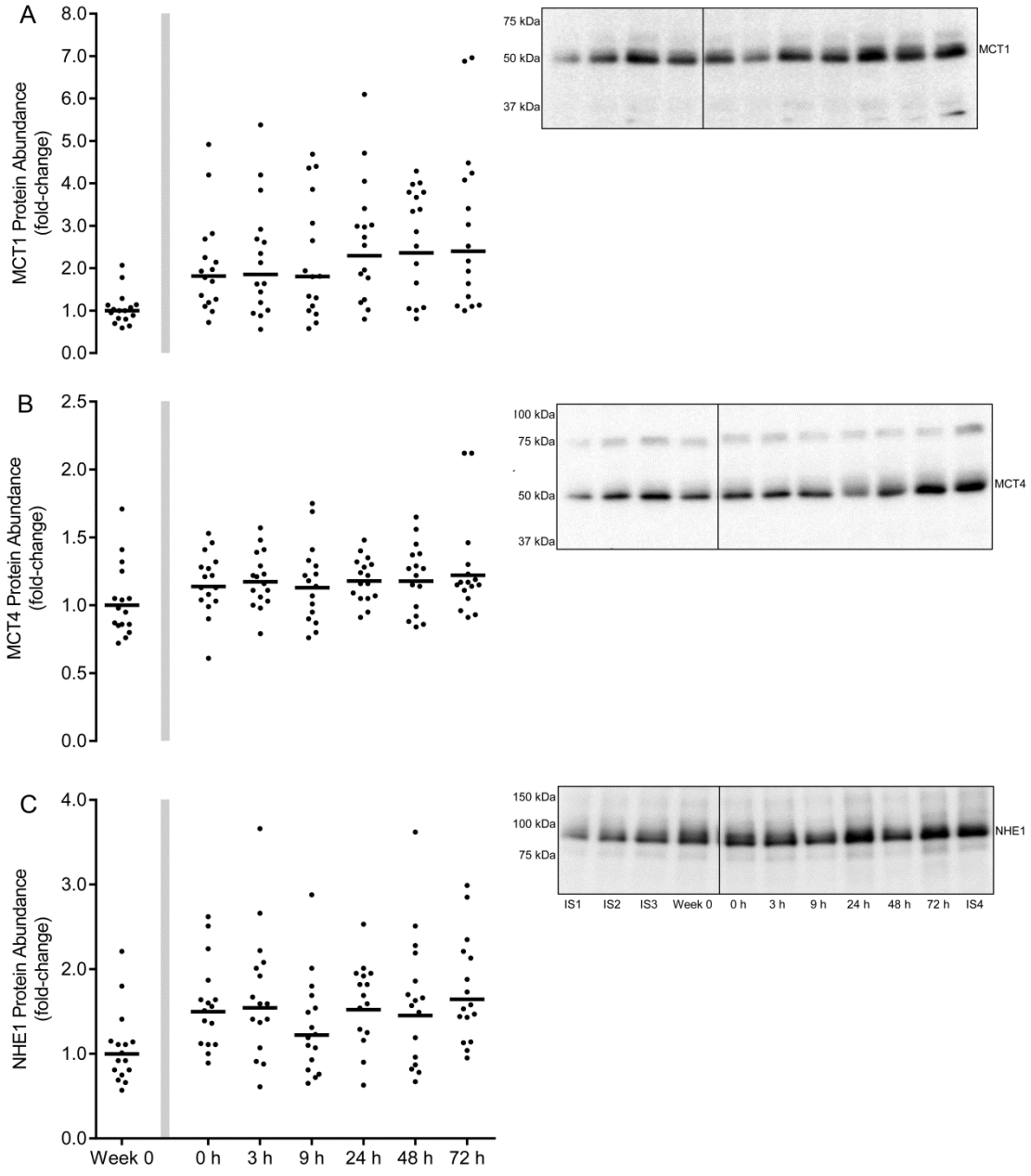


Figure 3.2 Representative immunoblots and protein abundance of (A) MCT1, (B) MCT4, and (C) NHE1 following a single bout of high-intensity interval exercise (HIE). Muscle samples were taken at rest before four weeks of HIE (Week 0), immediately after the final bout of HIE (0 h), and 3 h, 9 h, 24 h, 48 h, and 72 h post-HIE. Different concentrations of an internal standard (IS1–IS4) were also loaded on each gel. Individual data points and means (horizontal bars) are plotted. All data are fold-change relative to the corresponding Week 0 datum, but to illustrate variance at Week 0, individual Week 0 data are shown relative to the Week 0 geometric mean. Non-adjacent lanes from the same blots are indicated by a vertical line. CV of fold-change data for duplicate or triplicate gels: MCT1 (24%), MCT4 (17%), and NHE1 (20%).

3.3.2 Muscle Buffer Capacity

$\beta m_{in\ vitro}$ at Week 0 was 143.2 (13.4) mmol H^+•kg dm^{-1}•pH^{-1} and was greatest immediately after the end of the HIE bout (0 h) at 156.8 (9.3) mmol H^+•kg dm^{-1}•pH^{-1}, varying considerably over the subsequent 72 h (time main effect: $F_{6,50.5}$ = 4.74, P = 0.001). If a comparison of $\beta m_{in\ vitro}$ at Week 0 was made with any of the timepoints from 0 h to 72 h post-HIE, then the effects ranged from a moderate improvement at 0 h of 13.6 mmol H^+•kg dm^{-1}•pH^{-1} (90% CI 4.7 to 22.5), to a small decrease at 72 h of −5.4 mmol H^+•kg dm^{-1}•pH^{-1} (−10.1 to −0.7). Standardised ES and 90% CI for each timepoint compared to Week 0 are shown in Table 3.5.

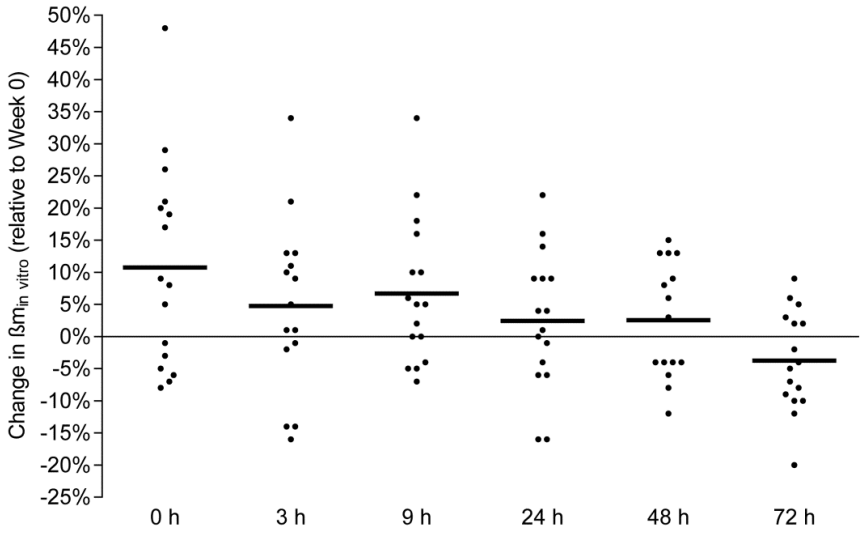


Figure 3.3 Percentage change relative to Week 0 of non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) following a single bout of high-intensity interval exercise (HIE). Muscle samples were taken at rest before 4 weeks of HIE (Week 0), immediately after the final bout of HIE (0 h), and 3 h, 9 h, 24 h, 48 h, and 72 h post-HIE. Individual data points and means (horizontal bars) are plotted. CV of duplicate or triplicate titrations was 7%.

Table 3.5 Magnitude-based inferences for non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$). Effect sizes and 90% confidence intervals are for the post−pre difference score at each timepoint following a single bout of high-intensity interval exercise (HIE).

Timepoint comparison	Effect Size	90% CI	Likelihood of a meaningful difference between timepoints	
			Probability	Inference
0 h – Week 0	0.97	0.33 to 1.60	97%	0 h greater than Week 0
3 h – Week 0	0.38	−0.21 to 0.97	70%	difference unclear
9 h – Week 0	0.62	0.16 to 1.08	94%	9 h greater than Week 0
24 h – Week 0	0.20	−0.26 to 0.66	50%	no meaningful difference
48 h – Week 0	−0.03	−0.56 to 0.51	29%	no meaningful difference
72 h – Week 0	−0.38	−0.72 to −0.05	82%	72 h lower than Week 0

3.3.3 Muscle pH

Compared to 3 h post-exercise, mean muscle pH was 0.27 pH units (90% CI: 0.20 to 0.35) lower immediately after the bout of HIE (time main effect: $F_{5,57.9} = 21.8$, $P < 0.001$). Mean pH at every other timepoint was between 7.02 and 7.08 (range 6.83–7.24).

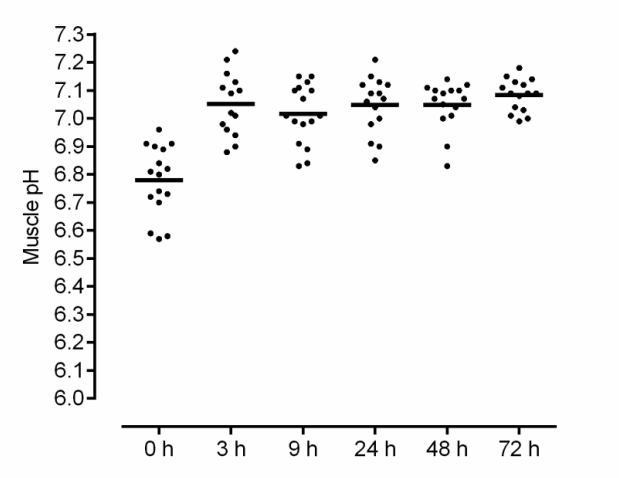


Figure 3.4 Muscle pH following a single bout of high-intensity interval exercise (HIE). Muscle samples were taken immediately post-exercise (0 h), and 3 h, 9 h, 24 h, 48 h, and 72 h post-exercise. Individual data points and means (horizontal bars) are plotted. CV of duplicate or triplicate measurements was 1%.

3.4 Discussion

The purpose of this study was to experimentally determine a timecourse for changes in H^+ transport protein content, and for $\beta m_{in\ vitro}$, following a single bout of high-intensity exercise in active men. The main finding is that MCT1 abundance was greater 24–72 h post-exercise, compared to the first 9 h. Similarly, NHE1 protein content was lower 9 h post-exercise compared to all other timepoints. In contrast, MCT4 abundance did not change over the 72-h measurement period. These results demonstrate that a biopsy taken less than 24 h after the final training session may provide an artefactually low indication of the changes in MCT1 or NHE1 protein content following a training intervention. A second major finding is that the between-day variation in $\beta m_{in\ vitro}$ was of greater magnitude than the mean effect of training studies reported to date.

MCT1 protein abundance was greater 24–72 h after a single bout of HIE, compared to 0, 3, and 9 h post-exercise, with no further increase after 24 h. These data fail to support the previously-hypothesised timecourse of Thomas *et al.* (467), who proposed MCT1 membrane content to decrease immediately after the onset of exercise, followed by a progressive increase in abundance commencing 2 h later (Figure 2.14). In the absence of a same day pre-exercise biopsy in the current study, it is unknown whether MCT1 content decreased in response to the exercise bout and then recovered to a similar abundance 24 h later, or if the greater content after

24 h reflected a delayed increase in protein synthesis following the exercise bout. A reduction in MCT1 abundance has been reported immediately after 45 s of cycling at 200% $\dot{V}O_{2peak}$ in humans (46), and after 2 × 5 min of electrical stimulation of rats (471). These analyses were performed on muscle membrane fractions and, therefore, with uncertain efficiency of protein extraction, cannot be directly compared to the crude cytosolic fractions analysed here. Others have also assayed the cytosolic fraction and found no change in MCT1 content immediately after, or 6–24 h after, a single bout of 3 × 30-s sprints, interspersed with 20 min of rest (129). Similarly, there was no change in cytosolic MCT1 protein content after 2 h cycling at 62% $\dot{V}O_{2peak}$ (182). Although the present data cannot provide an answer, a reduction in MCT1 content from the onset of exercise might be specific to the membrane fraction, but whether this is simply an artefact of inconsistent fractionation, or reflects a transient decrease in membrane content during short-duration, high-intensity exercise, is unknown at this time. Regardless of the cause, these data show that the timing of a post-training biopsy relative to the final bout of exercise may be a confounding factor in measurement of MCT1 abundance. It is recommended that the biopsy is performed a minimum of 24 h after the last training session.

Although it is uncertain if there was a decrease in MCT1 content from the onset of exercise, the main purpose of this study was to determine whether there were any changes in abundance during the 72 h post-exercise. The data presented here show that there was a delayed increase in protein content between 9 and 24 h post-exercise. Total protein synthesis has been reported to be reduced during skeletal muscle contractions, but the mechanisms for this are uncertain (399). One possible explanation for the lower MCT1 content during the first 9 h after exercise, compared to 24 h later, is that intracellular acidosis [pH 6.78 (0.12)] induced by exercise performed above the lactate threshold inhibited transcription, slowing protein resynthesis. Previous research has found that inducing acidosis over a 24-h period in rats resulted in inhibition of skeletal muscle protein synthesis (91). Although muscle pH was not measured in the latter study, arterial pH was maintained between 7.22 and 7.28 during the 24 h. No measurement of protein synthesis was made earlier than 24 h after induction of acidosis, so it remains unclear whether such prolonged acidosis can be compared to the current study. Nevertheless, a potential mechanism for inhibition of protein synthesis has been shown. Supporting data have been provided recently in humans, whereby greater MCT1 protein and mRNA expression were seen 6 h and 24 h after 3 × 30-s sprints performed under extracellular alkalosis ($NaHCO_3^-$) compared to placebo (129). While $NaHCO_3^-$ does not cross the sarcolemma to change pH_i (43, 129), by buffering extracellular H^+ there is a greater ionic gradient relative to the intracellular space (297, 397), likely enhancing H^+ efflux following each sprint. Therefore, exercise-induced acidosis may delay protein resynthesis due to inhibition of transcription, which has been estimated to be the primary contributor to increased protein

abundance for over 4,000 genes from a mouse cell line, rather than translation or catabolism (285).

In contrast to MCT1, there was little change in mean MCT4 content over the 72-h period following HIE. This supports similar findings following 3 × 30-s sprints (129), whereby MCT4 abundance was not affected by a single bout of exercise with or without $NaHCO_3^-$ ingestion. Together these data suggest the model proposed by Thomas *et al.* (467) does not hold for MCT4 abundance in cytosolic fractions, at least with these modalities of high-intensity exercise performed by moderately-trained men. MCT4 membrane protein content has been reported to decrease by 26% immediately after 45 s of maximal exercise (46); however, as the authors acknowledged this may have been an artefact of fractionation. Alternatively, one could hypothesise that exercise induced greater formation of basigin-bound MCT4 heterodimers (507), and the reduction in protein content was because of a failure to chemically break the disulphide bonds of the higher molecular weight (95 kDa) dimer. Direct evidence is required to confirm this. Another group have shown no change in MCT4 content after a 2-h bout of cycling at 62% of $\dot{V}O_{2peak}$ (182). In contrast, they found MCT4 to progressively increase 2 and 4 days after 5 h of similar exercise (179), and to gradually increase over a 16-h period of hourly, 6-min high-intensity cycling bouts (181). It seems likely both duration and intensity of exercise may affect post-exercise changes in MCT4 expression. In addition, increased MCT4 content during the 72 h following three consecutive days of moderate-intensity exercise has been shown to be more transient than MCT1 content, returning to baseline by the second day, whereas MCT1 content remained elevated (180). Therefore, it cannot be determined from the current study if there is an optimum time to take a representative biopsy for measurement of the MCT4 training response, or indeed, if a single timepoint can be representative of changes in a potentially transient protein.

MCT4 is upregulated *in vitro* by the hypoxia-inducible factor (HIF)-1α pathway (343, 476). Hence, it is possible that while MCT4 expression is induced during bouts of cellular hypoxia, transcription rapidly slows following removal of the hypoxic stimulus, with a subsequent delayed effect on protein synthesis. Therefore, cellular hypoxia during exercise (386) may potentially induce transient mRNA bursts (368), or hypoxia may stabilise MCT4 mRNA content (191), resulting in increased MCT4 content. But following removal of the stimulus of exercise and a subsequent return to cellular normoxia, MCT4 transcription might slow, and MCT4 protein resynthesis might then decrease. The implication of this speculation is that any measured change in MCT4 in response to training represents a temporary snapshot, rather than longer-term upregulation. This potentially explains the failure of most studies to observe any change in MCT4 protein content following exercise training. Although the current study failed to induce any change in mean MCT4 content, measuring protein content over

consecutive days post-exercise is recommended to provide better temporal resolution of potentially transient changes (143).

This is the first study to measure NHE1 protein content over a 72-h period following a single exercise bout. NHE1 content was stable across the measurement period, except for a decrease 9 h post-exercise. Given the sample size of 16, the CV of 16% for duplicate gels, and that all samples from a participant were run on the same gel, it is unlikely this 21% decrease in protein content, compared to 0 h, was artefactual. Furthermore, as a membrane-bound protein translated in the cytosol, NHE1 protein was unlikely to have been lost during the fractionation process. Therefore, assuming the reduced protein content was physiological, it is possible that exercise induced greater proteolysis, or a temporary reduction in translation rate, or perhaps, inhibition of transcription resulted in reduced protein synthesis (285). One potential explanation is that exercise-induced acidosis affected one or more components of protein turnover (91). Such a change may not be immediately apparent, depending on the balance between transcription rate, absolute mRNA content, translation, and proteolysis; hence, the delayed downstream effect on NHE1 abundance at 9 h but not 3 h post-exercise. Though there were no mRNA data, induced alkalosis, in contrast to a placebo, has previously been reported to result in increased NHE1 protein abundance 24 h following 3 × 30-s sprints, (129). It remains to be determined how transient is this proposed reduction in transcription, but the data here show the effect is less than 24 h. Therefore, it is recommended not to take a biopsy during the first 24 h post-exercise if seeking to measure the NHE1 response to exercise training.

This study has demonstrated for the first time that $\beta m_{in\ vitro}$ varies greatly in muscle samples taken over a 72-h period. In samples taken at rest over consecutive days from the same *vastus lateralis* muscle, the mean between-sample variability of 22 (8)%[114] was found to be much greater than the grand mean change for experimental groups from those published studies reporting an adaptation to training (mean; 90% CI: 6%; 3 to 9%, see Appendix H for all data). An inevitability of the homogenisation process is that the technique becomes a measure of the buffering capacity of a crude mixture of buffers from different intracellular compartments, as well as some extracellular proteins. Moreover, homogenisation causes almost total phosphagen hydrolysis (1, 306, 442), which results in inconsistencies in the phosphate profile of each sample (200). PCr is a poor buffer with a pK_a of 4.58, whereas P_i has a pK_a of 6.82 and is therefore an excellent buffer over the physiological pH range (200). In resting samples, without prior glycogenolysis the phosphate pool will consist almost entirely of P_i following homogenisation (1, 306, 442). In contrast, the phosphate profile of end-exercise samples will primarily consist of hexose monophosphates (pK_a: 6.11–6.13) (200), glycerol-3-phosphate (pK_a 6.67) (2), and with perhaps 20% as free P_i (54, 363). Following homogenisation, the remaining PCr will break down to form free P_i. Clearly, the βm of homogenate is influenced by

[114] Difference between the individual minimum and maximum titrations from 0 h to 72 h post-exercise.

sample preparation and may not be adequately representative of actual physicochemical buffering. It is also likely that $\beta m_{in\ vitro}$ of end-exercise samples more closely reflects actual physicochemical βm than resting samples. One implication arising from these data is that attribution of increases in $\beta m_{in\ vitro}$ to a given training intervention necessitates an effect at least as great as the between-sample variability of 22% found here.

3.5 Conclusions

MCT1 and NHE1 protein content have been shown here to be greater 24–72 h after a single bout of high-intensity exercise in active men, compare to the first 9 h post-exercise. This has important implications for biopsy timing in studies investigating the response to training of not only the H^+ transport proteins, but also other inducible genes (216). Failure to be cognisant of the transient changes in measured protein abundance following an exercise bout could result in an artefactual interpretation of adaptations to a training intervention. It is recommended post-training biopsies should be taken at least 24 h after the final training session, with daily resting biopsies taken 24–72 h post-exercise potentially providing even better temporal resolution (143). One of the limitations with the present study was the use of fractionated samples to measure protein abundance by western blot. It is possible that, by discarding the pellet after centrifugation and measuring protein abundance in the crude cytosolic fraction, some of the target protein of interest may have been lost (336). The sarcolemmal proteins measured are most likely to be found in the membrane fraction, and in the Golgi apparatus in the case of the MCTs, i.e., the cytosolic fraction. Therefore, it is probable that most, if not all, of these proteins were retained in the fraction measured. Nevertheless, without confirmation, fractionation remains a potential limitation of these protein data. Finally, the results presented for $\beta m_{in\ vitro}$ call into question the ecological validity of this technique for measuring changes in intracellular muscle buffer capacity. Although earlier attempts proved unsuccessful (442), it is recommended in future to attempt to chemically arrest phosphagen hydrolysis if possible, or alternatively correct for the change in the phosphate pool, as has previously been suggested (375). At the very least, to confidently conclude that a change in $\beta m_{in\ vitro}$ was due to an intervention requires an effect greater than the between-sample variability demonstrated here.

Chapter 4 Influence of training intensity on adaptations in acid/base transport proteins, muscle buffer capacity, and repeated-sprint ability

4.1 Introduction

Regulation of pH in skeletal muscle involves a balance between hydrogen ion (H^+) production, buffering, and transport. As exercise intensity increases there is a greater requirement for non-mitochondrial ATP turnover, leading to higher H^+ production. To minimise the potential reductions in intracellular pH (pH_i), the myocyte has a number of pH_i buffer systems. Physicochemical buffering entails H^+ uptake by weak bases, of which, the contribution from non-bicarbonate physicochemical buffers can be measured by titration ($\beta m_{in\ vitro}$) (229). The major physicochemical buffers are the histidine-based proteins and dipeptides, and inorganic phosphate (P_i). Transmembrane transport of acids (H^+) and bases (bicarbonate, HCO_3^-) across the sarcolemma provides an additional crucial component of muscle pH regulation (254). Despite their importance for pH_i regulation, the response to exercise training of most of the acid/base transport proteins has not been investigated.

The transmembrane acid/base transport proteins are located primarily in the sarcolemma, as well as in the T-tubules and sarcoplasmic reticulum (57). Of these proteins, the sodium/hydrogen exchangers (NHE) and the sodium-coupled bicarbonate transporters (NCBT) are thought to be the primary regulators of skeletal muscle pH_i at rest (248, 251). In addition, non-lactate-coupled H^+ transport predominates at low to moderate exercise intensities (16), and there is some evidence that the known skeletal muscle NHE isoform, NHE1, adapts to exercise training (232, 253, 327, 433). However, there are currently no studies on the NCBT response to training in humans, and limited protein evidence of the specific isoforms present in human skeletal muscle. The electrogenic sodium-bicarbonate cotransporters (NBCe1 and NBCe2) have been tentatively identified, but only using non-isoform-specific antibodies (252, 271, 360). During high-intensity exercise the largest portion of muscle H^+ transport is lactate-coupled, provided by the skeletal muscle isoforms of the lactate/H^+ symporters (monocarboxylate transporters: MCT1 and MCT4) (246, 250). Proper functioning of both MCTs requires their targeting to the membrane by the chaperone protein basigin (also called CD147 or EMMPRIN), with some evidence in cell models showing that catalytic activity of the MCTs is dependent on basigin (503). However, there is no protein evidence of basigin in human muscle and no research has investigated the basigin response to exercise training. There is also increasing *in vitro* evidence of a functional, and perhaps physical interaction (theorised transport metabolons), between each of these transport proteins and the cytosolic and sarcolemmal carbonic anhydrase (CA) isozymes (126, 269). CAII has been reported by some to directly bind to and enhance the transport activity of NBCe1 (23, 184), while limited evidence also supports a

similar role for CAIII (422) and for CAIV (6). Evidence of the CA protein response to training has not been shown for any of the isozymes to date.

Upregulation of MCT1 and MCT4 have been inconsistently found following training. While MCT1 appears to respond to a range of training intensities (40, 56, 79, 135, 327, 373), MCT4 adaptations have only been induced by high-intensity interval training (HIT) (79, 367, 373), and are typically of a smaller magnitude than for MCT1. However, several high-intensity training studies have failed to induce changes in either MCT1 or MCT4 (18, 47, 186, 187, 232, 354), or reported increased MCT1 but not MCT4 abundance (40, 327). Furthermore, some of those studies that failed to see MCT training adaptations have shown increased NHE1 abundance following training (187, 232, 253, 433, 468), even if the moderate magnitude and high variance in individual studies render some of the findings statistically uncertain. Together, these data suggest distinct stimuli may upregulate each of the transport proteins.

Increases in βm are also typically only found following HIT (29, 48, 139, 140, 496), but the published research remains equivocal regarding the best way to elicit such improvements. An increase in $\beta m_{in\ vitro}$ has been found after four weeks of 5-min HIT in endurance-trained cyclists (496), and after five weeks of 2-min HIT in well-trained male and female team-sport athletes (48, 140). In the latter study, a separate group who underwent work-matched continuous training showed no change in $\beta m_{in\ vitro}$, despite showing similar improvements in power at the lactate threshold (LT) and peak oxygen uptake ($\dot{V}O_{2peak}$) (140). The authors postulated that training intensity needs to be greater than the LT in order to provoke an adaptation in βm. However, a subsequent study from the same group found that five weeks of 2-min HIT at 100% $\dot{V}O_{2max}$ resulted in decreased $\beta m_{in\ vitro}$ (47). It is clear that further research is required to ascertain whether there is an optimal training intensity to improve βm.

It remains to be determined what the signalling factors are for upregulation of the acid/base transport proteins and muscle buffer capacity. It has been suggested that intracellular La^-/H^+ accumulation (128, 249, 495), or sustained H^+ production and subsequent efflux from the muscle (253, 466), may be important stimuli for some H^+ transporters and βm. If this is the case, then training interventions can be specifically designed with these factors in mind. For example, in order to increase La^-/H^+ accumulation during exercise, intervals of an intensity and duration that demand a large non-mitochondrial ATP turnover are necessary (241, 260). Balancing the volume and intensity of intervals to optimise adaptations is crucial. Earlier investigations into substrate depletion following short sprint intervals have reported an increasing relative contribution from oxidative phosphorylation for subsequent intervals (54, 168, 308). Too high an intensity, resulting in a precipitous early drop in pH_i, could conceivably cause inhibition of glycolytic enzyme activity while maintaining maximal PDH_a activity (363), leading to greater relative oxidative metabolism and a reduction in La^-/H^+ production.

Regardless of the cause, prior high-intensity exercise has been shown to inhibit glycolysis in a subsequent similar bout (12). With gene-specific and indeed fibre-type-specific transcriptional responses dependent on intensity and duration of exercise (216), manipulation of training intensity may also influence adaptations in acid/base transporter abundance.

The present study sought to provide the first comprehensive analysis of the acid/base transport protein response to exercise training. Having detected these proteins, it was possible to manipulate the bioenergetic cost of exercise by using different intensities of work-matched HIT, and thereby test the assumption that training intensity is a key factor in provoking upregulation of acid/base transport proteins and intracellular buffers in skeletal muscle. It was postulated that more intervals performed just above the LT would produce greater upregulation than fewer intervals closer to W_{peak}, and would subsequently lead to better improvements in exercise performance. This study also investigated if six weeks after stopping HIT there would be physiological detraining, characterised by a reduction in acid/base transport protein content and muscle buffer capacity.

4.2 Methods

4.2.1 Participants

Twenty-seven recreationally-active men gave written informed consent to participate in this study. Eleven participants withdrew during the familiarisation period or after the pre-training biopsy. Sixteen participants commenced training and completed the entire study [age: 23 (5) y; height: 179.8 (8.6) cm; mass: 80.2 (11.9) kg; mean (SD)]. Before the training intervention began, mean (SD) weekly training load was 1005 (592) arbitrary units, calculated from session RPE and training duration (162). All procedures were approved by the Victoria University Human Research Ethics Committee.

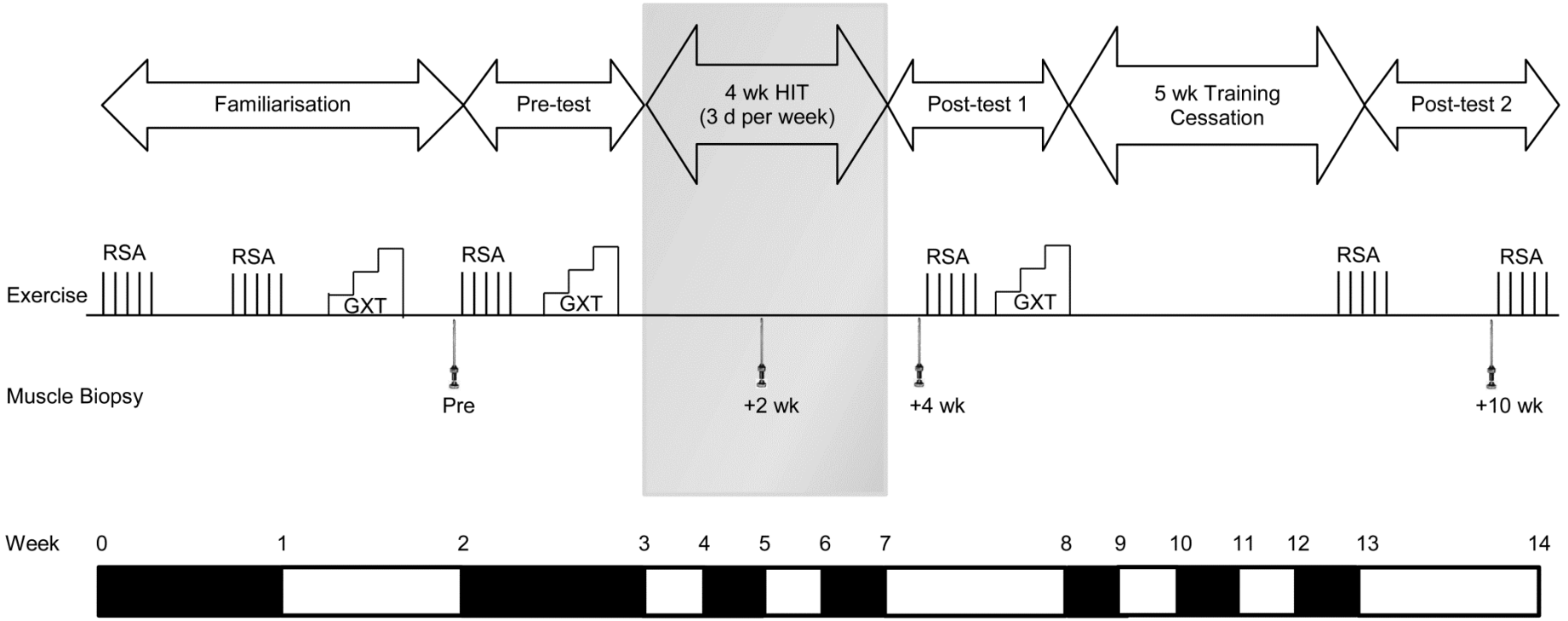


Figure 4.1 Experimental design. Abbreviations: GXT (graded-exercise test); HIT (high-intensity interval training); RSA (repeated-sprint ability test).

4.2.2 Experimental Design

The study employed a two-group parallel design (Figure 4.1). Participants performed two familiarisation trials of the repeated-sprint ability (RSA) test on separate days (86, 307, 313). On another day they performed familiarisation trials of the graded-exercise (GXT) and peak oxygen uptake ($\dot{V}O_{2peak}$) tests. In the week preceding the 10-week post-training test, participants performed a re-familiarisation trial of the RSA test.

Prior to the commencement of the four-week training intervention participants underwent a resting muscle biopsy, followed 5–10 min later by an RSA test. On a separate day each participant performed a GXT and a $\dot{V}O_{2peak}$ test. The same protocols were repeated post-training. Muscle biopsies were also taken halfway through the training intervention and six weeks after stopping HIT. To ascertain any potential physiological detraining response, an RSA test was performed six weeks post-training, 5–10 min after the final muscle biopsy.

Participants refrained from exercising and drinking alcohol 24 h prior to each of the trial days. All trials were performed in the morning (06.30–11.30) following an overnight fast, with participants recording and subsequently replicating a food diary for the 24 h prior to each exercise trial. Participants were asked to maintain their normal level of activity throughout the study and completed a bespoke web-based daily training diary to monitor compliance.

Following the pre-training tests, participants were ranked according to total work performed during the RSA test and allocated to one of two training groups. Type of training was then randomly allocated to the groups, as detailed in section 4.2.6.

4.2.3 Graded Exercise Test

GXTs were performed pre- and post-training to determine the LT, from venous blood samples, and peak aerobic power (W_{peak}). A 20 g IV cannula was inserted into an antecubital vein and samples were taken at rest and at the end of every stage during the GXT. Before each sample, 1–2 mL of blood was drawn into a dry syringe and discarded. Samples were then drawn into a 3 mL dry syringe and aliquoted into a microtube for instant analysis of lactate (2300 STAT Plus, YSI Inc., Yellow Springs, OH). The cannula was then flushed with a small volume of sterile saline.

The tests were performed on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands), using an intermittent protocol, with 4-min exercise stages and 30-s rest stages. The starting power (90–150 W) was ascertained from the familiarisation GXT, with the goal of minimising the number of stages to a maximum of ten, and the power was subsequently increased by 30 W every 4.5 min. Participants were required to maintain a set cadence of 70 rpm and consistent verbal encouragement was provided for the

latter stages. The test was terminated volitionally by the participant, or by the assessors when the participant could no longer maintain the required cadence (± 10 rpm) despite strong verbal encouragement. The LT was identified as the power at which venous blood lactate increased 1 mM above baseline (114), and was calculated using Lactate-E software (350). W_{peak} was calculated as previously reported (209, 273):

$$W_{peak} = W_{final} + \left(\frac{t}{240} \bullet 30\right)$$

where W_{final} was the power output of the last completed stage and *t* was the time in seconds of any final uncompleted stage.

4.2.4 Peak Oxygen Uptake Test

After the GXT, the participants performed a 5-min active recovery at 20 W on the cycle ergometer, followed by a square-wave $\dot{V}O_{2peak}$ test. This comprised a steady-state cycle to volitional fatigue at a supramaximal power output, equating to 105% of W_{peak} achieved during the GXT. A similar protocol has previously been reported to elicit $\dot{V}O_{2peak}$ values no different to that determined during either a ramp incremental test performed 5 min previously (401), or a GXT performed 3 min previously (423). Participants were advised to accelerate to 90–100 rpm at the commencement of a 5-s countdown, and to maintain a high, but not fixed cadence until volitional fatigue. Consistent verbal encouragement was provided throughout. Expired gases were analysed every 15 s using a custom-made metabolic cart. A two-point calibration of the gas analysers (S-3lA/II and CD-3A analysers, Ametek, PA, USA) was performed before each test using one certified gravimetric gas (16.1% O_2, 4.17% CO_2; BOC Gases, Chatswood, Australia) and ambient air. Ventilation was recorded every 15 s. The ventilometer (KL Engineering, Sunnyvale, CA, USA) was calibrated at the start of each day using a 3-L syringe (MedGraphics, St. Paul, MN). Peak $\dot{V}O_2$ was calculated as the mean of the two highest consecutive 15-s values.

4.2.5 Repeated-Sprint Ability Test

The RSA test was performed seated on the same cycle ergometer used for the GXT and followed a modified protocol of that previously reported (42, 44), comprising five 6-s maximal sprints, separated by 24 s of rest. Modification entailed using an isokinetic protocol to negate the effects of cadence on power output (102, 170, 499), and thereby isolate the fatigue-related power component. All sprints were limited to a maximum cadence of 115 rpm. During the recovery period participants remained stationary. Five seconds before the start of each sprint participants assumed the ready position – crank of the dominant leg at an angle of 45°. Initially, participants performed a 5-min steady-state warm-up at 75 W, followed by two 3-s practice

sprints at 90% of perceived maximal effort, separated by 24-s rest. A single criterion 6-s maximal sprint was then performed 90 s later, followed by 5 min of rest prior to the RSA test. To minimise pacing participants were required to achieve ≥ 90% of their criterion score in the first sprint of the 5 × 6-s test. If this criterion was not achieved, participants rested for a further five minutes before restarting the test. Before the first practice sprint, the single 6-s sprint, and the first sprint of the main test, participants accelerated to 115 rpm and stopped pedalling instantly, followed 24 s later by the requisite sprint. This ensured consistent velocity of the flywheel before each sprint. Strong verbal encouragement was given throughout. Total work and work decrement (fatigue index) were calculated from the raw data as has been previously reported (160).

4.2.6 High-Intensity Interval Training

The training intervention consisted of four weeks of supervised, work-matched HIT performed three times per week on an electromagnetically-braked cycle ergometer (Velotron, Racer-Mate, Seattle, WA). The training intensity for both groups was set initially to power at the LT, plus 20% (HITΔ20) or 90% (HITΔ90) of the difference (Δ) between power at the LT and W_{peak} [(LT) + (Δ%)(W_{peak} − LT)] (Table 4.1). For the first training session this was equivalent to 71 (4)% and 98 (0)% of W_{peak} for HITΔ20 and HITΔ90 respectively. Using a similar training plan to that previously reported (140), both groups performed a standardised 5-min steady-state warm-up at 75 W, followed by either 5–15 (HITΔ20) or 4–10 (HITΔ90) 2-min work intervals, interspersed with 1 min of passive recovery. Prior to allocation to group, training programmes were designed for each participant according to the HITΔ90 intervention, with a corresponding work-matched HITΔ20 intervention then being designed. Participants performed the requisite programme once allocated to their group. Training intensity increased by 5% for both groups for the fifth and eighth sessions in order to maintain the relative training stimulus. For the final training session all participants performed a standardised training session at the same relative intensity [(LT) + (40%)(W_{peak} − LT)] for characterisation of short-term post-exercise responses for a separate study (Chapter 3).

Table 4.1 High-intensity interval training programme performed by the HITΔ20 and HITΔ90 training groups.

Week	Session	HITΔ20 Group			HITΔ90 Group		
		Intervals	Relative Intensity†	External Power (W)	Intervals	Relative Intensity†	External Power (W)
1	1	5–6	20 (1)%	194 (18)	4	90 (1)%	280 (53)
	2	7–9	20 (1)%	194 (18)	6	90 (4)%	278 (54)
	3	9–10	20 (1)%	194 (18)	7	90 (2)%	279 (53)
2	4	10–12	20 (1)%	194 (18)	8	90 (5)%	277 (55)
	5	7–9	30 (2)%	204 (19)	6	110 (4)%	293 (56)
	6	10–12	30 (2)%	204 (19)	8	110 (5)%	292 (57)
3	7‡	12–15	30 (2)%	204 (18)	10	100 (3)%	288 (54)
	8	7–9	40 (5)%	214 (20)	6	120 (5)%	302 (56)
	9	9–10	40 (5)%	214 (20)	7	120 (6)%	302 (55)
4	10	11–13	40 (6)%	218 (19)	9	110 (9)%	295 (47)
	11	9–10	40 (5)%	214 (20)	7	110 (8)%	301 (54)
	12	7	40 (4)%	215 (19)	7	40 (3)%	229 (54)

†Relative intensity calculated as power at the lactate threshold (LT), plus *x*% of the difference (Δ) between power at the LT and peak aerobic power (Wpeak) [(LT) + (*x*%)(W_{peak} − LT)]. Data are mean (SD).

‡Muscle biopsy performed 2 or 3 d after training session 6, immediately prior to session 7.

4.2.7 Needle Muscle Biopsies

Muscle biopsies were taken from the belly of the *vastus lateralis*, approximately halfway between the knee and hip, using the needle biopsy technique modified with suction (149). Subsequent samples were taken approximately 1 cm from a previous biopsy site. An incision was made under local anaesthesia (1% Xylocaine) and a muscle sample taken using a Bergström needle. Samples were blotted on filter paper to remove blood and then immediately snap-frozen in liquid nitrogen and stored at −80°C until subsequent analyses. Muscle samples were taken from the non-dominant leg pre-training (Pre), immediately before the seventh training session (+2 wk), 2 or 3 d after the last training session (+4 wk), and 6 wk (+10 wk) after the final training session.

To ascertain any physiological detraining effects six weeks after stopping HIT, participants were required to assiduously maintain an online training diary in order to allow quantification of training. Failure to do this necessitated exclusion of their 10-wk post-training data. One participant was unavailable to undergo the +10 wk post-training biopsy and a further

two participants failed to adequately maintain a training diary; hence, n = 6 and n = 7 for HITΔ20 and HITΔ90 respectively at this timepoint.

4.2.8 Muscle Buffer Capacity

Non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) was measured on 2–3 mg dry mass (dm) of freeze-dried muscle samples using the titration technique (303). Samples were dissected free of visible blood and connective tissue (198), and homogenised on ice three times for 30 s in a 10 mM solution of the glycolytic inhibitor NaF (0.1 mL of NaF per 3 mg dm). pH measurements were performed at 37°C with a microelectrode (MI-410, Microelectrodes, Bedford, NH) connected to a pH meter (Lab 850, Schott Instruments GmbH, Mainz, Germany). After the initial pH measurement, homogenates were adjusted, if necessary, to pH 7.1–7.2 with 0.02 M NaOH and then titrated to pH 6.1–6.2 with the serial addition of 2 µL aliquots of 0.01 M HCl. A linear regression line was plotted and the number of moles of H^+ per kg dm required to change pH from 7.1–6.5 interpolated. $\beta m_{in\ vitro}$ was then expressed as mmol H^+•kg dm^{-1}•pH^{-1}. Titrations were performed up to five times per sample, depending on available tissue. The typical error (within-sample standard deviation) for repeat titrations was 9.8 mmol H^+•kg dm^{-1}•pH^{-1}, equating to a CV of 7%.

4.2.9 Quantitative Western Blotting

4.2.9.1 Muscle homogenate preparation

Approximately 30 mg of frozen muscle tissue was homogenised (Kontes Pellet Pestle, Kimble Chase, NJ, USA) in 1:20 dilution of ice-cold RIPA buffer (pH 7.4) containing: 0.15 M NaCl, 1% Triton-X100, 0.5% $C_{24}H_{39}NaO_4$, 0.05 M Tris, 0.1% SDS, 0.1 M EDTA, 1 µg•mL^{-1} aprotonin, 1 µg•mL^{-1} leupeptin, 1 mM benzamidine, 1 mM Na_3VO_4, 1 mM NaF, 5 mM $Na_4P_2O_7$, 1 mM DL-dithiothreitol (DTT), 1 mM phenylmethanesulfonyl fluoride (PMSF). Homogenates were rotated end-over-end for 60 min at 4°C and centrifuged twice at 15,000*g* for 10 min at 4°C. The supernatant was then collected and the pellet discarded.

NBCe1 was assayed in muscle tissue homogenised in a HEPES-sucrose buffer (pH 7.4) containing: 210 mM sucrose, 30 mM HEPES, 40 mM NaCl, 2 mM EGTA, 5 mM EDTA, 1% protease inhibitor cocktail (P8340, Sigma-Aldrich, St. Louis, MI), 1% phosphatase inhibitor cocktail (P5726, Sigma-Aldrich), 1 mM PMSF. Homogenates were prepared exactly as per the RIPA-buffered samples. Owing to insufficient muscle samples there were lower sample sizes for NBCe1 analysis.

4.2.9.2 Protein Assay

Protein content of muscle homogenate was measured in triplicate using a Bradford assay (Bio-Rad protein assay dye reagent concentrate, Bio-Rad Laboratories, Hercules, CA) against serial dilutions of bovine serum albumin (BSA, A9647, Sigma-Aldrich) standards.

4.2.9.3 Immunoblotting

RIPA-buffered homogenate was diluted 1:1 in 2X Laemmli buffer (0.125 M Tris, 4% SDS, 20% glycerol, 0.015% bromophenol blue, 10% 2-mercaptoethanol) and equal amounts of total protein (1–25 µg)[115] were loaded in different wells on 10% or 12% Tris-Glycine-HCl SDS-PAGE gels. For CAIV detection, homogenate was loaded on Criterion 4–12% Bis-Tris SDS-PAGE gels (Bio-Rad). For NBCe1, HEPES-sucrose buffered homogenate was diluted 3:1 in 4X Laemmli buffer and 20 µg of protein was loaded per well on Criterion 4–12% Bis-Tris SDS-PAGE gels. All samples for a particular participant were loaded in adjacent lanes on the same gel. Four to six different dilutions of a mixed-homogenate internal standard were also loaded on each gel and a calibration curve plotted of density against protein content. From the subsequent linear regression equation protein abundance was calculated from the measured band intensity for each sample on the gel (336). Coomassie blue (Phastgel Blue R-350, GE Healthcare, Rydalmere, Australia) staining of total protein was used as a loading control (491) and was similarly expressed relative to a calibration curve for each blot.

Gel electrophoresis ran for 70–90 min at 140 V for 10% gels and 4–12% gels, or 120–140 min at 100 V for 12% gels. Proteins were wet-transferred to a 0.2 µm polyvinylidene fluoride membrane at 100 V. Membranes were blocked for 60 min at room temperature in 5% non-fat dry milk (NFDM) diluted in Tris-buffered saline with 0.1% Tween-20 (TBST). Membranes were then washed in TBST and incubated – either at room temperature for 2 h or overnight at 4°C – with the appropriate primary antibody diluted in 5% BSA and 0.02% NaN_3 in TBST, or 5% NFDM in TBST for NBCe1 (see Table 4.2 for conditions). Following TBST washes the membranes were incubated in the relevant secondary antibody, diluted in 5% NFDM in TBST, for 90 min at room temperature.

After further washes membranes were incubated in chemiluminescent solution (1.25 mM luminol, 0.2 mM ρ-coumaric acid, 100 mM Tris pH 8.5, 0.009% H_2O_2) for 2 min and images were taken with a VersaDoc Imaging System (Bio-Rad) fitted with a CCD camera (Bio-Rad). Densitometry was performed with Image Lab 5.0 software (Bio-Rad) using the volume calculation (sum of all band intensities). Background correction was applied individually to each lane using a rolling-ball algorithm, with contrast and brightness adjustments applied homogenously to the entire image (171). Images are typically displayed with at least five bandwidths above and below the band of interest (344).

[115] CAIII proved to be highly abundant, with signal saturation on loading 5 µg. Homogenate was therefore diluted 1:8 in RIPA buffer (including inhibitors) and 1 µg was loaded to detect this protein.

4.2.9.4 Antibodies

Table 4.2 Details of primary and secondary antibodies used for western blotting

Primary Antibody	**Supplier/ Catalogue no./ Lot no.**	**[Primary]**	**[Secondary]**
Rabbit polyclonal anti-MCT1	Merck Millipore/ AB3540P/ 2136555	1:1,000	1:15,000
Rabbit polyclonal anti-MCT4	Merck Millipore/ AB3316P/ 2397059	1:1,000	1:20,000
Mouse monoclonal anti-NHE1	Merck Millipore/ MAB3140/ 2283852	1:500	1:7,500
Rabbit polyclonal NBCe1	Cell Signaling/ 11867/ 0001	1:500	1:5,000
Mouse monoclonal anti-basigin	Santa Cruz/ sc-21746/ K1913	1:200	1:7,500
Rabbit polyclonal CAII	Santa Cruz/ sc-25596/ F0611	1:1,250	1:30,000
Mouse monoclonal CAIII	Abnova/ H00000761-M02/ 12243-S1	1:2,500	1:40,000
Mouse polyclonal CAIV	Abnova/ H00000762-B02P/ 08325 WULz	1:500	1:10,000
Mouse polyclonal CAXIV	Abnova/ H00023632-B01P/ 08358 WULz	1:750	1:10,000
Secondary Antibody	**Supplier/ Catalogue no.**		
Goat anti-mouse IgG	Perkin Elmer/ NEF822001EA		
Goat anti-rabbit IgG	Perkin Elmer/ NEF812001EA		

4.2.10 Statistical Analyses

To detect a moderate difference in muscle buffer capacity (d = 0.8) using a mixed-model ANOVA at $\alpha = 0.05$ and $\beta = 0.2$ required $n = 8$ for each group. Measures of centrality and dispersion are means and standard deviations [mean (SD)]. To reduce bias from non-uniformity of error, data were log-transformed where heteroscedasticity was present (348), such as for western blot data. For these data, geometric mean (back-transformed mean of the log-transform) and back-transformed standard deviation are reported and plotted (geometric mean ×/÷ factor SD). Data were analysed using linear mixed models with, for example, 'time' (repeated-measure), 'group', and 'time×group' as fixed factors; and 'subject' and 'intercept' as random factors. Model fit was assessed by −2 log-likelihood (156). Uncertainty of effects are expressed as 90% confidence intervals (90% CI) and P values. The latter are presented as precise values unless $P < 0.001$ (118), and without arbitrarily defined significance thresholds (450). Effect sizes (ES) were assessed using Cohen's d, where ES thresholds were qualified as trivial < 0.2, small < 0.6, moderate < 1.2, large < 2.0, very large < 4.0, and extremely large ≥ 4.0 (225), and are reported as (ES; 90% CI) of the between-group difference (post − pre) scores. Effects were not considered meaningful if there was < 75% probability of being either substantially positive or substantially negative relative to the smallest worthwhile change (ES = 0.2), and were deemed unclear if there was a greater than 5% probability of being both

substantially positive and substantially negative (223, 225). Where no between-group differences were seen, pooled (ES; 90% CI) of the within-group difference (post − pre) scores are reported. Linear models were analysed using IBM SPSS Statistics V21 (IBM Corporation, Somers, NY, USA) and effect sizes and confidence intervals were calculated using custom Excel spreadsheets (223).

4.3 Results

4.3.1 Training Data

Both training groups performed similar volumes of total work throughout the training intervention, 22 MJ and 23 MJ for HITΔ20 and HITΔ90 respectively. There was 99% adherence to the training programme, with one participant in each group missing a single training session due to illness. The different metabolic cost for the two training intensities is shown by heart rate responses for a representative training session (11th) in Figure 4.2.

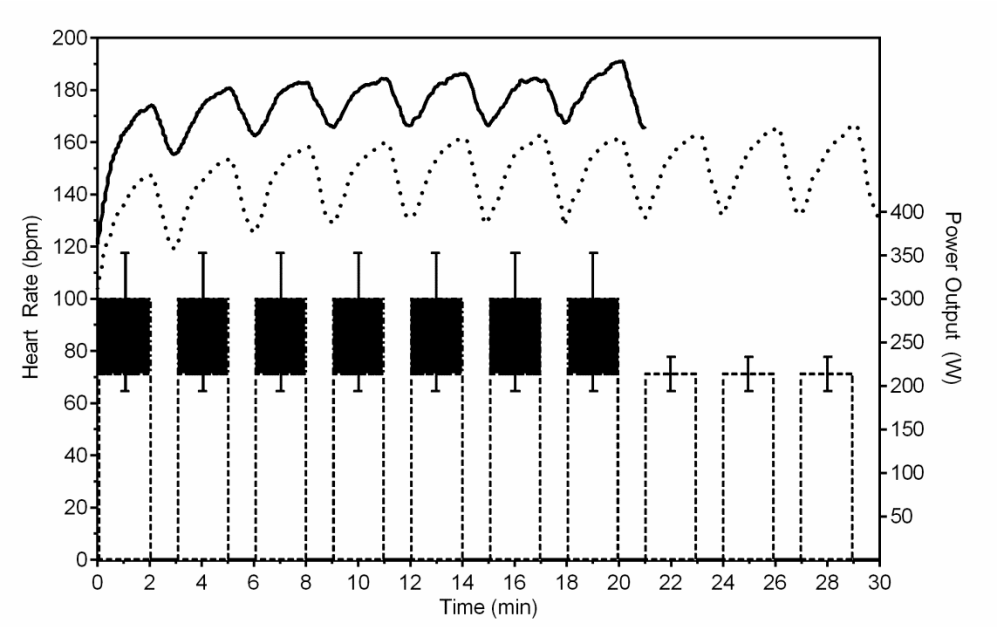


Figure 4.2 Mean heart rate during a representative training session (11) for the HITΔ20 (*n* = 8, dotted line) and HITΔ90 (*n* = 7, solid line) groups. Boxes show the mean (SD) power for HITΔ20 (white) and HITΔ90 (black). Exercise comprised 2-min intervals interspersed with 1 min of passive rest.

4.3.2 Protein Abundance

4.3.2.1 Basigin (CD147/EMMPRIN)

The predicted molecular mass for basigin-2 (isoform 1), the canonical basigin isoform and probable skeletal muscle isoform (289), is 42 kDa. Basigin has three *N*-glycosylation sites and functions in both a high- and a less-glycosylated form, giving a range of apparent molecular

masses (238, 463). Using a monoclonal antibody (sc-21746, Santa Cruz Biotechnology, Santa Cruz, CA), a single band was detected just above 37 kDa in muscle homogenate (Figure 4.3), and ~50 kDa in a Hep G2 cell lysate positive control (sc-2227, Santa Cruz). To confirm the presence of basigin, duplicate gels were run in parallel – same homogenate, same running and transfer tanks – and probed with the first basigin antibody and a second monoclonal basigin antibody (sc-46700, Santa Cruz). This second antibody had a poorer signal-to-noise ratio and detected a 50 kDa band and several weaker lower molecular mass bands, including just above 37 kDa (Figure 4.3), but did not detect any signal with the Hep G2 cell lysate. For additional confirmation, blots were reprobed using a different species (goat) basigin polyclonal antibody (sc-9757, Santa Cruz). This was performed following a 15-min incubation with H_2O_2 at 37°C to inactivate the peroxidase of the HRP-conjugated secondary antibody, using a previously reported protocol (425). Again, the antibody produced weak signal but did detect the > 37 kDa band in muscle homogenate and ~50 kDa band in Hep G2 cell lysate. With this supporting data, the single > 37 kDa band detected by the mouse monoclonal antibody (sc-21746) was quantified.

Overall basigin abundance changed in response to training (time main effect: $F_{3,37.2}$ = 4.47, P = 0.009), but there were unclear differences between groups (group×time interaction: $F_{3,37.2}$ = 1.78, P = 0.17) (Figure 4.4). Protein content was greater for HITΔ90 at +4 wk (ES: 1.49; 0.07 to 2.91), with no clear differences at +2 wk (ES: 0.64; −0.31 to 1.60) or +10 wk (ES: 0.69; −0.37 to 1.76). The difference at +4 wk was due to an increase in basigin content for HITΔ90 (1.18-fold ×/÷ 1.40) and a decrease for HITΔ20 (0.86-fold ×/÷ 1.41). Six weeks after stopping HIT, basigin decreased for both HITΔ90 (0.93-fold ×/÷ 1.33) and HITΔ20 (0.81-fold ×/÷ 1.14) below Pre values (pooled +10 wk ES: −0.64; −1.19 to −0.10).

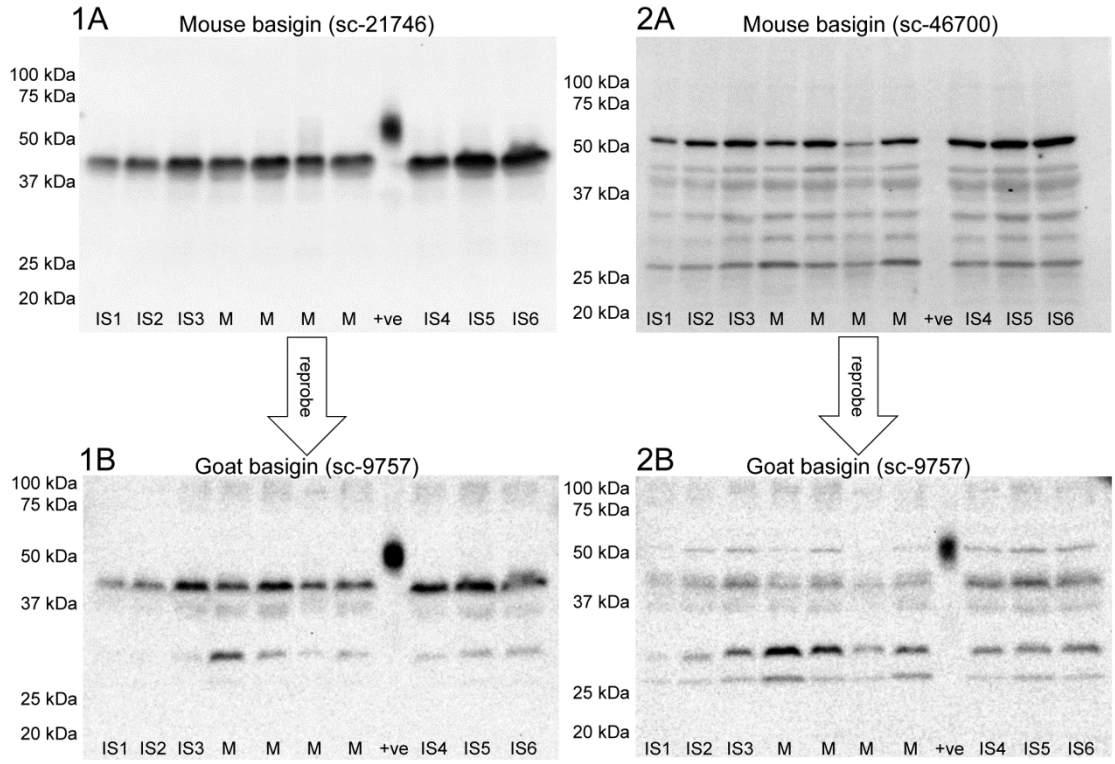


Figure 4.3 Basigin antibody validation. Using western blotting, two duplicate gels (1 and 2) were loaded with four lanes of skeletal muscle homogenate (M), different concentrations of an interval standard (IS1–IS6: 8–38 µg protein), and one lane of a Hep G2 cell lysate positive control (+ve). Following transfer, membrane 1A was immunoblotted with a mouse monoclonal basigin antibody (sc-21746) and membrane 2A was immunoblotted with a different mouse monoclonal basigin antibody (sc-46700). After imaging, the peroxidase of the HRP-conjugated secondary antibodies was inactivated by H_2O_2 incubation. Both membranes were reprobed (1B and 2B) with a goat polyclonal basigin antibody (sc-9757). See text for additional details on immunoblotting.

4.3.2.2 MCT1

The MCT1 antibody recognised a single band at approximately 50 kDa (Figure 4.4). MCT1 protein content increased for both groups (time main effect: $F_{3,40.2}$ = 13.00, P < 0.001), with no difference between the two groups (group×time interaction: $F_{3,40.2}$ = 0.08, P = 0.97, +4 wk ES: 0.25; −1.50 to 2.00). On pooling the data for the two groups, there was a moderate increase at +2 wk (ES: 0.60; −0.01 to 1.20) and a very large increase at +4 wk (ES: 2.49; 1.67 to 3.32). MCT1 abundance decreased six weeks after stopping HIT, but remained elevated above baseline (+10 wk ES: 0.90; 0.31 to 1.49).

4.3.2.3 MCT4

The MCT4 antibody recognised a strong band at ~50 kDa and a weaker band at ~75 kDa in some samples (Figure 4.4); only the 50 kDa band was quantified. Increases in MCT4 protein content were only evident for HITΔ20 (group×time interaction: $F_{3,42.1}$ = 4.5, P = 0.008). There was no difference between groups at +2 wk (ES: −0.22; −0.92 to 0.48), but at +4 wk MCT4 had increased for HITΔ20 relative to HITΔ90 (ES: 1.06; 0.29 to 1.83). The greater MCT4 abundance was lost six weeks after stopping HIT, with no clear difference between groups at +10 wk (ES: −0.30; −0.90 to 0.29), and the pooled data showing no difference compared to Pre (pooled ES: 0.02; −0.25 to 0.29).

4.3.2.4 NHE1

The NHE1 antibody recognised a single or a double band just below 100 kDa (Figure 4.4). Given that the predicted molecular mass of NHE1 is 91 kDa, it is likely the identified band(s) represent the non- or partially-glycosylated forms of the protein (113, 210). Both bands were quantified if present (252). There was an increase in NHE1 protein content for both groups (time main effect: $F_{3,44.2}$ = 11.35, P < 0.001), with no difference between the two groups (group×time interaction: $F_{3,44.2}$ = 0.45, P = 0.72, +4 wk ES: 0.03; −0.84 to 0.91). On pooling the data, there was no difference at +2 wk (pooled ES: 0.23; −0.21 to 0.67), but a large increase was evident at +4 wk (ES: 1.34; 0.93 to 1.75). There was still a clear effect at +10 wk (pooled ES: 0.58; 0.13 to 1.03), indicating that NHE1 abundance had not yet returned to baseline values.

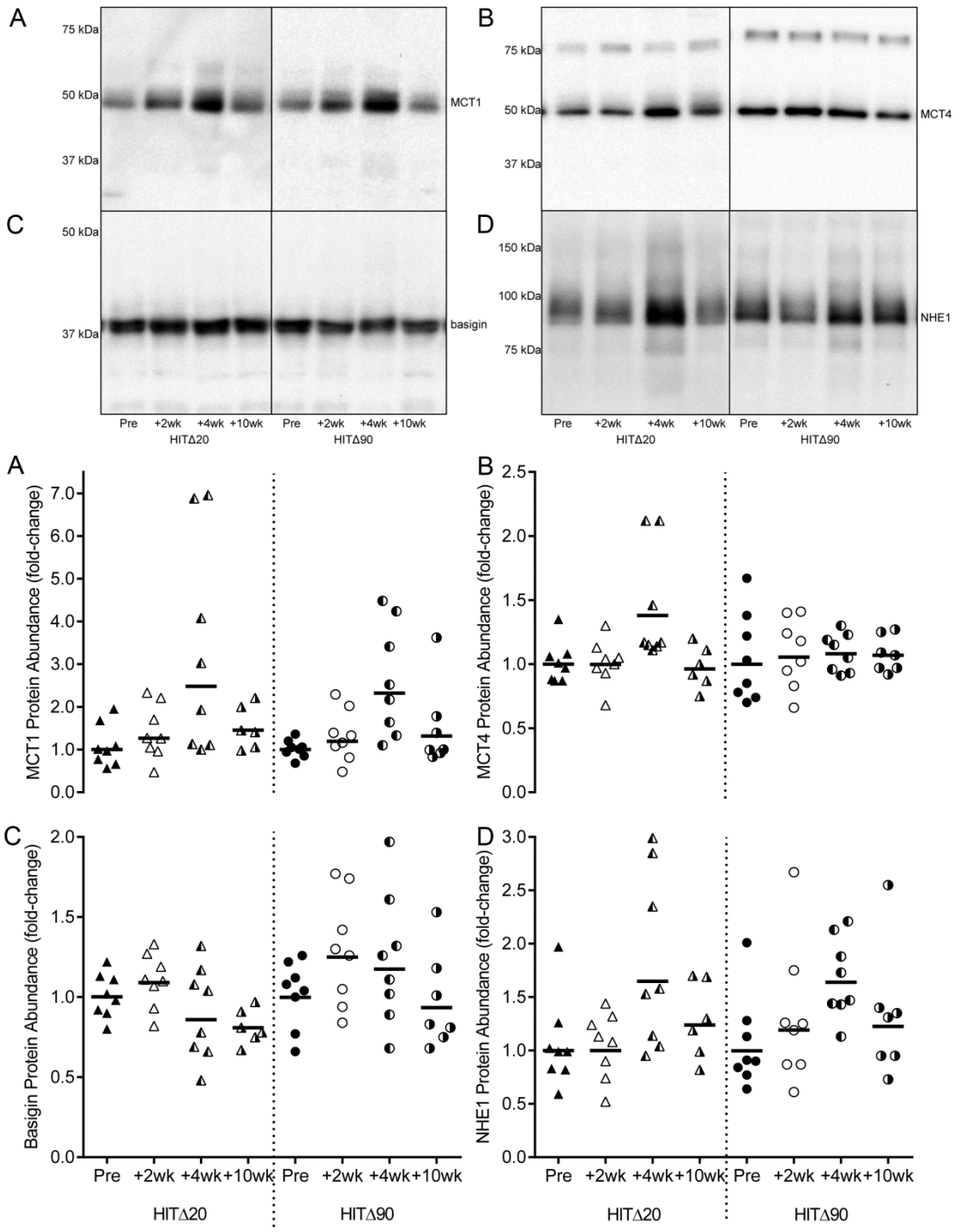


Figure 4.4 Representative immunoblots and protein abundance of (A) MCT1, (B) MCT4, (C) basigin, and (D) NHE1 before (Pre) and after 2 weeks (+2 wk) and 4 weeks (+4 wk) of high-intensity interval training (HIT), and 6 weeks after stopping HIT (+10 wk), for the HITΔ20 and HITΔ90 training groups. Individual data points and geometric means (horizontal bars) are plotted. Post-training data are calculated as fold-change relative to the corresponding Pre datum. To illustrate variance Pre, individual Pre data are shown relative to the Pre geometric mean for the respective groups. Separate blots (A, B, D) or non-adjacent lanes from the same blot (C) are indicated by vertical lines. CV of fold-change data for two to four gel repeats: MCT1 (21%), MCT4 (13%), basigin (22%), and NHE1 (21%).

4.3.2.5 NBCe1

The NBCe1 antibody was raised against a C-terminus sequence and recognised a band close to the predicted molecular mass of 121 kDa for the canonical B isoform (Figure 4.5). Stronger signal bands were also detected at lower molecular masses of about 50 kDa and 75 kDa. It is unknown if these bands represent as yet unidentified SLC4 proteins, or possibly proteolytic fragments of NBCe1, as has been suggested previously with other NBC antibodies (38, 421). In the absence of additional confirmation, only the ~120 kDa band was quantified. To avoid signal saturation from the lower molecular mass bands affecting quantification, membranes were cut at 75 kDa.

No clear differences were found in NBCe1 protein content between the groups (group×time interaction: $F_{3,16.7} = 0.82$, $P = 0.50$, +4 wk ES: 0.49; −0.91 to 1.88, +10 wk ES: 0.55; −0.86 to 1.95). NBCe1 increased in response to training (time main effect: $F_{3,16.7} = 12.3$, $P < 0.001$), with no change at +2 wk (pooled ES: 0.20; −0.35 to 0.75), but increased abundance at +4 wk (pooled ES: 1.31; 0.64 to 1.98).

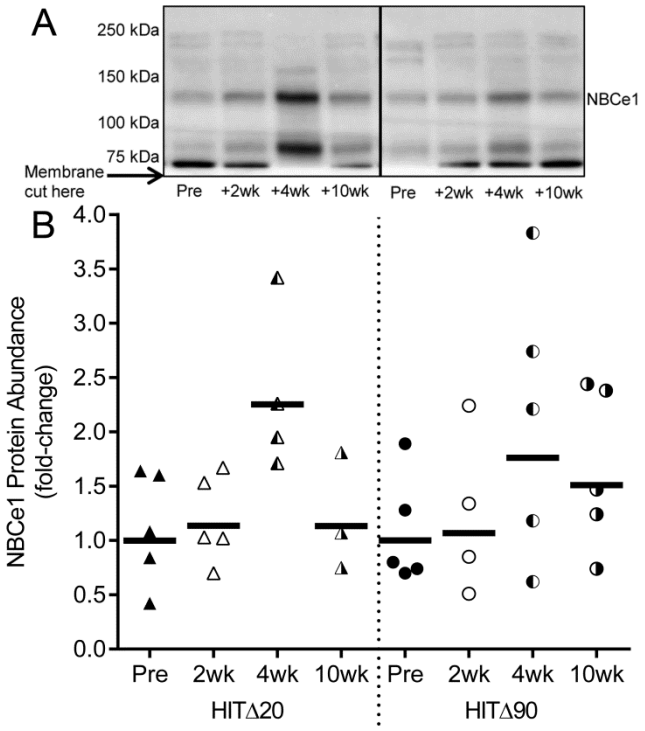


Figure 4.5 (A) Representative immunoblot and (B) NBCe1 protein abundance before (Pre) and after 2 weeks (+2 wk) and 4 weeks (+4 wk) of high-intensity interval training (HIT), and 6 weeks after stopping HIT (+10 wk), for the HITΔ20 and HITΔ90 training groups. Individual data points and geometric means (horizontal bars) are plotted. Post-training data are calculated as fold-change relative to the corresponding Pre datum. To illustrate variance Pre, individual Pre data are shown relative to the Pre geometric mean for the respective groups. Non-adjacent lanes from the same blot are indicated by a vertical line. CV of fold-change data for duplicate gels was 24%.

4.3.2.6 CAII

The CAII antibody recognised a single band just above 25 kDa (predicted molecular mass 29 kDa) (Figure 4.7). CAII content did not differ between the two groups (group×time interaction: $F_{3,37.7}$ = 0.54, *P* = 0.66, +4 wk ES: 0.15; −1.54 to 1.83). Overall, CAII increased after 4 weeks of training (time main effect: $F_{3,37.7}$ = 7.21, *P* = 0.001, pooled ES: 1.35; 0.55 to 2.15), but did not change after 2 weeks (pooled ES: −0.09; −0.60 to 0.41). A reduction in CAII content occurred 6 weeks after stopping HIT (pooled +10 wk ES: 0.15; −0.31 to 0.61).

4.3.2.7 CAIII

The CAIII antibody recognised a single band just above 25 kDa (predicted molecular mass 30 kDa) (Figure 4.7). There was no overall training effect for CAIII abundance (time main effect: $F_{3,37.6}$ = 0.46, *P* = 0.71, pooled +4 wk ES: 0.16; −0.35 to 0.67), but the two groups did respond differently to training (group×time interaction: $F_{3,37.6}$ = 3.03, *P* = 0.04). On comparing the change scores, the difference between groups was because of a small reduction in CAIII abundance at +2 wk for HITΔ90 of 0.86-fold ×/÷ 1.28, and a small increase for HITΔ20 of 1.09-fold ×/÷ 1.20 (ES: 1.01; 0.17 to 1.86). At +4 wk and +10 wk there was no difference between groups (+4 wk ES: −0.48; −1.54 to 0.57, +10 wk ES: −0.07; −0.79 to 0.64).

4.3.2.8 CAIV

There was low abundance of CAIV and consequently a poor signal-to-noise ratio. Multiple bands were detected, but the antibody recognised a single or double band at the predicted molecular mass of 35 kDa in the muscle homogenate and positive control (293T Cell Transient Overexpression Lysate, Abnova, Taipei, Taiwan). Similar to earlier reports (485), a strong band at ~75 kDa was recognised in the muscle homogenate but not the positive control (Figure 4.6). To test whether this band was a CAIV dimer, different concentrations of DTT (0.5 mM to 10 mM) were added to the homogenate in an attempt to break potential disulphide bonds. A similar approach proved successful in increasing CAIX monomer expression at the expense of dimer expression in recombinant proteins (218), but for the present experiment, additional DTT had no effect on the 75 kDa band (Figure 4.6). Therefore, only the 35 kDa band(s) were quantified (Figure 4.7).

CAIV content did not differ between the two groups (group×time interaction: $F_{3,28.3}$ = 0.74, *P* = 0.54, +4 wk ES: 0.20; −0.63 to 1.03). There were unclear changes in response to training (time main effect: $F_{3,28.3}$ = 1.72, *P* = 0.19). On pooling the data, protein content decreased at +2 wk (pooled ES: −0.30; −0.55 to −0.05), but was no different to baseline at +4 wk (pooled ES: 0.16; −0.23 to 0.55) and +10 wk (pooled ES: −0.15; −0.62 to 0.32).

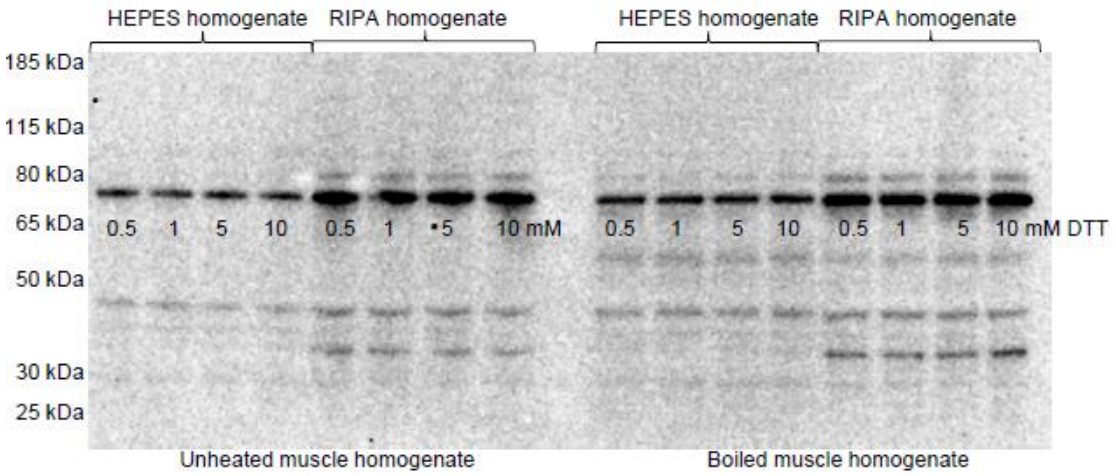


Figure 4.6 Effect of increasing concentration of DL-dithiothreitol (DTT) on bands detected by the CAIV antibody. To test whether a ~75 kDa band detected was a dimer of CAIV, different concentrations of DTT (0.5 to 10 mM) were added to RIPA or HEPES-sucrose buffered muscle homogenate in an attempt to break potential disulphide bonds. The homogenate was either unheated or boiled at 95°C for 5 min. See text for additional details on immunoblotting.

4.3.2.9 CAXIV

The CAXIV antibody recognised a single band below 50 kDa in the muscle homogenate (predicted molecular mass 38 kDa) and a positive control (293T Cell Transient Overexpression Lysate, Abnova) (Figure 4.7). CAXIV abundance did not differ between the two groups (group×time interaction: $F_{3,41.3}$ = 0.29, P = 0.83, +4 wk ES: 0.32; −0.30 to 0.94). Protein abundance increased for both groups in response to training (time main effect: $F_{3,41.3}$ = 12.3, P < 0.001). On pooling the data, there was greater protein content at +2 wk (pooled ES: 0.59; 0.14 to 1.04) and at +4 wk (pooled ES: 0.46; 0.16 to 0.76). These adaptations were lost 6 weeks after stopping HIT (pooled +10 wk ES: −0.41; −1.00 to 0.18).

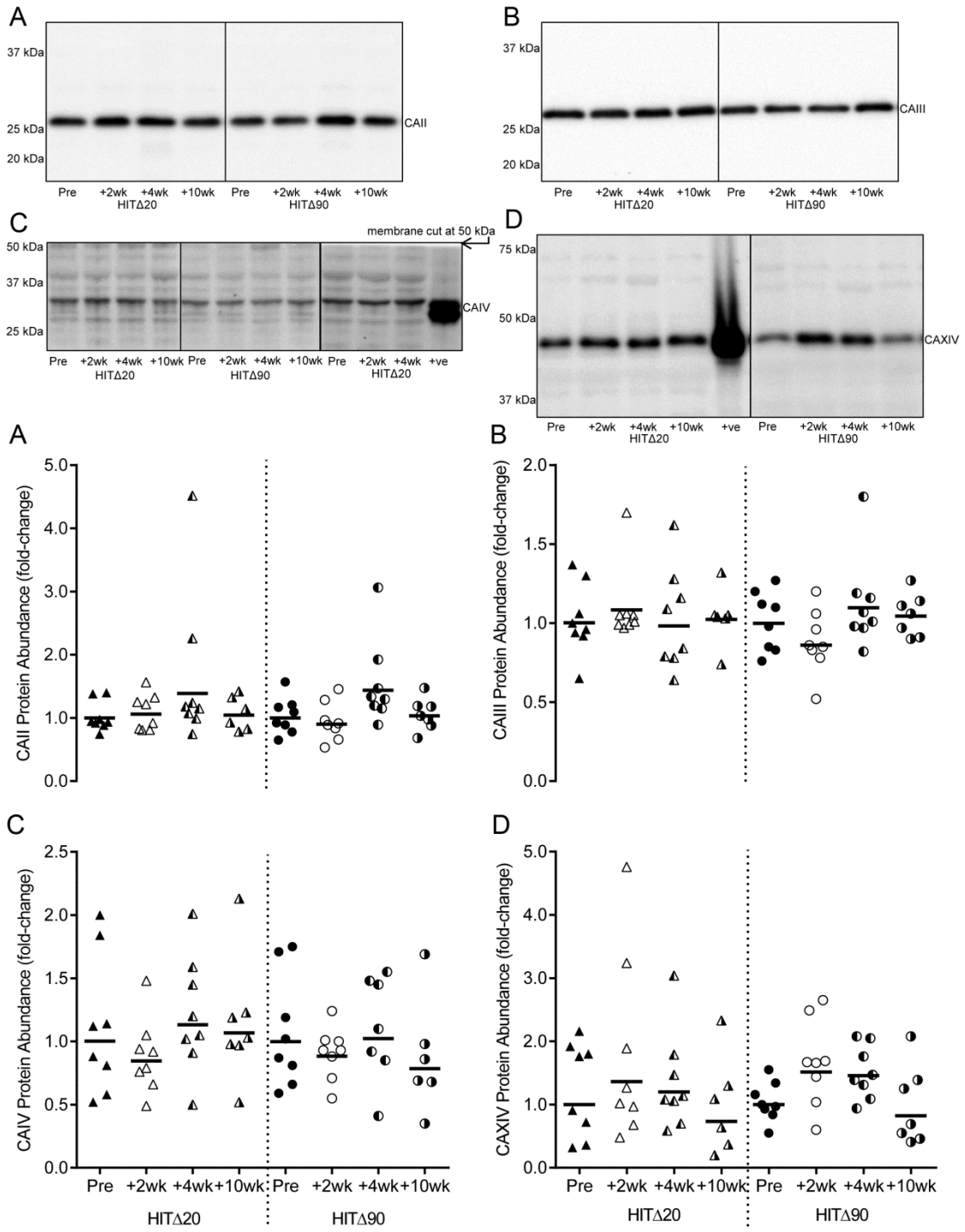


Figure 4.7 Representative immunoblots and protein abundance of (A) CAII, (B) CAIII, (C) CAIV, and (D) CAXIV before (Pre) and after 2 weeks (+2 wk) and 4 weeks (+4 wk) of high-intensity interval training (HIT), and 6 weeks after stopping HIT (+10 wk), for the HITΔ20 and HITΔ90 training groups. Individual data points and geometric means (horizontal bars) are plotted. Post-training data are calculated as fold-change relative to the corresponding Pre datum. To illustrate variance Pre, individual Pre data are shown relative to the Pre geometric mean for the respective groups. Positive controls (+ve) were loaded for CAIV and CAXIV. Non-adjacent lanes from the same blots (A, B) and from separate blots (C, D) are indicated by a vertical line. CV of fold-change data for two or three gel repeats: CAII (22%), CAIII (single gels), CAIV (single gels), and CAXIV (25%).

4.3.3 Muscle Buffer Capacity

$\beta m_{in\ vitro}$ differed little between groups (group×time interaction: $F_{3,44.2} = 0.48$, $P = 0.70$, +4 wk ES: 0.07; −0.64 to 0.78) (Figure 4.8). A change over time was seen ($F_{3,44.2} = 3.23$, $P = 0.03$), which, on pooling the data, indicated a small decrease following training at +4 wk (pooled ES: −0.41; −0.74 to −0.07). The pooled mean decrease in $\beta m_{in\ vitro}$ at +4 wk of −5.7 mmol H^+•kg dm^{-1}•pH^{-1} (90% CI −10.5 to −1.0) was less than the typical error of measurement of 9.8 mmol H^+•kg dm^{-1}•pH^{-1}. At +10 wk there was no clear difference in $\beta m_{in\ vitro}$ compared to Pre (pooled ES: −0.50; −1.18 to 0.18).

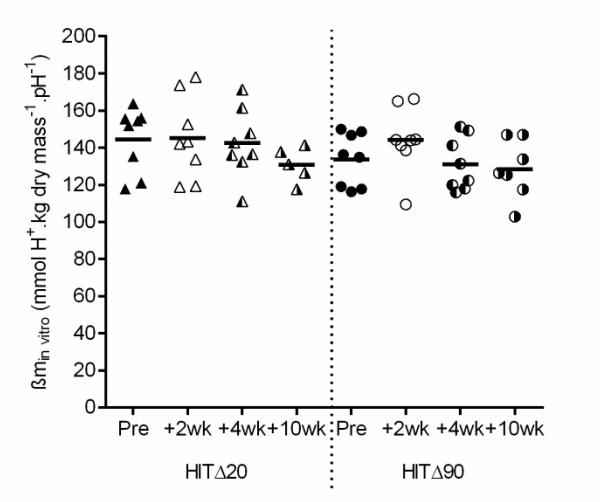


Figure 4.8 Non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) before (Pre) and after 2 weeks (+2 wk) and 4 weeks (+4 wk) of high-intensity interval training (HIT), and 6 weeks after stopping HIT (+10 wk), for the HITΔ20 and HITΔ90 training groups. Individual data points and means (horizontal bars) are plotted.

4.3.4 Repeated-Sprint Ability

Total work performed during the RSA test did not change in response to training (time main effect: $F_{2,17.8} = 0.97$, $P = 0.40$, +4 wk pooled ES: −0.04; −0.23 to 0.15) (Figure 4.9). There was no difference between the two groups for total work (group×time interaction: $F_{2,17.8} = 0.57$, $P = 0.58$, +4 wk ES: 0.19; −0.20 to 0.58); nor were there any meaningful differences comparing the change in work performed during each individual sprint after 4 weeks of HIT (Figure 4.10). There were also no group differences for the change in work decrement in response to training (group×time interaction: $F_{2,24.5} = 1.10$, $P = 0.36$, +4 wk ES: 0.42; −0.39 to 1.22) (Figure 4.9). Both groups improved similarly, with less work decrement after 4 weeks of HIT (time main effect: $F_{2,24.5} = 7.36$, $P = 0.003$). On pooling the data there was a small reduction in work decrement seen at +4 wk (pooled +4 wk ES: 0.59; 0.20 to 0.98), which was lost 6 weeks after stopping HIT (pooled +10 wk ES: 0.15; −0.29 to 0.59).

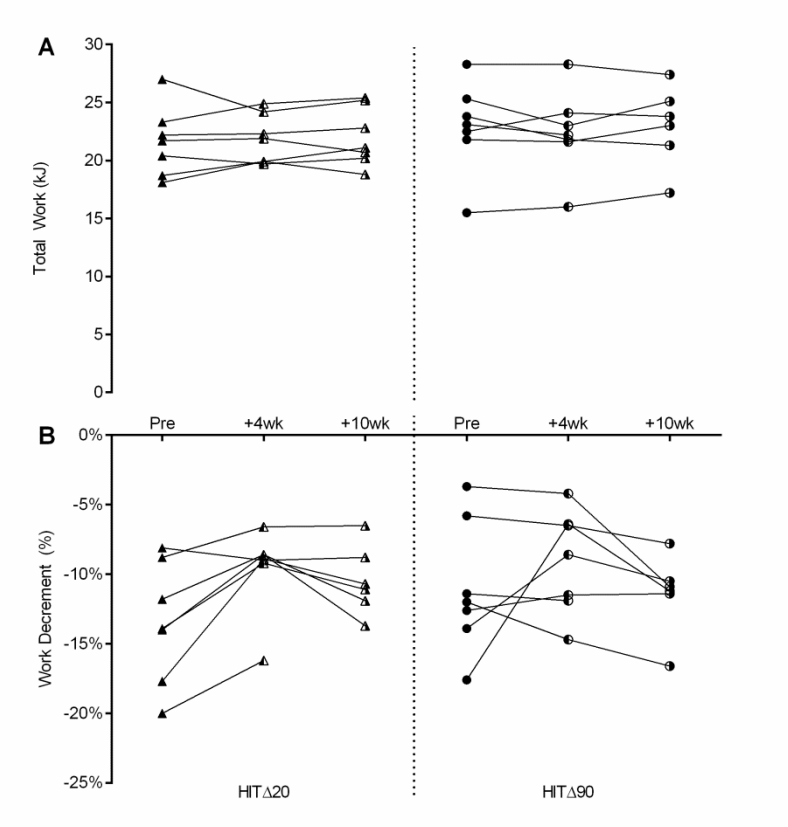


Figure 4.9 (A) Total work and (B) work decrement during a repeated-sprint ability test before (Pre) and after 4 weeks (+4 wk) of high-intensity interval training (HIT), and 6 weeks after stopping HIT (+10 wk), for the HITΔ20 and HITΔ90 training groups. Individual data points and means (horizontal bars) are plotted.

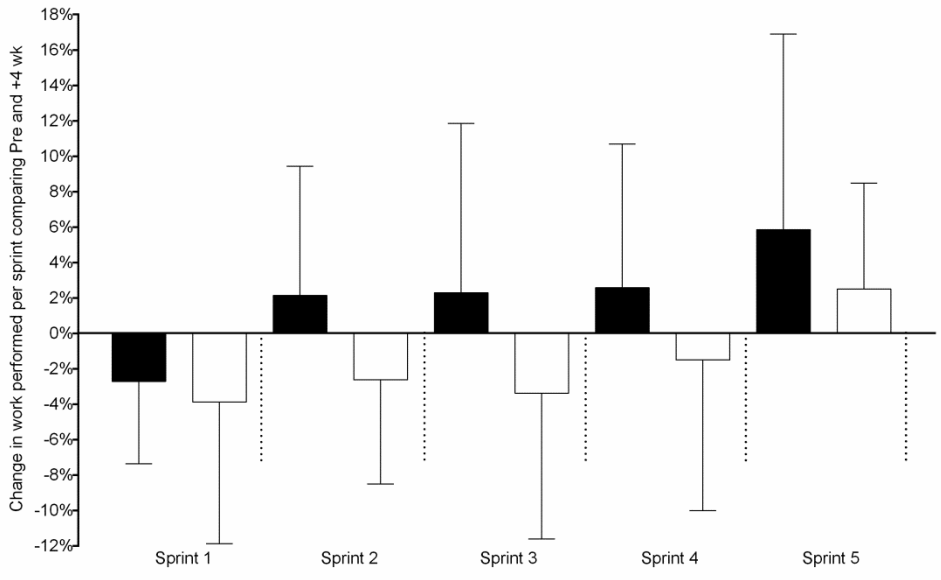


Figure 4.10 Comparison of the change in work performed per individual sprint during a repeated-sprint ability test before (Pre) and after 4 weeks (+4 wk) of high-intensity interval training for HITΔ20 (black) and HITΔ90 (white). Data are mean (SD) of the change from Pre to +4 wk.

4.3.5 Lactate Threshold and Peak Aerobic Power

Overall there was no meaningful increase in the LT after 4 weeks of training (time main effect: $F_{1,16}$ = 7.5, P = 0.02, pooled ES: 0.28; 0.06 to 0.49) (Figure 4.11). On comparing the two groups, the LT only increased for the HITΔ20 group (group×time interaction: $F_{1,16}$ = 5.85, P = 0.03, ES: 0.49; 0.11 to 0.87). There was no difference between the two groups in W_{peak} (group×time interaction: $F_{1,16} = 3.7 \times 10^{-4}$, P = 0.99, ES: 0.003; −0.30 to 0.31) (Figure 4.11). On pooling the data there was no meaningful change in W_{peak} in response to training (time main effect: $F_{1,16}$ = 6.51, P = 0.02, pooled ES: 0.20; 0.06 to 0.35).

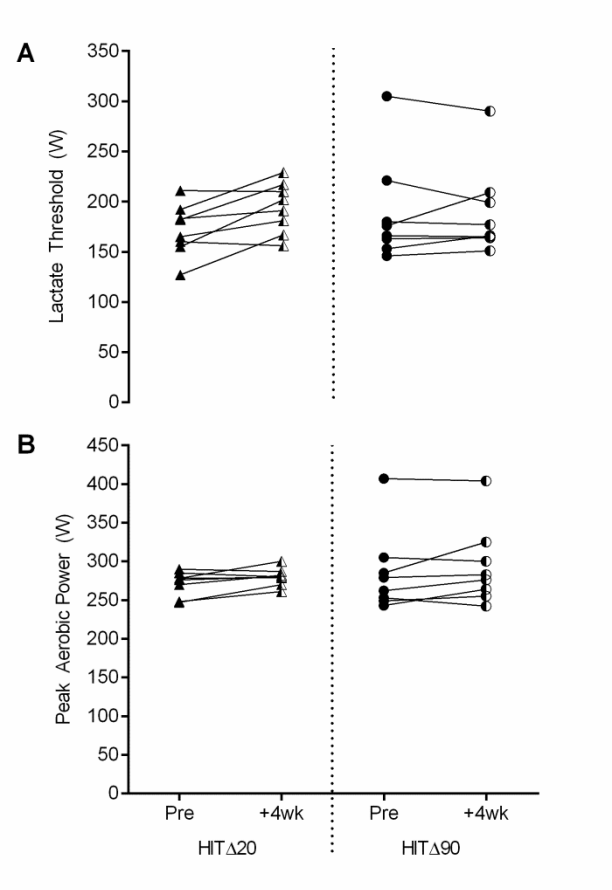


Figure 4.11 (A) Lactate threshold and (B) peak aerobic power during a graded-exercise test before (Pre) and after 4 weeks (+4 wk) of high-intensity interval training for the HITΔ20 and HITΔ90 training groups. Individual data points and means (horizontal bars) are plotted.

4.3.6 Peak Oxygen Uptake

There was no difference in $\dot{V}O_{2peak}$ between the two groups (group×time interaction: $F_{1,15}$ = 1.06, P = 0.32, ES: 0.27; −0.24 to 0.78), and there was no mean change following training (time main effect: $F_{1,15}$ = 0.007, P = 0.93, pooled ES: 0; −0.25 to 0.25) (Figure 4.12).

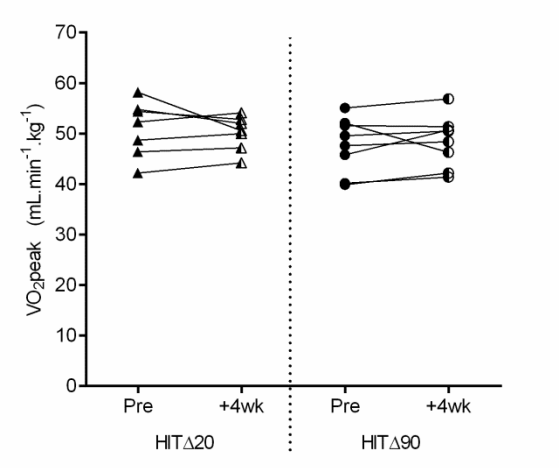


Figure 4.12 Peak oxygen uptake before (Pre) and after 4 weeks (+4 wk) of high-intensity interval training (HIT) for the HITΔ20 and HITΔ90 training groups. Individual data points and means (horizontal bars) are plotted.

4.4 Discussion

This study provides the first comprehensive investigation of the response to exercise training of the known skeletal muscle acid/base transport proteins. For the first time in humans, training-induced changes have been reported for the MCT chaperone protein basigin, NBCe1, and each of the skeletal muscle CA isozymes. The major finding is that the abundance of most proteins was increased following 4 weeks of HIT, but adaptations were typically not different between the two training intensities employed in this study. The exceptions were MCT4, whereby a mean increase in content was only found when a greater number of intervals were performed just above the LT, and basigin, where, in contrast, only intervals performed at an intensity close to W_{peak} were effective in eliciting adaptations. Regardless of training type, six sessions of HIT performed over the first two weeks provided an insufficient stimulus to provoke upregulation of most of the pH_i-regulatory proteins or of $\beta m_{in\ vitro}$. Furthermore, the transience of adaptations for most of the measured proteins was apparent from a detraining response six weeks after removing the physiological stress of HIT.

Despite being work-matched, after 4 weeks of training MCT4 increased for HITΔ20 (1.38-fold ×/÷ 1.32) but not HITΔ90 (1.05-fold ×/÷ 1.12). Increased MCT4 content has seldom been found to date, with the magnitude in those studies reporting a change less than 1.5-fold

(79, 367, 373). Therefore, the present data are consistent with some (79, 367, 373), but not most modalities of HIT (18, 40, 47, 150, 186, 187, 232, 324, 380, 433), inducing increases in MCT4. One proposed stimulus for upregulation of MCT4 is an increase in H^+ and/or La^- flux (466). Thus, a possible explanation for the greater increase for HITΔ20 may relate to a higher non-mitochondrial ATP turnover throughout this form of training. The initial high energetic cost of 2-min intervals performed at 98–104% of W_{peak} during the HITΔ90 protocol would have reduced pH_i (142), potentially inhibiting glycolysis and causing a reduction in La^-/H^+ production for sequential intervals (221, 363, 379, 444), and subsequently a reduction in La^-/H^+ efflux from the muscle. It can be hypothesised that intervals performed just above the LT in the HITΔ20 would have a consistently higher contribution from substrate level phosphorylation and therefore greater La^-/H^+ production, in part because of a lower initial reduction in pH_i, and lesser inactivation of Phos *a* and PFK activity (363, 444). Allosteric activation of PFK is apparent *in vivo* until very large decreases in pH (132), suggesting inhibition of glycolysis would not occur with the smaller reductions in pH_i likely with HITΔ20 – pH 6.84 (0.09) after the 7th interval of session 12 (data from Chap 3)[116]. Ultimately, the intensity and volume of intervals performed above the LT during HITΔ20 proved effective in provoking upregulation of MCT4 protein content, and this may have related to a high H^+/La^- production and efflux during exercise. However, direct evidence is required to confirm this hypothesis.

In contrast to MCT4, the large and variable increases in MCT1 abundance for both groups indicates that the intensity at which HIT was performed between the LT and W_{peak} did not influence upregulation, supporting previous research indicating that MCT1 and MCT4 have distinct signalling stimuli (58, 206). The magnitude of the increase for both groups at +4 wk was greater than has been reported to date, 2.48-fold ×/÷ 2.24 and 2.33-fold ×/÷ 1.69, for HITΔ20 and HITΔ90 respectively[117]. The modest increases at +2 wk for both groups indicate that the volume of HIT performed was important. MCT1 has previously demonstrated high plasticity in response to training intensities ranging from 7 consecutive days of continuous training at 60% $\dot{V}O_{2max}$ (56), to repeated 30-s sprint-interval training (SIT) (79, 327, 380), and repeated 6-s sprint training (RST) (327). The SIT and RST groups in the latter study showed similar improvements in MCT1 abundance despite training inducing greater increases in muscle $[H^+]$ and $[La^-]$ in the SIT group. Likewise, Hashimoto *et al.* (206) reported MCT1 expression in L6 cells to be induced by incubation in 10 mM and 20 mM $[La^-]$, with no meaningful difference between the different $[La^-]$ conditions. Therefore, the improvements for HITΔ20 and HITΔ90 support the existing data, which suggest exercise performed at progressively higher intensities above the LT, and resulting in greater La^-/H^+ accumulation, do not lead to greater increases in MCT1 abundance.

[116] There are no similar data for a HITΔ90 training session.

[117] These data are geometric means. The respective arithmetic means, which are more typically reported, were 3.4-fold and 2.6-fold for HITΔ20 and HITΔ90.

As a chaperone protein, basigin not only targets MCT1 and MCT4 to the membrane, but also maintains activity of these transporters (192). Despite its crucial function in La^-/H^+ transport, there has been only one report of basigin in human muscle (129), and none investigating the training response. Of the four known basigin isoforms, basigin-1 and basigin-4 are retina-specific (289), while basigin-2 and basigin-3 have predicted molecular masses of 42 and 19 kDa respectively (464). However, with three *N*-glycosylation sites there are a range of potential apparent molecular masses (238, 289, 340, 463). mRNA data has previously shown the 42 kDa canonical basigin-2 isoform to be the most highly-expressed human skeletal muscle isoform (289). In detecting a 55 kDa band using a goat polyclonal antibody, it is unclear which isoform of basigin was previously reported (129). Using the same antibody in the current study produced unclear data because of poor antibody-antigen affinity (blots 1B and 2B in Figure 4.3). To identify the specific basigin isoform, duplicate immunoblots were separately probed with two monoclonal mouse basigin antibodies, and then reprobed with the polyclonal goat basigin antibody, producing in all cases the same band above 37 kDa in muscle homogenate (Figure 4.3). Therefore, it is probable that the basigin-2 isoform was detected here.

Following this validation, quantification of the > 37 kDa band has shown for the first time that basigin can be upregulated with exercise training. Basigin abundance increased in response to training for HITΔ90 (1.18-fold ×/÷ 1.40) but not HITΔ20 (0.86-fold ×/÷ 1.41) at +4 wk, indicating that training intensity influenced changes. It is likely that there was higher La^- accumulation in the first intervals following HITΔ90 than HITΔ20, though La^-/H^+ production was probably greater throughout HITΔ20 because of the longer duration of exercise above the LT. This is consistent with previous research in L6 cells reporting an increasing dose-response upregulation of basigin protein expression following 1 h of incubation with 10 mM and 20 mM [La^-] (206). In contrast to MCT4, a high [La^-] rather than high sustained La^-/H^+ production may be necessary for basigin upregulation. Thus, it appears that while La^- accumulation is a stimulus for both basigin and MCT1, the threshold for provoking changes in basigin might be higher than for MCT1. More data is needed to test this supposition.

NHE1 abundance increased after 4 weeks of HIT by 1.65-fold ×/÷ 1.57 and 1.67-fold ×/÷ 1.30, for HITΔ20 and HITΔ90 respectively. No change was found after two weeks of training, indicating that 6 sessions of HIT provided insufficient stimulus to induce upregulation. Despite the different bioenergetic costs of exercise, training performed either above the LT or close to W_{peak} was sufficient to provoke similar adaptations in NHE1 after 4 weeks. Though high-intensity training has been shown to be important for the upregulation of NHE1 in humans (187, 232, 433) and rodents (249, 251), the present data suggest that any high-intensity training performed between the LT and W_{peak} may provide sufficient stimulus. It is difficult to make a direct comparison with data from other studies that have used different immunoblotting systems, especially given that the present study reports protein abundance relative to a

calibration curve of internal standards for each gel (336), whereas signal intensity in absorbance units has previously been reported. Using a calibration curve it was clear that NHE1 protein content, measured with the same antibody as previous studies, was almost directly proportional to chemiluminescent signal intensity over a narrow dynamic linear range of protein content. The magnitude of the training response here was greater than the modest and sometimes unclear increases that have been reported to date (1.15–1.35-fold) (232, 253, 327, 433). Given that some other studies have found little or no change with 6–9 weeks of different modalities of HIT (18, 186), it is probable that training status determines how readily upregulation of NHE1 abundance is stimulated (18). If high-intensity training is already a regular component of training, further increases in NHE1 content may be more difficult to achieve in the short term.

For the first time, this study has identified NBCe1 in human skeletal muscle using an isoform-specific antibody raised against the C-terminus of human NBCe1. Previous studies in human muscle have used a polyclonal antibody raised against a 54 amino acid sequence of the N-terminus of the NBCe1-A isoform (252, 271). A BLAST alignment (429) of this sequence of NBCe1-A[118] and four other putative skeletal muscle NCBTs (NBCe2, NBCn1, NBCn2, NDCBE) produced sequence identities ranging from 49% to 57% (Table 2.1). Given that the NCBTs have relatively similar predicted molecular masses between 116 and 140 kDa (360), previous immunoblotting data with this N-terminus antibody could not discriminate between isoforms. In addition, the response to training of any of the NCBT proteins has never been reported in humans before now. Despite the high individual variability and low sample sizes, from this preliminary evidence it seems that NBCe1 can be upregulated following HIT. Protein content increased similarly for HITΔ20 and HITΔ90, 2.25-fold $\times/\div$ 1.35 and 1.76-fold $\times/\div$ 2.06 respectively, suggesting that NBCe1 is not sensitive to moderate differences in training intensities between the LT and W_{peak}. The distinct bioenergetic status of the myocyte during different intensities of HIT, such as varying La^-/H^+ flux, may not be critical factors in provoking adaptations in NBCe1 content. The only other study to date that has investigated the NBC response to training, reported similar improvements in NBC content of the *soleus* membrane fraction after 5 weeks of HIT in rats receiving either sodium bicarbonate supplementation or a placebo prior to each training session (466). Sodium bicarbonate enhances H^+ efflux through increasing extracellular pH, with the resultant greater pH gradient relative to the intracellular space increasing transport activity (297, 397). These data seem to support the present contention that H^+ flux does not influence upregulation of NBC(e1) content.

Previous studies have shown that the skeletal muscle CA isozymes differentially respond to 8 weeks of hypoxia (252), or to a single bout of supramaximal exercise (319). However, no study has reported the response of any of the four cytosolic (CAII and CAIII) and

[118] NBCe1-B (121 kDa) is the canonical, widely express variant, and is probably the primary skeletal muscle isoform, whereas NBCe1-A (116 kDa) is found predominantly in the kidney (276, 396).

sarcolemmal (CAIV and CAXIV) isozymes to a training intervention. For the first time this study has demonstrated that the CAs can be upregulated following a short-term HIT intervention, though the intensity at which HIT was performed did not affect adaptations. Despite high individual variability, mean abundance of both CAII and CAXIV was found to increase following four weeks of HIT in both groups, but CAIII and CAIV changed little. While the relative importance of these changes *in vivo* is uncertain, *in vitro* research suggests there may be enhanced acid/base transport and intra/extracellular buffering (27). The extracellular facing sarcolemmal CAs catalyse buffering of H^+ in the interstitium by HCO_3^- (228), thereby maintaining a pH gradient relative to the intracellular space necessary for high MCT activity (172). Increasingly, evidence is showing that the role of the CA isozymes in pH regulation is not simply limited to a catalytic function in reversible hydration of CO_2. Despite some doubt about whether direct binding of CAs and acid/base transport protein occurs (65, 369), convincing *in vitro* data using animal and cell models indicate an important functional interaction between CAII, CAIV and MCT1/4 (22, 24-26, 455); between NBCe1 and CAII, CAIII, and CAIV (23, 422, 481); and possibly also between CAII, CAIV and NHE1 (286, 506). Greatest enhancement of transport activity by the CAs would be from increases in abundance of both cytosolic and sarcolemmal isozymes, as has been seen in this study with CAII and CAXIV. The extent to which transport activity is enhanced by CA protein content remains uncertain at this time, and given that there were no difference between the two training groups, the stimulus for CA upregulation requires further investigation.

$\beta m_{in\ vitro}$ decreased slightly for both groups following training, although the reduction was less than the typical error of measurement for the titration assay (9.8 mmol $H^+ \bullet kg\ dm^{-1} \bullet pH^{-1}$). Improved $\beta m_{in\ vitro}$ has previously been found following similar 2-min HIT intervals with short rest periods (48, 139, 140), but not when performed at 100% of $\dot{V}O_{2max}$ (47). Contrary to these data, and to the hypothesis of the present study, the failure to elicit increases in $\beta m_{in\ vitro}$ following two HIT protocols with distinct energetic costs suggests that training intensity above the LT does not influence adaptations. In addition, the ecological validity of the titration technique as a measure of muscle buffer capacity remains in doubt (see Chapter 3). The assay is a measure of the non-bicarbonate buffer capacity of homogenate that is a mixture of intracellular buffers and some extracellular proteins. Moreover, the homogenisation process is known to cause phosphagen hydrolysis (1, 306, 442), with inconsistencies in the phosphate pool of each sample introducing artefact into the measurement (200). It is possible that training adaptations reported to date for $\beta m_{in\ vitro}$ are indicative of methodological between-sample variance rather than physiological improvements in muscle buffer capacity. Methodological improvement, such as revisiting earlier attempts to inhibit phosphagen hydrolysis (375, 442), and subsequent validation of the technique, may be required.

Overall, mean aerobic capacity increased slightly, or not at all. There was no change in mean $\dot{V}O_{2peak}$ for either group following training. This is in contrast to the mean effect of HIT on $\dot{V}O_{2max}$ of 0.5 L•min^{-1} (95% CI: 0.43 to 0.60 L•min^{-1}) reported in a meta-analysis of similar populations (7). The meta-analysis did not include interventions of < 4 wk, but did note that studies with the lowest effect were typically of shorter duration. In the present study, it is likely that 12 sessions of HIT over four weeks was insufficient stimulus in active male participants to allow the primarily central adaptations necessary to improve $\dot{V}O_{2peak}$. Similarly, both groups demonstrated a lack of improvement in W_{peak}. While the group mean increases were small at ~3%, there were notable differences in the individual responses, ranging from −5 W to +23 W for HITΔ20, and −11 W to +40 W for HITΔ90. Meanwhile, mean increases in the LT were evident for HITΔ20 but not HITΔ90, 22 W (range: −5 to 47 W) and 1 W (range: −22 to 33 W), respectively. Once again the within-group differences demonstrated both high- and low-responders.

Previous research has shown that the ostensibly low or non-response to training holds for some, but not all measured variables in the same individuals (417, 470, 484). Similarly, in the present study some low-responders in, for example, W_{peak} or the LT, were comparatively high-responders to changes in specific target protein content. It remains unclear why there were low- and high-responders to training in both groups. The low-responders may have had initially high acid/base protein content, and by using a calibration curve of an internal standard on all gels as a reference, it was possible to compare protein content Pre to fold-change at +4 wk. Although a larger sample size would be required to robustly correlate the data, for some proteins, *viz*. NHE1, CAIII, and CAXIV, those with the highest protein content had the lowest response to training. And interestingly, the highest responders for one protein were typically the highest responders for other proteins. It is tempting to attribute high-response versus low-response to an initial comparatively untrained status, but while this is likely one deterministic factor, it does not sufficiently explain all of the variance in the data. It is highly probable that there are, as yet, unidentified genetic factors explaining some of the training response (300), but one unexplored possibility is that adaptations to training are random rather than primarily genetically determined. As has been suggested with individual response to drug treatment (424), rather than a certain percentage of participants responding to training 100% of the time, perhaps 100% of participants respond to training a certain percentage of the time. Only by repeating an intervention using the same participants after a sufficient washout period to facilitate detraining can this question be answered.

Consistent with the small changes in aerobic capacity, only modest improvements were seen in RSA, and there were no differences between groups. The large increases in acid/base transport protein abundance, but little or no change in performance, indicate a dissociation between pH_i regulation and exercise capacity. This supports previous data showing no change

in MCT1/4 or NHE1 abundance, despite improvements in sprint and endurance performance (18, 150, 433). The best evidence of improved RSA is an increase in total work performed and a reduction in percentage work decrement, i.e., greater fatigue resistance (160). Of the two, fatigue resistance is a less reliable measure of improved RSA than absolute work or power values (313, 356). The participants did show greater fatigue-resistance, but did not perform more work during the RSA test following four weeks of HIT. This was because less work was performed in the initial sprints post-training (Figure 4.10). Although a single criterion sprint was used prior to the 5 × 6-s sprints as a reference maximum, it is possible that a pacing strategy was maintained post-training. The latter sprints of an RSA test have previously been shown to have a greater $\dot{V}O_2$ than the first sprint (314). The failure to improve $\dot{V}O_{2peak}$ may have subsequently limited the capacity to sustain greater power for sequential sprints. Furthermore, previous studies showing improvements in RSA following similar HIT interventions have failed to control for the effects of cadence on power. Without fixing cadence it is not possible to discriminate changes in power output (or work) resulting from training adaptations, from the variation in maximal power dependent on the power-cadence relationship (170, 415). In the present study, an isokinetic protocol with cadence fixed at 115 rpm was employed to quantify changes in power and work due to training, independent of cadence. Therefore, the modest training adaptations achieved here may more accurately represent the efficacy of this type of HIT in improving RSA for an active male population. Further research is required to ascertain whether improvements in RSA can be achieved with just 4 weeks of HIT.

In addition to the training response, this study has shown that physiological detraining, characterised by a reduction in acid/base transport protein content, occurs within six weeks of removing the physiological stress of HIT. Notably, reversal of adaptations was not total for those proteins showing the largest training effect (i.e., MCT1 and NHE1). The present data support previous findings of detraining of MCT1 and MCT4 six weeks after repeated 30-s all-out sprints (79), as well as similar data in horses (266), and add to these data by showing rapid reversal of adaptations in basigin, NHE1, CAII, and CAXIV. The failure to elicit adaptations in $\beta m_{in\ vitro}$ means that it is not known whether a comparable rapid detraining would occur. Clearly, maintaining adaptations in pH_i-regulatory proteins requires the inclusion of some modality of high-intensity training. Given that sustaining weekly HIT session for consecutive macrocycles can lead to over-training (41), determining the minimum 'dose' of HIT to maintain adaptations, while avoiding adverse effects, will provide practical information in the future.

4.5 Conclusions

In summary, measurement of the adaptation to training of a comprehensive selection of proteins involved in pH_i regulation has been undertaken for the first time. Increased abundance

of most proteins was achieved after 4 weeks of HIT, but 6 sessions over the first 2 weeks provided an insufficient stimulus. It has also been shown that physiological detraining is rapid following the removal of a high-intensity training stimulus. Similar improvements in both groups indicate that, contrary to what was hypothesised, an ~40% difference in training intensity did not influence adaptations for most of the measured proteins. The exceptions were MCT4, which favourably adapted to a greater number of intervals performed just above the LT, rather than fewer intervals performed at or above W_{peak}, and basigin, which showed the opposite. It is likely that the differing energetic costs of training and subsequent variations in La^-/H^+ production and accumulation were important factors in explaining this response. The failure of either group to demonstrate improvements in $\beta m_{in\ vitro}$ suggests that, though improvements in $\beta m_{in\ vitro}$ can be achieved with HIT rather than moderate-intensity continuous training (140), the intensity at which HIT is performed, between the LT and W_{peak}, is not critical. Finally, the present study has shown only modest improvements, or none at all, in aerobic capacity and repeated-sprint ability following 4 weeks of HIT. Others have previously reported improvements in exercise capacity without parallel increases in H^+ transport protein abundance (18, 187, 468). It is clear from the present study that the converse is also true, increased protein abundance can be shown without any change in performance. Evidently there is a dissociation between protein abundance and performance, suggesting that the relative importance of the H^+ transporters in attenuating fatigue is small, at least for the specific performance tests used here.

Chapter 5 Influence of rest interval duration on adaptations in acid/base transport proteins, muscle buffer capacity, and repeated-sprint ability

5.1 Introduction

High-intensity exercise imposes a large proton (H^+) load on skeletal muscle through a high non-mitochondrial ATP turnover. Mitigation of reductions in intracellular pH (pH_i) entails uptake of H^+ by physicochemical buffers, such as histidyl-imidazole residues and inorganic phosphate (P_i) (81), and buffering of H^+ by metabolic reactions, in parallel with energy production (229). Acid/base transport across the sarcolemma by specific proteins provides an additional means to remove H^+ from the myocyte (254). To determine the importance of pH_i regulation for muscle function and exercise capacity, much research has focussed on the effects of exercise training on specific acid/base transporters and muscle buffer capacity (βm). Most of these studies have employed different modalities of high-intensity (HIT) or sprint interval training (SIT); however, equivocal findings to date indicate the stimuli required for upregulation of the individual components of pH_i regulation remain to be determined.

Increased abundance of the most important regulators of pH_i during high-intensity exercise (250), the monocarboxylate transport proteins (MCT1 and MCT4), has sometimes (40, 79, 327), but not always (18, 47, 187, 232), been reported following SIT or HIT. Apart from the data presented in Chapter 4, there are currently no other studies investigating the effect of SIT or HIT on the MCT1/4 chaperone protein basigin (CD147/EMMPRIN). Abundance of the known skeletal muscle sodium hydrogen exchanger isoform (NHE1) has been reported by one group to increase following some modalities of SIT or HIT (187, 232, 327, 433), although the same group have also found no change with ostensibly similar interventions (18, 468). In addition, the sodium-coupled bicarbonate transporter (NCBT) family of proteins may enhance βm through influx of bicarbonate (or equivalents) into the cell. Elevated sodium bicarbonate cotransporter (NBC) content (non-isoform specific antibody) has been shown in rats after 5 weeks of HIT (466), but similar NCBT data in humans have only been reported for the first time in Chapter 4. Furthermore, a functional, and perhaps physical interaction, has been demonstrated *in vitro* between each of these acid/base transport proteins and the skeletal muscle carbonic anhydrase (CA) isozymes (6, 23, 126, 269). Yet, no data currently exists on the response to SIT of any of the skeletal muscle CA isozymes – cytosolic CAII and CAIII, or sarcolemmal CAIV and CAXIV. Finally, non-bicarbonate physicochemical βm, measured by titration of homogenate ($\beta m_{in\ vitro}$), has occasionally been reported to increase in response to short-term HIT or SIT interventions (29, 48, 140, 496), but often there has been little change (8, 174, 196, 232, 349), or even a reduction in $\beta m_{in\ vitro}$ measured (47, 138).

Based on studies showing contrasting adaptations in acid/base transporter content and $\beta m_{in\ vitro}$ (232, 373), it is possible these components of pH_i regulation respond to separate

physiological stimuli. Additionally, studies reporting upregulation of some, but not all acid/base transporters (40, 187, 232, 433), indicate that there may be distinct stimuli for each of these proteins. Two factors proposed as being important are accumulation of intracellular La^-/H^+ (128, 161, 249, 495) and transmembrane flux of La^-/H^+ (466). If La^-/H^+ accumulation is important in provoking adaptations, then high-intensity work intervals, interspersed with short rest intervals, allowing insufficient time for pH_i and $[La^-]$ recovery, would be more favourable (495). Muscle pH recovers by ~50% in the first 4–6 min after single or multiple sprints (29, 53, 316), whereas pH has been shown to decrease even further in the first 90 s after a 30-s sprint (53). Therefore, shorter duration rest intervals will result in higher H^+ and La^- accumulation after sequential work intervals.

In contrast to their accumulation, transmembrane flux of La^-/H^+ is time-dependent, requiring sustained production of La^-/H^+, and their subsequent transport across the sarcolemma. An initial high-intensity work interval will produce a high La^- and H^+ concentration, but rest intervals less than 90 s, allowing limited pH_i recovery (53), may reduce glycogen phosphorylase (Phos) activity, while sustaining maximal pyruvate dehydrogenase (PDH_a) activity during subsequent intervals (363). Furthermore, PCr has been reported to be only 50% resynthesised 60 s after a single 30-s sprint (53), but to be almost full replenished after 4–6 min (53, 54, 90, 195). Consequently, shorter rest intervals will lead to reduced anaerobic glycolysis, greater oxidative phosphorylation, and thus lower La^-/H^+ production for sequential intervals. Therefore, to maximise La^-/H^+ production for each interval, and the subsequent transmembrane flux of La^-/H^+, requires longer rest intervals. This may then influence upregulation of the acid/base transporters and βm.

Given the uncertainty on how best to elicit improvements in pH_i-regulatory components, it is also not clear how detraining might be mitigated. Adaptations of H^+ transport proteins to training have been shown to be transient following cessation of HIT. In Chapter 4, almost total reversal of adaptations in all of the acid/base transporters was shown six weeks after completing 4 weeks of HIT. Reductions in MCT1 and MCT4 abundance have similarly been reported 6 weeks after stopping SIT, with a 20% reduction in MCT4 content evident after just 1 week (79). Furthermore, research suggests maintenance of other training-induced adaptations can be achieved, or dysregulation mitigated, by reducing the volume while maintaining the intensity of training (214, 215, 226, 227, 309, 427). It is probable that reversal of adaptations for some or all of the acid/base transport proteins can be similarly mitigated by a reduced volume of high-intensity training, but to date this hypothesis has not been tested.

The present study therefore compared two different recovery durations between 30-s intervals of work-matched SIT. The question addressed was whether manipulating the bioenergetic status of the muscle, through altering the duration of the rest interval, would differentially provoke upregulation of transmembrane acid/base transport protein abundance and

intracellular buffer capacity. Studies showing distinct upregulation of components of pH_i regulation indicate that they may respond to separate signalling stimuli (373). Based on these studies it was hypothesised that shorter rest intervals, which would be accompanied by greater La^-/H^+ accumulation, would be more favourable for upregulation of muscle buffer capacity. Conversely, acid/base transport protein abundance was hypothesised to improve more in response to longer rest intervals resulting in lower absolute La^-/H^+ accumulation, but greater La^-/H^+ production, and subsequently greater transmembrane La^-/H^+ flux. In addition, this study proposed that one day a week of SIT for 6 weeks, following a 4-week period of training 3 days per week, would be sufficient to maintain any potential adaptations in acid/base transport proteins and muscle buffer capacity.

5.2 Methods

5.2.1 Participants

Twenty-one recreationally-active women gave written informed consent to participate in this study. Six participants withdrew during the familiarisation period and one participant withdrew after the first SIT session. Fourteen participants completed the entire study [age: 24 (6) y; height: 164.6 (3.8) cm; mass: 62.9 (7.8) kg; mean (SD)]. Before the training intervention began, mean (SD) weekly training load was 1683 (1399) arbitrary units, calculated from session RPE and training duration (162). All procedures were approved by the Victoria University Human Research Ethics Committee.

5.2.2 Experimental Design

The study employed a two-group parallel design (Figure 5.1). Participants performed two familiarisation trials of the repeated-sprint ability (RSA) test on separate days (86, 307), and on another day they performed familiarisation trials of the graded-exercise (GXT) and peak oxygen uptake ($\dot{V}O_{2peak}$) tests. In the week preceding the 10-week post-training test, participants performed a re-familiarisation trial of the RSA test.

Following the pre-training tests, participants were ranked according to total work performed during the RSA test and allocated to one of two work-matched sprint interval training (SIT) groups, differing only in the duration of the rest component of the duty cycle. Rest interval duration was then randomly allocated to the groups – either 1-min rest intervals (Rest-1: $n = 7$) or 5-min rest intervals (Rest-5: $n = 7$).

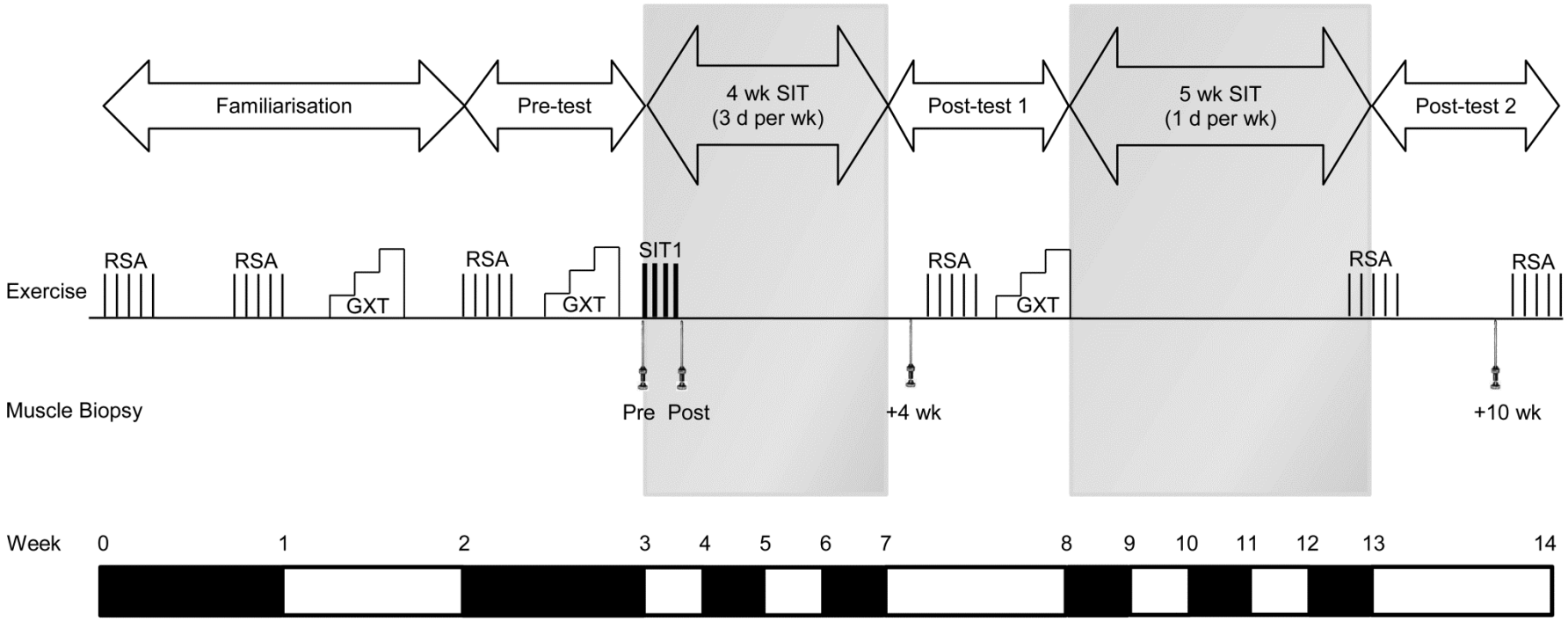


Figure 5.1 Experimental design. Abbreviations: GXT (graded-exercise test); RSA (repeated-sprint ability test); SIT1 (sprint-interval training session 1)

5.2.3 Single Bout of Sprint Interval Exercise

Short-term responses to a single bout of sprint-interval exercise were determined during the first training session (SIT1). The exercise bout consisted of 4 × 30-s sprints at 200% LT, with either 1 min or 5 min of rest between intervals as per group allocation. Muscle biopsies (see section 5.2.8) were taken at rest before exercise (Pre) and immediately following the final interval (Post).

Venous blood samples were taken at rest (before the muscle biopsy), and 2, 3, 5, 7, and 10 min after the final interval of the SIT session. A 22 g IV cannula was inserted into an antecubital vein. Before each sample the cannula was flushed with a small volume of sterile saline and then ~1 mL of blood was drawn into a dry syringe and discarded. Samples were taken with the participant recumbent on a plinth. Approximately 3 mL of blood was drawn into a heparinised syringe (Rapidlyte, Siemens Healthcare, Melbourne, Australia). Air was immediately removed from the sample by ejecting blood into the cap and carefully tapping the syringe. The syringe was then gently rolled to mix the sample thoroughly. Unless the sample was ready to be analysed without delay, the syringe was placed in an ice slurry to minimise changes in pH (146, 259), and subsequently analysed for blood pH (Rapidpoint 405, Siemens Healthcare, Melbourne, Australia). Prior to this, about 0.5 mL of blood was dispensed into a microtube and immediately analysed for lactate (2300 STAT Plus, YSI Inc., Yellow Springs, OH), or kept on ice for imminent analysis.

5.2.4 Repeated-Sprint Ability Test

The RSA test was performed seated on an electromagnetically-braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands) and followed a protocol modified from that previously reported (42, 44), comprising 5×6-s maximal sprints, separated by 24 s of rest. Modification entailed using an isokinetic protocol to negate the effects of cadence on power output (102, 170, 499) and thereby isolate the fatigue-related power component. All sprints were limited to a maximum cadence of 110 rpm. During the recovery period participants remained stationary. Five seconds before the start of each sprint participants assumed the ready position – crank of the dominant leg at an angle of 45°. Initially, participants performed a 5-min steady-state warm-up at 60 W, followed by 2 × 3-s practice sprints at 90% of perceived maximal effort, separated by 24 s of rest. A criterion 6-s maximal sprint was then performed 90 s later, followed by 5 min of rest prior to the RSA test. To minimise pacing participants were required to achieve ≥ 90% of their criterion score in the first sprint of the 5 × 6-s test. If this criterion was not achieved, participants rested for a further five minutes before restarting the test. Before the first practice sprint, the single 6-s sprint, and the first sprint of the 5×6-s test, participants accelerated to 110 rpm and stopped pedalling instantly, followed 24 s later by the

requisite sprint. This ensured similar velocity of the flywheel before each sprint. Consistent verbal encouragement was given throughout. Total work and work decrement were calculated from the raw data as has been previously reported (160).

5.2.5 Graded-Exercise Test

GXTs were performed pre- and post-training to determine the lactate threshold (LT) from venous blood samples (taken from an antecubital vein as described above). Peak aerobic power (W_{peak}) was also recorded. Venous blood samples were taken at rest and at the end of every stage during the GXT. Samples were drawn into a 3 mL dry syringe and aliquoted into a microtube for instant analysis of lactate (2300 STAT Plus, YSI Inc., Yellow Springs, OH). The tests were performed seated on the same ergometer used for the RSA test, employing an intermittent protocol, with 4-min exercise stages and 30-s rest stages. Beginning at 50 W, the power was subsequently increased by 25 W every 4.5 min and consistent verbal encouragement was provided for the latter stages. Participants were required to maintain a set cadence that was self-selected during the familiarisation trial and repeated for each of their subsequent GXTs. The test was terminated either volitionally by the participant, or by the assessors when the participant could no longer maintain the required cadence (± 10 rpm), despite strong verbal encouragement. The LT was identified as the power at which venous blood lactate increased by 1 mM above baseline (114), and was calculated using Lactate-E version 2.0 software (350). W_{peak} was calculated as previously reported (209, 273):

$$W_{peak} = W_{final} + \left(\frac{t}{240} \bullet 25\right)$$

where W_{final} was the power output of the last completed stage and *t* was the time in seconds of any final uncompleted stage.

5.2.6 Peak Oxygen Uptake Test

After the GXT, participants performed a 5-min active recovery at 20 W on the cycle ergometer, followed by a square-wave $\dot{V}O_{2peak}$ test. This comprised a steady-state cycle to volitional fatigue at a supramaximal power output, equating to 105% of W_{peak} achieved during the GXT. A similar protocol has previously been reported to elicit $\dot{V}O_{2peak}$ values no different to those determined during either a ramp incremental test performed 5 min previously (401), or a GXT performed 3 min previously (423). Participants were advised to accelerate to 90–100 rpm at the commencement of a 5-s countdown, and to maintain a high, but not fixed cadence until volitional fatigue. Consistent verbal encouragement was provided throughout. Expired gases were analysed every 15 s using a custom-made metabolic cart. A two-point calibration of the gas analysers (S-3lA/II and CD-3A analysers, Ametek, PA, USA) was performed before each

test using one certified gravimetric gas (16.1% O_2, 4.17% CO_2; BOC Gases, Chatswood, Australia) and ambient air. Ventilation was recorded every 15 s. The ventilometer (KL Engineering, Sunnyvale, CA, USA) was calibrated at the start of each day using a 3-L syringe (MedGraphics, St. Paul, MN). $\dot{V}O_{2peak}$ was calculated as the mean of the two highest consecutive 15-s values.

5.2.7 Sprint-Interval Training

The training intervention consisted of 10 weeks of supervised, work-matched SIT performed on an electromagnetically-braked cycle ergometer (Velotron, Racer-Mate, Seattle, WA). Participants trained three days per week for the first four weeks and, following one week of post-testing, they then trained one day per week for five weeks. The training intensity was initially set at 200% of the baseline LT (see Table 5.1), increasing to 305% of the baseline LT by week 4, which was equivalent to between 121 (14)% and 186 (17)% of baseline W_{peak} over the first 4 weeks. The 13th session training intensity was adjusted to 300% of the +4 wk LT, or where necessary to avoid a reduction in intensity, adjusted to up to 350% of the LT. This was equivalent to between 190 (13)% and 204 (14)% of the +4 wk W_{peak} during weeks 6 to 10. Both groups performed a standardised 5-min steady state warm-up at 60 W, followed by 4–10 × 30-s work intervals, interspersed with either 1 min (Rest-1) or 5 min (Rest-5) of passive recovery.

5.2.8 Needle Muscle Biopsies

Muscle biopsies were taken from the belly of the *vastus lateralis*, approximately halfway between the knee and hip, using the needle biopsy technique modified with suction (149). Subsequent samples were taken approximately 1 cm from a previous biopsy site. An incision was made under local anaesthesia (1% Xylocaine) and a muscle sample taken using a Bergström needle. Samples were blotted on filter paper to remove blood, before being immediately snap-frozen in liquid nitrogen and then stored at −80°C until subsequent analyses. Muscle samples were taken from the dominant leg before (Pre) and immediately after (Post) the first training session, 3 d after the twelfth training session (+4 wk), and 3 d after the seventeenth training session (+10 wk).

Table 5.1 Sprint-interval training programmes performed by the 1-min rest group (Rest-1) and 5-min rest group (Rest-5)

Week	Session	Intervals	Rest-1		Rest-5	
			Relative Intensity†	External Power (W)	Relative Intensity†	External Power (W)
1	1	4	200 (0)%	221 (33)	200 (0)%	223 (45)
	2	5	236 (25)%	262 (54)	236 (25)%	259 (32)
	3	6	250 (1)%	277 (41)	252 (5)%	280 (55)
2	4	7	252 (5)%	278 (39)	257 (10)%	285 (51)
	5	6	277 (5)%	306 (43)	273 (12)%	303 (52)
	6	8	279 (10)%	308 (41)	275 (13)%	304 (49)
3	7	9	291 (5)%	321 (45)	289 (13)%	319 (52)
	8	7	305 (6)%	337 (47)	301 (13)%	333 (57)
	9	9	305 (6)%	337 (47)	305 (17)%	336 (53)
4	10	10	305 (6)%	337 (47)	307 (22)%	339 (52)
	11	7	305 (6)%	337 (47)	308 (24)%	343 (56)
	12	6	305 (6)%	337 (47)	307 (22)%	339 (52)
Exercise performance tests and a muscle biopsy were performed in week 5						
6	13	6	300 (1)%	359 (42)	313 (22)%	347 (62)
7	14	8	309 (4)%	369 (44)	323 (22)%	358 (64)
8	15	7	319 (4)%	381 (45)	333 (22)%	369 (66)
9	16	9	323 (4)%	387 (46)	338 (22)%	375 (68)
10	17	6	323 (4)%	387 (46)	338 (22)%	375 (68)

†Relative intensity for weeks 1 to 4 was calculated as a percentage of the lactate threshold (LT) determined during the pre-training GXT. Relative intensity for weeks 6 to 10 was calculated as a percentage of the LT determined during the +4 wk GXT. Data are Mean (SD).

5.2.9 Muscle Buffer Capacity

Non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) was measured in duplicate on 2–3 mg dry mass (dm) of freeze-dried muscle samples using the titration technique (303). Samples were dissected free of visible blood and connective tissue (198), then homogenised on ice for 3 × 30 s in a 10 mM solution of the glycolytic inhibitor NaF (0.1 mL of NaF per 3 mg dm). pH measurements were performed at 37°C with a glass microelectrode (MI-410, Microelectrodes, Bedford, NH) connected to a pH meter (Lab 850, Schott Instruments GmbH, Mainz, Germany). After the initial pH measurement, homogenates were adjusted, if necessary, to pH 7.1–7.2 with 0.02 M NaOH, and then titrated to pH 6.1–6.2 with the serial addition of 2 µL of 0.01 M HCl. A linear regression line was plotted and the number of moles of H^+ per kg dm required to change pH from 7.1–6.5 interpolated. The typical error (within sample

standard deviation) for repeat titrations was 9.1 mmol H^+•kg dm^{-1}•pH^{-1}, equivalent to a CV of 6%.

Non-protein buffer capacity was also determined on the pre- and post-SIT1 samples using the titration technique (48, 361). Briefly, the homogenate was deproteinised with the addition of 0.03% 5-sulfosalicylic acid hydrate solution (100% w/v in 10 mM NaF), and centrifuged for 10 min at 1,000*g*. Following a similar procedure to above, the pH of the supernatant was adjusted to 7.1–7.2 with the minimum volume of strong concentration NaOH, and then titrated to pH 6.1–6.2 with the serial addition of 2 µL aliquots of 5 mM HCl. Non-protein $\beta m_{in\ vitro}$ was calculated as above, with the protein $\beta m_{in\ vitro}$ estimated from the difference between whole muscle and non-protein $\beta m_{in\ vitro}$. The typical error (within sample standard deviation) for repeat titrations was 5.4 mmol H^+•kg dm^{-1}•pH^{-1}, equivalent to a CV of 8%.

5.2.10 Quantitative Western Blotting

5.2.10.1 Muscle homogenate preparation

Approximately 30 mg of frozen muscle tissue was homogenised in a 1:20 dilution of ice-cold buffer (pH 7.4) containing: 0.15 M NaCl, 1% Triton-X100, 0.5% sodium deoxycholate, 0.05 M Tris, 0.1% SDS, 1 mM EDTA, 1X protease/phosphatase inhibitor cocktail (5872, Cell Signaling Technology, Danvers, MA, USA), and 1 mM phenylmethanesulfonyl fluoride (PMSF). Homogenates were rotated end-over-end for 60 min at 4°C and centrifuged twice at 15,000*g* for 10 min at 4°C. The supernatants were collected and the pellets discarded.

NBCe1 was assayed in muscle tissue homogenised in a HEPES-sucrose buffer (pH 7.4) containing: 210 mM sucrose, 30 mM HEPES, 40 mM NaCl, 2 mM EGTA, 5 mM EDTA, 1% protease inhibitor cocktail (P8340, Sigma-Aldrich, St. Louis, MI), 1% phosphatase inhibitor cocktail (P5726, Sigma-Aldrich), and 1 mM PMSF. Homogenates were otherwise prepared exactly as per the RIPA-buffered samples.

5.2.10.2 Protein Assay

Protein content of muscle homogenate was measured in triplicate using a Bradford assay (Bio-Rad protein assay dye reagent concentrate, Bio-Rad Laboratories, Hercules, CA) against serial dilutions of bovine serum albumin (BSA, A9647, Sigma-Aldrich) standards.

5.2.10.3 Immunoblotting

RIPA-buffered homogenate was diluted in 2X Laemmli buffer (0.125 M Tris, 4% SDS, 20% glycerol, 0.015% bromophenol blue, 10% 2-mercaptoethanol) and equal amounts of total

protein (1–25 µg)[119] were loaded in different wells on 10% or 12% Tris-Glycine-HCl SDS-PAGE gels. For CAIV detection, homogenate was loaded on Criterion 4–12% Bis-Tris SDS-PAGE gels (Bio-Rad). For NBCe1 detection, HEPES-sucrose buffered homogenate was diluted in 4X Laemmli buffer and 20 µg of protein was loaded in different wells on Criterion 4–12% Bis-Tris SDS-PAGE gels. Each sample for a participant was loaded on the same gel. Five or six different dilutions of a mixed-homogenate internal standard were also loaded on every gel and a calibration curve plotted of density against protein amount. From the subsequent linear regression equation, protein abundance was calculated from the measured band intensity for each sample on the gel (336). Coomassie blue (Phastgel Blue R-350, GE Healthcare, Rydalmere, Australia) staining of total protein was used as a loading control (491) and was similarly expressed relative to a calibration curve for each blot.

Gel electrophoresis ran for 60–80 min at 140 V for 10% gels and 4–12% gels, or 120–140 min at 100 V for 12% gels. Proteins were wet-transferred to a 0.2 µm polyvinylidene fluoride membrane at 100 V for 80 or 100 min. Membranes were blocked for 60 min at room temperature in 5% non-fat dry milk (NFDM) diluted in Tris-buffered saline with 0.1% Tween-20 (TBST). Membranes were then washed in TBST and incubated – either at room temperature for 2 h or overnight at 4°C – with the appropriate primary antibody diluted in 5% BSA and 0.02% NaN_3 in TBST, or in 5% NFDM in TBST for NBCe1 (see Table 5.2 for conditions). Following TBST washes the membranes were then incubated in the relevant secondary antibody, diluted in 5% NFDM in TBST, for 90 min at room temperature.

After further washes, membranes were incubated in chemiluminescent solution (1.25 mM luminol, 0.2 mM ρ-coumaric acid, 100 mM Tris pH 8.5, 0.009% H_2O_2) for 2 min and images were taken with a VersaDoc Imaging System (Bio-Rad, Hercules, CA) fitted with a CCD camera (Bio-Rad). Densitometry was performed with Image Lab 5.0 software (Bio-Rad) using the volume calculation (sum of all band intensities). Background correction was applied individually to each lane using a rolling-ball algorithm, with contrast and brightness adjustments applied homogenously to the entire image (171). Images are typically displayed with five bandwidths above and below the band of interest (344).

[119] CAIII proved to be highly abundant and had signal saturation on loading just 5 µg. Homogenate was therefore diluted 1:8 in RIPA buffer (including inhibitors) and 1 µg was loaded to detect this protein.

5.2.10.4 Antibodies

Table 5.2 Details of primary and secondary antibodies used for western blotting

Primary Antibody	**Supplier/ Catalogue no./ Lot no.**	**[Primary]**	**[Secondary]**
Rabbit polyclonal anti-MCT1	Merck Millipore/ AB3540P/ 2136555	1:1,000	1:15,000
Rabbit polyclonal anti-MCT4	Merck Millipore/ AB3316P/ 2397059	1:1,000	1:20,000
Mouse monoclonal anti-NHE1	Merck Millipore/ MAB3140/ 2283852	1:500	1:7,500
Rabbit polyclonal NBCe1	Cell Signaling/ 11867/ 0001	1:500	1:5,000
Mouse monoclonal anti-basigin	Santa Cruz/ sc-21746/ K1913	1:200	1:7,500
Rabbit polyclonal CAII	Santa Cruz/ sc-25596/ F0611	1:1,250	1:30,000
Mouse monoclonal CAIII	Abnova/ H00000761-M02/ 12243-S1	1:2,500	1:40,000
Mouse polyclonal CAIV	Abnova/ H00000762-B02P/ 08325 WULz	1:500	1:10,000
Mouse polyclonal CAXIV	Abnova/ H00023632-B01P/ 08358 WULz	1:750	1:10,000
Secondary Antibody	**Supplier/ Catalogue no.**		
Goat anti-mouse IgG	Perkin Elmer/ NEF822001EA		
Goat anti-rabbit IgG	Perkin Elmer/ NEF812001EA		

5.2.11 Statistical Analyses

To detect a moderate difference (Cohen's d = 0.8) in muscle buffer capacity using a mixed-model ANOVA at $\alpha = 0.05$ and $\beta = 0.2$ required $n = 8$ for each group. Measures of centrality and dispersion are means and standard deviations [mean (SD)]. To reduce bias from non-uniformity of error, data were log-transformed where heteroscedasticity was present (348), such as for western blot data. For these data, geometric mean (back-transformed mean of the log-transform) and back-transformed standard deviation are reported (geometric mean ×/÷ factor SD). Data were analysed using linear mixed models with, for example, 'time' (repeated-measure), 'group', and 'time×group' as fixed factors; and 'subject' and 'intercept' as random factors. Model fit was assessed by −2 log-likelihood (156). Uncertainty of effects are expressed as 90% confidence intervals (90% CI) and P values. The latter are presented as precise values unless $P < 0.001$ (118), and without arbitrarily defined significance thresholds (450). Effect sizes (ES) were assessed using Cohen's d, where ES thresholds were qualified as trivial < 0.2, small < 0.6, moderate < 1.2, large < 2.0, very large < 4.0, and extremely large ≥ 4.0 (225), and are reported as (ES; 90% CI) of the between-group difference (post − pre) scores. Effects were not considered meaningful if there was < 75% probability of being either substantially positive or substantially negative relative to the smallest worthwhile change (ES = 0.2), and were deemed unclear if there was a greater than 5% probability of being both

substantially positive and substantially negative (223, 225). Where no group differences were found, pooled (ES; 90% CI) of the within-group (time) difference scores are reported. Linear mixed models were analysed using IBM SPSS Statistics V21 (IBM Corporation, Somers, NY, USA) and effect sizes and confidence intervals were calculated using custom Excel spreadsheets (223).

5.3 Results

5.3.1 Training Data

Both training groups performed similar volumes of total work throughout the training intervention, 8.3 MJ and 8.1 MJ for Rest-1 and Rest-5 respectively. With only a single training session being missed by one participant, there was 99% training compliance.

5.3.2 First SIT Session (SIT1)

5.3.2.1 Muscle pH

Following SIT1, muscle pH decreased more for Rest-1 (Figure 5.2), 6.92 (0.11) to 6.53 (0.14), than Rest-5, 6.89 (0.13) to 6.63 (0.18) (group×time interaction: $F_{1,15}$ = 3.50, P = 0.08, ES: 1.07; −0.04 to 2.18).

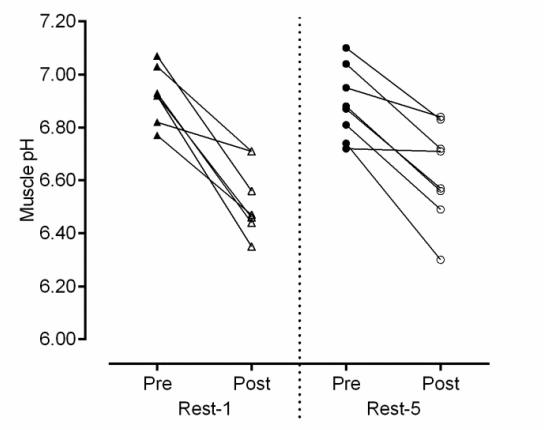


Figure 5.2 Muscle pH before (Pre) and immediately after (Post) a single bout of sprint-interval exercise for Rest-1 (triangles, n = 7) and Rest-5 (circles, n = 8). Individual data points are shown. CV of duplicate samples was 0.6%.

5.3.2.2 Venous Blood pH

Mean venous blood pH decreased from 7.36 (0.03) to 7.21 (0.07) following SIT1 (time main effect: $F_{5,68.4} = 34.1$, $P < 0.001$). There were no clear differences in pH between the two groups (group×time interaction: $F_{5,68.4} = 0.48$, $P = 0.79$), likely due to the high within-group variability (see Table 5.3 for magnitude and precision of effects).

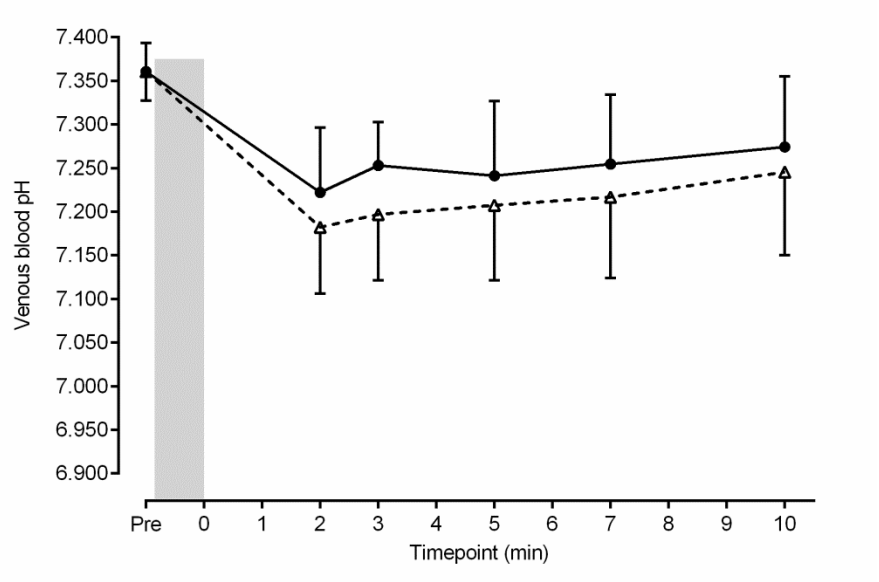


Figure 5.3 Venous blood pH following a single bout of sprint-interval exercise for Rest-1 (open triangles, *n* = 7) and Rest-5 (closed circles, *n* = 8). Samples were taken pre-exercise (Pre) and 2, 3, 5, 7, and 10 min post-exercise. Data are Mean (SD).

Table 5.3 Magnitude-based inferences for venous blood pH. Effect sizes and 90% confidence intervals are for the between-group difference scores (post − pre) at each timepoint following a single bout of sprint-interval exercise for Rest-1 and Rest-5.

Timepoint	Effect Size	90% CI	Likelihood of a meaningful difference between groups	
			Probability	Inference
+2 min	0.97	−1.16 to 3.10	74%	unclear
+3 min	1.47	−0.44 to 3.39	87%	unclear
+5 min	0.79	−1.66 to 3.24	66%	no meaningful difference
+7 min	0.92	−1.63 to 3.47	69%	no meaningful difference
+10 min	0.86	−1.53 to 3.24	68%	no meaningful difference

5.3.2.3 Venous Blood Lactate

Following SIT1, mean venous blood lactate concentration increased more for Rest-1 (group×time interaction: $F_{5,64.7} = 2.77$, $P = 0.025$). There were clear differences between the two groups at +5 min and +7 min (see Table 5.4 for magnitude of effects).

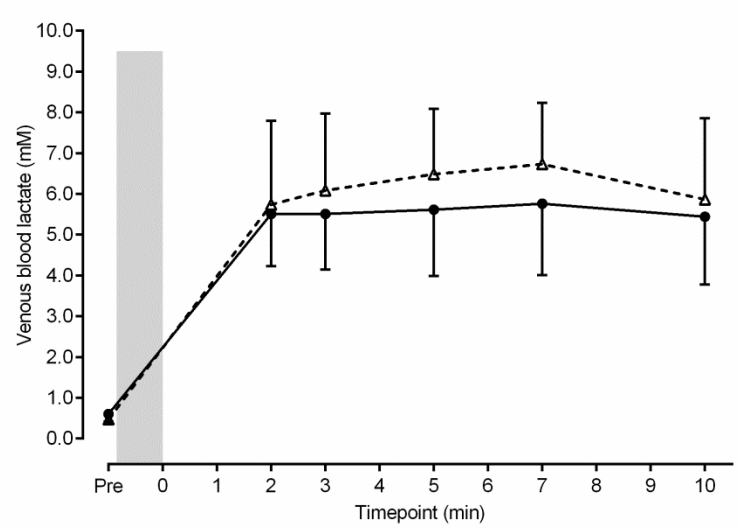


Figure 5.4 Venous blood lactate concentration following a single bout of sprint-interval exercise for Rest-1 (open triangles, *n* = 7) and Rest-5 (closed circles, *n* = 8). Samples were taken pre-exercise (Pre) and 2, 3, 5, 7, and 10 min post-exercise. Data are Mean (SD).

Table 5.4 Magnitude-based inferences for venous blood lactate concentrations. Effect sizes and 90% confidence intervals are for the between-group difference scores (post − pre) at each timepoint following a single bout of sprint-interval exercise for Rest-1 and Rest-5. Data were log-transformed because of non-uniformity of error.

Timepoint	Effect Size	90% CI	Likelihood of a meaningful difference between groups	
			Probability	Inference
+2 min	0.76	−0.62 to 2.14	76%	unclear
+3 min	0.97	−0.31 to 2.25	85%	unclear
+5 min	1.08	−0.03 to 2.20	91%	Rest-1 greater
+7 min	1.24	0.07 to 2.40	93%	Rest-1 greater
+10 min	0.84	−0.47 to 2.15	80%	unclear

5.3.2.4 Muscle Buffer Capacity

Mean $\beta m_{in\ vitro}$ did not change for either group following SIT1 (time main effect: $F_{1,15}$ = 0.008, P = 0.93, pooled ES: −0.03; −0.68 to 0.62). $\beta m_{in\ vitro}$ was lower Pre for Rest-5 than Rest-1, 146.7 (11.4) and 134.8 (9.5) mmol H^+•kg dm^{-1}•pH^{-1}, respectively (group main effect: $F_{1,15}$ = 14.4, P = 0.002, Pre ES: 1.06; 0.19 to 1.94). There was also no change in mean non-protein $\beta m_{in\ vitro}$ after SIT1 (time main effect: $F_{1,15}$ = 0.66, P = 0.43, pooled ES: 0.29; −0.34 to 0.92); nor was there a meaningful difference between groups (group main effect: $F_{1,15}$ = 1.92, P = 0.19, ES: 0.48; −0.88 to 1.84). Mean non-protein $\beta m_{in\ vitro}$ was 44% of total $\beta m_{in\ vitro}$ at rest, 62.6 (8.0) mmol H^+•kg dm^{-1}•pH^{-1}, with individual values ranging from 34% to 59% (see Figure 5.5 for all data).

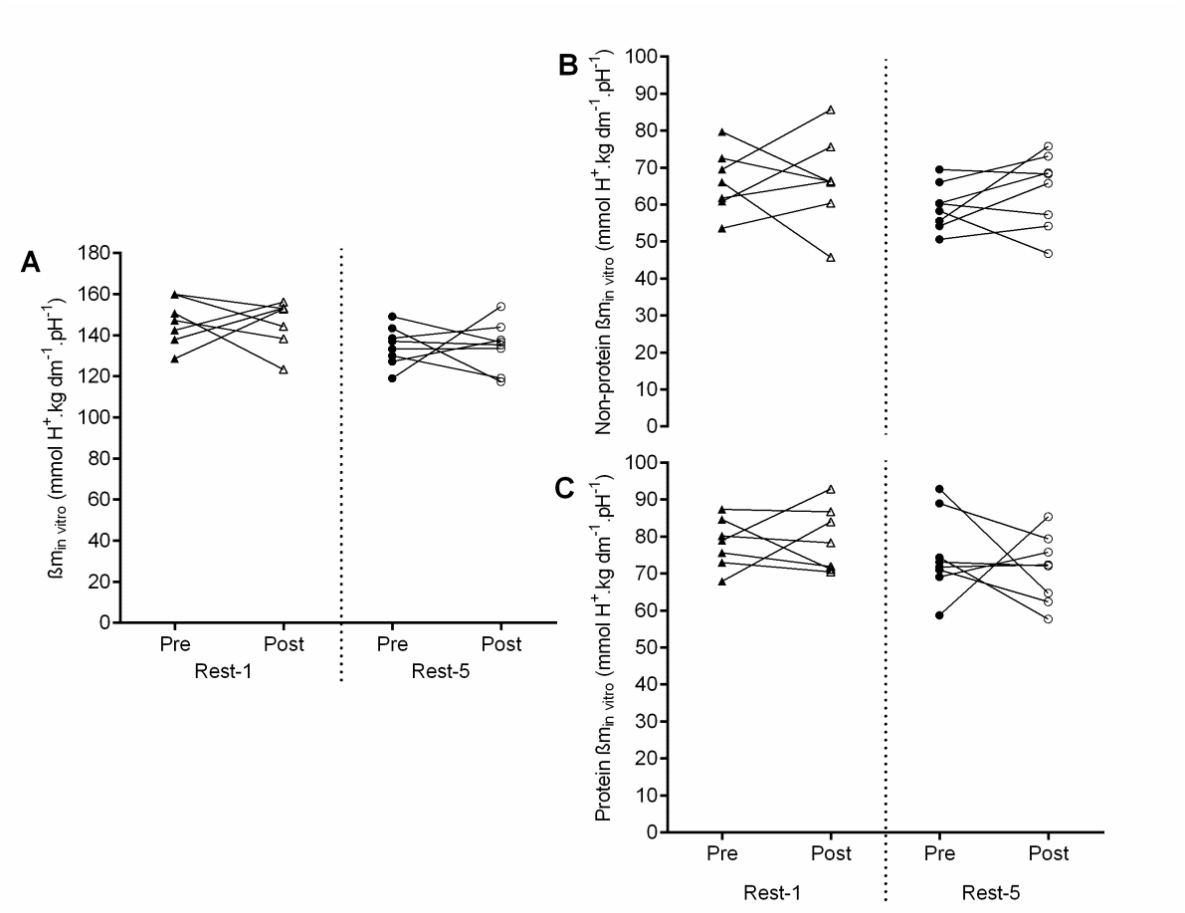


Figure 5.5 Non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) before (Pre) and immediately after (Post) a single bout of sprint-interval exercise for Rest-1 (triangles, n = 7) and Rest-5 (circles, n = 8). (A) Total $\beta m_{in\ vitro}$, (B) non-protein $\beta m_{in\ vitro}$, and (C) estimated protein $\beta m_{in\ vitro}$. CVs of duplicate titrations were 5% for total $\beta m_{in\ vitro}$ and 8% for non-protein $\beta m_{in\ vitro}$.

5.3.3 Transport Protein Abundance

5.3.3.1 MCT1

The MCT1 antibody recognised a single band at approximately 50 kDa (Figure 5.6). MCT1 protein content did not differ between the two groups (group×time interaction: $F_{2,25.2}$ = 0.17, P = 0.84, +4 wk ES: 0.04; −0.53 to 0.61). On pooling the data there was an increase at +4 wk of 1.18-fold ×/÷ 1.23 (time main effect: $F_{2,25.2}$ = 3.18 P = 0.06, ES: 0.44; 0.17 to 0.71). Comparing the pooled difference scores from +4 wk to +10 wk, i.e., when training switched from 3 d to 1 d per week, there was no change in mean MCT1 abundance (ES: −0.13; −0.50 to 0.24).

5.3.3.2 MCT4

The MCT4 antibody recognised a strong band at ~50 kDa and a weaker band at ~75 kDa in some samples (Figure 5.6); only the 50 kDa band was quantified. There were no differences between the two groups (group×time interaction: $F_{2,28.1}$ = 0.30, P = 0.74, +4 wk ES: 0.21; −0.61 to 1.03). Overall, mean MCT4 abundance did not change in response to training (time main effect: $F_{2, 28.1}$ = 0.46, P = 0.64, pooled +4 wk ES: 0.09; −0.30 to 0.48).

5.3.3.3 Basigin (CD147/EMMPRIN)

The basigin antibody detected a single band just above 37 kDa (Figure 5.6), likely the canonical basigin-2 isoform (*cf.* Chapter 4). Basigin content did not differ between the two groups (group×time interaction: $F_{2,28.3}$ = 0.20, P = 0.82, +4 wk ES: 0.06; −0.54 to 0.67). On pooling the data there were no meaningful changes (time main effect: $F_{2,28.3}$ = 3.04, P = 0.06, +4 wk ES: 0.30; 0.02 to 0.59, +10 wk ES: 0.02; −0.29 to 0.33).

5.3.3.4 NHE1

The NHE1 antibody recognised a single or a double band just below 100 kDa (Figure 5.6). The predicted molecular mass of NHE1 is 91 kDa, therefore it is probable that the identified band(s) represent non- or partially-glycosylated NHE1 protein (113, 210). As per previous reports, both bands were quantified if present (252). Overall there was an increase in NHE1 abundance following training (time main effect: $F_{2,25.3}$ = 4.00, P = 0.03, pooled +4 wk ES: 0.57; 0.23 to 0.90). The two groups responded differently to training (group×time interaction: $F_{2, 25.3}$ = 5.23, P = 0.013). There were similar increases at +4 wk of 1.11-fold ×/÷ 1.09 and 1.15-fold ×/÷ 1.22, for Rest-1 and Rest-5 respectively (ES: 0.16; −0.64 to 0.96), but when training switched to 1 d per week NHE1 decreased to baseline for Rest-1, while for

Rest-5 NHE1 increased by 1.44-fold ×/÷ 1.18 compared to baseline (+10 wk ES: 1.68; 0.71 to 2.66).

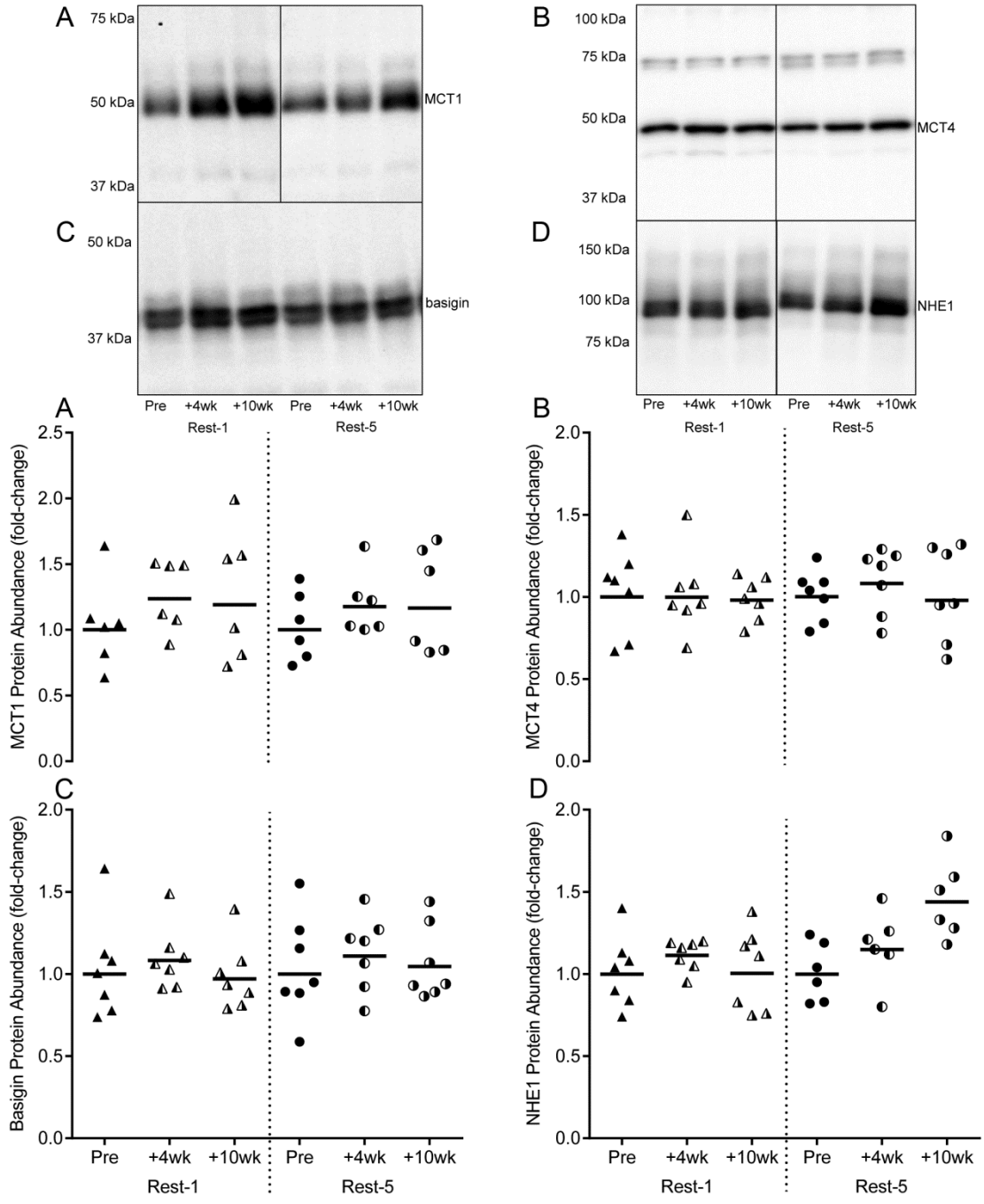


Figure 5.6 Representative immunoblots and protein abundance of (A) MCT1, (B) MCT4, (C) basigin, and (D) NHE1 before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual data points and geometric means (horizontal bars) are plotted. Post-training data are calculated as fold-change relative to the corresponding Pre datum. To illustrate variance at Pre, individual Pre data are shown relative to the Pre geometric mean for the respective groups. Non-adjacent lanes from the same blots are indicated by vertical lines (A, B, and D). CV of fold-change for duplicate or triplicate gels: MCT1 (16%), MCT4 (16%), basigin (9%), and NHE1 (15%).

5.3.3.5 NBCe1

The NBCe1 antibody recognised a band close to the predicted molecular mass of 121 kDa for the canonical NBCe1-B splice variant (Figure 5.7). Stronger signal bands of unknown origin were also detected at about 50 kDa and 75 kDa; only the ~120 kDa band was quantified (*cf.* Chapter 4). Overall there was no meaningful change in NBCe1 abundance following training (time main effect: $F_{2,17.8} = 0.23$, $P = 0.80$, pooled +4 wk ES: 0.15; −0.23 to 0.53). Mean NBCe1 content increased for Rest-1 (1.30-fold ×/÷ 1.55) and decreased for Rest-5 (0.89-fold ×/÷ 1.47) after 4 weeks of SIT due to high individual variability/outliers (group×time interaction: $F_{2,17.8} = 1.14$, $P = 0.34$, +4 wk ES: 0.64; −0.07 to 1.36). There was no meaningful difference between groups at +10 wk (ES: 0.42; −0.52 to 1.37).

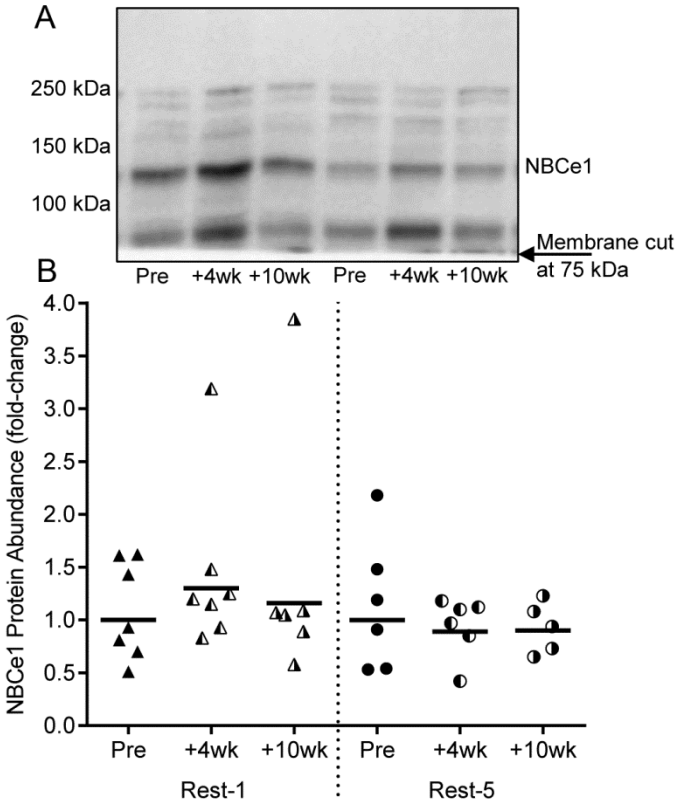


Figure 5.7 (A) Representative immunoblot and (B) NBCe1 protein abundance before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual data points and geometric means (horizontal bars) are plotted. Post-training data are calculated as fold-change relative to the corresponding Pre datum. To illustrate variance at Pre, individual Pre data are shown relative to the Pre geometric mean for the respective groups. CV of fold-change for duplicate gels was 26%.

5.3.3.6 CAII

The CAII antibody recognised a single band just above 25 kDa (Figure 5.8). There was no difference between groups in CAII content over time (group×time interaction: $F_{2,24.9}$ = 0.08, *P* = 0.92, +4 wk ES: 0.09; −0.55 to 0.73). On pooling the data CAII content decreased by 0.92-fold ×/÷ 1.11 following training (time main effect: $F_{2,24.9}$ = 3.79, *P* = 0.04, +4 wk ES: −0.47; −0.78 to −0.16).

5.3.3.7 CAIII

The CAIII antibody recognised a single band just above 25 kDa (Figure 5.8). There was no overall change in CAIII content for the two groups (time main effect: $F_{2,26.9}$ = 0.32, *P* = 0.73, pooled +4 wk ES: 0.19; −0.24 to 0.61). There was no difference between the two groups after four weeks training (group×time interaction: $F_{2,26.9}$ = 1.74, *P* = 0.20, ES: 0.26; −0.65 to 1.17). At +10 wk the difference between groups was unclear (ES: 1.04; −0.38 to 2.46).

5.3.3.8 CAIV

The CAIV antibody had poor signal-to-noise ratio but recognised a single or double band near the predicted molecular mass of 35 kDa (Figure 5.8) in the muscle homogenate and positive control (293T Cell Transient Overexpression Lysate, Abnova, Taipei, Taiwan). The CAIV response to training did not differ between the two groups (group×time interaction: $F_{2,18.6}$ = 0.26, *P* = 0.77, +4 wk ES: 0.01; −1.07 to 1.06). On pooling the data, CAIV content progressively decreased following training, 0.87-fold ×/÷ 1.40 and 0.79-fold ×/÷ 1.36 at +4 wk and +10 wk respectively (time main effect: $F_{2,18.6}$ = 3.50, *P* = 0.051, pooled +4 wk ES: −0.39; −0.86 to 0.08, +10 wk ES −0.72; −1.18 to −0.27).

5.3.3.9 CAXIV

The CAXIV antibody recognised a single band below 50 kDa in the muscle homogenate and the positive control (Figure 5.8). There was no difference between groups in the CAXIV response (group×time interaction: $F_{2,26.0}$ = 0.40, *P* = 0.68, +4 wk ES: 0.09; −0.95 to 1.13). Overall CAXIV abundance increased in response to training by 1.63-fold ×/÷ 1.66 (time main effect: $F_{2,26.0}$ = 5.77, *P* = 0.008, pooled +4 wk ES: 1.00; 0.51 to 1.48). After training switched to 1 d per week, mean CAXIV abundance decreased to baseline values (pooled +10 wk ES: 0.20; −0.42 to 0.83).

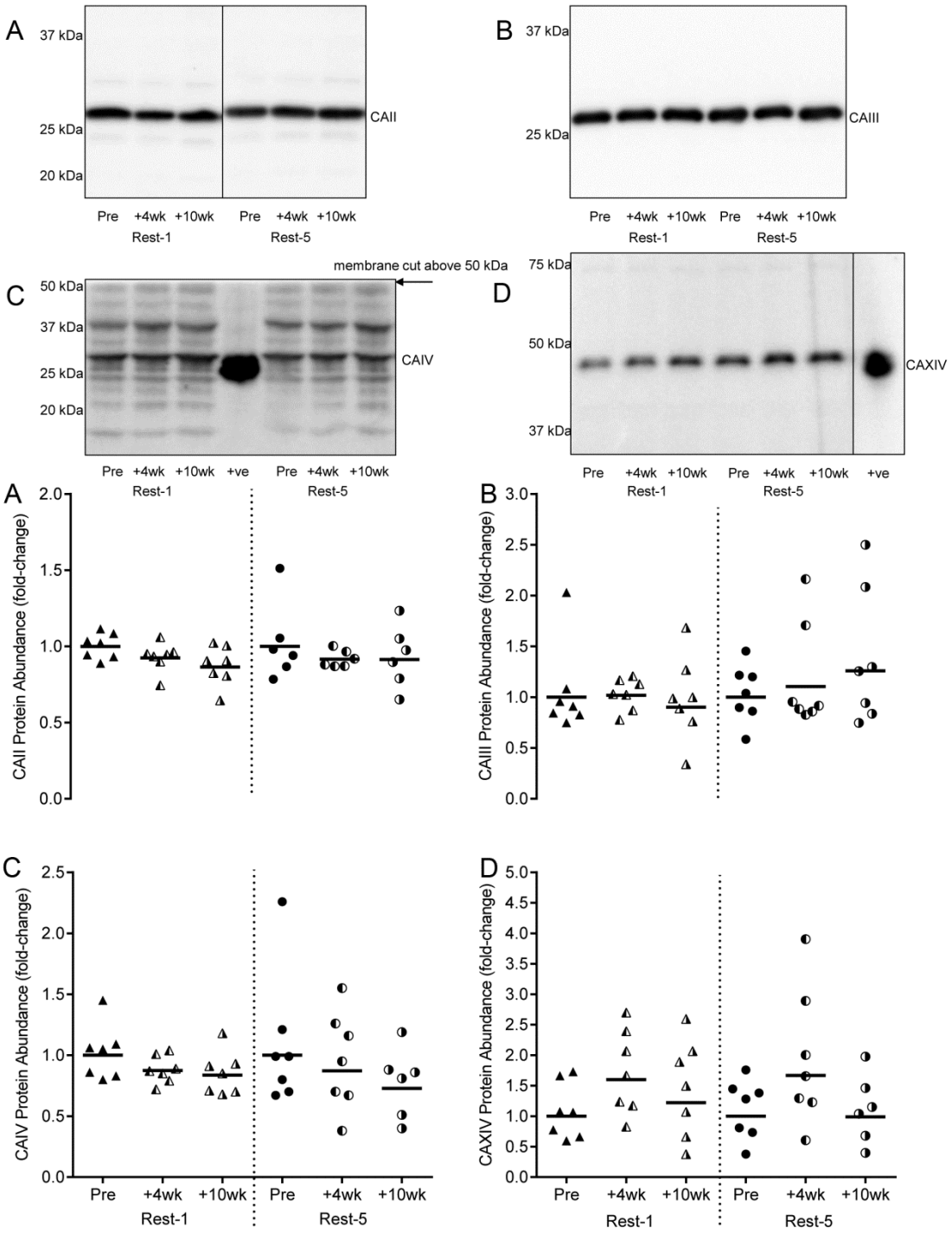


Figure 5.8 Representative immunoblots and protein abundance of (A) CAII, (B) CAIII, (C) CAIV, and (D) CAXIV before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual data points and geometric means (horizontal bars) are plotted. Post-training data are calculated as fold-change relative to the corresponding Pre datum. To illustrate variance at Pre, individual Pre data are shown relative to the Pre geometric mean for the respective groups. Positive controls (+ve) were loaded for CAIV and CAXIV. Non-adjacent lanes from the same blots are indicated by vertical lines (A and D). CV of fold-change for duplicate or triplicate gels: CAII (15%), CAIII (single gels), CAIV (23%), and CAXIV (29%).

5.3.4 Muscle Buffer Capacity

$\beta m_{in\ vitro}$ did not change in response to training for either group (time main effect: $F_{2,32.0} = 0.11$, $P = 0.89$, pooled +4 wk ES: 0.11; −0.29 to 0.50). As noted in section 5.3.2.1, $\beta m_{in\ vitro}$ was lower throughout for Rest-5 (group main effect: $F_{1,15} = 14.4$, $P = 0.002$, Pre ES: −1.06; −1.94 to −0.19).

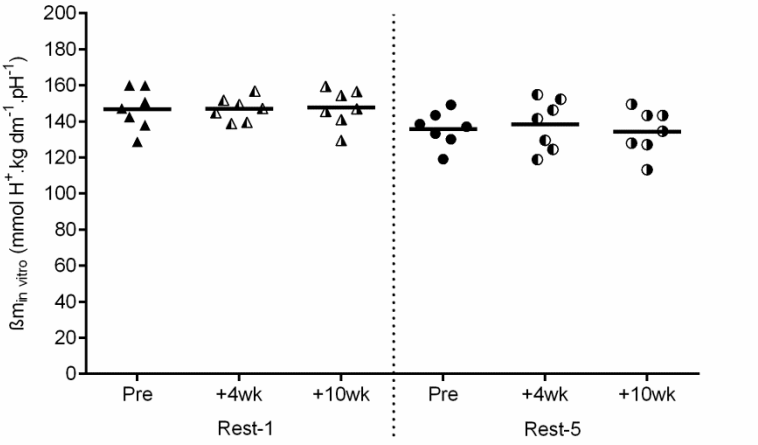


Figure 5.9 Non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual data points and means (horizontal bars) are plotted. CV of duplicate titrations = 7%.

5.3.5 Repeated-Sprint Ability

Total work performed during the RSA test improved only in the Rest-5 group (Figure 5.10) (group×time interaction: $F_{2,27.0} = 4.60$, $P = 0.02$, +4 wk ES: 0.51; 0.15 to 0.88). On switching to 1 d per week training, total work for Rest-5 decreased slightly from 13.9 (2.2) kJ to 13.5 (2.6) kJ, while for Rest-1 it did not change from 14.0 (2.1) kJ at +4 wk to 14.0 (2.5) kJ at +10 wk. Hence, there was still a clear difference between groups at +10 wk (ES: 0.42; −0.08 to 0.92). Comparing the change in work performed during each individual sprint (Figure 5.11), Rest-5 had better improvements for the last three sprints at +4 wk compared to Rest-1 (Table 5.5). For both groups work decrement during the RSA test improved following four weeks of training (time main effect: $F_{2,27.4} = 9.57$, $P = 0.001$, pooled +4 wk ES: 0.95; 0.42 to 1.47). There were no clear differences between the two groups (group×time interaction: $F_{2,27.4} = 1.84$, $P = 0.18$, +4 wk ES: 0.67; −0.38 to 1.72, +10 wk ES: 0.04; −1.04 to 1.12). There was no difference between the pooled +4 wk and +10 wk data, indicating that better work decrement was maintained with 1 d per week training (pooled ES: −0.20; −0.52 to 0.12).

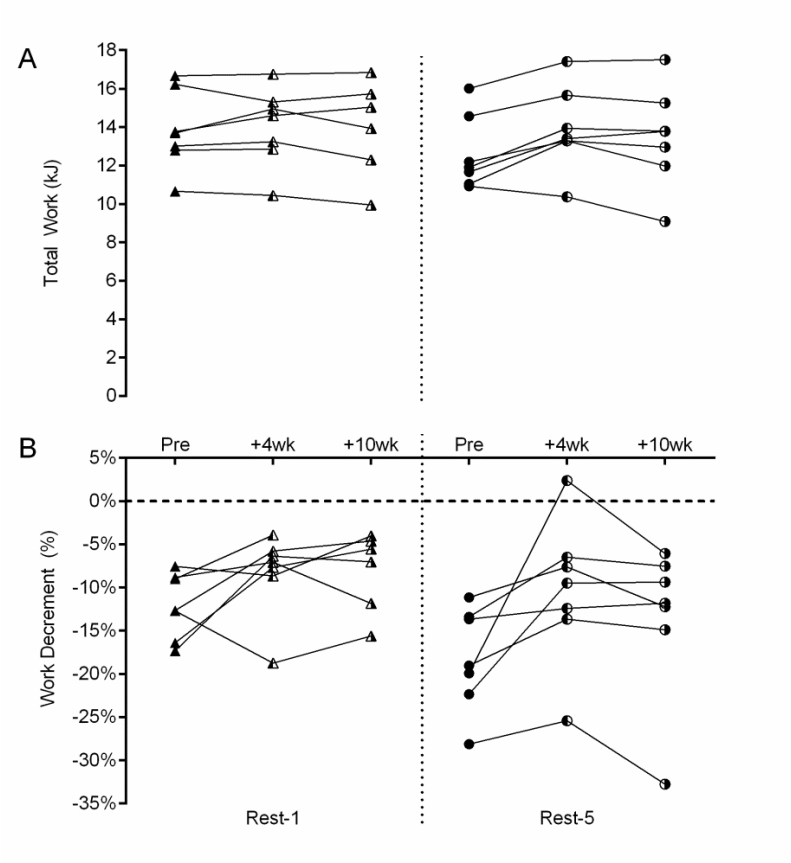


Figure 5.10 (A) Total work and (B) work decrement during a repeated-sprint ability test before (Pre) and after 4 weeks (+4 wk) and 10 weeks (+10 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual data points are plotted.

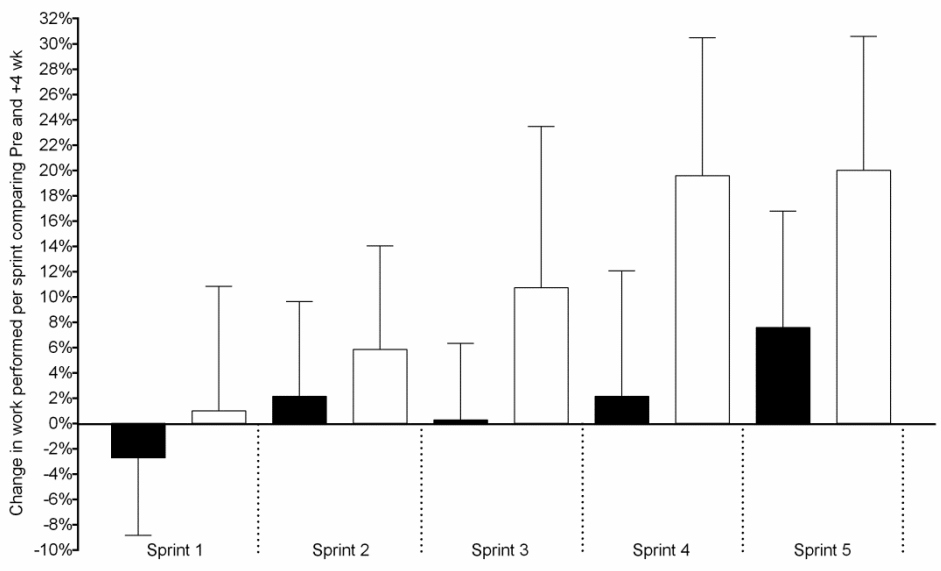


Figure 5.11 Comparison of the change in work performed per individual sprint during a repeated-sprint ability test (5 x 6 s) before (Pre) and after 4 weeks (+4 wk) of sprint interval training (SIT) for Rest-1 (black) and Rest-5 (white). Data are mean (SD) of the change from Pre to +4 wk.

Table 5.5 Magnitude-based inferences for the change in work performed per individual sprint during a repeated-sprint ability test (5 x 6 s) before (Pre) and after 4 weeks (+4 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Effect sizes and 90% confidence intervals are for the between-group (Rest-5 − Rest-1) difference scores (+4 wk − Pre) for each sprint.

Sprint no.	Between-group effect size	90% CI	Likelihood of a meaningful difference between groups	
			Probability	Inference
1	0.23	−0.28 to 0.74	54%	no meaningful difference
2	0.28	−0.15 to 0.71	63%	no meaningful difference
3	0.57	0.08 to 1.06	90%	Rest-5 greater
4	0.78	0.29 to 1.28	97%	Rest-5 greater
5	0.51	0.05 to 0.97	87%	Rest-5 greater

5.3.6 Lactate Threshold and Peak Aerobic Power

Power at the LT changed little in response to training for either group (time main effect: $F_{1,14}$ = 2.47, P = 0.14, ES: 0.24; −0.06 to 0.53). There was also no clear difference between the two groups (group×time interaction: $F_{1,14}$ = 1.79, P = 0.20, ES: 0.41; −0.19 to 1.00). As with the LT, mean W_{peak} did not change with training (time main effect: $F_{1,14}$ = 2.84, P = 0.11, pooled ES: 0.19; −0.02 to 0.40), nor was there a difference between the two groups (group×time interaction: $F_{1,14}$ = 0.23, P = 0.64, ES: 0.10; −0.32 to 0.52).

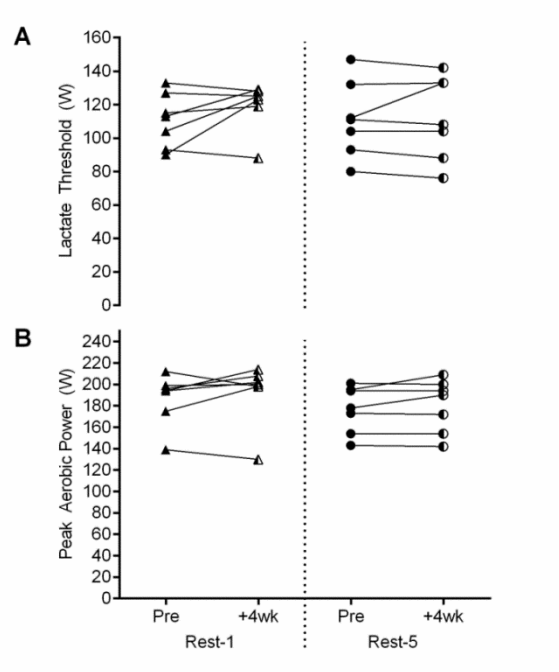


Figure 5.12 (A) Lactate threshold and (B) peak aerobic power during a graded-exercise test before (Pre) and after 4 weeks (+4 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual data are plotted.

5.3.7 Peak Oxygen Uptake

$\dot{V}O_{2peak}$ did not change differently between the two groups (group×time interaction: $F_{1,14}$ = 0.01, P = 0.91, ES: 0.03; −0.41 to 0.47). On pooling the data there was a small mean increase in $\dot{V}O_{2peak}$ of 2.2 mL•min^{-1}•kg^{-1} (90% CI: 1.0 to 3.3 mL•min^{-1}•kg^{-1}) (time main effect: $F_{1,14}$ = 12.00, P = 0.004, pooled ES: 0.38; 0.18 to 0.59).

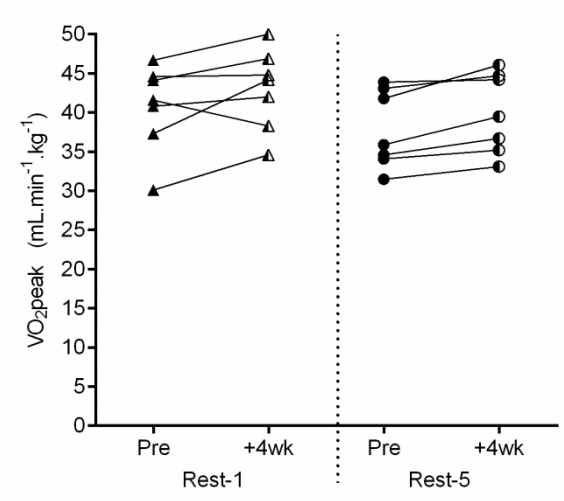


Figure 5.13 Peak oxygen uptake before (Pre) and after 4 weeks (+4 wk) of sprint interval training (SIT) for Rest-1 and Rest-5. Individual data points are plotted.

5.4 Discussion

Through manipulating the rest interval duration during SIT, this study investigated whether differences in muscle La^-/H^+ production or accumulation would elicit distinct adaptations in acid/base transport protein abundance and intracellular buffering. Maintenance of potential adaptations was also studied following a 6-week period during which weekly SIT sessions were reduced from 3 d to 1 d per week. The main finding was that upregulation of acid/base transporters did not differ between groups when training was 3 d per week, indicating that rest interval duration did not influence short-term adaptations within the muscle. Increased abundance of MCT1, NHE1, and CAXIV was seen after 4 weeks, with either no change or a decrease evident for MCT4, NBCe1, basigin, CAII, CAIII, and CAIV. Greater improvements in RSA were seen with Rest-5, while both groups had similarly modest improvements in aerobic capacity. In general, when training switched to 1 d per week, enhanced protein abundance was maintained, although disparate responses between groups were seen for NHE1, with further increases found for Rest-5.

There were small increases in MCT1 protein content for both Rest-1 (1.18-fold ×/÷ 1.26) and Rest-5 (1.17-fold ×/÷ 1.18) after 4 weeks of SIT. Greater abundance of MCT1 has been reported several times following similar short-term SIT interventions (40, 79, 253,

327, 373, 380), though others have failed to observe any such change (18, 186, 187, 232, 433). With the exception of Burgomaster *et al.* (79), where a large but variable increase of ~2.3-fold (90% CI: 0.6 to 3.9-fold) was found, the mean change from these studies is ~1.2-fold. Thus, the present study supports the existing research showing that, while increases in MCT1 protein content can be induced by SIT, the magnitude of change is typically small and variable.

In common with most studies, and regardless of rest interval duration, there were no changes in MCT4 protein content following 4 weeks of SIT. To date, only one study has reported an increase in MCT4 content following SIT (79). As with their MCT1 data, the effect after 6 weeks of training was large but highly variable (~1.5-fold; 90% CI: 1.1 to 1.9). It is not clear why only this one SIT study found MCT4 to increase, but a confounding variable might be initial training status. While their participants were moderately-trained or untrained, in those studies using more highly-trained participants there is typically little or no change in either MCT1 or MCT4 abundance (18, 150, 186, 187, 232, 468). And only one study has shown a clear increase in MCT4 but not MCT1, following polarised or threshold training in cyclists (345). One possible artefactual explanation for this latter outlier relates to their immunoblotting protocols. GAPDH is an unsuitable loading control because it may change with training and saturates at low protein concentrations (155, 480, 491). In addition, the poor fidelity of their representative blot for MCT4 indicates the novel antibody used may have been of low antigen-affinity. Ultimately, the failure to elicit any change in MCT abundance in the present study adds to the body of literature showing that upregulation of MCT4 is seldom induced following short-term training interventions.

While the signalling factors responsible for upregulation of the MCTs *in vivo* remain to be determined, the current study supports the existing *in vitro* evidence that they may respond to distinct stimuli (190, 206, 458). For example, La^- may be a signalling molecule for MCT1 but not MCT4, given increased content of only MCT1 has been shown in L6 cells 1 h after incubation with either 10 mM or 20 mM La^- (206). Although muscle [La^-] was not measured in the present study, muscle pH decreased substantially after the first bout of SIT (SIT1) for both groups, and was lower for Rest-1 than Rest-5 [6.53 (0.14) and 6.63 (0.18), respectively]. Similarly, venous blood [La^-] was higher for Rest-1 than Rest-5, five and 7 min after SIT1. Furthermore, two or three maximal 30-s sprints have been reported to result in muscle [La^-] of 30–40 mM, i.e., 100–130 mmol•kg dm^{-1} (54, 195, 379). Therefore, the present data are consistent with the results of Hashimoto *et al.* (206), and suggest that intracellular La^-/H^+ accumulation may also be a stimulus for MCT1 upregulation in human skeletal muscle. However, greater [La^-]/[H^+] does not appear to result in proportionally larger increases in MCT1 abundance. In contrast, the absence of any change in MCT4 abundance despite the large reductions in muscle pH, indicates that La^-/H^+ accumulation is unlikely to be a factor in MCT4 upregulation. And unless increases in MCT4 protein content require much higher [La^-] than

achieved here, and tested in the study of Hashimoto *et al.* (206), these data suggest La^- is not an important signalling molecule for MCT4.

One of the hypotheses of the current study was that longer rest intervals allowing for greater recovery of muscle La^-, H^+, and PCr, would lead to a greater non-mitochondrial ATP turnover for sequential intervals. The greater La^-/H^+ production during each interval, and consequently greater La^-/H^+ transport across the sarcolemma, was posited to prove more favourable for enhanced acid/base transport protein content. Contrary to this hypothesis, the length of the rest period did not influence upregulation of either MCT. This appears to contradict evidence from a rat study (466), whereby pre-exercise sodium bicarbonate supplementation provided over the course of 5 weeks of HIT, which would have enabled greater La^-/H^+ production and efflux (222), resulted in greater MCT4 content than a similarly-trained group who received a placebo. Aside from species and fibre-type differences, a stimulus other than high La^-/H^+ production likely explains the difference between studies. Ultimately, while MCT1 appears to adapt to training once there is a moderate increase in muscle [La^-] and [H^+], MCT4 is not sensitive to the same stimulus. Thus, those studies showing increased MCT4 abundance are in the minority, and the mechanisms determining changes remain uncertain.

MCT1 and MCT4 are targeted to the membrane by their chaperone protein basigin (264), and basigin appears essential for maintaining activity of both transporters (503). However, there are few data on the factors affecting upregulation of this protein in muscle. The present study provides the first investigation of the response of basigin to a SIT intervention, and there were no meaningful changes in protein abundance after 4 or 10 weeks of training for either group. Although muscle [La^-] was not measured in the current study, the large reductions in muscle pH for Rest-1 and Rest-5 [0.39 (0.16) and 0.26 (0.14), respectively], indicate that muscle [La^-] would have increased substantially following SIT. Therefore, these results contrast with those of Hashimoto *et al.* (206), who reported basigin protein content to increase by 1.85-fold to 2.78-fold after 1 h of La^- incubation in L6 cells, but to decrease after 6–48 h of incubation. There are important differences between a cell model and exercising human muscle. In particular, prolonged [La^-] of 10 or 20 mM are not physiological in human muscle *in vivo*. While accumulation of similar levels of intracellular La^- is typical of high-intensity exercise that alters the equilibrium of the lactate dehydrogenase reaction, activity of the MCTs causes La^- efflux from the cytosol, and there is eventual oxidation of La^- to pyruvate, or entry of La^- into the Cori cycle for gluconeogenesis (148). Thus, while prolonged high [La^-] may stimulate an increase in basigin content in L6 cells, the present data suggest the high [La^-] typical of SIT does not provoke increases in basigin content in exercising human muscle.

Second to the MCTs, NHE1 provides the most important contribution to H^+ efflux during high-intensity exercise (13, 16). NHE1 may also be functionally important during the recovery from acidosis (245, 508). This study found small increases in NHE1 abundance after

four weeks of SIT for Rest-1 (1.12-fold ×/÷ 1.09) and Rest-5 (1.13-fold ×/÷ 1.22). All of the research to date on the NHE1 protein response to training has emanated from Copenhagen (18, 186, 187, 232, 253, 327, 433, 468), and has used the same antibody as the current study. Mean fold-change following SIT/HIT interventions ranged from 0.95-fold (468) to 1.35-fold (433). The one study to include females, in a group consisting of both sexes, reported a 1.15-fold increase (186). Thus, it seems while NHE1 abundance responds to upregulation following HIT/SIT in different populations, the magnitude of any increase is typically small. An unexpected finding in the current study was a further increase in NHE1 content for Rest-5 of 1.25-fold ×/÷ 1.23, compared to +4 wk, after SIT switched to 1 d per week, and is of a greater magnitude than has been reported for NHE1 to date. In contrast, NHE1 abundance returned to baseline for Rest-1, indicating that the metabolic stress imposed by the two training regimens differentially regulated NHE1, but only when training volume was reduced. One study has shown NHE1 abundance to increase ~1.35-fold in elite footballers who stopped training for 2 weeks at the end of their season, in contrast to a group who performed 2 weeks of mixed HIT/SIT (468). In Chapter 4, total reversal of adaptations in NHE1 content were not yet apparent 6 weeks after the removing the physiological stress of HIT. It appears that, following a period of intensive training, a reduced training stimulus is favourable for NHE1 upregulation, but further evidence is required to confirm if and why this is so.

The NBCe1 protein response to a SIT intervention has been reported here for the first time. Although no changes in mean NBCe1 content were seen for either group, there were some variable individual responses. In Chapter 4, NBCe1 abundance was shown to increase in men undergoing 4 weeks of 2-min HIT intervals performed at intensities between the LT and W_{peak}. Using a non-isoform specific antibody, others have found NBC to increase in rat *soleus* following 5 weeks of 2-min HIT intervals at 80% of maximum velocity, regardless of whether rats received sodium bicarbonate or a placebo prior to each training session (466). By buffering extracellular H^+, sodium bicarbonate enhances H^+ efflux by creating a greater pH gradient relative to the intracellular space (297, 397). In the current study, 5 min of rest would have enabled greater H^+ efflux compared to 1 min of rest. Therefore, from these limited data it seems that H^+ efflux does not influence changes in NBC(e1) protein content. While there may be sex-specific responses comparing Chapter 4 and the present study (402), it seems that longer duration intervals of high-intensity training may be required to provoke adaptations in NBC(e1). The signalling factors required for upregulation of NBCe1 in human muscle *in vivo* are unknown, but *in vitro* research has shown that IRBIT[120] activates most of the NCBT proteins, except for NBCe2 (283, 360, 428). Whether IRBIT is important *in vivo* remains to be shown, but it is a potential target for future study.

[120] Inositol 1,4,5 trisphosphate receptor binding protein released with inositol 1,4,5 trisphosphate

There were mixed changes in each of the CA isozymes after four weeks of training. Of the cytosolic CAs, CAII content decreased slightly for both groups (~0.92-fold), whereas there was no change in CAIII content. There were disparate responses for the sarcolemmal CAs, with large increases in CAXIV abundance for both Rest-1 and Rest-5 (1.60-fold ×/÷ 1.54 and 1.67-fold ×/÷ 1.84 respectively), but a small decrease in CAIV content for both groups at +4 wk (0.87-fold ×/÷ 1.40). There are no published data on the CA response to SIT, although increases in CAII and CAXIV, but decreases or no change in CAIII or CAIV, were found following four weeks of 2-min HIT in men in Chapter 4. This limited evidence demonstrates the plasticity of the cytosolic and sarcolemmal CAs to training, with CAXIV seemingly most readily upregulated. The importance of increased abundance of CAXIV in this study is possibly related to a non-catalytic effect in enhancing acid/base transport activity. Investigations in rodents have reported that by knocking out CAXIV in mice (194), or by inhibiting extracellular CAs in rat muscle (498), lactic acid transport was reduced. Thus, the effect of increased CAXIV content in the current study may have been to enhance MCT activity, possibly through exchanging H^+ between protonatable residues extracellularly, as has been shown for CAII intracellularly (25, 455). Additional evidence is required to confirm this. Given that there were no differences between groups in the present study, despite differing metabolic stresses imposed by 1-min versus 5-min rest periods, nor in Chapter 4, despite differing intensities of training, there is little insight provided as to the potential mechanisms for upregulation of CA content. One possible inference is that H^+ accumulation and H^+ production are unimportant as potential stimuli. Training interventions using moderate-intensity continuous training, which induces little change in pH_i, could help support or refute this argument.

Finally, the reduction in CAIV may be an artefact of comparatively low abundance of this isozyme, or poor antibody-antigen affinity, resulting in low signal-to-noise ratio. Furthermore, as documented in Chapter 4, the antibody detected multiple bands, including a major band at 75 kDa. Though varying reducing conditions did not affect this band, it cannot be discounted that it was a CAIV dimer. Only the band(s) coinciding with the molecular mass of the positive control were quantified, but the presence of other splice variants or multimers remains a possibility.

There were no changes in $\beta m_{in\ vitro}$ for either group following 4 or 10 weeks of SIT. Based on previous research, it was postulated that H^+ accumulation might be a stimulus for upregulation of $\beta m_{in\ vitro}$ (495). It followed that $\beta m_{in\ vitro}$ might improve more for Rest-1 because the shorter rest periods would prevent recovery of pH_i between intervals. As expected, muscle pH was lower for Rest-1 than Rest-5, but given there were no improvements for either group, the low pH_i associated with both types of training was not a factor in improving $\beta m_{in\ vitro}$. These data add to the existing evidence showing improvements in $\beta m_{in\ vitro}$ are typically not found with short-duration work intervals (8, 196, 232, 349).

There was also no change in mean $\beta m_{in\ vitro}$ for either group immediately after the first bout of 4 × 30-s all-out sprints (SIT1). A small reduction in $\beta m_{in\ vitro}$ because of lower phosphate buffering might theoretically be expected post-exercise, due to accumulation of hexose monophosphates and glycerol-3-phosphate, and consequently lower free P_i content following sample homogenisation. Contrary to this, mean non-protein $\beta m_{in\ vitro}$ changed little following SIT1, though responses were random, suggesting methodological rather than physiological variation. Lower $\beta m_{in\ vitro}$ has been reported immediately after 45-s maximal cycling, cycling at 120% $\dot{V}O_{2max}$ to fatigue, and after 6 × 4-s maximal sprints (46, 48). In the latter study non-protein $\beta m_{in\ vitro}$ measured by titration was unchanged, thus the lower total $\beta m_{in\ vitro}$ was inferred to be due to reduced protein buffering. There is some doubt over whether a sufficient reduction in the content of carnosine or protein-bound histidine could have occurred after such a short period of exercise (254). Though others have shown a reduction in $\beta m_{in\ vitro}$ in rat muscle two days after lengthening contractions (372), this modality of exercise would be expected to cause efflux of intracellular proteins into the extracellular space. In the current study, with no change in either total $\beta m_{in\ vitro}$ or non-protein $\beta m_{in\ vitro}$, there was consequently no change in estimated protein $\beta m_{in\ vitro}$. The present data question whether a short-term reduction in protein buffering is likely immediately following high-intensity exercise. Given the methodological limitations of the titration technique (*cf.* Chapter 3), without measuring both protein and non-protein $\beta m_{in\ vitro}$ directly, it is uncertain whether the sum of the two titrations would be equal to total $\beta m_{in\ vitro}$. Further validation of the technique is required to confirm whether or not actual buffering capacity can be reduced by such short-duration exercise.

It was anticipated that performance improvements would be specific to training that more closely matched the substrate usage of the exercise test. Hence, the Rest-1 training programme, having a greater ATP demand from oxidative phosphorylation due to lesser PCr resynthesis and, potentially, inhibition of the glycolytic enzymes phosphofructokinase (PFK) and Phos *a* (94, 221, 363), was postulated to result in improved aerobic capacity but not RSA. Conversely, the predicted greater non-mitochondrial ATP turnover during Rest-5 training was anticipated to produce improvements in substrate usage likely to benefit RSA. However, not all of the performance improvements were as expected. RSA did improve more for Rest-5, with greater total work performed and better fatigue resistance. This was not due to improved single (i.e., first) sprint performance, but to progressively greater total work performed in sequential sprints at +4 wk compared to Pre (Figure 5.11). Rest-1 had no improvement in total work, but did have improved fatigue resistance because of a lower reduction in total work for the final sprints. Greater total work probably relates to an enhanced anaerobic capacity providing greater relative substrate level phosphorylation for serial sprints, in part because of improved PCr resynthesis (47). Better fatigue resistance more likely reflects that the ability to sustain power for later sprints is dependent on oxidative rather than glycolytic capacity (314). Accordingly,

both groups displayed similarly modest improvements in $\dot{V}O_{2peak}$ of ~6%, or 2.2 mL•min^{-1}•kg^{-1} (90% CI: −0.2 to 4.6 mL•min^{-1}•kg^{-1}), and 2.1 mL•min^{-1}•kg^{-1} (1.0 to 3.1 mL•min^{-1}•kg^{-1}), for Rest-1 and Rest-5 respectively. These data are less than the aggregate reported in a meta-analysis of SIT studies of 8%, or 3.6 mL•min^{-1}•kg^{-1} (175), but greater than the 3.6% improvement in a subgroup analysis of non-athletic active females, reported in a separate meta-analysis (497). It appears that short-duration SIT interventions produce only moderate improvements in $\dot{V}O_{2peak}$ in active women, possibly due to insufficient time to achieve the primarily central adaptations determining $\dot{V}O_{2peak}$. Finally, there were no group mean improvements in the LT or W_{peak} for Rest-1, nor was there for Rest-5. However, there was notable individual variability within both groups, with some participants showing large improvements in the LT and/or W_{peak}, whereas others were low- or non-responders to training. Both groups had high- and low-responders, suggesting that some individuals may respond regardless of the training stimulus, but others less readily achieve performance improvements, at least after just 4 weeks of training. While initial training status no doubt influences the magnitude of any training response, there are other factors determining adaptations that require clarification (300).

For the first time in humans, this study has shown that adaptations in some acid/base transport proteins can be maintained by a reduction in training volume, but not intensity, for a subsequent 6-week period. The increases in protein content elicited by four weeks of SIT performed three days per week were maintained during one day per week training, with the exception of CAXIV. Increased abundance of MCT1 was maintained by both groups training one day per week, though there was individual variation. This supports earlier data in horses showing that reversal of adaptations in MCT1 following HIT was prevented by 6 weeks of moderate-intensity training (266). Although loss of adaptations for both MCT1 and MCT4 is rapid following removal or reduction of a training stimulus (Chapter 4 and reference 79), it is evident that a minimal volume of training is needed to maintain MCT1 content. Whether this is the same for MCT4 cannot be answered by the present study because of the absence of any adaptations, but it may be more likely dependent on inclusion of high-intensity training than MCT1 (266). As there was little or no improvement with training for three of the four CA isozymes it is unknown whether loss of adaptations for CAII/III/IV can be similarly mitigated. However, the data for CAXIV showed clear detraining at +10 wk, indicating a reduced volume, but maintained intensity of SIT, did not provide adequate stimulus to prevent reversal of adaptations for this protein. An unexpected finding was that NHE1 protein content increased further for Rest-5 after switching to one day per week training. Why this occurred is uncertain, but it may be that NHE1 requires longer recovery between the stress of training to optimise adaptations. Finally, improved performance was evident during the RSA test for both groups, but to different degrees. On comparing total work and work decrement at +10 wk and +4 wk

there were no differences, demonstrating that where RSA was enhanced after 4 weeks of training, it was maintained with just one day per week of training at a similar intensity. This shows that once performance adaptations are achieved with a short period of intensified training, they can be readily maintained with a lower volume of high-intensity training.

5.5 Conclusions

In summary, this study has demonstrated selective upregulation of acid/base transport proteins after 4 weeks of SIT. The similar adaptations of two work-matched training groups, differing only in relief intervals of 1 or 5 minutes, indicates duration of recovery does not influence short-term adaptations in these proteins, at least in an active female population. The absence of improvements in $\beta m_{in\ vitro}$ for either group re-emphasises that muscle buffer capacity is typically not improved with short-duration work intervals such as those performed here. Four weeks of SIT produced only modest improvements in $\dot{V}O_{2peak}$, indicating there was insufficient training load to produce the primarily central adaptations that determine $\dot{V}O_{2peak}$. Overall, there was little change in mean LT or W_{peak}, but evidence of high- and low-responders in both groups merits further exploration. There were better improvements in RSA for Rest-5, likely reflecting the greater anaerobic training stimulus provided by longer rest periods. Finally, maintaining the physiological stimulus of training intensity, but reducing the volume of training, was sufficient to mitigate reversal of adaptations for acid/base transport proteins and RSA. The implication from this research is that rest intervals of 5 minutes during SIT lead to better improvements in RSA than 1-minute rest intervals, and these improvements can be sustained by reducing volume but not intensity of training.

Chapter 6 Summary and Conclusions

6.1 Summary of Main Findings

This thesis investigated components of skeletal muscle pH_i regulation following different modalities of high-intensity intermittent exercise. In study 1, the protein content of specific H^+ transporters, and changes in $\beta m_{in\ vitro}$, were measured during the 72 h following a single bout of HIT. These data showed that MCT1 protein content was greater 24 h post-exercise, compared to the first 9 h post-exercise, while NHE1 content was lower 9 h post-exercise compared to all other timepoints. This indicated that post-training biopsies for the subsequent studies should be at least 24 h after the final training session. High variability of $\beta m_{in\ vitro}$ over the 72 h post-exercise showed that the between-sample variability was greater than the mean effect of published studies.

The first objective of study 2 was to validate immunoblotting techniques for detection of a comprehensive selection of acid/base transport proteins. Having achieved this, the response of the major isoforms of each transport family to four weeks of HIT in active men was studied, including, for the first time, basigin, NBCe1, and each of the four CA isozymes. Increased protein abundance was evident for MCT1, NHE1, NBCe1, CAII, and CAXIV, but there were decreases in CAIII and CAIV. In comparing two work-matched intensities of HIT, performed either above the LT (HITΔ20) or close to W_{peak} (HITΔ90)[121], improvements in most of these proteins were not influenced by intensity, with the exception of MCT4 and basigin, while $\beta m_{in\ vitro}$ did not change. And performance improvements were modest for both groups. Six weeks after stopping training, reversal of adaptations for most of the acid/base transport proteins was evident, although the proteins with the largest increase with training, namely MCT1 and NHE1, remained elevated above baseline.

In study 3, changes in components of pH_i regulation were measured in response to four weeks of SIT in active women, comparing two work-matched groups who differed only in rest interval duration – 1 min (Rest-1) versus 5 min (Rest-5). Compared to four weeks of HIT in study 2, the effect of training on acid/base protein abundance was of a lower magnitude following SIT, and evident with fewer proteins (MCT1, NHE1, and CAXIV). There was once again no change in $\beta m_{in\ vitro}$. RSA improved most in the 5-min rest group, while modest changes in aerobic capacity were similar for the two groups. Finally, a subsequent 6-week period of one day per week SIT, performed at the same intensity for both groups, was sufficient to maintain most adaptations in acid/base transporters and in RSA.

[121] The training intensity for both groups was above the lactate threshold (LT), set initially to power at the LT, plus either 20% (HITΔ20) or 90% (HITΔ90) of the difference (Δ) between power at the LT and peak aerobic power (W_{peak}).

6.2 Changes in Acid/Base Transport Protein Content with Training

Notwithstanding sex differences between studies, the greatest improvements in acid/base transport protein abundance were found following four weeks of 2-min HIT intervals in study 2, rather than four weeks of 30-s SIT intervals in study 3. It was also clear from study 2 that two weeks of HIT provided an insufficient stimulus to induce any adaptations in pH_i regulation. The following sections summarise the changes in each of these proteins.

6.2.1 MCT1 and MCT4

The increases in MCT1 abundance in study 2, following HIT performed at intensities between the LT and W_{peak}, were of a greater magnitude[122] than has been reported to date, although there was high individual variability (Figure 6.1). In contrast, the small increases for both groups in study 3 are more typical of the magnitude of changes found following SIT, with the exception of the study of Burgomaster *et al.* (79). MCT1 protein has been reported to increase in response to high [La^-] *in vitro* (206), and appears to increase following exercise resulting in intracellular La^-/H^+ accumulation. However, the large reductions in muscle pH following both modalities of SIT in the current study indicate that adaptations are not directly proportional to the magnitude of H^+ accumulation. It is likely the duration of exercise bouts performed influences adaptations, suggesting sustained production of La^-/H^+ may also be an important stimulus.

In common with most training interventions to date, there were no changes in MCT4 content following SIT, regardless of rest-interval duration. There was also no change for HITΔ90, but increased abundance was found for HITΔ20. Thus, a greater number of longer duration intervals performed above the LT, but not close to maximal aerobic power, provided the physiological stress to induce changes in MCT4 protein. This suggests that absolute intracellular concentrations of La^- or H^+ do not stimulate MCT4, but perhaps duration of La^-/H^+ flux does. MCT4 is upregulated by hypoxia *in vitro* (191), and cellular hypoxia may induce MCT4 expression *in vivo*, though simulated hypoxic environments have failed to provoke any changes (100, 252, 324, 355). Given the HIF-1α protein is constitutively expressed and degrades rapidly in normoxia (37, 237), it is possible MCT4 expression is more transient than for MCT1. Therefore, the increased MCT4 content for HITΔ20, and those few studies showing similar increases (79, 367, 373), may simply represent a snapshot of elevated protein content.

[122] The forest plot is a mixture of arithmetic means and geometric means, depending on how data were reported in the original papers. If arithmetic means were reported for the data in study 2, the magnitude of the increase would be even greater for HITΔ20 and HITΔ90, 3.4-fold and 2.6-fold, respectively

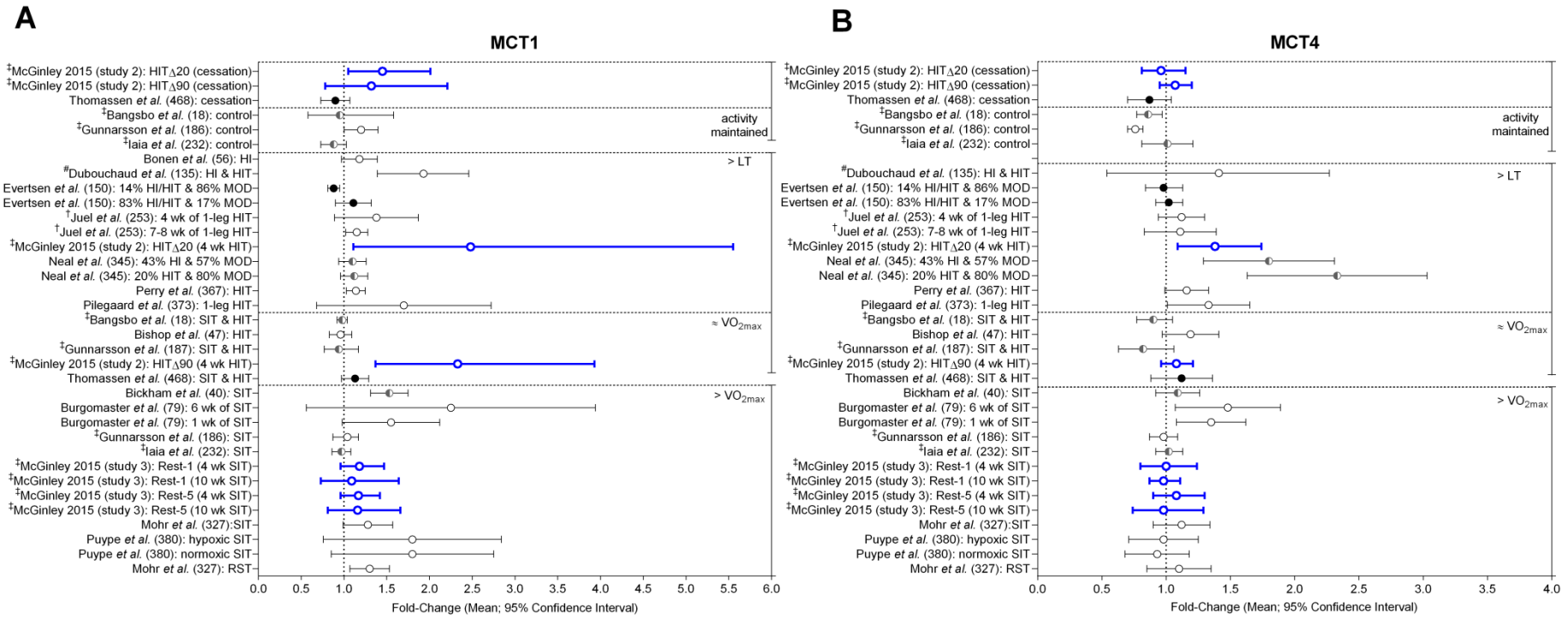


Figure 6.1 Forest plot of mean fold-change and 95% confidence intervals (CI) for MCT1 and MCT4 protein content following different training interventions. The figure is a duplication of Figure 2.13 from the Literature Review, with the inclusion of data from study 2 and study 3 of this thesis (blue lines). Abbreviations: MOD (moderate-intensity continuous training), HI (high-intensity continuous training), HICT (high-intensity concurrent training), HIT (high-intensity interval training), LT (lactate threshold), RST (repeated-sprint training), SIT (sprint-interval training), VO_{2max} (maximal oxygen uptake). Training status: ● elite, ◐ well-trained, ○ recreationally active. See Figure 2.13 caption for additional details.

6.2.2 Basigin (CD147/EMMPRIN)

This thesis has provided the first data on the basigin response to exercise training. SIT did not induce any changes in basigin content, whereas greater abundance was found for HITΔ90 but not HITΔ20. From the large reductions in muscle pH following SIT, it can be assumed that, in contrast to evidence from L6 cells (206), absolute [La^-] does not appear to be a signalling factor for basigin *in vivo*. This may reflect an invalid comparison of 1 h of constant high [La^-] in cells, with the fluctuating [La^-] in contracting muscle *in vivo*. Despite a transient increase in [La^-] post-exercise, La^-/H^+ efflux by the MCTs will reduce [La^-] within 10–20 minutes (213, 258), in contrast to a cell model.

6.2.3 NHE1

Four weeks of SIT induced small increases in NHE1 abundance, whereas the increases following four weeks of HIT were greater than has been reported to date (Figure 6.2). A confounding factor in comparing studies is that training status seems to influence upregulation of NHE1 (18). Therefore, the improvements reported in this thesis may reflect a comparatively novel high-intensity training load for these recreationally-active participants. While the signalling factors responsible for upregulation of NHE1 are not clear, high-intensity training appears to be necessary for increased abundance. An interesting finding from study 3 was that NHE1 content of the Rest-5 group increased further when training switched to one day per week for five weeks. This was unexpected, and assuming it was not artefactual, may indicate that NHE1 requires longer recovery between the high-intensity sessions to optimise adaptations. Further evidence is required to test this supposition.

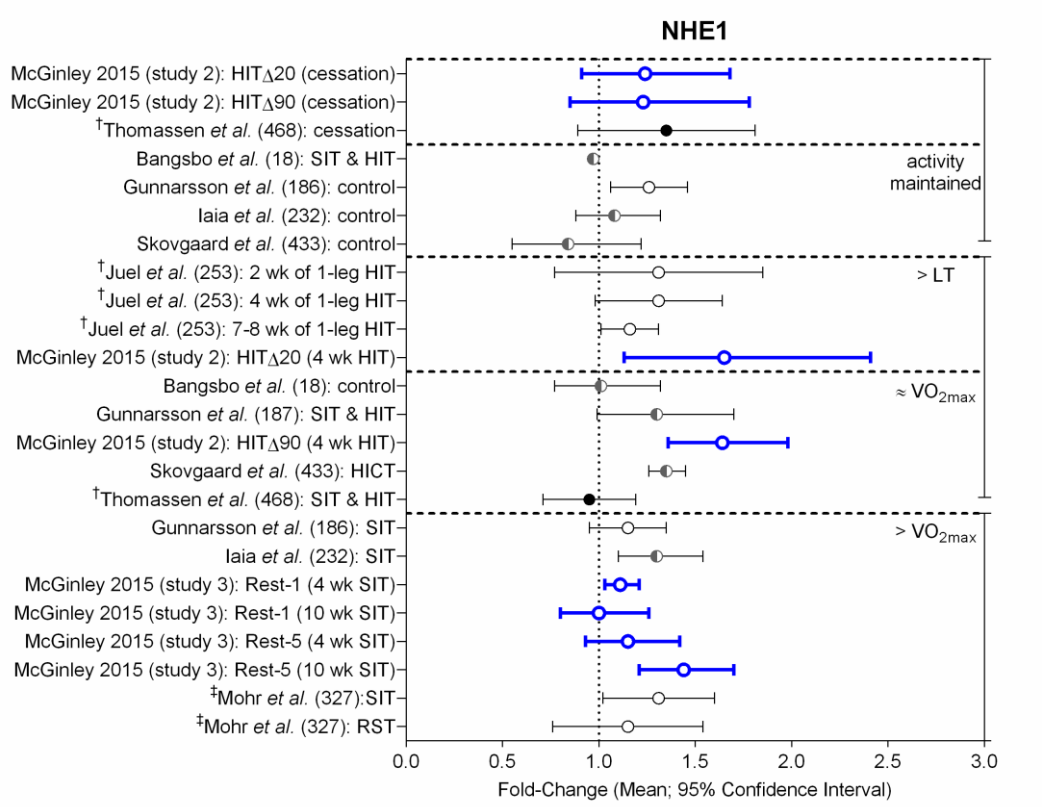


Figure 6.2 Forest plot of mean fold-change and 95% confidence intervals (CI) for NHE1 protein content following different training interventions. The figure is a duplication of Figure 2.15 from the Literature Review, with the inclusion of data from study 2 and study 3 of this thesis (blue lines). Abbreviations: HICT (high-intensity concurrent training), HIT (high-intensity interval training), LT (lactate threshold), RST (repeated-sprint training), SIT (sprint-interval training), VO_{2max} (maximal oxygen uptake). Training status: ● elite, ◐ well-trained, ○ recreationally active. See Figure 2.15 caption for additional details.

6.2.4 NBCe1

This thesis has detected NBCe1 using an isoform-specific antibody for the first time, and provides the first evidence of the NBCe1 response to exercise training. Although there was high variability, perhaps because of poor antibody-antigen affinity, NBCe1 content increased following four weeks of HIT, but not SIT. The intensity at which HIT was performed between the LT and W_{peak} did not affect adaptations, showing that upregulation of NBCe1 was not influenced by moderate differences in training intensity. Although the signalling factors for NBCe1 cannot be educed from these preliminary data, the lack of change following SIT suggests that H^+ accumulation was not a stimulus. IRBIT[123] has been shown *in vitro* to activate most of the NCBT proteins (283, 360, 428), and is a potential target for future research.

[123] Inositol 1,4,5 trisphosphate receptor binding protein released with inositol 1,4,5 trisphosphate

6.2.5 Carbonic Anhydrases

Studies 2 and 3 provide the first evidence of the plasticity of the four skeletal muscle CA isozymes to training (Figure 6.3). The sarcolemmal CAXIV isozyme demonstrated the greatest responsiveness to training, increasing following four weeks of both HIT and SIT. Cytosolic CAII abundance increased following HIT, but decreased following SIT, whereas there were no changes or decreases in cytosolic CAIII and sarcolemmal CAIV protein content. Once again, HIT proved more effective in eliciting adaptions in pH_i-regulatory proteins than SIT. Although little inference can be drawn from these studies alone as to mechanisms for regulation of the CAs, once can speculate on the effect of changes in protein content.

The present studies confirm that CAIII is highly abundant in skeletal muscle isoform (172), requiring loading of just 1 µg of total protein during SDS-PAGE to avoid saturation of the signal on immunoblotting. It is possible CAIII does not readily change with training because it is so highly abundant. Abundance of the other cytosolic isozyme, CAII, increased following four weeks of HIT, but decreased slightly after four weeks of SIT. Extrapolating from *in vitro* research it can be proposed that increased CAII content would enhance MCT activity by shuttling H^+ between protonatable residues on the MCTs and CAs (25, 455). In addition, CAII could catalytically enhance NBCe1 activity, potentially by forming transport metabolons (23), and facilitating transport of HCO_3^- (or CO_3^{2-} or $NaCO_3^-$) into the cell to buffer H^+.

CAIV abundance did not change in study 2 and decreased in study 3. It is uncertain why this was so, but an artefactual explanation cannot be ruled out given the poor antibody-antigen affinity shown here. It may also be that CAIV is of low abundance and therefore difficult to detect in crude cytosolic fractions. More robust data is required to make any inference on this protein. And although there is less information on the synergy between CAXIV and the acid/base transporters than there is for the cytosolic CAs, it is possible the increased CAXIV found in both studies would have provided a similar extracellular function to CAII, acting as an intramolecular shuttle in exchanging H^+ with the MCTs for subsequent buffering in the interstitium by HCO_3^- (498). Direct evidence is required to confirm this.

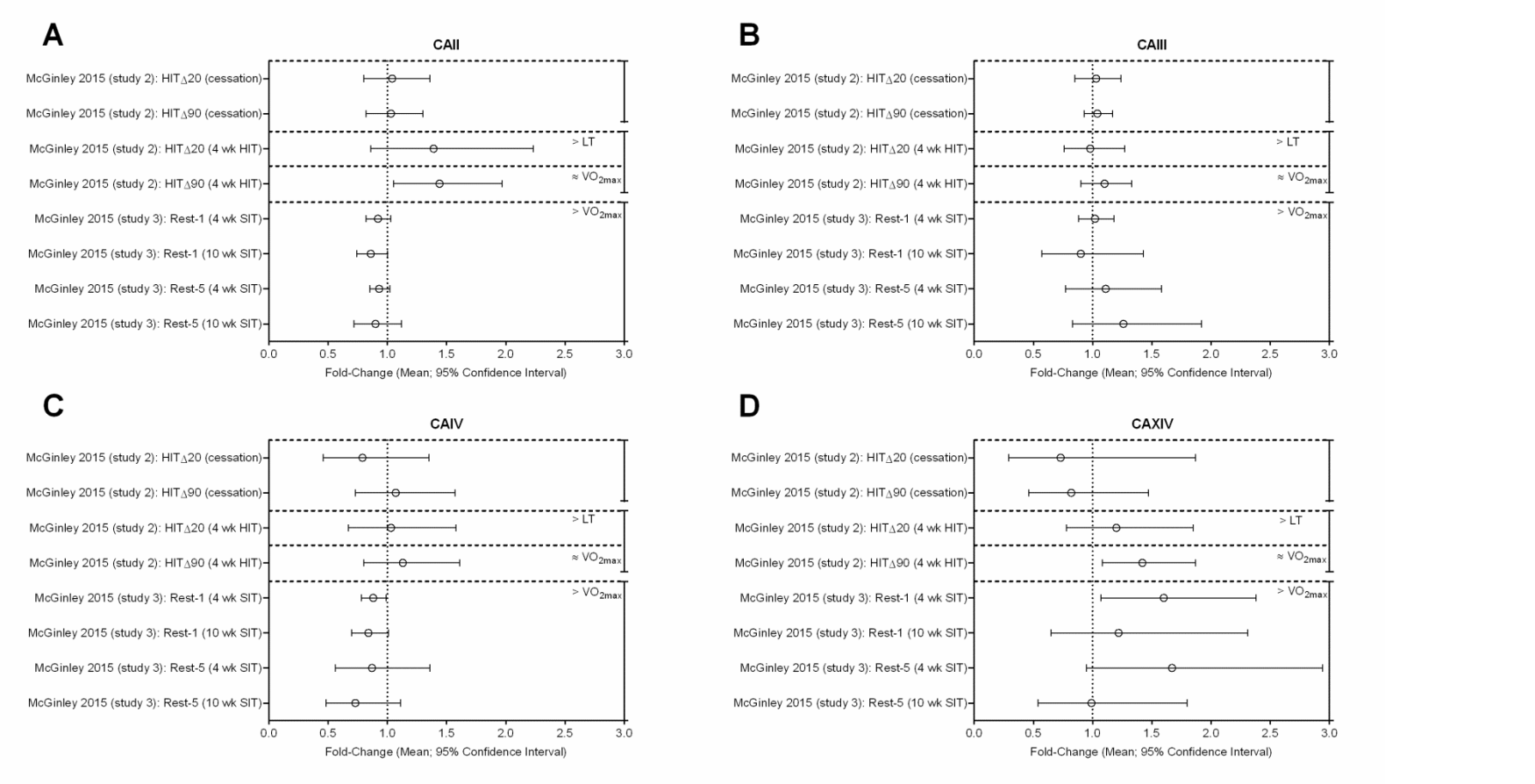


Figure 6.3 Forest plot of mean fold-change and 95% confidence intervals (CI) for the four carbonic anhydrase (CA) isozymes measured in studies 2 and 3 of this thesis, cytosolic CAII and CAIII, sarcolemmal CAIV and CAXIV. Date are geometric mean (95% CI). Abbreviations: Abbreviations: HIT (high-intensity interval training), LT (lactate threshold), SIT (sprint-interval training), VO_{2max} (maximal oxygen uptake).

6.3 Muscle Buffer Capacity

There were no improvements in $\beta m_{in\ vitro}$ following any of the training modalities employed in the studies described in this thesis (Figure 6.4). These data support the published research evincing no benefit of short-duration SIT intervals on $\beta m_{in\ vitro}$. Contrary to the hypothesis of study 2, the intensity at which HIT was performed between the LT and W_{peak} did not influence adaptations. The factors determining changes in muscle buffer capacity following training are therefore not clarified by this research; however, study 1 has raised some doubts about the ecological validity of the titration assay as a measure of intracellular buffer capacity. The high vararibilty between samples taken from the same *vastus lateralis* over a 72-h period showed that the reported effects of training are typically lower than the sample-to-sample variability. The change in $\beta m_{in\ vitro}$ due to variable phosphagen hydrolysis may be greater than the expected effect of training, indicating the technique requires further improvement and validation in future.

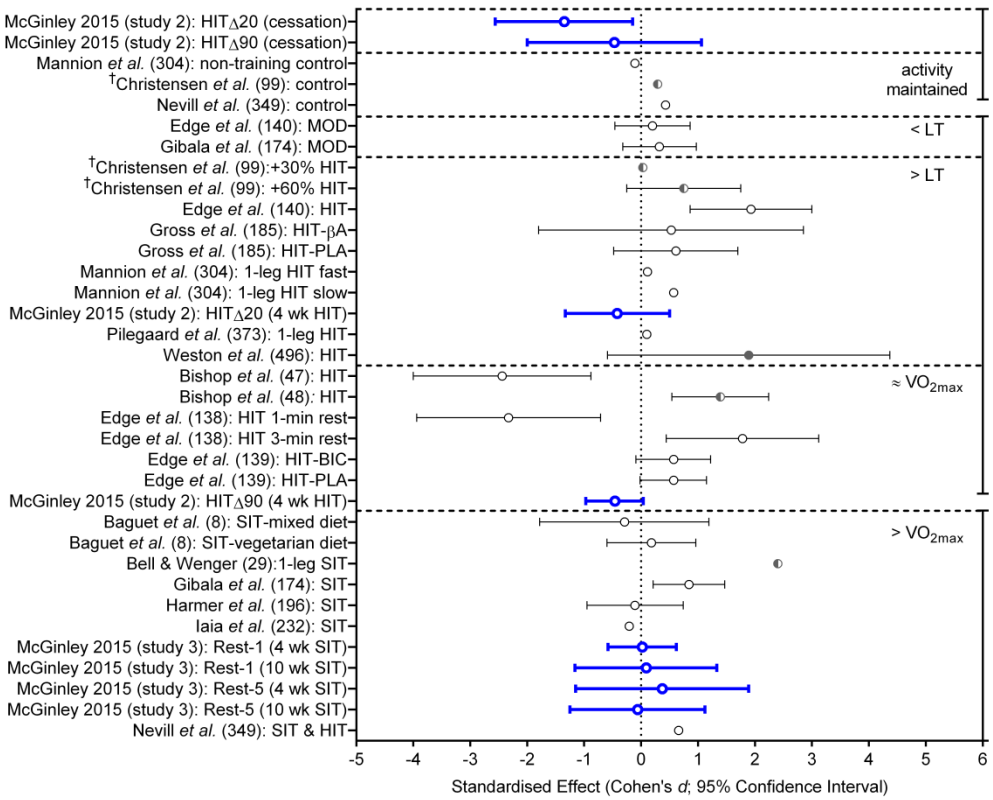


Figure 6.4 Forest plot of Cohen's *d* effect size and 95% confidence intervals for $\beta m_{in\ vitro}$ following different training interventions. The figure is a duplication of Figure 2.11 from the Literature Review, with the inclusion of data from study 2 and study 3 of this thesis (blue lines). Abbreviations: βA (β-alanine supplementation), BIC (sodium bicarbonate supplementation), HIT (high-intensity interval training), LT (lactate threshold), MOD (moderate-intensity continuous), PLA (placebo), SIT (sprint-interval training), VO_{2max} (maximal oxygen uptake). †No details given on modality of HIT. Training status: ● highly-trained, ◐ well-trained, ○ recreationally active. See Figure 2.11 caption for additional details.

6.4 Exercise Performance

Overall, improvements in exercise capacity in both training studies were modest, and there appeared to be a dissociation between changes in pH_i regulation and changes in performance. Four weeks of HIT or SIT induced small mean changes in aerobic capacity, suggesting that four weeks of training provided insufficient stimulus to provoke adaptations in already active participants. RSA improved to varying degrees following both HIT and SIT, with the longer rest periods for Rest-5 in study 3 resulting in better improvements, most probably because of the greater anaerobic stimulus. The small improvements after four weeks of HIT may indicate that previous reports of improved RSA following similar training interventions were confounded by the failure to isolate the effects of cadence on power, from the potential effects of training on power. The isokinetic protocols used in this thesis controlled for cadence, and therefore may be more representative of physiological adaptations than earlier research. Finally, there were high- and low-responders to training for all measured variables, which might in future be part explained by genetic factors, though it is probable there were a multitude of additional factors influencing adaptations (300).

6.5 Detraining and Training Maintenance

Study 2 and study 3 collectively provide strong evidence that reversal of adaptations for the acid/base transport proteins occurs quite rapidly, but adaptations can be maintained by exercising at a high intensity and with a reduced volume of training. A reversal of adaptations in those proteins with the greatest magnitude increases, namely MCT1 and NHE1, was not total six weeks after stopping training. And NHE1 protein content increased further for the Rest-5 group when SIT switched to one day per week. As noted above, it is possible that NHE1 adapts more to longer recovery between sessions of SIT; however, further investigation is needed to determine whether this finding is repeatable. In contrast, performing SIT one day per week provided insufficient stimulus to mitigate reductions in CAXIV abundance. Finally, improvements in RSA were maintained after six weeks of reduced training volume. By showing it is not necessary to sustain large volumes of high-intensity training for consecutive macrocycles, these data have implications for the periodisation of training programmes.

6.6 Limitations of this Research

- A fundamental weakness of the research presented in this thesis is that it is primarily descriptive in nature. While mechanisms for upregulation of the various acid/base transporters were proposed, without direct measurement of, for example, muscle La^- and H^+ during and after exercise, these remain speculative. Measuring changes in substrates and metabolites following a representative training session, or estimating the maximal accumulated O_2 deficit from the $\dot{V}O_2$ versus power output relationship, or perhaps comparing the first and last sessions, could have provided some invaluable data to support more robust conclusions. Inferences have been made on the basis that there were contrasting bioenergetic costs of different intensities of HIT, or through having differing rest interval durations during SIT, but no direct measurements were made to support these assumptions. In addition, calculation of La^-/H^+ production and subsequent flux, through measurement of arteriovenous differences (13), and using microdialysis to measure interstitial changes at the exercising muscle (453), may have helped support or falsify some of the suppositions contained in this thesis.
- This thesis adds to the body of research showing a dissociation between changes in pH_i regulation and changes in exercise performance, in particular in the more highly trained (18, 150, 186, 187, 232, 468). However, the functional relevance of the acid/base transporters and muscle buffer capacity for exercise capacity, or their potential role in mitigating fatigue, is uncertain, and has not been addressed by the current research. While this can be estimated by correlating changes in protein content with changes in performance, for high precision of estimation, sample sizes of 30 or more are probably needed (*cf.* Figure 2.10).
- Four weeks of HIT or SIT elicited only modest performance gains in the two training studies and may have provided insufficient training stimulus to thoroughly investigate adaptations in pH_i regulation. Without a larger effect of training on performance, it was not possible to relate reversal of adaptations in protein content to potential reductions in performance. Similarly, the modest improvements in performance, in particular aerobic capacity, indicate that longer interventions were needed to observe larger effects of training in these active populations.
- Fractionation of muscle prior to immunoblotting may have introduced error to measurement of protein content in this thesis, and indeed all previous research into the acid/base transport proteins. If cellular distribution of proteins varies at different timepoints following exercise, measuring protein content in crude cytosolic and, especially, membrane fractions, risks discarding some of the target protein. Recent novel techniques for measuring protein content in unspun whole muscle homogenate, or in single muscle fibres (336), are recommended in future.

- A limitation of study 1 was the failure to take a resting muscle biopsy on the same day as the high-intensity exercise bout. The initial rationale was to minimise the number of biopsies taken, of which, there were already nine. It was reasoned that the response of the H^+ transporters over a 72-h period *following* exercise was of primary interest, and therefore a same day baseline measurement of protein content was not essential. In hindsight, this was a poor decision. A resting biopsy on the same day as exercise could have answered whether MCT1 and NHE1 protein content decreased following exercise, and subsequently returned to pre-exercise levels 24–72 h later, or if there was a delayed increase in protein synthesis in response to a single exercise bout. Furthermore, without a pre-exercise biopsy it is unknown whether $\beta m_{in\ vitro}$ increased immediately post-exercise and decreased thereafter. One might assume that the highest $\beta m_{in\ vitro}$ post-exercise in study 1 was because of greater phosphate buffering, but study 3 showed no change in $\beta m_{in\ vitro}$ immediately after a bout of SIT. It is therefore unclear how the post-exercise $\beta m_{in\ vitro}$ data fits in with the sample-to-sample variability of the titration assay. Finally, it would also be essential to have a same-day resting biopsy for potential future measurement of changes in mRNA content post-exercise.
- Short-term changes in protein content post-exercise in the first study may have been an artefact of post-translational modification, such that antibodies may not have bound to target proteins that had been modified by the cellular stress of exercise. NHE1 can be both phosphorylated and glycosylated, and although the MCTs are not glycosylated (188), they do form heterodimers with glycosylated basigin, the expression of which has been shown to decrease 6 h after SIT (129). Therefore, it is unclear whether the cause of reduced protein abundance was, for example, inhibited transcription (285), or increased protein carbonylation (129), or was an artefact of lower antibody binding affinity for post-translationally modified protein sequences.
- Measuring target protein content in homogenised wet muscle potentially leads to contamination by erythrocyte content of the same protein, notably the MCTs and CAs (70). While samples were carefully blotted free of blood prior to snap-freezing in liquid nitrogen, it is possible that erythrocyte contamination affected results. Freeze-drying muscle and dissecting samples free of blood prior to preparation for immunoblotting, as others have done for some of these target proteins, e.g., Bangsbo *et al.* (18), may be advisable.
- Despite demonstrating in the literature review that existing NBC protein evidence in human muscle cannot be confidently determined to be the NBCe1 isoform without further validation, this thesis relied on apparent molecular mass to identify NBCe1. Unfortunately, full length recombinant NBCe1 protein could not be sourced, nor could a reliable overexpression lysate. The antibody used in this thesis was raised against the C-

terminus of the NBCe1 protein and deemed by Cell Signaling to be isoform-specific, unlike the previously used N-terminus antibody, which is described by Merck Millipore as being raised against a sequence common to most NBC isoforms. And the N-terminus shows greater sequence similarity between SLC4 proteins. Nevertheless, while it is probable that NBCe1 was the isoform detected here, without further validation it is not certain.

- The first study has raised doubts about the ecological validity of the $\beta m_{in\ vitro}$ technique because of the large between-sample variability compared to the typical effect of training. In addition, because of the failure to observe group mean changes in either training study, it was not possible to confidently determine the effects of different training modalities on actual muscle buffer capacity, or the subsequent effects of training cessation or reduced training volume.

6.7 Suggested Future Research

- To investigate the cause of reduced NHE1 abundance protein abundance following a single bout of exercise. Possible causes include post-exercise acidosis inhibiting transcription, or alternatively there may be reduced translation, or potentially post-transcriptional or post-translational mechanisms affected.
- To determine in unfractionated muscle homogenate whether MCT1 protein content decreases immediately after the onset of exercise, or if there is a delayed increased in protein synthesis 24 h later.
- While this study has measured changes in protein content, the resultant effect on protein activity is unknown. Current data on MCT1/4 and NHE1 activity are primarily in sarcolemmal giant vesicles, and there are some data on changes in La^- and H^+ efflux from contracting muscle, but there are no *in vivo* data relating changes in abundance of specific transporters to changes in their activity. Therefore, the functional relevance of increased protein abundance remains to be determined.
- Although there are some immunohistochemical data (374), most evidence of fibre-type specificity of the MCTs is by correlation of their protein content with myosin heavy chain isoform content. These data have low precision because of small sample size, but may also be prone to spurious correlation if the same housekeeping protein was used as a loading control for the two correlated proteins, in particular when using a bivariate correlation. Therefore, single-fibre western blot analysis of the MCTs, and indeed the other acid/base regulatory proteins, should clarify whether they are fibre-type specific. Furthermore, single-fibre analysis could reveal whether changes in MCT content are fibre-type specific, and have potentially been hidden in the noise of mixed homogenate

analysis to date. There may also be compartmentalisation of both MCTs in type I and type II muscle fibres, including in subsarcolemmal mitochondria (510), and single-fibre analysis could, at least, help falsify this supposition.

- There are some *in vitro* data on the importance of the NHE1 for pH recovery following acidification. One could extrapolate from this that NHE1 is functionally important for pH_i recovery following high-intensity exercise, but direct evidence is needed to provide insight into the role of NHE1. Furthermore, transcriptional evidence indicates there are likely additional NHE isoforms in human skeletal muscle, namely plasmalemmal NHE-2, -4, and -5, and intracellular NHE-7 and -8 (134, 167). Validation of antibodies to detect these putative isoforms by immunoblotting should be a first step in future investigation of the NHE proteins.
- There is some tentative evidence of NBCe2 protein in human skeletal muscle (271), but unfortunately, failure to validate a custom-made NBCe2 antibody meant that the current thesis was unable to confirm this. Future investigation of the NCBTs should aim to identify all of the possible isoforms, namely, NBCe2, NBCn1, NBCn2, and NDCBE, and determine their response to exercise. Furthermore, in immunoblotting for NBCe1 in this study, unidentified bands were observed at 50 and 75 kDa. It is possible that these represented proteolytic fragments of NBCe1 (38, 421), or alternatively, unknown splice variants of NBCe1 or other SLC4 proteins. Verification of the identity of these bands, and measurement of NBCe1 in all post-translationally modified forms, should provide more comprehensive data on this protein.
- The functional relevance of the NCBTs during and after exercise seems to be intrinsically linked with that of the sarcolemmal and cytosolic CAs. However, there are currently no data in humans to confirm this. Determining the functional relevance of both of these protein families in skeletal muscle should prove insightful in future.
- Immunoblotting of CAIV produced multiple bands, probably because of low abundance in the crude cytosolic fractions analysed, as well as poor antibody-antigen affinity. More robust data could be provided by using a chemiluminescent detection system of greater sensitivity, thereby increasing the signal-to-noise ratio, and validating a CAIV antibody of greater affinity.
- Further validation of the $\beta m_{in\ vitro}$ assay is recommended before physiological changes can be distinguished from artefacts of the measurement technique. One possibility is to revisit earlier attempts to develop a method for chemical inhibition of phosphagen hydrolysis due to homogenisation, possibly using EGTA for calcium chelation and nitrilotriacetic acid as a metabolic inhibitor (376, 442). An alternative is to measure content of the phosphate pool following homogenisation, and correct $\beta m_{in\ vitro}$ for changes in specific phosphates.

- It is unclear from this study whether HIT is an effective means for improving RSA, or whether previous reports of larger effects of HIT on RSA were, in part, artefacts of the power-cadence relationship and their failure to control cadence. Future research should therefore confirm whether measured improvements in absolute and sustained power output are physiological adaptations. This could be achieved by determining optimal cadence through torque-velocity tests, before and after HIT interventions, and conducting isokinetic RSA tests at individually-determined optimal cadences.
- Investigating individual responses to training, both on a molecular level and for exercise performance, should prove more insightful than simply interrogating the group mean response. Though genetic factors must be important in determining adaptations, no studies to date have tried to repeat an intervention after sufficient washout, using the same participants, in an effort to see if the training response is random. Essentially, without controlling for participants' activity, nutrition, and sleep, over every hour of the investigation, in essence accounting for the Hawthorne effect, there may be too much noise in the data from external factors to attribute the training response to a small percentage change in total activity. There are high-responders and low-responders to training, but individuals are not low-responders for every outcome variable. One question that has never been answered is whether the same individuals respond to training every time, or are there a percentage of random responders or non-responders to training? To paraphrase Senn (424) on drug treatments, do 70% of individuals respond to training 100% of the time, or do 100% of individuals respond to training 70% of the time. Repeat interventions using the same participants, and with a sufficient wash-out period, would help address this question.

References

1. Adams, G. R., Foley, J. M. & Meyer, R. A. (1990). Muscle buffer capacity estimated from pH changes during rest-to-work transitions. *J Appl Physiol,* 69, 968-72.
2. Alberty, R. A. (2005). Introduction to Apparent Equilibrium Constants. *Thermodynamics of Biochemical Reactions.* John Wiley & Sons, Inc., pp. 1-17.
3. Allen, D. G., Lamb, G. D. & Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev,* 88, 287-332.
4. Allen, D. G., Lamb, G. D. & Westerblad, H. (2008). Impaired calcium release during fatigue. *J Appl Physiol,* 104, 296-305.
5. Altman, D. G. & Bland, J. M. (2011). How to obtain the confidence interval from a P value. *BMJ,* 343, d2090. 10.1136/bmj.d2090.
6. Alvarez, B. V., Loiselle, F. B., Supuran, C. T., Schwartz, G. J. & Casey, J. R. (2003). Direct extracellular interaction between carbonic anhydrase IV and the human NBC1 sodium/bicarbonate co-transporter. *Biochemistry,* 42, 12321-9.
7. Bacon, A. P., Carter, R. E., Ogle, E. A. & Joyner, M. J. (2013). VO_2max trainability and high intensity interval training in humans: a meta-analysis. *PloS ONE,* 8, e73182. 10.1371/journal.pone.0073182.
8. Baguet, A., Everaert, I., De Naeyer, H., Reyngoudt, H., Stegen, S., Beeckman, S., Achten, E., Vanhee, L., Volkaert, A., Petrovic, M., Taes, Y. & Derave, W. (2011). Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity. *Eur J Appl Physiol,* 111, 2571-80.
9. Bakardjiev, A. & Bauer, K. (1994). Transport of β-alanine and biosynthesis of carnosine by skeletal muscle cells in primary culture. *Eur J Biochem,* 225, 617-23.
10. Baker, S. K., McCullagh, K. J. & Bonen, A. (1998). Training intensity-dependent and tissue-specific increases in lactate uptake and MCT-1 in heart and muscle. *J Appl Physiol,* 84, 987-94.
11. Bangsbo, J. & Mizuno, M. Morphological and metabolic alterations in soccer players with detraining and retraining and their relation to performance. *In:* REILLY, T., LEES, A., DAVIDS, K. & MURPHY, W. J., (eds). Science and Football : Proceedings of the First World Congress of Science and Football, 13 – 17 April 1987 Liverpool. E. & F.N. Spon, 114-124.

12. Bangsbo, J., Graham, T., Johansen, L., Strange, S., Christensen, C. & Saltin, B. (1992). Elevated muscle acidity and energy production during exhaustive exercise in humans. *Am J Physiol Regul Integr Comp Physiol,* 263, R891-9.

13. Bangsbo, J., Johansen, L., Graham, T. & Saltin, B. (1993). Lactate and H^+ effluxes from human skeletal muscles during intense, dynamic exercise. *J Physiol,* 462, 115-33.

14. Bangsbo, J., Johansen, L., Quistorff, B. & Saltin, B. (1993). NMR and analytic biochemical evaluation of CrP and nucleotides in the human calf during muscle contraction. *J Appl Physiol,* 74, 2034-9.

15. Bangsbo, J., Madsen, K., Kiens, B. & Richter, E. A. (1996). Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J Physiol,* 495, 587-96.

16. Bangsbo, J., Juel, C., Hellsten, Y. & Saltin, B. (1997). Dissociation between lactate and proton exchange in muscle during intense exercise in man. *J Physiol,* 504, 489-99.

17. Bangsbo, J. & Juel, C. (2006). Counterpoint: lactic acid accumulation is a disadvantage during muscle activity. *J Appl Physiol,* 100, 1412-3; discussion 1413-4.

18. Bangsbo, J., Gunnarsson, T. P., Wendell, J., Nybo, L. & Thomassen, M. (2009). Reduced volume and increased training intensity elevate muscle Na^+-K^+ pump α_2-subunit expression as well as short- and long-term work capacity in humans. *J Appl Physiol,* 107, 1771-80.

19. Bate-Smith, E. C. (1938). The buffering of muscle in rigor; protein, phosphate and carnosine. *J Physiol,* 92, 336-343.

20. Beaver, W. L., Wasserman, K. & Whipp, B. J. (1986). Bicarbonate buffering of lactic acid generated during exercise. *J Appl Physiol,* 60, 472-8.

21. Becker, H. M., Bröer, S. & Deitmer, J. W. (2004). Facilitated lactate transport by MCT1 when coexpressed with the sodium bicarbonate cotransporter (NBC) in *Xenopus* oocytes. *Biophys J,* 86, 235-47.

22. Becker, H. M., Hirnet, D., Fecher-Trost, C., Sültemeyer, D. & Deitmer, J. W. (2005). Transport activity of MCT1 expressed in *Xenopus* oocytes is increased by interaction with carbonic anhydrase. *J Biol Chem,* 280, 39882-9.

23. Becker, H. M. & Deitmer, J. W. (2007). Carbonic anhydrase II increases the activity of the human electrogenic Na^+/HCO_3^- cotransporter. *J Biol Chem,* 282, 13508-21.

24. Becker, H. M. & Deitmer, J. W. (2008). Nonenzymatic Proton Handling by Carbonic Anhydrase II during H^+-Lactate Cotransport via Monocarboxylate Transporter 1. *J Biol Chem,* 283, 21655-21667.

25. Becker, H. M., Klier, M., Schüler, C., McKenna, R. & Deitmer, J. W. (2011). Intramolecular proton shuttle supports not only catalytic but also noncatalytic function of carbonic anhydrase II. *Proc Natl Acad Sci U S A,* 108, 3071-6.

26. Becker, H. M., Heidtmann, H., Noor, I. S., Dietz, S., Klier, M., Boone, C. D., McKenna, R. & Deitmer, J. W. (2014). Proton-driven metabolite transport and how it is facilitated by carbonic anhydrases. *Acta Physiol (Oxf),* 210, 18.

27. Becker, H. M., Klier, M. & Deitmer, J. W. (2014). Carbonic Anhydrases and Their Interplay with Acid/Base-Coupled Membrane Transporters. *In:* FROST, S. C. & MCKENNA, R. (eds.) *Carbonic Anhydrase: Mechanism, Regulation, Links to Disease, and Industrial Applications.* Netherlands: Springer, pp. 105-134.

28. Bedford, T. G., Tipton, C. M., Wilson, N. C., Oppliger, R. A. & Gisolfi, C. V. (1979). Maximum oxygen consumption of rats and its changes with various experimental procedures. *J Appl Physiol Respir Environ Exerc Physiol,* 47, 1278-83.

29. Bell, G. J. & Wenger, H. A. (1988). The effect of one-legged sprint training on intramuscular pH and nonbicarbonate buffering capacity. *Eur J Appl Physiol Occup Physiol,* 58, 158-64.

30. Belton, R. J., Chen, L., Mesquita, F. S. & Nowak, R. A. (2008). Basigin-2 Is a Cell Surface Receptor for Soluble Basigin Ligand. *J Biol Chem,* 283, 17805-17814.

31. Ben-Zaken, S., Eliakim, A., Nemet, D., Rabinovich, M., Kassem, E. & Meckel, Y. (2015). Differences in MCT1 A1470T polymorphism prevalence between runners and swimmers. *Scand J Med Sci Sports,* 25, 365-71.

32. Benton, C. R., Campbell, S. E., Tonouchi, M., Hatta, H. & Bonen, A. (2004). Monocarboxylate transporters in subsarcolemmal and intermyofibrillar mitochondria. *Biochem Biophys Res Commun,* 323, 249-53.

33. Benton, C. R., Yoshida, Y., Lally, J., Han, X. X., Hatta, H. & Bonen, A. (2008). PGC-1α increases skeletal muscle lactate uptake by increasing the expression of MCT1 but not MCT2 or MCT4. *Physiol Genomics,* 35, 45-54.

34. Bergman, B. C., Wolfel, E. E., Butterfield, G. E., Lopaschuk, G. D., Casazza, G. A., Horning, M. A. & Brooks, G. A. (1999). Active muscle and whole body lactate kinetics after endurance training in men. *J Appl Physiol,* 87, 1684-1696.

35. Bergström, J., Fürst, P., Norée, L. O. & Vinnars, E. (1974). Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol,* 36, 693-7.

36. Bermes II, E. W. & Young, D. S. (1996). General Laboratory Techniques and Procedures. *In:* BURTIS, C. A. & ASHWOOD, E. R. (eds.) *Tietz Fundamentals of Clinical Chemistry*. 4th ed. Philadelphia, PA: W.B. Saunders, p. 21.

37. Berra, E., Roux, D., Richard, D. E. & Pouysségur, J. (2001). Hypoxia-inducible factor-1α (HIF-1α) escapes O_2-driven proteasomal degradation irrespective of its subcellular localization: nucleus or cytoplasm. *EMBO Rep*, 2, 615-20.

38. Bevensee, M. O., Schmitt, B. M., Choi, I., Romero, M. F. & Boron, W. F. (2000). An electrogenic Na^+-HCO_3^- cotransporter (NBC) with a novel COOH-terminus, cloned from rat brain. *Am J Physiol Cell Physiol*, 278, C1200-11.

39. Bexfield, N. A., Parcell, A. C., Nelson, W. B., Foote, K. M. & Mack, G. W. (2009). Adaptations to high-intensity intermittent exercise in rodents. *J Appl Physiol*, 107, 749-54.

40. Bickham, D. C., Bentley, D. J., Le Rossignol, P. F. & Cameron-Smith, D. (2006). The effects of short-term sprint training on MCT expression in moderately endurance-trained runners. *Eur J Appl Physiol*, 96, 636-43.

41. Billat, V. L., Flechet, B., Petit, B., Muriaux, G. & Koralsztein, J. P. (1999). Interval training at VO_{2max}: effects on aerobic performance and overtraining markers. *Med Sci Sports Exerc*, 31, 156-63.

42. Bishop, D., Spencer, M., Duffield, R. & Lawrence, S. (2001). The validity of a repeated sprint ability test. *J Sci Med Sport*, 4, 19-29.

43. Bishop, D., Edge, J., Davis, C. & Goodman, C. (2004). Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. *Med Sci Sports Exerc*, 36, 807-13.

44. Bishop, D., Edge, J. & Goodman, C. (2004). Muscle buffer capacity and aerobic fitness are associated with repeated-sprint ability in women. *Eur J Appl Physiol*, 92, 540-7.

45. Bishop, D. & Edge, J. (2006). Determinants of repeated-sprint ability in females matched for single-sprint performance. *Eur J Appl Physiol*, 97, 373-9.

46. Bishop, D., Edge, J., Thomas, C. & Mercier, J. (2007). High-intensity exercise acutely decreases the membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle. *J Appl Physiol*, 102, 616-21.

47. Bishop, D., Edge, J., Thomas, C. & Mercier, J. (2008). Effects of high-intensity training on muscle lactate transporters and postexercise recovery of muscle lactate and hydrogen ions in women. *Am J Physiol Regul Integr Comp Physiol*, 295, R1991-8.

48. Bishop, D., Edge, J., Mendez-Villanueva, A., Thomas, C. & Schneiker, K. (2009). High-intensity exercise decreases muscle buffer capacity via a decrease in protein buffering in human skeletal muscle. *Pflügers Arch,* 458, 929-36.

49. Bishop, D. J., Thomas, C., Moore-Morris, T., Tonkonogi, M., Sahlin, K. & Mercier, J. (2010). Sodium bicarbonate ingestion prior to training improves mitochondrial adaptations in rats. *Am J Physiol Endocrinol Metab,* 299, E225-33.

50. Bishop, D. J. (2011). Comments on Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. Confusion concerning the lactate proton ratio: a problem of definition? *J Appl Physiol,* 110, 1494-5.

51. Blanchard, E. M., Pan, B. S. & Solaro, R. J. (1984). The effect of acidic pH on the ATPase activity and troponin Ca^{2+} binding of rabbit skeletal myofilaments. *J Biol Chem,* 259, 3181-6.

52. Blanchard, E. M. & Solaro, R. J. (1984). Inhibition of the activation and troponin calcium binding of dog cardiac myofibrils by acidic pH. *Circ Res,* 55, 382-91.

53. Bogdanis, G. C., Nevill, M. E., Boobis, L. H., Lakomy, H. K. & Nevill, A. M. (1995). Recovery of power output and muscle metabolites following 30 s of maximal sprint cycling in man. *J Physiol,* 482 (Pt 2), 467-80.

54. Bogdanis, G. C., Nevill, M. E., Boobis, L. H. & Lakomy, H. K. (1996). Contribution of phosphocreatine and aerobic metabolism to energy supply during repeated sprint exercise. *J Appl Physiol,* 80, 876-84.

55. Boldyrev, A. A., Aldini, G. & Derave, W. (2013). Physiology and pathophysiology of carnosine. *Physiol Rev,* 93, 1803-45.

56. Bonen, A., McCullagh, K. J., Putman, C. T., Hultman, E., Jones, N. L. & Heigenhauser, G. J. (1998). Short-term training increases human muscle MCT1 and femoral venous lactate in relation to muscle lactate. *Am J Physiol Endocrinol Metab,* 274, E102-7.

57. Bonen, A., Miskovic, D., Tonouchi, M., Lemieux, K., Wilson, M. C., Marette, A. & Halestrap, A. P. (2000). Abundance and subcellular distribution of MCT1 and MCT4 in heart and fast-twitch skeletal muscles. *Am J Physiol Endocrinol Metab,* 278, E1067-77.

58. Bonen, A., Tonouchi, M., Miskovic, D., Heddle, C., Heikkila, J. J. & Halestrap, A. P. (2000). Isoform-specific regulation of the lactate transporters MCT1 and MCT4 by contractile activity. *Am J Physiol Endocrinol Metab,* 279, E1131-8.

59. Bonen, A. (2001). The expression of lactate transporters (MCT1 and MCT4) in heart and muscle. *Eur J Appl Physiol,* 86, 6-11.

60. Bonen, A., Heynen, M. & Hatta, H. (2006). Distribution of monocarboxylate transporters MCT1-MCT8 in rat tissues and human skeletal muscle. *Appl Physiol Nutr Metab,* 31, 31-9.

61. Böning, D., Strobel, G., Beneke, R. & Maassen, N. (2005). Lactic acid still remains the real cause of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol,* 289, R902-R903.

62. Böning, D. & Maassen, N. (2011). Comments on Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. No evidence for the Counterpoint position. *J Appl Physiol,* 110, 1493-4.

63. Boron, W. F. (1978). Active control of intracellular pH. *Respir Physiol,* 33, 59-62.

64. Boron, W. F. (2004). Regulation of intracellular pH. *Adv Physiol Educ,* 28, 160-79.

65. Boron, W. F. (2010). Evaluating the role of carbonic anhydrases in the transport of HCO_3^--related species. *Biochim Biophys Acta,* 1804, 410-21.

66. Boyarsky, G., Ganz, M. B., Cragoe, E. J., Jr. & Boron, W. F. (1990). Intracellular-pH dependence of Na-H exchange and acid loading in quiescent and arginine vasopressin-activated mesangial cells. *Proc Natl Acad Sci U S A,* 87, 5921-4.

67. Broberg, S. & Sahlin, K. (1989). Adenine nucleotide degradation in human skeletal muscle during prolonged exercise. *J Appl Physiol,* 67, 116-22.

68. Broch-Lips, M., Overgaard, K., Praetorius, H. A. & Nielsen, O. B. (2007). Effects of extracellular HCO_3^- on fatigue, pH_i, and K^+ efflux in rat skeletal muscles. *J Appl Physiol,* 103, 494-503.

69. Brooks, G. A. & White, T. P. (1978). Determination of metabolic and heart rate responses of rats to treadmill exercise. *J Appl Physiol Respir Environ Exerc Physiol,* 45, 1009-15.

70. Brooks, G. A., Brown, M. A., Butz, C. E., Sicurello, J. P. & Dubouchaud, H. (1999). Cardiac and skeletal muscle mitochondria have a monocarboxylate transporter MCT1. *J Appl Physiol,* 87, 1713-8.

71. Brooks, G. A. (2000). Intra- and extra-cellular lactate shuttles. *Med Sci Sports Exerc,* 32, 790-9.

72. Brooks, G. A. (2002). Lactate shuttle - between but not within cells? *J Physiol,* 541, 333-334.

73. Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *J Physiol,* 587, 5591-600.

74. Brooks, G. A. (2010). Master regulator or readout: the wisdom of distributed control. Focus on "Pyruvate suppresses PGC1α expression and substrate utilization despite increased respiratory chain content in C2C12 myotubes". *Am J Physiol Cell Physiol,* 299, C216-7.

75. Brooks, G. A. (2010). What does glycolysis make and why is it important? *J Appl Physiol,* 108, 1450-1.

76. Bruton, J. D., Lännergren, J. & Westerblad, H. (1998). Effects of CO_2-induced acidification on the fatigue resistance of single mouse muscle fibers at 28°C. *J Appl Physiol,* 85, 478-83.

77. Buchheit, M. & Laursen, P. B. (2013). High-intensity interval training, solutions to the programming puzzle. Part II: anaerobic energy, neuromuscular load and practical applications. *Sports Med,* 43, 927-54.

78. Burgomaster, K. A., Hughes, S. C., Heigenhauser, G. J., Bradwell, S. N. & Gibala, M. J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol,* 98, 1985-90.

79. Burgomaster, K. A., Cermak, N. M., Phillips, S. M., Benton, C. R., Bonen, A. & Gibala, M. J. (2007). Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am J Physiol Regul Integr Comp Physiol,* 292, R1970-6.

80. Burgomaster, K. A., Howarth, K. R., Phillips, S. M., Rakobowchuk, M., Macdonald, M. J., McGee, S. L. & Gibala, M. J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol,* 586, 151-60.

81. Burton, R. F. (1978). Intracellular buffering. *Respir Physiol,* 33, 51-8.

82. Cady, E. B., Elshove, H., Jones, D. A. & Moll, A. (1989). The metabolic causes of slow relaxation in fatigued human skeletal muscle. *J Physiol,* 418, 327-37.

83. Cady, E. B., Jones, D. A., Lynn, J. & Newham, D. J. (1989). Changes in force and intracellular metabolites during fatigue of human skeletal muscle. *J Physiol,* 418, 311-25.

84. Cairns, S. P. (2006). Lactic acid and exercise performance : culprit or friend? *Sports Med,* 36, 279-91.

85. Cairns, S. P. & Lindinger, M. I. (2008). Do multiple ionic interactions contribute to skeletal muscle fatigue? *J Physiol,* 586, 4039-54.

86. Capriotti, P. V., Sherman, W. M. & Lamb, D. R. (1999). Reliability of power output during intermittent high-intensity cycling. *Med Sci Sports Exerc,* 31, 913-5.

87. Carpenter, L., Poole, R. C. & Halestrap, A. P. (1996). Cloning and sequencing of the monocarboxylate transporter from mouse Ehrlich Lettré tumour cell confirms its identity as MCT1 and demonstrates that glycosylation is not required for MCT1 function. *Biochim Biophys Acta,* 1279, 157-163.

88. Carr, A. J., Hopkins, W. G. & Gore, C. J. (2011). Effects of acute alkalosis and acidosis on performance: a meta-analysis. *Sports Med,* 41, 801-14.

89. Carreau, A., El Hafny-Rahbi, B., Matejuk, A., Grillon, C. & Kieda, C. (2011). Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med,* 15, 1239-1253.

90. Casey, A., Constantin-Teodosiu, D., Howell, S., Hultman, E. & Greenhaff, P. L. (1996). Metabolic response of type I and II muscle fibers during repeated bouts of maximal exercise in humans. *Am J Physiol Endocrinol Metab,* 271, E38-43.

91. Caso, G., Garlick, B. A., Casella, G. A., Sasvary, D. & Garlick, P. J. (2004). Acute metabolic acidosis inhibits muscle protein synthesis in rats. *Am J Physiol Endocrinol Metab,* 287, E90-6.

92. Chase, P. B. & Kushmerick, M. J. (1988). Effects of pH on contraction of rabbit fast and slow skeletal muscle fibers. *Biophys J,* 53, 935-46.

93. Chasiotis, D., Hultman, E. & Sahlin, K. (1982). Acidotic depression of cyclic AMP accumulation and phosphorylase *b* to *a* transformation in skeletal muscle of man. *J Physiol,* 335, 197-204.

94. Chasiotis, D., Sahlin, K. & Hultman, E. (1982). Regulation of glycogenolysis in human muscle at rest and during exercise. *J Appl Physiol,* 53, 708-15.

95. Cheetham, M. E., Boobis, L. H., Brooks, S. & Williams, C. (1986). Human muscle metabolism during sprint running. *J Appl Physiol,* 61, 54-60.

96. Chi, M. M. Y., Hintz, C. S., Coyle, E. F., Martin, W. H., Ivy, J. L., Nemeth, P. M., Holloszy, J. O. & Lowry, O. H. (1983). Effects of Detraining on Enzymes of Energy-Metabolism in Individual Human-Muscle Fibers. *Am J Physiol Cell Physiol,* 244, C276-C287.

97. Chin, E. R. & Allen, D. G. (1998). The contribution of pH-dependent mechanisms to fatigue at different intensities in mammalian single muscle fibres. *J Physiol,* 512 (Pt 3), 831-40.

98. Choi, I., Hu, L., Rojas, J. D., Schmitt, B. M. & Boron, W. F. (2003). Role of glycosylation in the renal electrogenic Na^+-HCO_3^- cotransporter (NBCe1). *Am J Physiol Renal Physiol,* 284, F1199-206.

99. Christensen, T., Bagger, M., Juel, C., Højlund, K. & Pedersen, P. K. Effect of intensified running training on muscular buffering capacity and muscular Na^+/H^+ exchanger. *In:* AVELA, J., KOMI, P. & KOMULAINEN, J., (eds). 5th Annual Congress of the European College of Sport Science, 19 – 23 July 2000 Jyväskylä, Finland.

100. Clark, S. A., Aughey, R. J., Gore, C. J., Hahn, A. G., Townsend, N. E., Kinsman, T. A., Chow, C. M., McKenna, M. J. & Hawley, J. A. (2004). Effects of live high, train low hypoxic exposure on lactate metabolism in trained humans. *J Appl Physiol,* 96, 517-25.

101. Clarke, S. J., Khaliulin, I., Das, M., Parker, J. E., Heesom, K. J. & Halestrap, A. P. (2008). Inhibition of mitochondrial permeability transition pore opening by ischemic preconditioning is probably mediated by reduction of oxidative stress rather than mitochondrial protein phosphorylation. *Circ Res,* 102, 1082-90.

102. Coast, J. R., Cox, R. H. & Welch, H. G. (1986). Optimal Pedaling Rate in Prolonged Bouts of Cycle Ergometry. *Med Sci Sport Exer,* 18, 225-230.

103. Cochran, A. J., Percival, M. E., Tricarico, S., Little, J. P., Cermak, N., Gillen, J. B., Tarnopolsky, M. A. & Gibala, M. J. (2014). Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations. *Exp Physiol,* 99, 782-91.

104. Coles, L., Litt, J., Hatta, H. & Bonen, A. (2004). Exercise rapidly increases expression of the monocarboxylate transporters MCT1 and MCT4 in rat muscle. *J Physiol,* 561, 253-61.

105. Conley, K. E., Blei, M. L., Richards, T. L., Kushmerick, M. J. & Jubrias, S. A. (1997). Activation of glycolysis in human muscle in vivo. *Am J Physiol Cell Physiol,* 273, C306-15.

106. Conley, K. E., Kemper, W. F. & Crowther, G. J. (2001). Limits to sustainable muscle performance: interaction between glycolysis and oxidative phosphorylation. *J Exp Biol,* 204, 3189-94.

107. Conley, K. E. (2005). Determination of muscle pH and glycolytic flux by magnetic resonance spectroscopy in contracting human skeletal muscle may have systematic errors. *Am J Physiol Cell Physiol,* 289, C230; author reply C231.

108. Constantin-Teodosiu, D., Greenhaff, P. L., McIntyre, D. B., Round, J. M. & Jones, D. A. (1997). Anaerobic energy production in human skeletal muscle in intense contraction: a comparison of ^{31}P magnetic resonance spectroscopy and biochemical techniques. *Exp Physiol,* 82, 593-601.

109. Cooke, R., Franks, K., Luciani, G. B. & Pate, E. (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J Physiol,* 395, 77-97.

110. Cordat, E. & Casey, J. R. (2009). Bicarbonate transport in cell physiology and disease. *Biochem J,* 417, 423-39.

111. Costill, D. L., Sharp, R. L., Fink, W. J. & Katz, A. (1982). Determination of human muscle pH in needle-biopsy specimens. *J Appl Physiol,* 53, 1310-3.

112. Costill, D. L., Fink, W. J., Hargreaves, M., King, D. S., Thomas, R. & Fielding, R. (1985). Metabolic Characteristics of Skeletal Muscle during Detraining from Competitive Swimming. *Med Sci Sport Exer,* 17, 339-343.

113. Counillon, L., Pouysségur, J. & Reithmeier, R. A. (1994). The Na^+/H^+ exchanger NHE-1 possesses N- and O-linked glycosylation restricted to the first N-terminal extracellular domain. *Biochemistry,* 33, 10463-9.

114. Coyle, E. F., Martin, W. H., Ehsani, A. A., Hagberg, J. M., Bloomfield, S. A., Sinacore, D. R. & Holloszy, J. O. (1983). Blood Lactate Threshold in Some Well-Trained Ischemic-Heart-Disease Patients. *J Appl Physiol,* 54, 18-23.

115. Cunningham, J. N., Jr., Carter, N. W., Rector, F. C., Jr. & Seldin, D. W. (1971). Resting transmembrane potential difference of skeletal muscle in normal subjects and severely ill patients. *J Clin Invest,* 50, 49-59.

116. Cupeiro, R., Benito, P. J., Maffulli, N., Calderon, F. J. & Gonzalez-Lamuno, D. (2010). MCT1 genetic polymorphism influence in high intensity circuit training: a pilot study. *J Sci Med Sport,* 13, 526-30.

117. Cupeiro, R., Gonzalez-Lamuno, D., Amigo, T., Peinado, A. B., Ruiz, J. R., Ortega, F. B. & Benito, P. J. (2012). Influence of the MCT1-T1470A polymorphism (rs1049434) on blood lactate accumulation during different circuit weight trainings in men and women. *J Sci Med Sport,* 15, 541-547.

118. Curran-Everett, D. & Benos, D. J. (2007). Guidelines for reporting statistics in journals published by the American Physiological Society: the sequel. *Adv Physiol Educ,* 31, 295-8.

119. Davies, N. W. (1990). Modulation of ATP-sensitive K^+ channels in skeletal muscle by intracellular protons. *Nature,* 343, 375-7.

120. Dawson, B., Goodman, C., Lawrence, S., Preen, D., Polglaze, T., Fitzsimons, M. & Fournier, P. (1997). Muscle phosphocreatine repletion following single and repeated short sprint efforts. *Scand J Med Sci Sports,* 7, 206-13.

121. Dawson, M. J., Gadian, D. G. & Wilkie, D. R. (1977). Contraction and recovery of living muscles studies by ^{31}P nuclear magnetic resonance. *J Physiol,* 267, 703-35.

122. Dawson, M. J., Gadian, D. G. & Wilkie, D. R. (1980). Mechanical relaxation rate and metabolism studied in fatiguing muscle by phosphorus nuclear magnetic resonance. *J Physiol,* 299, 465-84.

123. de Ruiter, C. J., Jones, D. A., Sargeant, A. J. & de Haan, A. (1999). Temperature effect on the rates of isometric force development and relaxation in the fresh and fatigued human adductor pollicis muscle. *Exp Physiol,* 84, 1137-50.

124. De Ruiter, C. J. & De Haan, A. (2000). Temperature effect on the force/velocity relationship of the fresh and fatigued human adductor pollicis muscle. *Pflügers Arch,* 440, 163-170.

125. Degroot, M., Massie, B. M., Boska, M., Gober, J., Miller, R. G. & Weiner, M. W. (1993). Dissociation of [H^+] from fatigue in human muscle detected by high time resolution ^{31}P-NMR. *Muscle Nerve,* 16, 91-8.

126. Deitmer, J. W. & Becker, H. M. (2013). Transport metabolons with carbonic anhydrases. *Front Physiol,* 4, 291.

127. Deitmer, J. W. & Thomas, R. C. (2014). Hundred years buffer theory by Koppel & Spiro: Cellular functions of protons and bicarbonate. *Acta Physiol (Oxf),* 210, 17.

128. Dela, F., Holten, M. & Juel, C. (2004). Effect of resistance training on Na,K pump and Na^+/H^+ exchange protein densities in muscle from control and patients with type 2 diabetes. *Pflügers Arch,* 447, 928-33.

129. Delfour-Peyrethon, R., Bishop, D. J., Hanon, C., Oliveira, R. S. F., McGinley, C., Molinari, N., Mercier, J., Lambert, K. & Thomas, C. (2015). Role of pre-exercise alkalosis on acute exercise-induced changes in sarcolemmal proteins involved in human muscle pH. *Manuscript submitted for publication*.

130. Delpire, E. (2014). How carbonic anhydrases and pH buffers facilitate the movement of carbon dioxide through biological membranes. *Am J Physiol Cell Physiol*.

131. Dimmer, K. S., Friedrich, B., Lang, F., Deitmer, J. W. & Broer, S. (2000). The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. *Biochem J,* 350, 219-27.

132. Dobson, G. P., Yamamoto, E. & Hochachka, P. W. (1986). Phosphofructokinase control in muscle: nature and reversal of pH-dependent ATP inhibition. *Am J Physiol Regul Integr Comp Physiol,* 250, R71-6.

133. Donaldson, S. K. B. & Hermansen, L. (1978). Differential, direct effects of H^+ on Ca^{2+}-activated force of skinned fibers from the soleus, cardiac and adductor magnus muscles of rabbits. *Pflügers Arch,* 376, 55-65.

134. Donowitz, M., Tse, C. M. & Fuster, D. (2013). SLC9/NHE gene family, a plasma membrane and organellar family of Na^+/H^+ exchangers. *Mol Aspects Med,* 34, 236-251.

135. Dubouchaud, H., Butterfield, G. E., Wolfel, E. E., Bergman, B. C. & Brooks, G. A. (2000). Endurance training, expression, and physiology of LDH, MCT1, and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab,* 278, E571-9.

136. Durnin, J. V. G. A. (1996). Energy requirements: general principles. *Eur J Clin Nutr,* 50 Suppl 1, S2-9; discussion 9-10.

137. Edge, J., Bishop, D., Goodman, C. & Dawson, B. (2005). Effects of high- and moderate-intensity training on metabolism and repeated sprints. *Med Sci Sports Exerc,* 37, 1975-82.

138. Edge, J. (2006). *Effects of exercise training on muscle buffer capacity and H^+ regulation.* PhD thesis, University of Western Australia.

139. Edge, J., Bishop, D. & Goodman, C. (2006). Effects of chronic $NaHCO_3$ ingestion during interval training on changes to muscle buffer capacity, metabolism, and short-term endurance performance. *J Appl Physiol,* 101, 918-25.

140. Edge, J., Bishop, D. & Goodman, C. (2006). The effects of training intensity on muscle buffer capacity in females. *Eur J Appl Physiol,* 96, 97-105.

141. Edge, J., Bishop, D., Hill-Haas, S., Dawson, B. & Goodman, C. (2006). Comparison of muscle buffer capacity and repeated-sprint ability of untrained, endurance-trained and team-sport athletes. *Eur J Appl Physiol,* 96, 225-34.

142. Edge, J., Eynon, N., McKenna, M. J., Goodman, C. A., Harris, R. C. & Bishop, D. J. (2013). Altering the rest interval during high-intensity interval training does not affect muscle or performance adaptations. *Exp Physiol,* 98, 481-490.

143. Egan, B., O'Connor, P. L., Zierath, J. R. & O'Gorman, D. J. (2013). Time Course Analysis Reveals Gene-Specific Transcript and Protein Kinetics of Adaptation to Short-Term Aerobic Exercise Training in Human Skeletal Muscle. *PloS ONE,* 8, e74098. 10.1371/journal.pone.0074098.

144. Egan, B. & Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab,* 17, 162-84.

145. Eggleton, M. G. & Eggleton, P. (1933). Carnosine in amphibian voluntary muscle. *Exp Physiol,* 23, 391-402.

146. Eichhorn, J. H. (1985). Inaccuracy in Blood Gas pH Measurements Caused by the Blood Sample. *J Med Technol,* 2, 23-26.

147. Elustondo, P. A., White, A. E., Hughes, M. E., Brebner, K., Pavlov, E. & Kane, D. A. (2013). Physical and Functional Association of Lactate Dehydrogenase (LDH) with Skeletal Muscle Mitochondria. *J Biol Chem*, 288, 25309-25317.

148. Emhoff, C. A., Messonnier, L. A., Horning, M. A., Fattor, J. A., Carlson, T. J. & Brooks, G. A. (2013). Direct and indirect lactate oxidation in trained and untrained men. *J Appl Physiol,* 115, 829-38.

149. Evans, W. J., Phinney, S. D. & Young, V. R. (1982). Suction applied to a muscle biopsy maximizes sample size. *Med Sci Sports Exerc,* 14, 101-2.

150. Evertsen, F., Medbø, J. I. & Bonen, A. (2001). Effect of training intensity on muscle lactate transporters and lactate threshold of cross-country skiers. *Acta Physiol Scand,* 173, 195-205.

151. Eydoux, N., Py, G., Lambert, K., Dubouchaud, H., Préfaut, C. & Mercier, J. (2000). Training does not protect against exhaustive exercise-induced lactate transport capacity alterations. *Am J Physiol Endocrinol Metab,* 278, E1045-52.

152. Fabiato, A. & Fabiato, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiace and skeletal muscles. *J Physiol,* 276, 233-55.

153. Favero, T. G., Zable, A. C., Bowman, M. B., Thompson, A. & Abramson, J. J. (1995). Metabolic end products inhibit sarcoplasmic reticulum Ca^{2+} release and [^{3}H]ryanodine binding. *J Appl Physiol,* 78, 1665-72.

154. Fedotovskaya, O. N., Mustafina, L. J., Popov, D. V., Vinogradova, O. L. & Ahmetov, I. I. (2014). A common polymorphism of the MCT1 gene and athletic performance. *Int J Sports Physiol Perform,* 9, 173-80.

155. Ferguson, R. E., Carroll, H. P., Harris, A., Maher, E. R., Selby, P. J. & Banks, R. E. (2005). Housekeeping proteins: A preliminary study illustrating some limitations as useful references in protein expression studies. *Proteomics,* 5, 566-571.

156. Field, A. P. (2013). Discovering statistics using IBM SPSS statistics : and sex and drugs and rock 'n' roll. 4th ed. Los Angeles: Sage, pp. 825-826.

157. Fishbein, W. N., Merezhinskaya, N. & Foellmer, J. W. (2002). Relative distribution of three major lactate transporters in frozen human tissues and their localization in unfixed skeletal muscle. *Muscle Nerve,* 26, 101-12.

158. Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. *Physiol Rev,* 74, 49-94.

159. Fitts, R. H. (2008). The cross-bridge cycle and skeletal muscle fatigue. *J Appl Physiol,* 104, 551-8.

160. Fitzsimons, M., Dawson, B., Ward, D. & Wilkinson, A. (1993). Cycling and running tests of repeated sprint ability. *Aust J Sci Med Sport,* 25, 82-87.

161. Fliegel, L. & Fröhlich, O. (1993). The Na^+/H^+ Exchanger - an Update on Structure, Regulation and Cardiac Physiology. *Biochem J,* 296, 273-285.

162. Foster, C. (1998). Monitoring training in athletes with reference to overtraining syndrome. *Med Sci Sport Exer,* 30, 1164-1168.

163. Freeman, P. R. (1993). The role of *P*-values in analysing trial results. *Stat Med,* 12, 1443-52; discussion 1453-8.

164. Fuchs, F., Reddy, Y. & Briggs, F. N. (1970). The interaction of cations with the calcium-binding site of troponin. *Biochim Biophys Acta,* 221, 407-9.

165. Fürst, P., Jonsson, A., Josephson, B. & Vinnars, E. (1970). Distribution in muscle and liver vein protein of ^{15}N administered as ammonium acetate to man. *J Appl Physiol,* 29, 307-12.

166. Furugen, A., Kobayashi, M., Narumi, K., Watanabe, M., Otake, S., Itagaki, S. & Iseki, K. (2011). AMP-activated protein kinase regulates the expression of monocarboxylate transporter 4 in skeletal muscle. *Life Sci,* 88, 163-8.

167. Fuster, D. G. & Alexander, R. T. (2014). Traditional and emerging roles for the SLC9 Na^+/H^+ exchangers. *Pflügers Arch,* 466, 61-76.

168. Gaitanos, G. C., Williams, C., Boobis, L. H. & Brooks, S. (1993). Human muscle metabolism during intermittent maximal exercise. *J Appl Physiol,* 75, 712-9.

169. Garcia, C. K., Goldstein, J. L., Pathak, R. K., Anderson, R. G. & Brown, M. S. (1994). Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle. *Cell,* 76, 865-73.

170. Gardner, A. S., Martin, D. T., Jenkins, D. G., Dyer, I., Van Eiden, J., Barras, M. & Martin, J. C. (2009). Velocity-specific fatigue: quantifying fatigue during variable velocity cycling. *Med Sci Sports Exerc,* 41, 904-11.

171. Gassmann, M., Grenacher, B., Rohde, B. & Vogel, J. (2009). Quantifying Western blots: pitfalls of densitometry. *Electrophoresis,* 30, 1845-55.

172. Geers, C. & Gros, G. (2000). Carbon dioxide transport and carbonic anhydrase in blood and muscle. *Physiol Rev,* 80, 681-715.

173. Gevers, W. (1977). Generation of protons by metabolic processes in heart cells. *J Mol Cell Cardiol,* 9, 867-74.

174. Gibala, M. J., Little, J. P., van Essen, M., Wilkin, G. P., Burgomaster, K. A., Safdar, A., Raha, S. & Tarnopolsky, M. A. (2006). Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol,* 575, 901-11.

175. Gist, N. H., Fedewa, M. V., Dishman, R. K. & Cureton, K. J. (2014). Sprint interval training effects on aerobic capacity: a systematic review and meta-analysis. *Sports Med,* 44, 269-79.

176. Gladden, L. B. (2007). Is there an intracellular lactate shuttle in skeletal muscle? *J Physiol,* 582, 899.

177. 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, 275-86.

178. Gray, K. A., Yates, B., Seal, R. L., Wright, M. W. & Bruford, E. A. (2015). genenames.org: the HGNC resources in 2015. *Nucleic Acids Res,* 43, D1079-85.

179. Green, H., Halestrap, A., Mockett, C., O'Toole, D., Grant, S. & Ouyang, J. (2002). Increases in muscle MCT are associated with reductions in muscle lactate after a single exercise session in humans. *Am J Physiol Endocrinol Metab,* 282, E154-60.

180. Green, H. J., Bombardier, E., Duhamel, T. A., Stewart, R. D., Tupling, A. R. & Ouyang, J. (2008). Metabolic, enzymatic, and transporter responses in human muscle during three consecutive days of exercise and recovery. *Am J Physiol Regul Integr Comp Physiol,* 295, R1238-50.

181. Green, H. J., Duhamel, T. A., Holloway, G. P., Moule, J. W., Ranney, D. W., Tupling, A. R. & Ouyang, J. (2008). Rapid upregulation of GLUT-4 and MCT-4 expression during 16 h of heavy intermittent cycle exercise. *Am J Physiol Regul Integr Comp Physiol,* 294, R594-600.

182. Green, H. J., Duhamel, T. A., Smith, I. C., Rich, S. M., Thomas, M. M., Ouyang, J. & Yau, J. E. (2011). Muscle metabolic, enzymatic and transporter responses to a session of prolonged cycling. *Eur J Appl Physiol,* 111, 827-37.

183. Gross, E., Hawkins, K., Abuladze, N., Pushkin, A., Cotton, C. U., Hopfer, U. & Kurtz, I. (2001). The stoichiometry of the electrogenic sodium bicarbonate cotransporter NBC1 is cell-type dependent. *J Physiol,* 531, 597-603.

184. Gross, E., Pushkin, A., Abuladze, N., Fedotoff, O. & Kurtz, I. (2002). Regulation of the sodium bicarbonate cotransporter kNBC1 function: role of Asp^{986}, Asp^{988} and kNBC1-carbonic anhydrase II binding. *J Physiol,* 544, 679-85.

185. Gross, M., Boesch, C., Bolliger, C. S., Norman, B., Gustafsson, T., Hoppeler, H. & Vogt, M. (2014). Effects of beta-alanine supplementation and interval training on physiological determinants of severe exercise performance. *Eur J Appl Physiol,* 114, 221-34.

186. Gunnarsson, T. P. & Bangsbo, J. (2012). The 10-20-30 training concept improves performance and health profile in moderately trained runners. *J Appl Physiol,* 113, 16-24.

187. Gunnarsson, T. P., Christensen, P. M., Thomassen, M., Nielsen, L. R. & Bangsbo, J. (2013). Effect of intensified training on muscle ion kinetics, fatigue development, and repeated short-term performance in endurance-trained cyclists. *Am J Physiol Regul Integr Comp Physiol,* 305, R811-21.

188. Halestrap, A. P. & Price, N. T. (1999). The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem J,* 343 Pt 2, 281-99.

189. Halestrap, A. P. & Meredith, D. (2004). The SLC16 gene family-from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. *Pflügers Arch,* 447, 619-28.

190. Halestrap, A. P. (2012). The monocarboxylate transporter family--Structure and functional characterization. *IUBMB life,* 64, 1-9.

191. Halestrap, A. P. & Wilson, M. C. (2012). The monocarboxylate transporter family--role and regulation. *IUBMB life,* 64, 109-19.

192. Halestrap, A. P. (2013). Monocarboxylic acid transport. *Compr Physiol,* 3, 1611-43.

193. Halestrap, A. P. (2013). The SLC16 gene family - structure, role and regulation in health and disease. *Mol Aspects Med,* 34, 337-49.

194. Hallerdei, J., Scheibe, R. J., Parkkila, S., Waheed, A., Sly, W. S., Gros, G., Wetzel, P. & Endeward, V. (2010). T tubules and surface membranes provide equally effective

pathways of carbonic anhydrase-facilitated lactic acid transport in skeletal muscle. *PloS ONE,* 5, e15137. 10.1371/journal.pone.0015137.

195. Hargreaves, M., McKenna, M. J., Jenkins, D. G., Warmington, S. A., Li, J. L., Snow, R. J. & Febbraio, M. A. (1998). Muscle metabolites and performance during high-intensity, intermittent exercise. *J Appl Physiol,* 84, 1687-91.

196. Harmer, A. R., McKenna, M. J., Sutton, J. R., Snow, R. J., Ruell, P. A., Booth, J., Thompson, M. W., Mackay, N. A., Stathis, C. G., Crameri, R. M., Carey, M. F. & Eager, D. M. (2000). Skeletal muscle metabolic and ionic adaptations during intense exercise following sprint training in humans. *J Appl Physiol,* 89, 1793-803.

197. Harris, J. A. & Benedict, F. G. (1918). A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci U S A,* 4, 370-3.

198. Harris, R. C., Snow, D. H., Katz, A. & Sahlin, K. (1989). Effect of freeze-drying on measurements of pH in biopsy samples of the middle gluteal muscle of the horse: comparison of muscle pH to the pyruvate and lactate content. *Equine Vet J,* 21, 45-7.

199. Harris, R. C., Marlin, D. J., Dunnett, M., Snow, D. H. & Hultman, E. (1990). Muscle buffering capacity and dipeptide content in the thoroughbred horse, greyhound dog and man. *Comp Biochem Physiol A Comp Physiol,* 97, 249-51.

200. Harris, R. C., Tallon, M. J., Dunnett, M., Boobis, L., Coakley, J., Kim, H. J., Fallowfield, J. L., Hill, C. A., Sale, C. & Wise, J. A. (2006). The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids,* 30, 279-89.

201. Harris, R. C., Edge, J., Kendrick, I. P., Bishop, D., Goodman, C. & Wise, J. A. (2007). The effect of very high interval training on the carnosine content and buffering capacity of V lateralis from humans. *Faseb J,* 21, A944.

202. Harris, R. C., Jones, G., Hill, C. A., Kendrick, L. P., Boobis, L., Kim, C. K., Kim, H. J., Dang, V. H., Edge, J. & Wise, J. A. (2007). The carnosine content of V lateralis in vegetarians and omnivores. *FASEB J,* 21, A944.

203. Harris, R. C., Wise, J. A., Price, K. A., Kim, H. J., Kim, C. K. & Sale, C. (2012). Determinants of muscle carnosine content. *Amino Acids,* 43, 5-12.

204. Hashimoto, T., Masuda, S., Taguchi, S. & Brooks, G. A. (2005). Immunohistochemical analysis of MCT1, MCT2 and MCT4 expression in rat plantaris muscle. *J Physiol,* 567, 121-9.

205. Hashimoto, T., Hussien, R. & Brooks, G. A. (2006). Colocalization of MCT1, CD147, and LDH in mitochondrial inner membrane of L6 muscle cells: evidence of a

mitochondrial lactate oxidation complex. *Am J Physiol Endocrinol Metab,* 290, E1237-44.

206. Hashimoto, T., Hussien, R., Oommen, S., Gohil, K. & Brooks, G. A. (2007). Lactate sensitive transcription factor network in L6 cells: activation of MCT1 and mitochondrial biogenesis. *FASEB J,* 21, 2602-12.

207. Hashimoto, T. & Brooks, G. A. (2008). Mitochondrial lactate oxidation complex and an adaptive role for lactate production. *Med Sci Sports Exerc,* 40, 486-94.

208. Hatta, H., Tonouchi, M., Miskovic, D., Wang, Y., Heikkila, J. J. & Bonen, A. (2001). Tissue-specific and isoform-specific changes in MCT1 and MCT4 in heart and soleus muscle during a 1-yr period. *Am J Physiol Endocrinol Metab,* 281, E749-56.

209. Hawley, J. A. & Noakes, T. D. (1992). Peak power output predicts maximal oxygen uptake and performance time in trained cyclists. *Eur J Appl Physiol Occup Physiol,* 65, 79-83.

210. Haworth, R. S., Fröhlich, O. & Fliegel, L. (1993). Multiple carbohydrate moieties on the Na^+/H^+ exchanger. *Biochem J,* 289 (Pt 3), 637-40.

211. Heisler, N. (2004). Buffering and H^+ ion dynamics in muscle tissues. *Respir Physiol Neurobiol,* 144, 161-72.

212. Hermansen, L. & Osnes, J. B. (1972). Blood and muscle pH after maximal exercise in man. *J Appl Physiol,* 32, 304-8.

213. Hermansen, L. & Vaage, O. (1977). Lactate disappearance and glycogen synthesis in human muscle after maximal exercise. *Am J Physiol,* 233, E422-9.

214. Hickson, R. C. & Rosenkoetter, M. A. (1981). Reduced training frequencies and maintenance of increased aerobic power. *Med Sci Sports Exerc,* 13, 13-6.

215. Hickson, R. C., Foster, C., Pollock, M. L., Galassi, T. M. & Rich, S. (1985). Reduced training intensities and loss of aerobic power, endurance, and cardiac growth. *J Appl Physiol,* 58, 492-9.

216. Hildebrandt, A. L., Pilegaard, H. & Neufer, P. D. (2003). Differential transcriptional activation of select metabolic genes in response to variations in exercise intensity and duration. *Am J Physiol Endocrinol Metab,* 285, E1021-7.

217. Hill, C. A., Harris, R. C., Kim, H. J., Harris, B. D., Sale, C., Boobis, L. H., Kim, C. K. & Wise, J. A. (2007). Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. *Amino Acids,* 32, 225-33.

218. Hilvo, M., Baranauskiene, L., Salzano, A. M., Scaloni, A., Matulis, D., Innocenti, A., Scozzafava, A., Monti, S. M., Di Fiore, A., De Simone, G., Lindfors, M., Jänis, J., Valjakka, J., Pastoreková, S., Pastorek, J., Kulomaa, M. S., Nordlund, H. R., Supuran, C. T. & Parkkila, S. (2008). Biochemical characterization of CA IX, one of the most active carbonic anhydrase isozymes. *J Biol Chem,* 283, 27799-809.

219. Hochachka, P. W. & Mommsen, T. P. (1983). Protons and anaerobiosis. *Science,* 219, 1391-7.

220. Hochachka, P. W. & Somero, G. N. (2002). *Biochemical adaptation: mechanism and process in physiological evolution,* New York, Oxford University Press.

221. Hollidge-Horvat, M. G., Parolin, M. L., Wong, D., Jones, N. L. & Heigenhauser, G. J. (1999). Effect of induced metabolic acidosis on human skeletal muscle metabolism during exercise. *Am J Physiol Endocrinol Metab,* 277, E647-58.

222. Hollidge-Horvat, M. G., Parolin, M. L., Wong, D., Jones, N. L. & Heigenhauser, G. J. (2000). Effect of induced metabolic alkalosis on human skeletal muscle metabolism during exercise. *Am J Physiol Endocrinol Metab,* 278, E316-29.

223. Hopkins, W. G. (2006). Spreadsheets for analysis of controlled trials with adjustment for a predictor. *Sportscience,* 10, 46-50.

224. Hopkins, W. G. (2007). A spreadsheet for deriving a confidence interval, mechanistic inference and clinical inference from a P value. *Sportscience,* 11, 16-20.

225. Hopkins, W. G., Marshall, S. W., Batterham, A. M. & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc,* 41, 3-13.

226. Houmard, J. A., Costill, D. L., Mitchell, J. B., Park, S. H., Hickner, R. C. & Roemmich, J. N. (1990). Reduced training maintains performance in distance runners. *Int J Sports Med,* 11, 46-52.

227. Houmard, J. A., Tyndall, G. L., Midyette, J. B., Hickey, M. S., Dolan, P. L., Gavigan, K. E., Weidner, M. L. & Dohm, G. L. (1996). Effect of reduced training and training cessation on insulin action and muscle GLUT-4. *J Appl Physiol,* 81, 1162-8.

228. Hulikova, A., Harris, A. L., Vaughan-Jones, R. D. & Swietach, P. (2012). Acid-extrusion from tissue: the interplay between membrane transporters and pH buffers. *Curr Pharm Des,* 18, 1331-7.

229. Hultman, E. & Sahlin, K. (1980). Acid-base balance during exercise. *Exerc Sport Sci Rev,* 8, 41-128.

230. Hultman, E. & Sjöholm, H. (1983). Energy metabolism and contraction force of human skeletal muscle in situ during electrical stimulation. *J Physiol,* 345, 525-32.

231. Hultman, E., Del Canale, S. & Sjöholm, H. (1985). Effect of induced metabolic acidosis on intracellular pH, buffer capacity and contraction force of human skeletal muscle. *Clin Sci (Lond),* 69, 505-10.

232. Iaia, F. M., Thomassen, M., Kolding, H., Gunnarsson, T., Wendell, J., Rostgaard, T., Nordsborg, N., Krustrup, P., Nybo, L., Hellsten, Y. & Bangsbo, J. (2008). Reduced volume but increased training intensity elevates muscle Na^+-K^+ pump α_1-subunit and NHE1 expression as well as short-term work capacity in humans. *Am J Physiol Regul Integr Comp Physiol,* 294, R966-74.

233. Iaia, F. M. & Bangsbo, J. (2010). Speed endurance training is a powerful stimulus for physiological adaptations and performance improvements of athletes. *Scand J Med Sci Sports,* 20 Suppl 2, 11-23.

234. Inesi, G. & Hill, T. L. (1983). Calcium and proton dependence of sarcoplasmic reticulum ATPase. *Biophys J,* 44, 271-80.

235. Ioannidis, J. P. A., Ntzani, E. E., Trikalinos, T. A. & Contopoulos-Ioannidis, D. G. (2001). Replication validity of genetic association studies. *Nat Genet,* 29, 306-309.

236. Ioannidis, J. P. A. & Trikalinos, T. A. (2005). Early extreme contradictory estimates may appear in published research: The Proteus phenomenon in molecular genetics research and randomized trials. *J Clin Epidemiol,* 58, 543-549.

237. Jewell, U. R. & Gassmann, M. (2001). Mammalian gene expression in hypoxic conditions. *Zoology,* 104, 192-7.

238. Jia, L., Zhou, H., Wang, S., Cao, J., Wei, W. & Zhang, J. (2006). Deglycosylation of CD147 down-regulates Matrix Metalloproteinase-11 expression and the adhesive capability of murine hepatocarcinoma cell HcaF in vitro. *IUBMB life,* 58, 209-16.

239. Jóhannsson, E., Lunde, P. K., Heddle, C., Sjaastad, I., Thomas, M. J., Bergersen, L., Halestrap, A. P., Blackstad, T. W., Ottersen, O. P. & Sejersted, O. M. (2001). Upregulation of the cardiac monocarboxylate transporter MCT1 in a rat model of congestive heart failure. *Circulation,* 104, 729-34.

240. Jones, D. A., de Ruiter, C. J. & de Haan, A. (2006). Change in contractile properties of human muscle in relationship to the loss of power and slowing of relaxation seen with fatigue. *J Physiol,* 576, 913-22.

241. Jones, N. L. (1980). Hydrogen ion balance during exercise. *Clin Sci (Lond),* 59, 85-91.

242. Jones, N. L. & Heigenhauser, G. J. (1996). Getting rid of carbon dioxide during exercise. *Clin Sci (Lond)*, 90, 323-35.

243. Jones, N. L. (2008). An obsession with CO_2. *Appl Physiol Nutr Metab*, 33, 641-650.

244. Jubrias, S. A., Crowther, G. J., Shankland, E. G., Gronka, R. K. & Conley, K. E. (2003). Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle *in vivo*. *J Physiol*, 553, 589-99.

245. Juel, C. (1988). Intracellular pH recovery and lactate efflux in mouse soleus muscles stimulated in vitro: the involvement of sodium/proton exchange and a lactate carrier. *Acta Physiol Scand*, 132, 363-71.

246. Juel, C. (1996). Lactate/proton co-transport in skeletal muscle: regulation and importance for pH homeostasis. *Acta Physiol Scand*, 156, 369-74.

247. Juel, C. (1997). Lactate-proton cotransport in skeletal muscle. *Physiol Rev*, 77, 321-58.

248. Juel, C. (1998). Muscle pH regulation: role of training. *Acta Physiol Scand*, 162, 359-66.

249. Juel, C. (1998). Skeletal muscle Na^+/H^+ exchange in rats: pH dependency and the effect of training. *Acta Physiol Scand*, 164, 135-40.

250. Juel, C. & Halestrap, A. P. (1999). Lactate transport in skeletal muscle - role and regulation of the monocarboxylate transporter. *J Physiol*, 517, 633-42.

251. Juel, C. (2000). Expression of the Na^+/H^+ exchanger isoform NHE1 in rat skeletal muscle and effect of training. *Acta Physiol Scand*, 170, 59-63.

252. Juel, C., Lundby, C., Sander, M., Calbet, J. A. L. & van Hall, G. (2003). Human skeletal muscle and erythrocyte proteins involved in acid-base homeostasis: adaptations to chronic hypoxia. *J Physiol*, 548, 639-48.

253. Juel, C., Klarskov, C., Nielsen, J. J., Krustrup, P., Mohr, M. & Bangsbo, J. (2004). Effect of high-intensity intermittent training on lactate and H^+ release from human skeletal muscle. *Am J Physiol Endocrinol Metab*, 286, E245-51.

254. Juel, C. (2008). Regulation of pH in human skeletal muscle: adaptations to physical activity. *Acta Physiol (Oxf)*, 193, 17-24.

255. Junge, W. & McLaughlin, S. (1987). The role of fixed and mobile buffers in the kinetics of proton movement. *Biochim Biophys Acta*, 890, 1-5.

256. Kanduc, D. (2012). Homology, similarity, and identity in peptide epitope immunodefinition. *J Pept Sci*, 18, 487-94.

257. Kao, L., Sassani, P., Azimov, R., Pushkin, A., Abuladze, N., Peti-Peterdi, J., Liu, W., Newman, D. & Kurtz, I. (2008). Oligomeric structure and minimal functional unit of the electrogenic sodium bicarbonate cotransporter NBCe1-A. *J Biol Chem,* 283, 26782-94.

258. Karlsson, J. (1971). Lactate and phosphagen concentrations in working muscle of man with special reference to oxygen deficit at the onset of work. *Acta Physiol Scand Suppl,* 358, 1-72.

259. Kelman, G. R. & Nunn, J. F. (1966). Nomograms for correction of blood P_{O_2}, P_{CO_2}, pH, and base excess for time and temperature. *J Appl Physiol,* 21, 1484-90.

260. Kemp, G. (2005). Lactate accumulation, proton buffering, and pH change in ischemically exercising muscle. *Am J Physiol Regul Integr Comp Physiol,* 289, R895-901.

261. Kemp, G. J. & Radda, G. K. (1994). Quantitative interpretation of bioenergetic data from ^{31}P and ^{1}H magnetic resonance spectroscopic studies of skeletal muscle: an analytical review. *Magn Reson Q,* 10, 43-63.

262. Kemp, G. J., Roussel, M., Bendahan, D., Le Fur, Y. & Cozzone, P. J. (2001). Interrelations of ATP synthesis and proton handling in ischaemically exercising human forearm muscle studied by ^{31}P magnetic resonance spectroscopy. *J Physiol,* 535, 901-28.

263. Kiilerich, K. (2010). *PDH regulation in skeletal muscle.* PhD thesis, University of Copenhagen.

264. Kirk, P., Wilson, M. C., Heddle, C., Brown, M. H., Barclay, A. N. & Halestrap, A. P. (2000). CD147 is tightly associated with lactate transporters MCT1 and MCT4 and facilitates their cell surface expression. *EMBO J,* 19, 3896-904.

265. Kitaoka, Y., Machida, M., Takemasa, T. & Hatta, H. (2011). Expression of monocarboxylate transporter (MCT) 1 and MCT4 in overloaded mice plantaris muscle. *J Physiol Sci,* 61, 467-72.

266. Kitaoka, Y., Masuda, H., Mukai, K., Hiraga, A., Takemasa, T. & Hatta, H. (2011). Effect of training and detraining on monocarboxylate transporter (MCT) 1 and MCT4 in Thoroughbred horses. *Exp Physiol,* 96, 348-55.

267. Kitaoka, Y., Endo, Y., Mukai, K., Aida, H., Hiraga, A., Takemasa, T. & Hatta, H. (2013). Effect of acute exercise on monocarboxylate transporters 1 and 4 in untrained and trained Thoroughbreds. *Am J Vet Res,* 74, 642-7.

268. Kitaoka, Y., Takahashi, Y., Machida, M., Takeda, K., Takemasa, T. & Hatta, H. (2014). Effect of AMPK activation on monocarboxylate transporter (MCT)1 and MCT4 in denervated muscle. *J Physiol Sci,* 64, 59-64.

269. Klier, M., Andes, F. T., Deitmer, J. W. & Becker, H. M. (2014). Intracellular and extracellular carbonic anhydrases cooperate non-enzymatically to enhance activity of monocarboxylate transporters. *J Biol Chem,* 289, 2765-75.

270. Knuth, S. T., Dave, H., Peters, J. R. & Fitts, R. H. (2006). Low cell pH depresses peak power in rat skeletal muscle fibres at both 30°C and 15°C: implications for muscle fatigue. *J Physiol,* 575, 887-99.

271. Kristensen, J. M., Kristensen, M. & Juel, C. (2004). Expression of Na^+/HCO_3^- co-transporter proteins (NBCs) in rat and human skeletal muscle. *Acta Physiol Scand,* 182, 69-76.

272. Kristensen, M., Albertsen, J., Rentsch, M. & Juel, C. (2005). Lactate and force production in skeletal muscle. *J Physiol,* 562, 521-6.

273. Kuipers, H., Verstappen, F. T., Keizer, H. A., Geurten, P. & van Kranenburg, G. (1985). Variability of aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med,* 6, 197-201.

274. Kurtz, I., Petrasek, D. & Tatishchev, S. (2004). Molecular mechanisms of electrogenic sodium bicarbonate cotransport: structural and equilibrium thermodynamic considerations. *J Membr Biol,* 197, 77-90.

275. Kurtz, I., Kraut, J., Ornekian, V. & Nguyen, M. K. (2008). Acid-base analysis: a critique of the Stewart and bicarbonate-centered approaches. *Am J Physiol Renal Physiol,* 294, F1009-31.

276. Kurtz, I. (2014). NBCe1 as a model carrier for understanding the structure-function properties of Na^+-coupled SLC4 transporters in health and disease. *Pflügers Arch,* 466, 1501-16.

277. Kushmerick, M. J. (1997). Multiple equilibria of cations with metabolites in muscle bioenergetics. *Am J Physiol,* 272, C1739-47.

278. Lamb, G. D., Recupero, E. & Stephenson, D. G. (1992). Effect of myoplasmic pH on excitation-contraction coupling in skeletal muscle fibres of the toad. *J Physiol,* 448, 211-24.

279. Lamb, G. D. & Stephenson, D. G. (1994). Effects of intracellular pH and $[Mg^{2+}]$ on excitation-contraction coupling in skeletal muscle fibres of the rat. *J Physiol,* 478, 331-9.

280. Lamb, G. D. & Stephenson, D. G. (2006). Point: lactic acid accumulation is an advantage during muscle activity. *J Appl Physiol,* 100, 1410-2; discussion 1414.

281. Larsen, R. G., Maynard, L. & Kent, J. A. (2014). High-intensity interval training alters ATP pathway flux during maximal muscle contractions in humans. *Acta Physiol (Oxf),* 211, 147-60.

282. Laursen, P. B. & Jenkins, D. G. (2002). The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes. *Sports Med,* 32, 53-73.

283. Lee, S. K., Boron, W. F. & Parker, M. D. (2012). Relief of autoinhibition of the electrogenic Na-HCO_3 cotransporter NBCe1-B: role of IRBIT vs. amino-terminal truncation. *Am J Physiol Cell Physiol,* 302, C518-26.

284. Lenz, G. R. & Martell, A. E. (1964). Metal Complexes of Carnosine. *Biochemistry,* 3, 750-3.

285. Li, J. J. & Biggin, M. D. (2015). Gene expression. Statistics requantitates the central dogma. *Science,* 347, 1066-7.

286. Li, X., Alvarez, B., Casey, J. R., Reithmeier, R. A. & Fliegel, L. (2002). Carbonic anhydrase II binds to and enhances activity of the Na^+/H^+ exchanger. *J Biol Chem,* 277, 36085-91.

287. Li, X., Liu, Y., Alvarez, B. V., Casey, J. R. & Fliegel, L. (2006). A novel carbonic anhydrase II binding site regulates NHE1 activity. *Biochemistry,* 45, 2414-24.

288. Li, X., Prins, D., Michalak, M. & Fliegel, L. (2013). Calmodulin-dependent binding to the NHE1 cytosolic tail mediates activation of the Na^+/H^+ exchanger by Ca^{2+} and endothelin. *Am J Physiol Cell Physiol,* 305, C1161-9.

289. Liao, C. G., Kong, L. M., Song, F., Xing, J. L., Wang, L. X., Sun, Z. J., Tang, H., Yao, H., Zhang, Y., Wang, L., Wang, Y., Yang, X. M., Li, Y. & Chen, Z. N. (2011). Characterization of Basigin Isoforms and the Inhibitory Function of Basigin-3 in Human Hepatocellular Carcinoma Proliferation and Invasion. *Mol Cell Biol,* 31, 2591-2604.

290. Lin, R. Y., Vera, J. C., Chaganti, R. S. & Golde, D. W. (1998). Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. *J Biol Chem,* 273, 28959-65.

291. Lindinger, M. I., Kowalchuk, J. M. & Heigenhauser, G. J. (2005). Applying physicochemical principles to skeletal muscle acid-base status. *Am J Physiol Regul Integr Comp Physiol,* 289, R891-4; author reply R904-910.

292. Lindinger, M. I. (2006). Lactic acid accumulation is an advantage/disadvantage during muscle activity. *J Appl Physiol,* 100, 2100.

293. Lindinger, M. I. (2011). Lactate: metabolic fuel or poison. *Exp Physiol,* 96, 1099-1100.

294. Lindinger, M. I. & Heigenhauser, G. J. (2011). Comments on Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. Calculations of Robergs support the view of Vinnakota and Kushmerick. *J Appl Physiol,* 110, 1493.

295. Linossier, M. T., Dormois, D., Perier, C., Frey, J., Geyssant, A. & Denis, C. (1997). Enzyme adaptations of human skeletal muscle during bicycle short-sprint training and detraining. *Acta Physiol Scand,* 161, 439-445.

296. Liu, Y., Qin, X., Wang, D. K., Guo, Y. M., Gill, H. S., Morris, N., Parker, M. D., Chen, L. M. & Boron, W. F. (2013). Effects of optional structural elements, including two alternative amino termini and a new splicing cassette IV, on the function of the sodium-bicarbonate cotransporter NBCn1 (SLC4A7). *J Physiol,* 591, 4983-5004.

297. Mainwood, G. W. & Worsley-Brown, P. (1975). The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J Physiol,* 250, 1-22.

298. Mainwood, G. W. & Renaud, J. M. (1985). The effect of acid-base balance on fatigue of skeletal muscle. *Can J Physiol Pharmacol,* 63, 403-16.

299. Malley, W. J. (2005). *Clinical blood gases : assessment and intervention,* St. Louis, Mo., Elsevier Saunders.

300. Mann, T. N., Lamberts, R. P. & Lambert, M. I. (2014). High responders and low responders: factors associated with individual variation in response to standardized training. *Sports Med,* 44, 1113-24.

301. Manning Fox, J. E., Meredith, D. & Halestrap, A. P. (2000). Characterisation of human monocarboxylate transporter 4 substantiates its role in lactic acid efflux from skeletal muscle. *J Physiol,* 529, 285-293.

302. Mannion, A. F., Jakeman, P. M., Dunnett, M., Harris, R. C. & Willan, P. L. T. (1992). Carnosine and Anserine Concentrations in the Quadriceps Femoris Muscle of Healthy Humans. *Eur J Appl Physiol Occup Physiol,* 64, 47-50.

303. Mannion, A. F., Jakeman, P. M. & Willan, P. L. (1993). Determination of human skeletal muscle buffer value by homogenate technique: methods of measurement. *J Appl Physiol,* 75, 1412-8.

304. Mannion, A. F., Jakeman, P. M. & Willan, P. L. (1994). Effects of isokinetic training of the knee extensors on high-intensity exercise performance and skeletal muscle buffering. *Eur J Appl Physiol Occup Physiol,* 68, 356-61.

305. Marcinek, D. J., Kushmerick, M. J. & Conley, K. E. (2010). Lactic acidosis in vivo: testing the link between lactate generation and H^+ accumulation in ischemic mouse muscle. *J Appl Physiol,* 108, 1479-86.

306. Marlin, D. J. & Harris, R. C. (1991). Titrimetric determination of muscle buffering capacity (βm_{titr}) in biopsy samples. *Equine Vet J,* 23, 193-7.

307. Martin, J. C., Diedrich, D. & Coyle, E. F. (2000). Time course of learning to produce maximum cycling power. *Int J Sports Med,* 21, 485-7.

308. McCartney, N., Spriet, L. L., Heigenhauser, G. J., Kowalchuk, J. M., Sutton, J. R. & Jones, N. L. (1986). Muscle power and metabolism in maximal intermittent exercise. *J Appl Physiol,* 60, 1164-9.

309. McConell, G. K., Costill, D. L., Widrick, J. J., Hickey, M. S., Tanaka, H. & Gastin, P. B. (1993). Reduced training volume and intensity maintain aerobic capacity but not performance in distance runners. *Int J Sports Med,* 14, 33-7.

310. McCullagh, K. J., Poole, R. C., Halestrap, A. P., Tipton, K. F., O'Brien, M. & Bonen, A. (1997). Chronic electrical stimulation increases MCT1 and lactate uptake in red and white skeletal muscle. *Am J Physiol Endocrinol Metab,* 273, E239-46.

311. McCullagh, K. J. A., Poole, R. C., Halestrap, A. P., O'Brien, M. & Bonen, A. (1996). Role of the lactate transporter (MCT1) in skeletal muscles. *Am J Physiol Endocrinol Metab,* 271, E143-50.

312. McDermott, J. C. & Bonen, A. (1993). Endurance Training Increases Skeletal Muscle Lactate Transport. *Acta Physiol Scand,* 147, 323-327.

313. McGawley, K. & Bishop, D. (2006). Reliability of a 5 x 6-s maximal cycling repeated-sprint test in trained female team-sport athletes. *Eur J Appl Physiol,* 98, 383-93.

314. McGawley, K. & Bishop, D. J. (2015). Oxygen uptake during repeated-sprint exercise. *J Sci Med Sport,* 18, 214-8.

315. McGowan, C. M., Golland, L. C., Evans, D. L., Hodgson, D. R. & Rose, R. J. (2002). Effects of prolonged training, overtraining and detraining on skeletal muscle metabolites and enzymes. *Equine Vet J Suppl,* 257-63.

316. Mendez-Villanueva, A., Edge, J., Suriano, R., Hamer, P. & Bishop, D. (2012). The Recovery of Repeated-Sprint Exercise Is Associated with PCr Resynthesis, while

Muscle pH and EMG Amplitude Remain Depressed. *PloS ONE,* 7, e51977. 10.1371/journal.pone.0051977.

317. Merezhinskaya, N., Fishbein, W. N., Davis, J. I. & Foellmer, J. W. (2000). Mutations in MCT1 cDNA in patients with symptomatic deficiency in lactate transport. *Muscle Nerve,* 23, 90-97.

318. Merezhinskaya, N. & Fishbein, W. N. (2009). Monocarboxylate transporters: past, present, and future. *Histol Histopathol,* 24, 243-64.

319. Messonnier, L., Kristensen, M., Juel, C. & Denis, C. (2007). Importance of pH regulation and lactate/H^+ transport capacity for work production during supramaximal exercise in humans. *J Appl Physiol,* 102, 1936-44.

320. Metzger, J. M. & Moss, R. L. (1988). Depression of Ca^{2+} insensitive tension due to reduced pH in partially troponin-extracted skinned skeletal muscle fibers. *Biophys J,* 54, 1169-73.

321. Metzger, J. M. & Moss, R. L. (1990). Calcium-sensitive cross-bridge transitions in mammalian fast and slow skeletal muscle fibers. *Science,* 247, 1088-90.

322. Metzger, J. M. & Moss, R. L. (1990). pH modulation of the kinetics of a Ca^{2+}-sensitive cross-bridge state transition in mammalian single skeletal muscle fibres. *J Physiol,* 428, 751-64.

323. Metzger, J. M. & Moss, R. L. (1990). Effects of tension and stiffness due to reduced pH in mammalian fast- and slow-twitch skinned skeletal muscle fibres. *J Physiol,* 428, 737-50.

324. Millet, G., Bentley, D. J., Roels, B., Mc Naughton, L. R., Mercier, J. & Cameron-Smith, D. (2014). Effects of intermittent training on anaerobic performance and MCT transporters in athletes. *PloS ONE,* 9, e95092. 10.1371/journal.pone.0095092.

325. Miyauchi, T., Masuzawa, Y. & Muramatsu, T. (1991). The basigin group of the immunoglobulin superfamily: complete conservation of a segment in and around transmembrane domains of human and mouse basigin and chicken HT7 antigen. *J Biochem,* 110, 770-4.

326. Mizuno, M., Juel, C., Bro-Rasmussen, T., Mygind, E., Schibye, B., Rasmussen, B. & Saltin, B. (1990). Limb skeletal muscle adaptation in athletes after training at altitude. *J Appl Physiol,* 68, 496-502.

327. Mohr, M., Krustrup, P., Nielsen, J. J., Nybo, L., Rasmussen, M. K., Juel, C. & Bangsbo, J. (2007). Effect of two different intense training regimens on skeletal muscle ion

transport proteins and fatigue development. *Am J Physiol Regul Integr Comp Physiol*, 292, R1594-602.

328. Mollica, J. P., Oakhill, J. S., Lamb, G. D. & Murphy, R. M. (2009). Are genuine changes in protein expression being overlooked? Reassessing Western blotting. *Anal Biochem*, 386, 270-275.

329. Montgomery, H. E., Marshall, R., Hemingway, H., Myerson, S., Clarkson, P., Dollery, C., Hayward, M., Holliman, D. E., Jubb, M., World, M., Thomas, E. L., Brynes, A. E., Saeed, N., Barnard, M., Bell, J. D., Prasad, K., Rayson, M., Talmud, P. J. & Humphries, S. E. (1998). Human gene for physical performance. *Nature*, 393, 221-2.

330. Moore, R. L., Thacker, E. M., Kelley, G. A., Musch, T. I., Sinoway, L. I., Foster, V. L. & Dickinson, A. L. (1987). Effect of training/detraining on submaximal exercise responses in humans. *J Appl Physiol*, 63, 1719-24.

331. Mootha, V. K., Bunkenborg, J., Olsen, J. V., Hjerrild, M., Wisniewski, J. R., Stahl, E., Bolouri, M. S., Ray, H. N., Sihag, S., Kamal, M., Patterson, N., Lander, E. S. & Mann, M. (2003). Integrated Analysis of Protein Composition, Tissue Diversity, and Gene Regulation in Mouse Mitochondria. *Cell*, 115, 629-640.

332. Morrow, J. R., Jr. & Jackson, A. W. (1993). How "significant" is your reliability? *Res Q Exerc Sport*, 64, 352-5.

333. Mujika, I. & Padilla, S. (2000). Detraining: loss of training-induced physiological and performance adaptations. Part I: short term insufficient training stimulus. *Sports Med*, 30, 79-87.

334. Mujika, I. & Padilla, S. (2000). Detraining: loss of training-induced physiological and performance adaptations. Part II: Long term insufficient training stimulus. *Sports Med*, 30, 145-54.

335. Mujika, I. (2010). Intense training: the key to optimal performance before and during the taper. *Scand J Med Sci Sports*, 20 Suppl 2, 24-31.

336. Murphy, R. M. & Lamb, G. D. (2013). Important considerations for protein analyses using antibody based techniques: down-sizing Western blotting up-sizes outcomes. *J Physiol*, 591, 5823-31.

337. Musa-Aziz, R., Occhipinti, R. & Boron, W. F. (2014). Evidence from simultaneous intracellular- and surface-pH transients that carbonic anhydrase II enhances CO_2 fluxes across *Xenopus* oocyte plasma membranes. *Am J Physiol Cell Physiol*, 307, C791-813.

338. Musa-Aziz, R., Occhipinti, R. & Boron, W. F. (2014). Evidence from simultaneous intracellular- and surface-pH transients that carbonic anhydrase IV enhances CO_2 fluxes across *Xenopus* oocyte plasma membranes. *Am J Physiol Cell Physiol,* 307, C814-40.

339. Mutch, B. J. & Banister, E. W. (1983). Ammonia metabolism in exercise and fatigue: a review. *Med Sci Sports Exerc,* 15, 41-50.

340. Nakai, M., Chen, L. & Nowak, R. A. (2006). Tissue distribution of basigin and monocarboxylate transporter 1 in the adult male mouse: A study using the wild-type and basigin gene knockout mice. *Anat Rec Part A,* 288a, 527-535.

341. Nakamaru, Y. & Schwartz, A. (1972). The influence of hydrogen ion concentration on calcium binding and release by skeletal muscle sarcoplasmic reticulum. *J Gen Physiol,* 59, 22-32.

342. Nakamura, N., Tanaka, S., Teko, Y., Mitsui, K. & Kanazawa, H. (2005). Four Na^+/H^+ exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. *J Biol Chem,* 280, 1561-1572.

343. Narumi, K., Kobayashi, M., Otake, S., Furugen, A., Takahashi, N., Ogura, J., Itagaki, S., Hirano, T., Yamaguchi, H. & Iseki, K. (2012). Regulation of human monocarboxylate transporter 4 in skeletal muscle cells: the role of protein kinase C (PKC). *Int J Pharm,* 428, 25-32.

344. NCB Editorial (2004). Gel slicing and dicing: a recipe for disaster. *Nat Cell Biol,* 6, 275.

345. Neal, C. M., Hunter, A. M., Brennan, L., O'Sullivan, A., Hamilton, D. L., De Vito, G. & Galloway, S. D. (2013). Six weeks of a polarized training-intensity distribution leads to greater physiological and performance adaptations than a threshold model in trained cyclists. *J Appl Physiol,* 114, 461-71.

346. Nelson, C. R. & Fitts, R. H. (2014). Effects of low cell pH and elevated inorganic phosphate on the pCa-force relationship in single muscle fibers at near-physiological temperatures. *Am J Physiol Cell Physiol,* 306, C670-8.

347. Neufer, P. D. (1989). The effect of detraining and reduced training on the physiological adaptations to aerobic exercise training. *Sports Med,* 8, 302-20.

348. Nevill, A. (1997). Why the analysis of performance variables recorded on a ratio scale will invariably benefit from a log transformation. *J Sport Sci,* 15, 457-8.

349. Nevill, M. E., Boobis, L. H., Brooks, S. & Williams, C. (1989). Effect of training on muscle metabolism during treadmill sprinting. *J Appl Physiol,* 67, 2376-82.

350. Newell, J., Higgins, D., Madden, N., Cruickshank, J., Einbeck, J., McMillan, K. & McDonald, R. (2007). Software for calculating blood lactate endurance markers. *J Sport Sci,* 25, 1403-1409.

351. Nguyen, M. K., Kao, L. Y. & Kurtz, I. (2011). Defining the buffering process by a triprotic acid without relying on Stewart-electroneutrality considerations. *Theor Biol Med Model,* 8.

352. Nielsen, H. B., Hein, L., Svendsen, L. B., Secher, N. H. & Quistorff, B. (2002). Bicarbonate attenuates intracellular acidosis. *Acta Anaesthesiol Scand,* 46, 579-84.

353. Nielsen, O. B., de Paoli, F. & Overgaard, K. (2001). Protective effects of lactic acid on force production in rat skeletal muscle. *J Physiol,* 536, 161-6.

354. Nordsborg, N., Bangsbo, J. & Pilegaard, H. (2003). Effect of high-intensity training on exercise-induced gene expression specific to ion homeostasis and metabolism. *J Appl Physiol,* 95, 1201-6.

355. Nordsborg, N. B., Siebenmann, C., Jacobs, R. A., Rasmussen, P., Diaz, V., Robach, P. & Lundby, C. (2012). Four weeks of normobaric "live high-train low" do not alter muscular or systemic capacity for maintaining pH and K^+ homeostasis during intense exercise. *J Appl Physiol,* 112, 2027-36.

356. Oliver, J. L. (2009). Is a fatigue index a worthwhile measure of repeated sprint ability? *J Sci Med Sport,* 12, 20-3.

357. Orlowski, J. & Grinstein, S. (2004). Diversity of the mammalian sodium/proton exchanger SLC9 gene family. *Pflügers Arch,* 447, 549-565.

358. Ørtenblad, N., Westerblad, H. & Nielsen, J. (2013). Muscle glycogen stores and fatigue. *J Physiol,* 591, 4405-13.

359. Parker, M. D., Musa-Aziz, R., Rojas, J. D., Choi, I., Daly, C. M. & Boron, W. F. (2008). Characterization of human SLC4A10 as an electroneutral Na/HCO_3 cotransporter (NBCn2) with Cl^- self-exchange activity. *J Biol Chem,* 283, 12777-12788.

360. Parker, M. D. & Boron, W. F. (2013). The divergence, actions, roles, and relatives of sodium-coupled bicarbonate transporters. *Physiol Rev,* 93, 803-959.

361. Parkhouse, W. S., McKenzie, D. C., Hochachka, P. W. & Ovalle, W. K. (1985). Buffering capacity of deproteinized human vastus lateralis muscle. *J Appl Physiol,* 58, 14-7.

362. Parkhouse, W. S., Dobson, G. P., Belcastro, A. N. & Hochachka, P. W. (1987). The Role of Intermediary Metabolism in the Maintenance of Proton and Charge Balance during Exercise. *Mol Cell Biochem,* 77, 37-47.

363. Parolin, M. L., Chesley, A., Matsos, M. P., Spriet, L. L., Jones, N. L. & Heigenhauser, G. J. (1999). Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise. *Am J Physiol,* 277, E890-900.

364. Pate, E., Bhimani, M., Franks-Skiba, K. & Cooke, R. (1995). Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for fatigue. *J Physiol,* 486, 689-94.

365. Pedersen, T. H., Nielsen, O. B., Lamb, G. D. & Stephenson, D. G. (2004). Intracellular acidosis enhances the excitability of working muscle. *Science,* 305, 1144-7.

366. Péronnet, F. & Aguilaniu, B. (2006). Lactic acid buffering, nonmetabolic CO_2 and exercise hyperventilation: a critical reappraisal. *Respir Physiol Neurobiol,* 150, 4-18.

367. Perry, C. G., Heigenhauser, G. J., Bonen, A. & Spriet, L. L. (2008). High-intensity aerobic interval training increases fat and carbohydrate metabolic capacities in human skeletal muscle. *Appl Physiol Nutr Metab,* 33, 1112-23.

368. Perry, C. G. R., Lally, J., Holloway, G. P., Heigenhauser, G. J. F., Bonen, A. & Spriet, L. L. (2010). Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J Physiol,* 588, 4795-4810.

369. Piermarini, P. M., Kim, E. Y. & Boron, W. F. (2007). Evidence against a direct interaction between intracellular carbonic anhydrase II and pure C-terminal domains of SLC4 bicarbonate transporters. *J Biol Chem,* 282, 1409-21.

370. Pilegaard, H., Juel, C. & Wibrand, F. (1993). Lactate Transport Studied in Sarcolemmal Giant Vesicles from Rats - Effect of Training. *Am J Physiol,* 264, E156-E160.

371. Pilegaard, H., Bangsbo, J., Richter, E. A. & Juel, C. (1994). Lactate transport studied in sarcolemmal giant vesicles from human muscle biopsies: relation to training status. *J Appl Physiol,* 77, 1858-62.

372. Pilegaard, H. & Asp, S. (1998). Effect of prior eccentric contractions on lactate/H^+ transport in rat skeletal muscle. *Am J Physiol Endocrinol Metab,* 274, E554-E559.

373. Pilegaard, H., Domino, K., Noland, T., Juel, C., Hellsten, Y., Halestrap, A. P. & Bangsbo, J. (1999). Effect of high-intensity exercise training on lactate/H^+ transport capacity in human skeletal muscle. *Am J Physiol Endocrinol Metab,* 276, E255-61.

374. Pilegaard, H., Terzis, G., Halestrap, A. & Juel, C. (1999). Distribution of the lactate/H^+ transporter isoforms MCT1 and MCT4 in human skeletal muscle. *Am J Physiol Endocrinol Metab,* 276, E843-8.

375. Pörtner, H. O. (1990). Determination of intracellular buffer values after metabolic inhibition by fluoride and nitrilotriacetic acid. *Respir Physiol,* 81, 275-88.

376. Pörtner, H. O., Boutilier, R. G., Tang, Y. & Toews, D. P. (1990). Determination of Intracellular pH and PCO_2 after Metabolic Inhibition by Fluoride and Nitrilotriacetic Acid. *Respir Physiol,* 81, 255-274.

377. Puigserver, P. & Spiegelman, B. M. (2003). Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): transcriptional coactivator and metabolic regulator. *Endocr Rev,* 24, 78-90.

378. Pushkin, A., Abuladze, N., Lee, I., Newman, D., Hwang, J. & Kurtz, I. (1999). Cloning, tissue distribution, genomic organization, and functional characterization of NBC3, a new member of the sodium bicarbonate cotransporter family. *J Biol Chem,* 274, 16569-16575.

379. Putman, C. T., Jones, N. L., Lands, L. C., Bragg, T. M., Hollidge-Horvat, M. G. & Heigenhauser, G. J. (1995). Skeletal muscle pyruvate dehydrogenase activity during maximal exercise in humans. *Am J Physiol,* 269, E458-68.

380. Puype, J., Van Proeyen, K., Raymackers, J. M., Deldicque, L. & Hespel, P. (2013). Sprint interval training in hypoxia stimulates glycolytic enzyme activity. *Med Sci Sports Exerc,* 45, 2166-74.

381. Rahn, H., Reeves, R. B. & Howell, B. J. (1975). Hydrogen ion regulation, temperature, and evolution. *Am Rev Respir Dis,* 112, 165-72.

382. Ranatunga, K. W. (1987). Effects of acidosis on tension development in mammalian skeletal muscle. *Muscle Nerve,* 10, 439-45.

383. Ranatunga, K. W., Sharpe, B. & Turnbull, B. (1987). Contractions of a human skeletal muscle at different temperatures. *J Physiol,* 390, 383-95.

384. Rasmussen, H. N., van Hall, G. & Rasmussen, U. F. (2002). Lactate dehydrogenase is not a mitochondrial enzyme in human and mouse vastus lateralis muscle. *J Physiol,* 541, 575-80.

385. Rice, P., Longden, I. & Bleasby, A. (2000). EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet,* 16, 276-7.

386. Richardson, R. S., Noyszewski, E. A., Kendrick, K. F., Leigh, J. S. & Wagner, P. D. (1995). Myoglobin O_2 Desaturation during Exercise - Evidence of Limited O_2 Transport. *J Clin Invest,* 96, 1916-1926.

387. Richardson, R. S., Duteil, S., Wary, C., Wray, D. W., Hoff, J. & Carlier, P. G. (2006). Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. *J Physiol,* 571, 415-424.

388. Rietjens, G. J. W. M., Keizer, H. A., Kuipers, H. & Saris, W. H. M. (2001). A reduction in training volume and intensity for 21 days does not impair performance in cyclists. *Br J Sports Med,* 35, 431-4.

389. Robergs, R., Hutchinson, K., Hendee, S., Madden, S. & Siegler, J. (2005). Influence of pre-exercise acidosis and alkalosis on the kinetics of acid-base recovery following intense exercise. *Int J Sport Nutr Exerc Metab,* 15, 59-74.

390. Robergs, R. A., Ghiasvand, F. & Parker, D. (2004). Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol,* 287, R502-16.

391. Robergs, R. A., Ghiasvand, F. & Parker, D. (2005). Reply - Lingering construct of lactic acidosis. *Am J Physiol Regul Integr Comp Physiol,* 289, R904-R910.

392. Robergs, R. A. (2011). Counterpoint: Muscle lactate and H^+ production do not have a 1:1 association in skeletal muscle. *J Appl Physiol,* 110, 1489-91.

393. Robin, E. D. (1961). Of men and mitochondria — intracellular and subcellular acid-base relations. *N Engl J Med,* 265, 780-5.

394. Robin, E. D., Wilson, R. J. & Bromberg, P. A. (1961). Intracellular acid-base relations and intracellular buffers. *Ann N Y Acad Sci,* 92, 539-46.

395. Romero, M. F., Hediger, M. A., Boulpaep, E. L. & Boron, W. F. (1997). Expression cloning and characterization of a renal electrogenic Na^+/HCO_3^- cotransporter. *Nature,* 387, 409-413.

396. Romero, M. F., Chen, A. P., Parker, M. D. & Boron, W. F. (2013). The SLC4 family of bicarbonate (HCO_3^-) transporters. *Mol Aspects Med,* 34, 159-82.

397. Roos, A. & Boron, W. F. (1981). Intracellular pH. *Physiol Rev,* 61, 296-434.

398. Roots, H., Ball, G., Talbot-Ponsonby, J., King, M., McBeath, K. & Ranatunga, K. W. (2009). Muscle fatigue examined at different temperatures in experiments on intact mammalian (rat) muscle fibers. *J Appl Physiol,* 106, 378-384.

399. Rose, A. J. & Richter, E. A. (2009). Regulatory mechanisms of skeletal muscle protein turnover during exercise. *J Appl Physiol,* 106, 1702-1711.

400. Ross, A. & Leveritt, M. (2001). Long-term metabolic and skeletal muscle adaptations to short-sprint training: Implications for sprint training and tapering. *Sports Med,* 31, 1063-1082.

401. Rossiter, H. B., Kowalchuk, J. M. & Whipp, B. J. (2006). A test to establish maximum O_2 uptake despite no plateau in the O_2 uptake response to ramp incremental exercise. *J Appl Physiol,* 100, 764-770.

402. Roth, S. M., Ferrell, R. E., Peters, D. G., Metter, E. J., Hurley, B. F. & Rogers, M. A. (2002). Influence of age, sex, and strength training on human muscle gene expression determined by microarray. *Physiol Genomics,* 10, 181-190.

403. Sahlin, K., Harris, R. C. & Hultman, E. (1975). Creatine kinase equilibrium and lactate content compared with muscle pH in tissue samples obtained after isometric exercise. *Biochem J,* 152, 173-80.

404. Sahlin, K., Alvestrand, A., Bergström, J. & Hultman, E. (1977). Intracellular pH and bicarbonate concentration as determined in biopsy samples from the quadriceps muscle of man at rest. *Clin Sci Mol Med,* 53, 459-66.

405. Sahlin, K. (1978). Intracellular pH and energy metabolism in skeletal muscle of man. With special reference to exercise. *Acta Physiol Scand Suppl,* 455, 1-56.

406. Sahlin, K., Alvestrand, A., Brandt, R. & Hultman, E. (1978). Intracellular pH and bicarbonate concentration in human muscle during recovery from exercise. *J Appl Physiol,* 45, 474-80.

407. Sahlin, K., Edström, L., Sjöholm, H. & Hultman, E. (1981). Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. *Am J Physiol Cell Physiol,* 240, C121-6.

408. Sahlin, K. & Ren, J. M. (1989). Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction. *J Appl Physiol,* 67, 648-54.

409. Sahlin, K. & Broberg, S. (1990). Adenine nucleotide depletion in human muscle during exercise: causality and significance of AMP deamination. *Int J Sports Med,* 11 Suppl 2, S62-7.

410. Sahlin, K., Fernstrom, M., Svensson, M. & Tonkonogi, M. (2002). No evidence of an intracellular lactate shuttle in rat skeletal muscle. *J Physiol,* 541, 569-74.

411. Sahlin, K. (2005). Determination of muscle pH and glycolytic flux by magnetic resonance spectroscopy in contracting human skeletal muscle may have systematic errors. *Am J Physiol Cell Physiol,* 289, C230; author reply C231.

412. Sahlin, K. (2011). Comments on Point:Counterpoint: Muscle lactate and H^+ production do/do not have a 1:1 association in skeletal muscle. Why add complexity/confusion to a simple issue? *J Appl Physiol,* 110, 1494.

413. Sahlin, K. (2014). Muscle energetics during explosive activities and potential effects of nutrition and training. *Sports Med,* 44 Suppl 2, S167-73.

414. Sale, C., Saunders, B. & Harris, R. C. (2010). Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance. *Amino Acids,* 39, 321-33.

415. Sargeant, A. J., Hoinville, E. & Young, A. (1981). Maximum Leg Force and Power Output during Short-Term Dynamic Exercise. *J Appl Physiol,* 51, 1175-1182.

416. Sawczuk, M., Banting, L. K., Cięszczyk, P., Maciejewska-Karłowska, A., Zarębska, A., Leońska-Duniec, A., Jastrzębski, Z., Bishop, D. J. & Eynon, N. (2015). MCT1 A1470T: A novel polymorphism for sprint performance? *J Sci Med Sport,* 18, 114-118.

417. Scharhag-Rosenberger, F., Walitzek, S., Kindermann, W. & Meyer, T. (2012). Differences in adaptations to 1 year of aerobic endurance training: individual patterns of nonresponse. *Scand J Med Sci Sports,* 22, 113-8.

418. Scheuermann, B. W., Kowalchuk, J. M., Paterson, D. H. & Cunningham, D. A. (2000). Carbonic anhydrase inhibition delays plasma lactate appearance with no effect on ventilatory threshold. *J Appl Physiol,* 88, 713-21.

419. Scheuermann, B. W., Kowalchuk, J. M., Paterson, D. H., Taylor, A. W. & Green, H. J. (2000). Muscle metabolism during heavy-intensity exercise after acute acetazolamide administration. *J Appl Physiol,* 88, 722-9.

420. Schiaffino, S. & Reggiani, C. (2011). Fiber types in mammalian skeletal muscles. *Physiol Rev,* 91, 1447-531.

421. Schmitt, B. M., Biemesderfer, D., Romero, M. F., Boulpaep, E. L. & Boron, W. F. (1999). Immunolocalization of the electrogenic Na^+-HCO_3^- cotransporter in mammalian and amphibian kidney. *Am J Physiol,* 276, F27-38.

422. Schueler, C., Becker, H. M., McKenna, R. & Deitmer, J. W. (2011). Transport activity of the sodium bicarbonate cotransporter NBCe1 is enhanced by different isoforms of carbonic anhydrase. *PloS ONE,* 6, e27167. 10.1371/journal.pone.0027167.

423. Sedgeman, D., Dalleck, L., Clark, I. E., Jamnick, N. & Pettitt, R. W. (2013). Analysis of square-wave bouts to verify VO_{2max}. *Int J Sports Med,* 34, 1058-62.

424. Senn, S. (2004). Individual response to treatment: is it a valid assumption? *BMJ,* 329, 966-8.

425. Sennepin, A. D., Charpentier, S., Normand, T., Sarré, C., Legrand, A. & Mollet, L. M. (2009). Multiple reprobing of Western blots after inactivation of peroxidase activity by its substrate, hydrogen peroxide. *Anal Biochem,* 393, 129-31.

426. Seraydarian, K., Mommaerts, W. F. H. M. & Wallner, A. (1962). The amount and compartmentalization of adenosine diphosphate in muscle. *Biochim Biophys Acta,* 65, 443-60.

427. Shepley, B., MacDougall, J. D., Cipriano, N., Sutton, J. R., Tarnopolsky, M. A. & Coates, G. (1992). Physiological effects of tapering in highly trained athletes. *J Appl Physiol,* 72, 706-11.

428. Shirakabe, K., Priori, G., Yamada, H., Ando, H., Horita, S., Fujita, T., Fujimoto, I., Mizutani, A., Seki, G. & Mikoshiba, K. (2006). IRBIT, an inositol 1,4,5-trisphosphate receptor-binding protein, specifically binds to and activates pancreas-type Na^+/HCO_3^- cotransporter 1 (pNBC1). *Proc Natl Acad Sci U S A,* 103, 9542-7.

429. Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D. & Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol,* 7, 539.

430. Siggaard-Andersen, O. & Fogh-Andersen, N. (1995). Base excess or buffer base (strong ion difference) as measure of a non-respiratory acid-base disturbance. *Acta Anaesthesiol Scand Suppl,* 107, 123-8.

431. Simoneau, J. A., Lortie, G., Boulay, M. R., Marcotte, M., Thibault, M. C. & Bouchard, C. (1987). Effects of 2 High-Intensity Intermittent Training-Programs Interspaced by Detraining on Human Skeletal-Muscle and Performance. *Eur J Appl Physiol Occup Physiol,* 56, 516-521.

432. Sjøgaard, G. (1996). Potassium and fatigue: the pros and cons. *Acta Physiol Scand,* 156, 257-64.

433. Skovgaard, C., Christensen, P. M., Larsen, S., Andersen, T. R., Thomassen, M. & Bangsbo, J. (2014). Concurrent speed endurance and resistance training improves performance, running economy, and muscle NHE1 in moderately trained runners. *J Appl Physiol,* 117, 1097-109.

434. Smith, M. B. (2011). *Organic chemistry : an acid-base approach,* Boca Raton, FL, Taylor & Francis.

435. Soleimani, M. & Burnham, C. E. (2001). Na^+:HCO_3^- cotransporters (NBC): cloning and characterization. *J Membr Biol,* 183, 71-84.

436. Somero, G. N. (1981). pH-Temperature Interactions on Proteins: Principles of Optimal pH and Buffer System Design. *Mar Biol Lett,* 2, 163-178.

437. Somero, G. N. (1995). Proteins and temperature. *Annu Rev Physiol,* 57, 43-68.

438. Sostaric, S. M., Skinner, S. L., Brown, M. J., Sangkabutra, T., Medved, I., Medley, T., Selig, S. E., Fairweather, I., Rutar, D. & McKenna, M. J. (2006). Alkalosis increases muscle K^+ release, but lowers plasma [K^+] and delays fatigue during dynamic forearm exercise. *J Physiol,* 570, 185-205.

439. Spencer, M., Dawson, B., Goodman, C., Dascombe, B. & Bishop, D. (2008). Performance and metabolism in repeated sprint exercise: effect of recovery intensity. *Eur J Appl Physiol,* 103, 545-552.

440. Spriet, L. L., Matsos, C. G., Peters, S. J., Heigenhauser, G. J. & Jones, N. L. (1985). Effects of acidosis on rat muscle metabolism and performance during heavy exercise. *Am J Physiol,* 248, C337-47.

441. Spriet, L. L., Lindinger, M. I., Heigenhauser, G. J. & Jones, N. L. (1986). Effects of alkalosis on skeletal muscle metabolism and performance during exercise. *Am J Physiol,* 251, R833-9.

442. Spriet, L. L., Söderlund, K., Thomson, J. A. & Hultman, E. (1986). pH measurement in human skeletal muscle samples: effect of phosphagen hydrolysis. *J Appl Physiol,* 61, 1949-54.

443. Spriet, L. L., Söderlund, K., Bergström, M. & Hultman, E. (1987). Skeletal muscle glycogenolysis, glycolysis, and pH during electrical stimulation in men. *J Appl Physiol,* 62, 616-21.

444. Spriet, L. L., Lindinger, M. I., McKelvie, R. S., Heigenhauser, G. J. & Jones, N. L. (1989). Muscle glycogenolysis and H^+ concentration during maximal intermittent cycling. *J Appl Physiol,* 66, 8-13.

445. Spriet, L. L. (1991). Phosphofructokinase activity and acidosis during short-term tetanic contractions. *Can J Physiol Pharmacol,* 69, 298-304.

446. Spriet, L. L., Howlett, R. A. & Heigenhauser, G. J. (2000). An enzymatic approach to lactate production in human skeletal muscle during exercise. *Med Sci Sports Exerc,* 32, 756-63.

447. St-Amand, J., Yoshioka, M., Nishida, Y., Tobina, T., Shono, N. & Tanaka, H. (2012). Effects of mild-exercise training cessation in human skeletal muscle. *Eur J Appl Physiol,* 112, 853-869.

448. Stephens, T. J., McKenna, M. J., Canny, B. J., Snow, R. J. & McConell, G. K. (2002). Effect of sodium bicarbonate on muscle metabolism during intense endurance cycling. *Med Sci Sports Exerc,* 34, 614-21.

449. Stephenson, D. G., Lamb, G. D. & Stephenson, G. M. (1998). Events of the excitation-contraction-relaxation (E-C-R) cycle in fast- and slow-twitch mammalian muscle fibres relevant to muscle fatigue. *Acta Physiol Scand,* 162, 229-45.

450. Sterne, J. A. & Davey Smith, G. (2001). Sifting the evidence-what's wrong with significance tests? *BMJ,* 322, 226-31.

451. Stewart, P. A. (1978). Independent and dependent variables of acid-base control. *Respir Physiol,* 33, 9-26.

452. Stewart, P. A. (1983). Modern quantitative acid-base chemistry. *Can J Physiol Pharmacol,* 61, 1444-61.

453. Street, D., Bangsbo, J. & Juel, C. (2001). Interstitial pH in human skeletal muscle during and after dynamic graded exercise. *J Physiol,* 537, 993-998.

454. Street, D., Nielsen, J. J., Bangsbo, J. & Juel, C. (2005). Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. *J Physiol,* 566, 481-9.

455. Stridh, M. H., Alt, M. D., Wittmann, S., Heidtmann, H., Aggarwal, M., Riederer, B., Seidler, U., Wennemuth, G., McKenna, R., Deitmer, J. W. & Becker, H. M. (2012). Lactate flux in astrocytes is enhanced by a non-catalytic action of carbonic anhydrase II. *J Physiol,* 590, 2333-51.

456. Stroka, D. M., Burkhardt, T., Desbaillets, I., Wenger, R. H., Neil, D. A., Bauer, C., Gassmann, M. & Candinas, D. (2001). HIF-1 is expressed in normoxic tissue and displays an organ-specific regulation under systemic hypoxia. *FASEB J,* 15, 2445-53.

457. Sullivan, M. J., Saltin, B., Negro-Vilar, R., Duscha, B. D. & Charles, H. C. (1994). Skeletal muscle pH assessed by biochemical and ^{31}P-MRS methods during exercise and recovery in men. *J Appl Physiol,* 77, 2194-200.

458. Summermatter, S., Santos, G., Pérez-Schindler, J. & Handschin, C. (2013). Skeletal muscle PGC-1α controls whole-body lactate homeostasis through estrogen-related receptor α-dependent activation of LDH B and repression of LDH A. *Proc Natl Acad Sci U S A,* 110, 8738-43.

459. Supuran, C. T. (2008). Carbonic anhydrases—an overview. *Curr Pharm Des,* 14, 603-14.

460. Sutton, J. R., Jones, N. L. & Toews, C. J. (1981). Effect of pH on muscle glycolysis during exercise. *Clin Sci (Lond),* 61, 331-8.

461. Swietach, P., Leem, C. H., Spitzer, K. W. & Vaughan-Jones, R. D. (2014). Pumping Ca^{2+} up H^{+} gradients: a cytoplasmic Ca^{2+}-H^{+} exchanger without a membrane. *J Physiol,* 592, 3179-3188.

462. Takimoto, M., Takeyama, M. & Hamada, T. (2013). Possible involvement of AMPK in acute exercise-induced expression of monocarboxylate transporters MCT1 and MCT4 mRNA in fast-twitch skeletal muscle. *Metabolism,* 62, 1633-40.

463. Tang, W., Chang, S. B. & Hemler, M. E. (2004). Links between CD147 function, glycosylation, and caveolin-1. *Mol Biol Cell,* 15, 4043-4050.

464. The UniProt Consortium (2015). UniProt: a hub for protein information. *Nucleic Acids Res,* 43, D204-D212.

465. Thomas, C., Perrey, S., Lambert, K., Hugon, G., Mornet, D. & Mercier, J. (2005). Monocarboxylate transporters, blood lactate removal after supramaximal exercise, and fatigue indexes in humans. *J Appl Physiol,* 98, 804-9.

466. Thomas, C., Bishop, D., Moore-Morris, T. & Mercier, J. (2007). Effects of high-intensity training on MCT1, MCT4, and NBC expressions in rat skeletal muscles: influence of chronic metabolic alkalosis. *Am J Physiol Endocrinol Metab,* 293, E916-22.

467. Thomas, C., Bishop, D. J., Lambert, K., Mercier, J. & Brooks, G. A. (2012). Effects of acute and chronic exercise on sarcolemmal MCT1 and MCT4 contents in human skeletal muscles: current status. *Am J Physiol Regul Integr Comp Physiol,* 302, R1-R14.

468. Thomassen, M., Christensen, P. M., Gunnarsson, T. P., Nybo, L. & Bangsbo, J. (2010). Effect of 2-wk intensified training and inactivity on muscle Na^{+}-K^{+} pump expression, phospholemman (FXYD1) phosphorylation, and performance in soccer players. *J Appl Physiol,* 108, 898-905.

469. Thompson, L. V., Balog, E. M. & Fitts, R. H. (1992). Muscle fatigue in frog semitendinosus: role of intracellular pH. *Am J Physiol,* 262, C1507-12.

470. Timmons, J. A., Knudsen, S., Rankinen, T., Koch, L. G., Sarzynski, M., Jensen, T., Keller, P., Scheele, C., Vollaard, N. B., Nielsen, S., Åkerström, T., MacDougald, O. A., Jansson, E., Greenhaff, P. L., Tarnopolsky, M. A., van Loon, L. J., Pedersen, B. K., Sundberg, C. J., Wahlestedt, C., Britton, S. L. & Bouchard, C. (2010). Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. *J Appl Physiol,* 108, 1487-96.

471. Tonouchi, M., Hatta, H. & Bonen, A. (2002). Muscle contraction increases lactate transport while reducing sarcolemmal MCT4, but not MCT1. *Am J Physiol Endocrinol Metab,* 282, E1062-9.

472. Toole, B. P. (2003). Emmprin (CD147), a cell surface regulator of matrix metalloproteinase production and function. *Curr Top Dev Biol,* 54, 371-89.

473. Trivedi, B. & Danforth, W. H. (1966). Effect of pH on the kinetics of frog muscle phosphofructokinase. *J Biol Chem,* 241, 4110-2.

474. Trump, M. E., Heigenhauser, G. J., Putman, C. T. & Spriet, L. L. (1996). Importance of muscle phosphocreatine during intermittent maximal cycling. *J Appl Physiol,* 80, 1574-80.

475. Ui, M. (1966). A role of phosphofructokinase in pH-dependent regulation of glycolysis. *Biochim Biophys Acta,* 124, 310-22.

476. Ullah, M. S., Davies, A. J. & Halestrap, A. P. (2006). The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1 α-dependent mechanism. *J Biol Chem,* 281, 9030-7.

477. van Hall, G., Saltin, B., van der Vusse, G. J., Söderlund, K. & Wagenmakers, A. J. M. (1995). Deamination of amino acids as a source for ammonia production in human skeletal muscle during prolonged exercise. *J Physiol,* 489 (Pt 1), 251-61.

478. van Hall, G. (2010). Lactate kinetics in human tissues at rest and during exercise. *Acta Physiol (Oxf),* 199, 499-508.

479. Van Slyke, D. D. (1922). On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer solution. *J Biol Chem,* 52, 525-570.

480. Vigelsø, A., Dybboe, R., Hansen, C. N., Dela, F., Helge, J. W. & Guadalupe Grau, A. (2015). GAPDH and β-actin protein decreases with aging, making Stain-Free technology a superior loading control in Western blotting of human skeletal muscle. *J Appl Physiol,* 118, 386-94.

481. Villafuerte, F. C., Swietach, P., Youm, J. B., Ford, K., Cardenas, R., Supuran, C. T., Cobden, P. M., Rohling, M. & Vaughan-Jones, R. D. (2014). Facilitation by intracellular carbonic anhydrase of Na^+-HCO_3^- co-transport but not Na^+/H^+ exchange activity in the mammalian ventricular myocyte. *J Physiol,* 592, 991-1007.

482. Vinnakota, K. C. & Kushmerick, M. J. (2011). Point: Muscle lactate and H^+ production do have a 1:1 association in skeletal muscle. *J Appl Physiol,* 110, 1487-9; discussion 1497.

483. Virkki, L. V., Wilson, D. A., Vaughan-Jones, R. D. & Boron, W. F. (2002). Functional characterization of human NBC4 as an electrogenic Na^+-HCO_3^- cotransporter (NBCe2). *Am J Physiol Cell Physiol,* 282, C1278-89.

484. Vollaard, N. B., Constantin-Teodosiu, D., Fredriksson, K., Rooyackers, O., Jansson, E., Greenhaff, P. L., Timmons, J. A. & Sundberg, C. J. (2009). Systematic analysis of adaptations in aerobic capacity and submaximal energy metabolism provides a unique insight into determinants of human aerobic performance. *J Appl Physiol,* 106, 1479-86.

485. Waheed, A., Zhu, X. L., Sly, W. S., Wetzel, P. & Gros, G. (1992). Rat skeletal muscle membrane associated carbonic anhydrase is 39-kDa, glycosylated, GPI-anchored CA IV. *Arch Biochem Biophys,* 294, 550-6.

486. Wakabayashi, S., Shigekawa, M. & Pouyssegur, J. (1997). Molecular physiology of vertebrate Na^+/H^+ exchangers. *Physiol Rev,* 77, 51-74.

487. Wang, X. M., Levi, A. J. & Halestrap, A. P. (1994). Kinetics of the Sarcolemmal Lactate Carrier in Single Heart-Cells Using BCECF to Measure pH_i. *Am J Physiol-Heart C,* 267, H1759-H1769.

488. Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M. & Barton, G. J. (2009). Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics,* 25, 1189-1191.

489. Wattanapermpool, J., Reiser, P. J. & Solaro, R. J. (1995). Troponin I isoforms and differential effects of acidic pH on soleus and cardiac myofilaments. *Am J Physiol,* 268, C323-30.

490. Weintraub, W. H. & Machen, T. E. (1989). pH regulation in hepatoma cells: roles for Na-H exchange, Cl-HCO_3 exchange, and Na-HCO_3 cotransport. *Am J Physiol Gastrointest Liver Physiol,* 257, G317-27.

491. Welinder, C. & Ekblad, L. (2011). Coomassie Staining as Loading Control in Western Blot Analysis. *J Proteome Res,* 10, 1416-1419.

492. Westerblad, H. & Allen, D. G. (1996). Slowing of relaxation and $[Ca^{2+}]_i$ during prolonged tetanic stimulation of single fibres from *Xenopus* skeletal muscle. *J Physiol,* 492 (Pt 3), 723-36.

493. Westerblad, H., Bruton, J. D. & Lännergren, J. (1997). The effect of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *J Physiol,* 500, 193-204.

494. Westerblad, H., Allen, D. G. & Lännergren, J. (2002). Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol Sci,* 17, 17-21.

495. Weston, A. R., Wilson, G. R., Noakes, T. D. & Myburgh, K. H. (1996). Skeletal muscle buffering capacity is higher in the superficial vastus than in the soleus of spontaneously running rats. *Acta Physiol Scand,* 157, 211-6.

496. Weston, A. R., Myburgh, K. H., Lindsay, F. H., Dennis, S. C., Noakes, T. D. & Hawley, J. A. (1997). Skeletal muscle buffering capacity and endurance performance after high-intensity interval training by well-trained cyclists. *Eur J Appl Physiol Occup Physiol,* 75, 7-13.

497. Weston, M., Taylor, K. L., Batterham, A. M. & Hopkins, W. G. (2014). Effects of low-volume high-intensity interval training (HIT) on fitness in adults: a meta-analysis of controlled and non-controlled trials. *Sports Med,* 44, 1005-17.

498. Wetzel, P., Hasse, A., Papadopoulos, S., Voipio, J., Kaila, K. & Gros, G. (2001). Extracellular carbonic anhydrase activity facilitates lactic acid transport in rat skeletal muscle fibres. *J Physiol,* 531, 743-56.

499. Williams, C. A., Doust, J. H. & Hammond, A. (2006). Power output and VO_2 responses during 30 s maximal isokinetic cycle sprints at different cadences in comparison to the Wingate test. *Isokinet Exerc Sci,* 14, 327-333.

500. Wilson, J. R., McCully, K. K., Mancini, D. M., Boden, B. & Chance, B. (1988). Relationship of muscular fatigue to pH and diprotonated P_i in humans: a ^{31}P-NMR study. *J Appl Physiol,* 64, 2333-9.

501. Wilson, M. C., Jackson, V. N., Heddle, C., Price, N. T., Pilegaard, H., Juel, C., Bonen, A., Montgomery, I., Hutter, O. F. & Halestrap, A. P. (1998). Lactic acid efflux from white skeletal muscle is catalyzed by the monocarboxylate transporter isoform MCT3. *J Biol Chem,* 273, 15920-6.

502. Wilson, M. C., Meredith, D. & Halestrap, A. P. (2002). Fluorescence resonance energy transfer studies on the interaction between the lactate transporter MCT1 and CD147 provide information on the topology and stoichiometry of the complex in situ. *J Biol Chem,* 277, 3666-72.

503. Wilson, M. C., Meredith, D., Fox, J. E. M., Manoharan, C., Davies, A. J. & Halestrap, A. P. (2005). Basigin (CD147) is the target for organomercurial inhibition of monocarboxylate transporter isoforms 1 and 4: The ancillary protein for the insensitive MCT2 is embigin (gp70). *J Biol Chem,* 280, 27213-27221.

504. Wiseman, R. W., Beck, T. W. & Chase, P. B. (1996). Effect of intracellular pH on force development depends on temperature in intact skeletal muscle from mouse. *Am J Physiol Cell Physiol,* 271, C878-86.

505. Wroblewski, K., Spalthoff, S., Zimmerman, U. J., Post, R. L., Sanger, J. W. & Forster, R. E. (2005). The role of carbonic anhydrase in the recovery of skeletal muscle from anoxia. *J Appl Physiol,* 99, 488-98.

506. Wu, Q., Pierce, W. M., Jr. & Delamere, N. A. (1998). Cytoplasmic pH responses to carbonic anhydrase inhibitors in cultured rabbit nonpigmented ciliary epithelium. *J Membr Biol,* 162, 31-8.

507. Xu, D. & Hemler, M. E. (2005). Metabolic activation-related CD147-CD98 complex. *Mol Cell Proteomics,* 4, 1061-71.

508. Yeung, E. W., Bourreau, J. P., Allen, D. G. & Ballard, H. J. (2002). Effect of eccentric contraction-induced injury on force and intracellular pH in rat skeletal muscles. *J Appl Physiol,* 92, 93-9.

509. Yoshida, T. & Watari, H. (1993). ^{31}P-nuclear magnetic resonance spectroscopy study of the time course of energy metabolism during exercise and recovery. *Eur J Appl Physiol Occup Physiol,* 66, 494-9.

510. Yoshida, Y., Holloway, G. P., Ljubicic, V., Hatta, H., Spriet, L. L., Hood, D. A. & Bonen, A. (2007). Negligible direct lactate oxidation in subsarcolemmal and intermyofibrillar mitochondria obtained from red and white rat skeletal muscle. *J Physiol,* 582, 1317-35.

511. Zoll, J., Ponsot, E., Dufour, S., Doutreleau, S., Ventura-Clapier, R., Vogt, M., Hoppeler, H., Richard, R. & Flück, M. (2006). Exercise training in normobaric hypoxia in endurance runners. III. Muscular adjustments of selected gene transcripts. *J Appl Physiol,* 100, 1258-66.

Appendices

Appendix A Study 1 & 2 Participant Documentation

Information to Participants Involved in Research

You are invited to participate

You are invited to participate in a research project entitled:

Effects of modulating intensity during high-intensity interval training on muscle buffer capacity in males

This project is being conducted by a student researcher, Cian McGinley, as part of a PhD study at Victoria University, under the supervision of Prof. David Bishop from the Faculty of Arts, Education, and Human Development.

Project explanation

The main aim of the project is to compare the effects of different intensities of high-intensity interval training on repeated-sprint ability and muscle buffer capacity. The ability to regulate muscle acidity, known as buffering, is thought to play a role in delaying fatigue during high-intensity exercise. This is potentially of particular importance during team sports, for example, which typically involve repeated bouts of short-duration, high-intensity exercise. This study will also seek to determine how quickly improvements in muscle buffer capacity are achieved, as well as profiling the time-course of reversibility of these improvements. We will also investigate whether small variations in your genes are associated with the changes that you experience following training; to do this we require only a small blood sample.

What will I be asked to do?

Time commitment

You as the volunteer will be asked to give between 20 and 30 hours of your time over a maximum of 13 weeks from commencement of the study. For the first two weeks of the study we will require between 30 minutes and one hour of your time on six separate days. Over the subsequent four weeks we will require between 20 and 30 minutes of your time on three weekdays per week to perform the exercise training. On the day of the final training session we will require your time intermittently over a 9-hour period. You will then be needed on four of the following five days for either 30, 60, or 90 minutes. Finally, we will conduct a follow-up test seven weeks after the final training session and will require one hour of your time on this day.

Health screening and familiarisation with exercise

After filling out an informed consent form and exclusion criteria questionnaires, we will then begin a familiarisation period during which you will become used to the exercise tests that you will be asked to perform. We want you to be comfortable with all of the procedures before the study begins, and we need you to perform to the peak of your performance ability, so you will need practice at this. You will complete four familiarisation sessions, each taking between 30 minutes and one hour.

Pre-test period

The exercise tests you became familiarised with will be repeated on three separate days over approximately one week. The first test is called a maximal oxygen uptake test and involves cycling on a stationary cycle ergometer. Following a warm-up, you will cycle continuously as the intensity increases every minute. The test ends when you can no longer keep up the required pace. Throughout this test you will wear a mouth piece which we will use to analyse the air you breathe out. This will allow us to determine your maximal oxygen uptake, essentially a measure of your aerobic fitness.

On the same day, and following one hour of rest, you will perform a graded exercise test. This also involves cycling on a stationary cycle ergometer. Following a warm-up, you will cycle for four-minute stages of progressively increasing intensity. These stages are separated by 30 second rest periods, during which a small blood sample will be taken from a vein in your arm. The total time required to complete this test will be no longer than one hour. This test will be used to set the intensity at which you will be training.

The final exercise test will be conducted on a separate day, and is a test of repeated-sprint ability. This requires you to cycle for six seconds 'all-out', for five repetitions, each separated by 24 seconds of rest. The exercise test will last approximately 15 minutes, including a warm-up. The repeated-sprint ability test is preceded by a muscle biopsy (explained below), and we will also take several small blood samples from a vein in your arm for one hour following exercise. Blood samples will be taken from an arm vein using a cannulation technique, meaning that a needle only needs to be inserted once.

Training period

The exercise programme will last for four weeks. During this period you will train three times per week (e.g., Monday, Wednesday, Friday). The exercise will be performed on a cycle ergometer, and will initially involve four intervals of two minute exercise, with one minute of rest between each interval. As you progress the number of intervals will increase, so the duration of each training session will vary between 15 and 30 minutes. Halfway through the training programme you will be asked to undergo a muscle biopsy (explained below). This will allow us to test how quickly you are adapting to the training.

Post-test period

On the final day of training you will undergo muscle biopsies (explained below) immediately after exercise, and three and eight hours later. You will then be asked to undergo one muscle biopsy one, two, and three days later, all at the same time of day. This will enable us to examine changes in your muscle following a single bout of exercise. Following the last of these biopsies, you will perform the test of repeated-sprint ability, and we will again take venous blood samples for one hour after exercise using the venous cannulation technique. Finally, two days later you will repeat the maximal oxygen uptake and graded exercise tests.

Follow-up

We then ask that you stop any high-intensity interval training for a period of seven weeks, although you can maintain your normal level of activity during this time. After seven weeks we will ask you to return for one more muscle biopsy, immediately followed by a test of repeated-sprint ability.

Muscle biopsy

You will undergo a total of nine muscle biopsies, including three on one day. The purpose of a muscle biopsy is so that we can examine the muscle in detail using a number of techniques we have available. A qualified and experienced doctor will perform each muscle biopsy. The technique involves first cleaning the skin above the part of your thigh where the biopsy is taken. A local anaesthetic is then injected into the skin where the biopsy will be taken. This may cause a slight stinging sensation. Once the numbness has set in a small incision is made with a scalpel, and the steel biopsy needle is inserted about 2-5 centimetres into your thigh muscle, and a small amount of muscle is taken out (maximum of 0.4 grammes). The procedure takes about 5-10 seconds. Pressure is then applied to the muscle to ensure there is minimal swelling. You will undergo one muscle biopsy two days before the training begins, one biopsy two weeks into training, three biopsies on the final training day, and one biopsy per day on each of the following three days. A final biopsy will be performed seven weeks after the final training session.

What will I gain from participating?

From participating in this study you can expect to gain significant improvements in your fitness. You will train in state-of-the-art facilities by qualified sport scientists with years of experience. We will give you feedback on your performance in the various tests, allowing you to better understand your own fitness. From your participation in this project you will gain experience and knowledge on how this type of study is undertaken, which might be beneficial for your own understanding of the human body.

How will the information I give be used?

All information gathered in this trial is highly confidential, and will be stored under secure conditions. You should be aware that data gathered during the study will be used in a PhD thesis and in scientific publications, but at no stage will names or personal details be disclosed. All we ask is that the information may be used anonymously in the preparation of scientific reports for presentation at scientific congresses or in refereed publications.

What are the potential risks of participating in this project?

- The Victoria University Human Research Ethics Committee has assessed all risks associated with each test procedure. Documents explaining these risks are available if requested.
- The exercise bouts might result in muscle soreness and stiffness; however, this will subside completely within a couple of days.
- The muscle biopsy might result in a slight muscle ache for one or two days after each biopsy. The biopsy occasionally leads to bruising around the incision area, and the incision will leave a small mark that should fade over time. Some people have reported that a small scar is visible on their leg when their skin tans, even years after the biopsy. We would suggest you consider this possibility before volunteering for this study. There is also a very small but real possibility that you could experience more serious complications as a result of the biopsy. It is possible that your muscle could swell up and there could be bleeding in the muscle. We will minimise this possibility by applying pressure to the biopsy area after each biopsy, and by monitoring you while you rest after each biopsy. We will also show you how to treat the muscle should it swell up or bleed later on after you have left the laboratory.
- With both the muscle biopsy and the blood samples – as with all surgical procedures – there is a small possibility of infection. We will, of course, endeavour to keep this risk to a minimum through using sterile equipment and appropriate procedures.
- The blood sampling might lead to some bruising, although this will fade after a few days.
- Sometimes people become distressed and anxious at the thought of a muscle biopsy. We will endeavour to minimise any anxiety by explaining the procedure clearly beforehand. Please remember you are free to withdraw from the study at any time, and you do not have to provide us with a reason. If you are feeling distressed and would like to discuss this with someone, you can avail of the services of a Victoria University psychologist, Dr Harriet Speed:

  Tel: (03) 9919 5412
  Email:harriet.speed@vu.edu.au

How will this project be conducted?

All volunteers will initially be screened for cardiovascular risk factors and any health issues that might prevent them from participating in this study. Once the investigators and the volunteer are satisfied that the experiment has been explained in full, and following a period of reflection, you will be asked to complete an informed consent document. The study will then be conducted over a 13-week period, following the protocol described above.

Who is conducting the study?

Institute of Sport, Exercise & Active Living (ISEAL), Victoria University

Chief Investigator: Prof. David Bishop
Tel: (03) 9919 9471
Email: david.bishop@vu.edu.au

PhD Researcher: Mr. Cian McGinley
Tel: 0449 817 237
Email: cian.mcginley@vu.edu.au

Any queries about your participation in this project may be directed to the Chief Investigator listed above.

If you have any queries or complaints about the way you have been treated, you may contact:

Research Ethics and Biosafety Manager
Victoria University Human Research Ethics Committee
Victoria University
PO Box 14428
Melbourne, VIC 8001
Tel: (03) 9919 4148

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

CONSENT FORM FOR PARTICIPANTS INVOLVED IN RESEARCH

INFORMATION TO PARTICIPANTS
We would like to invite you to be a part of a study into:

Effects of modulating intensity during high-intensity interval training on muscle buffer capacity in males

The main aim of this project is to compare the effects of different intensities of high-intensity interval training on repeated-sprint ability and muscle buffer capacity. This is potentially of particular importance during team sports, for example, which typically involve repeated bouts of short-duration, high-intensity exercise. The study will be conducted over a 13-week period, requiring between 20 and 30 hours of your time in total. After two weeks of preliminary testing, exercise training will be conducted three times per week for four weeks, with a follow-up test seven weeks later. The procedures include three different exercise performance tests, drawing of venous blood sample by cannulation, and a total of nine muscle biopsies. Training will initially involve four repetitions of two minute cycling intervals, each separated by one minute of rest. As with any exercise there is the potential for muscle or joint injury. This risk will be minimised by performance of appropriate warm-up and supervision of all exercise bouts. High-intensity exercise will result in breathlessness and elevated heart rate. Some people will occasionally feel light-headed or nauseous after this type of exercise. These feelings will pass quickly however. The blood sampling might lead to some bruising, although this will fade over subsequent days. The muscle biopsy might result in a slight muscle ache for one or two days after each biopsy. The biopsy occasionally leads to bruising around the incision area, and the incision will leave a small mark that should fade over time. In addition, some people might experience anxiety prior to the muscle biopsy. We will aim to alleviate this by explaining the procedure in full beforehand. Finally, with both the muscle biopsy and the blood samples – as with all surgical procedures – there is a small possibility of infection. We will, of course, endeavour to keep this risk to a minimum through using sterile equipment and appropriate procedures.

CERTIFICATION BY SUBJECT

I, _____

of _____

certify that I am at least 18 years old and that I am voluntarily giving my consent to participate in the study:

'Effects of modulating intensity during high-intensity interval training on muscle buffer capacity in males' being conducted at Victoria University by: *Prof. David Bishop*

I certify that the objectives of the study, together with any risks and safeguards associated with the procedures listed hereunder to be carried out in the research, have been fully explained to me by:

Mr. Cian McGinley

and that I freely consent to participation involving the below mentioned procedures:

- High-intensity interval training
- Graded exercise test
- Maximal oxygen uptake test
- Repeated-sprint ability
- Venous cannulation (blood sample)
- Needle muscle biopsy

(please turn over page)

I certify that I have had the opportunity to have any questions answered and that I understand that I can withdraw from this study at any time, and that this withdrawal will not jeopardise me in any way.

I have been informed that the information I provide will be kept confidential.

Signed: _____

Date: _____

Any queries about your participation in this project may be directed to the researcher:

Prof. David Bishop
(03) 9919 9471
Email: david.bishop@vu.edu.au

If you have any queries or complaints about the way you have been treated, you may contact:

Research Ethics and Biosafety Manager
Victoria University Human Research Ethics Committee
Victoria University
PO Box 14428
Melbourne, VIC 8001
Tel: (03) 9919 4148

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

Risk Factor Assessment Questionnaire

Investigators: Prof. David Bishop
Mr. Cian McGinley

Volunteer Name _____
(Please print)

In order to be eligible to participate in the experiment investigating:

Effects of modulating intensity during high-intensity interval training on muscle buffer capacity in males,

you are required to complete the following questionnaire, which is designed to assess the risk of you having a cardiovascular event during an exhaustive exercise bout. Please take your time in answering the following questions. Answer them as best you can. If you have any queries regarding any of the questions please contact one of the investigators before answering it.

MEDICAL HISTORY

In the past have you ever had (tick No or Yes):

Medical Condition	NO	YES	Medical Condition	NO	YES
Heart Attack	❑	❑	Congenital Heart Disease	❑	❑
Chest Pain (angina)	❑	❑	Disease of Arteries/Veins	❑	❑
Heart Murmur	❑	❑	Asthma	❑	❑
Heart Rhythm Disturbance	❑	❑	Lung Disease (e.g. emphysema)	❑	❑
Heart Valve Disease	❑	❑	Epilepsy	❑	❑
Stroke	❑	❑	Injuries to back, knees, ankles	❑	❑

Please list any prescribed medications being taken:

_____

_____

SYMPTOMS DURING OR AFTER EXERCISE

As a result of exercise, have you ever experienced any of the following (tick No or Yes):

Symptom during exercise	NO	YES	Symptom during exercise	NO	YES
Pain or discomfort in the chest, back, arm, or jaw	❑	❑	Palpitations (heart rhythm disturbance)	❑	❑
Severe shortness of breath or problems with breathing during mild exertion	❑	❑	Pain in the legs during mild exertion	❑	❑
Dizziness, nausea, or fainting	❑	❑	Severe heat exhaustion	❑	❑

(please turn over page)

CARDIOVASCULAR RISK FACTORS

Do you have (tick NO, YES or circle ? for DON'T KNOW)

Cardiovascular Risk Factors	**NO**	**YES**	**DON'T KNOW**	
High Blood Pressure	❑	❑	?	
High Blood Cholesterol/Triglycerides	❑	❑	?	
Diabetes	❑	❑	?	
Current Smoker	❑	❑	Average/day =	
Ex-smoker	❑	❑	Average/day =	
Do you drink alcohol regularly?	❑	❑	Average/day =	drinks

	NO	**YES**
Do you know of any other reason why you should not undergo physical activity? This might include severe asthma, diabetes, a current or recent sports injury, or serious illness.	❑	❑

I, _____ declare that the above information is correct at the time of completing this questionnaire

Date ……/……/…….

Please Note: If your health changes so that you can then answer YES to any of the above questions, tell one of the experimenters.

Volunteer

Signed _____

Print _____ (BLOCK CAPITALS)

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

Muscle Biopsy & Venous Cannulation Questionnaire

Investigators: Prof. David Bishop
Mr. Cian McGinley

Volunteer Name (Please print) _____

In order to be eligible to participate in the experiment investigating:

Effects of modulating intensity during high-intensity interval training on muscle buffer in males

you are required to complete the following questionnaire, which is designed to assess the risk of you having a an adverse event during muscle or venous blood sampling. Please take your time in answering the following questions. Answer them as best you can. If you have any queries regarding any of the questions please contact one of the investigators before answering it.

	Yes	No	Don't Know
1. Have you or your family suffered from any tendency to bleed excessively (e.g. haemophilia) or bruise very easily? If yes, please elaborate _____	❑	❑	❑
2. Are you taking any drugs which will affect blood clotting? If yes, please elaborate _____	❑	❑	❑
3. Are you allergic to xylocaine, the local anaesthetic to be employed during the muscle biopsy? If yes, please elaborate _____	❑	❑	❑
4. Do you have any skin allergies? If yes, please elaborate _____	❑	❑	❑
5. Have you any other allergies? If yes, please elaborate _____	❑	❑	❑
6. Have you ever fainted when you had an injection or blood sample taken? If yes, please elaborate _____	❑	❑	❑
7. Do you or other members of your family have Raynaud's disease, or suffer from very poor circulation in the fingers, leading to painful fingers that turn white/blue? If yes, please elaborate _____	❑	❑	❑

I, _____ declare that the above information is correct at the time of completing this questionnaire

Date ……/……/…….

Volunteer
Signed _____
Print _____ (BLOCK CAPITALS)

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

MEMO

TO Professor David Bishop
Institute of Sport and Active Living
Victoria University
Footscray Park Campus

DATE 17/1/2012

FROM Dr Anthony Watt
Acting Chair
Victoria University Human Research Ethics Committee

SUBJECT Ethics Application – HRETH 11/289

Dear Professor Bishop,

Thank you for resubmitting this application for ethical approval of the project entitled:

***HRETH 11/289** Effects of modulating intensity during high-intensity interval training on muscle buffer capacity in males (HREC 11/181)

The proposed research project has been accepted and deemed to meet the requirements of the National Health and Medical Research Council (NHMRC) 'National Statement on Ethical Conduct in Human Research (2007)' by the Victoria University Human Research Ethics Committee. Approval has been granted from 17th January 2012 to 8th December 2013.

Continued approval of this research project by the Victoria University Human Research Ethics Committee (VUHREC) is conditional upon the provision of a report within 12 months of the above approval date (by **17th January 2013**) or upon the completion of the project (if earlier). A report proforma may be downloaded from the VUHREC web site at: http://research.vu.edu.au/hrec.php.

Please note that the Human Research Ethics Committee must be informed of the following: any changes to the approved research protocol, project timelines, any serious events or adverse and/or unforeseen events that may affect continued ethical acceptability of the project. In these unlikely events, researchers must immediately cease all data collection until the Committee has approved the changes. Researchers are also reminded of the need to notify the approving HREC of changes to personnel in research projects via a request for a minor amendment.

On behalf of the Committee, I wish you all the best for the conduct of the project.

Kind regards,

Dr Anthony Watt
Acting Chair
Victoria University Human Research Ethics Committee

Appendix B Study 3 Participant Documentation

Information to Participants Involved in Research

You are invited to participate

You are invited to participate in a research project entitled:

Effects of modulating rest interval during high-intensity interval training on muscle buffer capacity

This project is being conducted by a student researcher, Cian McGinley, as part of a PhD study at Victoria University, under the supervision of Prof. David Bishop from the College of Sport and Exercise Science.

Project explanation

The main aim of the project is to compare the effects of different rest intervals during high-intensity interval training on repeated-sprint ability and muscle buffer capacity. The ability to regulate muscle acidity, known as buffering, is thought to play a role in delaying fatigue during high-intensity exercise. This may be of importance during team sports, for example, which typically involve repeated bouts of short-duration, high-intensity exercise. This study will also seek to determine if a period of reduced training volume is sufficient to maintain potential training adaptations. We will also take a small blood sample, and extract and analyse your DNA, in order to investigate whether small variations in your genes are associated with the changes in both muscle characteristics and performance that you might experience following training.

What will I be asked to do?

Time commitment
You as the volunteer will be asked to give between 20 and 30 hours of your time over 12-14 weeks from commencement of the study (please see the diagram below). For the first two weeks of the study we will require between 30 minutes and one hour of your time on five separate days. Over the subsequent four weeks we will require between 15 and 60 minutes of your time on three weekdays per week to perform the exercise training. You will then be needed on three of the following seven days for between 30 and 60 minutes. For six weeks you will then train only once per week, again between 15 and 60 minutes. Finally, at the end of training we will conduct tests on two separate days requiring 30 minutes of your time per day.

Health screening and familiarisation with exercise
After filling out an informed consent form and exclusion criteria questionnaires, we will then begin a familiarisation period during which you will become used to the exercise tests that you will be asked to perform. We want you to be comfortable with all of the procedures before the study begins, and we need you to perform to the peak of your performance ability, so you will need practice at this. You will complete three familiarisation sessions, each taking between 30 minutes and 90 minutes.

Pre-test period
The exercise tests you became familiarised with will be repeated on two separate days two days apart. The first test is called a graded-exercise test and involves cycling on a stationary cycle ergometer. Following a warm-up, you will cycle for four-minute stages of progressively increasing intensity. These stages are separated by 30 second rest periods, during which a small blood sample will be taken from a vein in your arm. The total time required to complete this test will be no longer than one hour. This test will be used to set the intensity at which you will be training.

Five minutes after the end of the graded-exercise test, you will perform a maximal oxygen uptake test on the same cycle ergometer. For this test you will cycle continuously at an intensity slightly greater than that at which you stopped during the graded-exercise test. The test ends when you can no longer keep up the required pace, and will take no longer than five minutes to complete. Throughout this test you will wear a mouth piece which we will use to analyse the air you breathe out. This will allow us to determine your maximal oxygen uptake, essentially a measure of your aerobic fitness.

The final exercise test will be conducted on a separate day, and is a test of repeated-sprint ability. This requires you to cycle for six seconds 'all-out', for five repetitions, each separated by 24 seconds of rest. This exercise test will last approximately 25 minutes, including a warm-up and cool-down.

Training period
The exercise programme will last for ten weeks. During this period you will train three times per week (e.g., Monday, Wednesday, Friday) for four weeks, followed by once a week training for six weeks. The exercise will be performed on a cycle ergometer, and will initially involve four intervals of 30 seconds exercise, with either short duration (e.g. 90 seconds) or longer duration (e.g. four minutes) rest periods between each interval. As you progress the number of intervals will increase, so the duration of each training session will vary between 15 and 60 minutes.
You will undergo a muscle biopsy (please see below for description) before and immediately after the first training session.

In addition, during a separate training session in the first two weeks, we will measure your oxygen uptake using the same procedure described above for the maximal oxygen uptake test. We will also take a small blood sample from a vein in your arm to measure the acidity and concentration of lactate in your blood. This will enable us to quantify the type of energy you are expending during the training session.

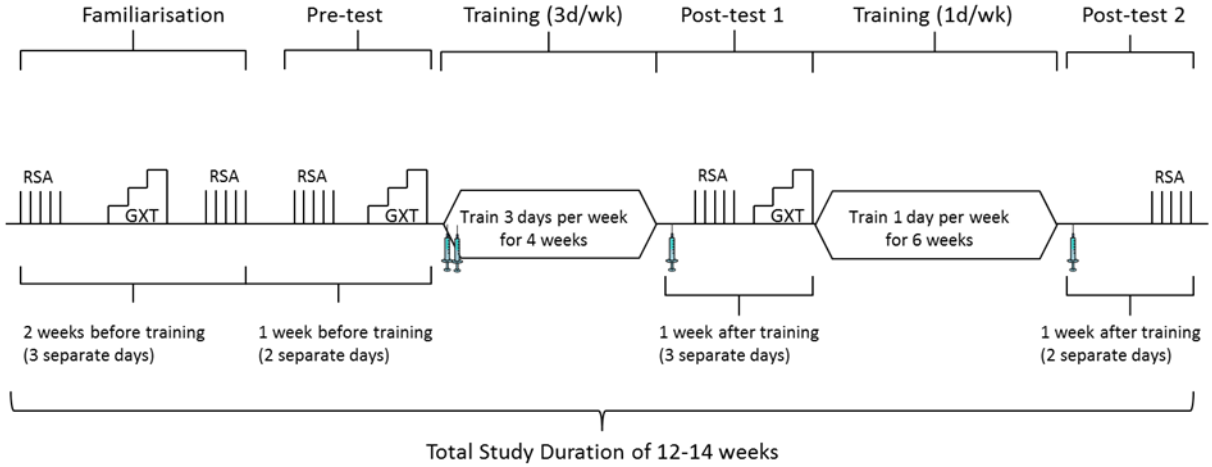
Post-test period 1
Three days after completion of the first 4 weeks of training, you will undergo a muscle biopsy (see below). Two days later you will again perform the test of repeated-sprint ability. A further two days later you will repeat the graded exercise and maximal oxygen uptake tests.

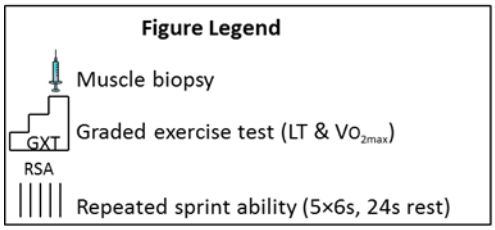
Post-test period 2
Two days after completion of the final training session, you will undergo a muscle biopsy (see below). Three days later you will again perform the test of repeated-sprint ability.

Muscle biopsies
You will undergo a total of four muscle biopsies. On the first day of training you will undergo muscle biopsies before and immediately after exercise. There will be a muscle biopsy three days after the twelfth training session. Six week later there will be one final biopsy after the final training session. The purpose of a muscle biopsy is so that we can examine the muscle in detail using a number of techniques we have available. A qualified and experienced doctor (Dr. Mitch Anderson) will perform each muscle biopsy. The technique involves first cleaning the skin above the part of your thigh where the biopsy is taken. A local anaesthetic is then injected into the skin where the biopsy will be taken. This will cause a slight stinging sensation. Once the numbness has set in a small incision is made with a scalpel, and the steel biopsy needle is inserted about 2-5 centimetres into your thigh muscle, and a small amount of muscle is taken out (maximum of 0.6 grammes). The biopsy takes about 5-10 seconds and you will feel some pressure in your leg and possibly some pain during the procedure. Pressure is then applied to the muscle to ensure there is minimal swelling.

Muscle Analysis
While most of the muscle analysis will be performed at Victoria University, a small portion of your muscle biopsy samples may be sent to Professor Kathryn North and colleagues of the Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne to be analysed for enzyme activity and protein expression. Samples will contain alphanumeric codes and the researchers there will have no access to any of your personal details.

Outline of Study Design and Time Commitment





What will I gain from participating?

Although we cannot guarantee it, from participating in this study you might gain significant improvements in your fitness. You will train in state-of-the-art facilities by qualified sport scientists with years of experience. We will give you feedback on your performance in the various tests, allowing you to better understand your own fitness. From your participation in this project you will gain experience and knowledge on how this type of study is undertaken, which might be beneficial for your own understanding of the human body.

How will the information I give be used?

All information gathered in this trial is highly confidential, and will be stored under secure conditions. You should be aware that data gathered during the study will be used in a PhD thesis and in scientific publications, but at no stage will names or personal details be disclosed. The samples and data will not be released for any other use without your prior consent, unless required by law. All we ask is that the information may be used anonymously in the preparation of scientific reports for presentation at scientific congresses or in refereed publications.

Please note that the data from this study may be combined with the data from other studies to increase the participant numbers and to generate additional scientific manuscripts. If you do not want your data to be combined with data from other similar types of studies investigating whether small variations in genes are associated with training-induced changes in both muscle characteristics and performance, then please tick the box below.

❑ I do not wish my data to be combined with data from other similar studies

In addition, we might ask your permission to take still photos or video images of the test procedures for use in research presentations. This will only be undertaken with your prior permission, with all images anonymised to maintain your privacy.

What are the potential risks of participating in this project?

- The Victoria University Human Research Ethics Committee has assessed all risks associated with each test procedure. Documents explaining these risks are available if requested.
- The exercise bouts might result in muscle soreness and stiffness; however, this will subside completely within a couple of days.
- The exercise bouts might result in feelings of dizziness, nausea, or possibly result in fainting. All of the investigators have current CPR and First Aid certificates. In the event of you feeling unwell we will typically move you to a bed and raise your legs to allow blood to return your upper body from your legs. We will monitor you until you feel better, which normally takes only a few minutes.
- The muscle biopsy might result in a muscle ache for one or two days after each biopsy, somewhat like the feeling of a 'corky'. The biopsy occasionally leads to bruising around the incision area, and the incision will leave a small mark that should fade over time. Some people have reported that a small scar is visible on their leg when their skin tans, even years after the biopsy. We would suggest you consider this possibility before volunteering for this study. There is also a very small but real possibility that you could experience more serious complications as a result of the biopsy. It is possible that your muscle could swell up and there could be bleeding in the muscle. We will minimise this possibility by applying pressure to the biopsy area after each biopsy, and by monitoring you while you rest after each biopsy. We will also show you how to treat the muscle should it swell up or bleed later on after you have left the laboratory.
- With both the muscle biopsies and the blood samples – as with all surgical procedures – there is a small possibility of infection. We will, of course, endeavour to keep this risk to a minimum through using sterile equipment and appropriate procedures.
- The blood sampling might lead to some bruising, although this will fade after a few days.
- With both the muscle biopsies and the blood samples, you might also feel faint or dizzy. In this case we will follow the procedures already described above for managing your symptoms.
- Sometimes people become distressed and anxious at the thought of a muscle biopsy. We will endeavour to minimise any anxiety by explaining the procedure clearly beforehand. Please remember you are free to withdraw from the study at any time, and you do not have to provide us with a reason. If you are feeling distressed and would like to discuss this with someone, you can avail of the services of a Victoria University psychologist:

Dr. Harriet Speed:
Tel: (03) 9919 5412
email: harriet.speed@vu.edu.au

How will this project be conducted?

All volunteers will initially be screened for cardiovascular risk factors and any health issues that might prevent them from participating in this study. Once the investigators and the volunteer are satisfied that the experiment has been explained in full, and following a period of reflection, you will be asked to complete an informed consent document. The study will then be conducted over a 12-14-week period, following the protocol described above.

Who is conducting the study?

College of Sport and Exercise Science, Victoria University

Chief Investigator: Prof. David Bishop
Tel: (03) 9919 9471
email: david.bishop@vu.edu.au

PhD Researcher: Mr. Cian McGinley
Tel: 0449 817 237
email: cian.mcginley@vu.edu.au

Any queries about your participation in this project may be directed to the Chief Investigator listed above.

If you have any queries or complaints about the way you have been treated, you may contact:

Research Ethics and Biosafety Manager
Victoria University Human Research Ethics Committee
Victoria University
PO Box 14428
Melbourne, VIC 8001
Tel: (03) 9919 4148

CONSENT FORM FOR PARTICIPANTS INVOLVED IN RESEARCH

INFORMATION TO PARTICIPANTS
We would like to invite you to be a part of a study into:

Effects of modulating rest interval during high-intensity interval training on muscle buffer capacity

The main aim of this project is to compare the effects of different rest intervals during high-intensity interval training on repeated-sprint ability and muscle buffer capacity. The study will be conducted over a 12-week period, requiring between 20 and 30 hours of your time in total. After two weeks of preliminary testing, exercise training will be conducted three times per week for four weeks, followed by once a week for six weeks.

CERTIFICATION BY PARTICIPANT
I, _____

of _____ (suburb)

certify that I am at least 18 years old and that I am voluntarily giving my consent to participate in the study:

'Effects of modulating rest interval during high-intensity interval training on muscle buffer capacity' being conducted at Victoria University by: *Prof. David Bishop*

I certify that the objectives of the study, together with any risks and safeguards associated with the procedures listed hereunder to be carried out in the research, have been fully explained to me by:

Mr. Cian McGinley

and that I freely consent to participation involving the below mentioned procedures:

- ☐ High-intensity interval training
- ☐ Graded exercise test
- ☐ Maximal oxygen uptake test
- ☐ Repeated-sprint ability test
- ☐ Venous cannulation (blood sample)
- ☐ Needle muscle biopsy

I certify that I have had the opportunity to have any questions answered and that I understand that I can withdraw from this study at any time, and that this withdrawal will not jeopardise me in any way.

I have been informed that the information I provide will be kept confidential.

Signed:_____

Date: _____

Any queries about your participation in this project may be directed to the researcher:
Prof. David Bishop
(03) 9919 9471
Email: david.bishop@vu.edu.au

If you have any queries or complaints about the way you have been treated, you may contact:
Research Ethics and Biosafety Manager
Victoria University Human Research Ethics Committee
Victoria University
PO Box 14428
Melbourne, VIC 8001
Tel: (03) 9919 4148

CONSENT FORM FOR FUTURE USE OF DATA

INFORMATION TO PARTICIPANTS

We would like to request your permission to use any data, blood, and muscle samples obtained during this study for future use. The development of new techniques and technologies in the future might enable further analysis to be performed that could provide new insights into the field of exercise performance. In addition, this data might be used in future publications.

CERTIFICATION BY PARTICIPANT

I, _____

of _____ (suburb)

certify that I am at least 18 years old and that I am voluntarily giving my consent to have my data, blood, and muscle samples to be stored for potential future analysis and subsequent publication of this data.

I certify that the purposes and implications of storing this data and tissue have been explained to me by:

Mr. Cian McGinley

and that I freely consent to having my data, blood, and tissue samples stored at the College of Sport and Exercise Science, Footscray Park, Victoria University.

I certify that I have had the opportunity to have any questions answered and I have been informed that the data, blood, and tissue that I provide will be kept confidential.

Signed: _____

Date: _____

Any queries about your participation in this project may be directed to the researcher:
Prof. David Bishop
(03) 9919 9471
Email: david.bishop@vu.edu.au

If you have any queries or complaints about the way you have been treated, you may contact:

Research Ethics and Biosafety Manager
Victoria University Human Research Ethics Committee
Victoria University
PO Box 14428
Melbourne, VIC 8001
Tel: (03) 9919 4148

VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

MEMO

TO Professor David Bishop
College of Sport and Exercise Science
Victoria University

DATE 6/02/2013

FROM Dr Deborah Zion
Chair
Victoria University Human Research Ethics Committee

SUBJECT Ethics Application – HRETH 12/332

Dear Professor Bishop

Thank you for submitting this application for ethical approval of the project entitled:

HRETH 12/332 Effects of modulating rest interval during high-intensity training on muscle buffer capacity

The proposed research project has been accepted and deemed to meet the requirements of the National Health and Medical Research Council (NHMRC) 'National Statement on Ethical Conduct in Human Research (2007)' by the Victoria University Human Research Ethics Committee. Approval has been granted from **6 February 2013 to 6 February 2015.**

Continued approval of this research project by the Victoria University Human Research Ethics Committee (VUHREC) is conditional upon the provision of a report within 12 months of the above approval date (**6 February 2014**) or upon the completion of the project (if earlier). A report proforma may be downloaded from the VUHREC web site at: http://research.vu.edu.au/hrec.php.

Please note that the Human Research Ethics Committee must be informed of the following: any changes to the approved research protocol, project timelines, any serious events or adverse and/or unforeseen events that may affect continued ethical acceptability of the project. In these unlikely events, researchers must immediately cease all data collection until the Committee has approved the changes. Researchers are also reminded of the need to notify the approving HREC of changes to personnel in research projects via a request for a minor amendment. It should also be noted that it is the Chief Investigators' responsibility to ensure the research project is conducted in line with the recommendations outlined in the National Health and Medical Research Council (NHMRC) 'National Statement on Ethical Conduct in Human Research (2007).'

On behalf of the Committee, I wish you all the best for the conduct of the project.

Kind regards,

Dr Deborah Zion
Chair
Victoria University Human Research Ethics Committee

Appendix C Study 1 (Chapter 3) Results

C-1 Participant Anthropometric Data

Participant	Age (y)	Height (cm)	Mass (kg)	$\dot{V}O_{2peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)
1	23	183.3	103.0	39.9
2	30	166.6	69.8	47.6
3	20	176.2	67.8	52.1
4	19	176.4	74.5	45.8
5	19	185.1	76.9	51.6
6	21	175.8	76.0	49.6
7	30	193.7	88.9	55.1
8	28	192.1	92.8	40.2
9	22	181.8	74.3	52.3
10	20	169.3	70.3	54.4
11	18	171.4	74.3	46.4
12	20	179.4	73.8	48.7
13	19	176.9	74.3	58.2
14	23	172.9	71.9	54.8
15	31	196.3	106.2	37.1
16	26	179.3	87.8	42.2
Mean	**23**	**179.8**	**80.2**	**48.5**
SD	**4**	**8.6**	**11.9**	**6.2**

C-2 Western Blotting

MCT1 abundance relative to an internal standard calibration curve and corrected for total protein

Participant	Gel no.	Pre-HIT	+0 h	+3 h	+9 h	+24 h	+48 h	+72 h	+72 h duplicate
1	1	0.65	1.42	1.12	1.26	1.86	2.08	0.99	1.19
	2	0.65	1.35	0.98	1.25	2.05	1.62	1.03	
2	1	0.69	3.24	1.78	2.94	3.27	1.39	2.11	2.65
	2	0.67	3.23	1.84	2.23	3.14	1.34	1.89	2.47
	3	0.50	2.67	1.81	3.55	2.34	1.20	1.77	
3	1	0.75	1.00	1.25	0.87	1.27	2.24	1.64	1.42
	2	0.55	0.76	0.87	0.32	2.61	2.15	1.73	
4	1	0.85	1.38	0.99	0.70	1.63	3.84	2.93	3.11
	2	0.77	2.02	0.52	0.41	1.29	3.45	3.31	2.87
	3	0.89	2.24	0.84	0.68	1.78	2.70	5.15	
5	1	0.59	1.33	1.95	2.36	3.24	1.51	2.29	2.22
	2	0.45	1.46	2.03	2.21	3.09	1.95	2.14	
6	1	0.43	0.59	0.50	0.92	0.80	1.69	0.40	0.51
	2	0.66	0.61	0.68	0.91	1.02	1.68	0.77	0.62
	3	0.17	0.18	0.09	0.45	0.42	1.68	0.24	
7	1	0.79	1.06	1.42	1.39	2.13	1.11	1.91	2.25
	2	0.68	0.39	0.70	0.52	0.76	0.37	1.13	
8	1	0.35	0.55		1.14	0.40	2.05	0.55	0.42
	2	0.78	0.94	1.72	1.78	0.80	2.36	1.14	1.09
	3	0.61	0.72	1.30	1.67	0.57	1.93	0.70	
9	1	1.02	1.32	0.88	1.06	1.03	1.32	1.03	
	2	1.30	1.45	1.15	1.25	0.84	1.18	1.28	
10	1	0.37	1.22	0.61	0.93	1.02	1.10	2.60	1.99
	2	0.48	2.16	1.11	2.05	1.70	2.19	4.32	3.17
	3	0.30	1.46	0.75	1.48	1.22	1.66	1.89	
11	1	1.21	1.07	0.91	0.81	1.75	1.11	1.34	1.27
	2	1.48	0.88	0.61	0.74	1.65	1.06	1.72	
12	1	0.28	0.63	1.75	1.72	1.00	0.53	0.71	0.65
	2	0.60	0.92	1.65	1.98	1.29	0.95	0.88	0.86
	3	0.49	0.78	2.36	2.27	1.18	0.79	1.09	
13	1	0.70	1.18	1.68	2.01	1.87	2.46	4.23	3.76
	2	0.64	1.20	1.92	2.09	2.11	2.63	5.33	
14	1	0.73	1.23	3.44	0.68	2.89	1.70	2.29	2.72
	2	0.58	1.18	3.16	0.93	2.10	1.68	2.45	2.28
	3	0.29	0.75	2.02	0.54	1.50	0.66	1.67	
15	1	0.58	2.23	0.88	1.37	0.87	0.84	0.83	0.82
	2	0.90	1.96	0.89	1.30	0.89	0.71	0.83	
16	1	0.62	1.39	2.22	0.78	2.56	3.05	2.31	2.45
	2	0.66	1.25	1.34	0.89	1.54	2.24	1.58	1.61
	3	0.82	1.41	1.36	0.65	1.63		2.38	

MCT4 abundance relative to an internal standard calibration curve and corrected for total protein

Participant	Gel no.	Pre-HIT	+0 h	+3 h	+9 h	+24 h	+48 h	+72 h	+72 h duplicate
1	1	0.91	1.07	1.02	1.23	1.48	1.49	1.16	1.33
	2	1.00	0.99	1.31	1.03	1.05	1.49	1.17	0.89
2	1	1.08	1.19	1.10	1.33	1.20	1.12	1.08	1.32
	2	2.34	1.42	1.06	1.33	1.39	1.08	1.55	1.70
	3	1.24	1.57	1.54	1.79	1.81	1.81	1.44	1.36
3	1	1.61	1.80	1.48	1.49	1.27	1.44	1.54	1.76
	2	2.15	2.16	2.36	2.33	3.07	3.05	2.50	2.29
4	1	0.78	0.98	0.84	0.97	0.85	0.59	0.79	0.74
	2	0.98	1.00	1.11	1.02	1.00	1.03	0.92	0.82
5	1	1.09	1.27	1.33	1.46	1.46	1.74	1.24	1.34
	2	1.23	1.33	1.48	1.53	1.49	1.20	1.28	1.01
6	1	0.72	1.33	1.30	1.38	1.17	1.33	0.83	0.96
	2	0.87	1.09	1.19	1.30	1.18	1.28	1.06	1.05
7	1	1.42	0.73	2.15	1.15	1.69	1.81	1.48	1.70
	2	1.32	0.95	1.92	1.03	1.66	1.53	1.38	1.73
8	1	0.74	1.14	0.78	1.46	0.69	1.05	0.66	0.67
	2	0.93	1.29	0.85	1.46	0.83	1.23	0.86	1.02
9	1	0.84	0.96	0.92	0.82	0.92	0.82	0.94	0.82
	2	1.48	2.02	1.40	1.19	1.52	1.12	1.80	1.80
10	1	0.80	1.00	0.86	1.13	1.07	1.25	1.03	1.32
	2	1.47	1.82	1.83	1.39	1.55	1.41	1.49	1.35
	3	0.98	1.15	1.51	0.95	0.95	1.05	1.25	1.00
11	1	1.21	1.27	0.92	0.85	1.09	1.07	1.90	1.93
	2	1.86	1.63	2.11	1.13	2.97	1.34	2.62	7.31
	3	1.29	1.60	1.46	1.31	1.36	1.43	2.40	2.35
12	1	1.39	1.62	1.12	1.24	1.20	1.29	1.47	1.34
	2	0.73	1.07	0.94	1.10	1.13	0.76	1.06	0.99
	3	0.69	1.01	1.19	1.09	0.92	0.72	0.86	0.85
13	1	0.73	0.96	0.85	1.19	1.10	1.19	1.97	2.08
	2	1.17	1.35	1.26	1.48	1.47	1.27	2.20	1.83
14	1	1.12	1.00	1.77	1.20	1.25	1.11	1.40	1.24
	2	1.16	1.27	1.46	1.11	1.73	1.54	1.38	1.35
15	1	0.94	1.33	1.31	1.25	1.09	1.30	1.31	1.45
16	1	1.29	1.33	1.28	1.19	1.37	1.28	1.22	1.28
	2	0.80	0.85	0.93	0.70	0.88	1.36	1.05	1.06

NHE1 abundance relative to an internal standard calibration curve and corrected for total protein

Participant	Gel no.	Pre-HIT	Pre-HIT duplicate	+0 h	+3 h	+9 h	+24 h	+48 h	+72 h	+72 h duplicate
1	1	0.68		1.11	0.93	1.01	1.72	1.11	1.08	0.92
2	1	0.68		1.70	1.36	1.95	1.36	1.12	1.18	1.16
3	1	0.56		1.14	1.55	0.89		2.75	1.60	1.33
	2	0.60		1.03	1.27	0.56		2.14	1.71	1.35
4	1	0.82			0.57	0.53	1.05	1.44	1.40	1.53
	2	0.44		0.70	0.54	0.37	0.58	1.33	1.00	0.81
5	1	0.96		1.34	1.53	1.48	1.48	1.14	2.39	1.71
6	1	0.48		0.90	0.68	0.97	0.88	1.21	0.69	0.69
7	1	1.51		1.50	1.61	1.41	1.89	1.23	1.92	2.39
8	1	0.68	1.01	0.81	1.68	1.00	0.82	1.56	1.03	0.75
	2	0.86	0.86	1.09	1.59	0.87	0.72	1.60	1.14	0.94
9	1	0.93		1.03	0.85	0.71	0.59	0.89	0.92	0.85
10	1	0.48		1.13	0.72	0.80	1.08	1.19	1.60	1.60
	2	0.48		1.11	1.07	0.90	0.90	0.86	1.28	1.32
	3	0.70		1.50	1.00	1.12	1.23	0.79	2.23	1.98
11	1	2.38		1.49	1.42	1.10	2.23	1.25	2.66	1.57
	2	1.32		1.80	0.85	1.30	2.08	1.23	2.00	2.17
12	1	0.93		1.26	2.46	1.67	1.69	0.72	1.44	1.40
13	1	0.96		1.45	2.12	1.25	1.61	1.50	2.39	2.10
14	1	0.74	0.87	1.14	2.64	0.93	1.46	1.44	2.01	1.57
	2	0.83	0.65	1.28	3.04	0.91	1.56	0.87	2.58	2.67
15	1	1.08	1.29	3.11	1.88	1.45	1.88	1.03	1.39	1.08
16	1	0.77		1.26	1.08	0.62	1.50	1.75	1.22	1.21

C-3 Muscle pH

Participant	Sample	+0 h	+3 h	+9 h	+24 h	+48 h	+72 h
1	1 2	6.80	6.88	6.84	6.91	6.83	7.18 7.18
2	1 2	6.73	6.90	6.83 6.83	6.69	6.59	7.25
3	1 2	6.72	no sample	6.89 6.93	6.99 6.97	7.00	7.12 7.17
4	1 2 3	6.58	7.03 7.01	7.01	7.00	6.97 6.85 6.87	7.12
5	1 2	6.70	7.06 6.82	6.99	7.04	7.07	7.07
6	1 2	6.57	7.10	7.10	7.07	7.05	7.10 7.10
7	1 2	6.82	no sample	6.98	6.85	7.07	7.00 7.03
8	1	6.74	7.01	6.99	6.90	7.11	no sample
9	1 2	6.81	7.21	7.15	7.14 7.03	7.09	7.03
10	1 2	6.91	7.16	7.15	7.12	7.20 7.00	7.12
11	1 2	6.91	7.09	7.11	7.15	7.14	7.03 7.04
12	1	6.59	7.11	7.01	7.12	7.10	no sample
13	1 2	6.96	7.12 6.84	7.10	7.13	7.12 6.96	7.00
14	1	6.89	7.24	7.07	7.21	7.01	no sample
15	1 2 3	6.84	7.00 6.90 6.97	6.89	7.06	7.10	7.25 7.14
16	1 2	6.90	7.13	7.13	7.08 7.10	7.12	7.05

C-4 Non-Bicarbonate Muscle Buffer Capacity

$\beta m_{in\ vitro}$ (mmol H^+•kg dm^{-1}•pH^{-1})

Participant	Titration	Pre-HIT	+0 h	+3 h	+9 h	+24 h	+48 h	+72 h
1	1	120.8	174.6	158.2	157.9	143.1	135.0	120.9
	2	114.5						118.6
2	1	143.1	159.5	145.5	128.8	144.6	150.8	119.9
	2	123.2			149.8			
3	1	145.0	160.7		143.1	170.2	157.5	129.3
	2	152.1		no sample	140.2	173.7		148.6
	3							149.7
4	1	134.9	160.5	152.1	165.0	154.1	155.1	117.9
	2	133.1					135.6	124.3
	3	136.5						113.8
5	1	131.0	151.3	168.5	132.8	134.5	124.2	129.6
	2	113.2		136.3				142.5
	3	139.7		165.7				
	4	135.4						
6	1	122.3	159.6	139.3	145.2	128.6	139.2	115.7
	2	124.4						112.8
7	1	128.7	166.3	146.5	132.7	110.8	127.5	100.0
	2	136.0						112.2
8	1	145.9	150.6	135.5	145.9	148.6	148.2	147.6
	2	159.8						162.3
	3	167.5						
9	1	177.8	166.9	151.2	160.4	142.7	155.0	158.3
	2	120.2				131.4		164.8
	3	115.1						125.6
10	1	144.1	143.5	153.7	167.2	149.4	123.6	152.9
	2	156.5			143.4		151.6	142.6
	3	166.2						
11	1	154.9	150.2	131.2	163.0	168.9	149.0	140.4
	2	156.3						
12	1	133.8	146.5	148.3	162.2	160.1	159.9	154.8
	2	159.5						147.9
	3	148.4						
13	1	189.2	150.4	163.0	171.5	151.7	149.3	161.5
	2	150.5					160.1	181.1
	3	150.5						
	4	154.3						
14	1	142.3	142.5	130.9	145.5	153.5	105.8	136.4
	2	161.5						139.1
15	1	160.6	166.8	129.8	146.2	152.2	140.6	145.6
	2	143.9		167.7				166.4
	3	155.8		155.8				
16	1	162.4	158.9	159.3	167.0	127.0	155.5	151.4
	2	137.5						135.9
	3	143.9						130.2
	4	161.7						

Appendix D Study 2 (Chapter 4) Results

D-1 Participant Anthropometric Data

Group	Participant	Age (y)	Height (cm)	Mass (kg)	$\dot{V}O_{2peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)
HITΔ20	**1**	22	181.8	74.3	52.3
	2	20	169.3	70.3	54.4
	3	18	171.4	74.3	46.4
	4	20	179.4	73.8	48.7
	5	19	176.9	74.3	58.2
	6	23	172.9	71.9	54.8
	7	31	196.3	106.2	37.1
	8	26	179.3	87.8	42.2
Mean		**22**	**178.4**	**79.1**	**49.3**
SD		**4**	**8.4**	**12.2**	**7.1**
HITΔ90	**9**	23	183.3	103.0	39.9
	10	30	166.6	69.8	47.6
	11	20	176.2	67.8	52.1
	12	19	176.4	74.5	45.8
	13	19	185.1	76.9	51.6
	14	21	175.8	76.0	49.6
	15	30	193.7	88.9	55.1
	16	28	192.1	92.8	40.2
Mean		**24**	**181.1**	**81.2**	**47.7**
SD		**5**	**9.1**	**12.4**	**5.5**

D-2 Repeated-Sprint Ability Test

Total Work (kJ)

HITΔ20

Participant	Pre	+4 wk	+10 wk
1	23.34	24.94	25.42
2	18.67	19.94	21.11
3	18.06	19.87	18.83
4	22.23	22.27	22.76
5	20.35	19.68	20.19
6	21.69	21.95	20.68‖
7	27.84‡	no test	no test
8	26.96	24.22	25.17
Mean	21.61	21.84	22.02
SD	3.03	2.15	2.52

HITΔ90

Participant	Pre	+4 wk	+10 wk
9	22.46	24.06	23.80
10	15.47	15.96	17.23
11	18.72†	17.79†	16.53†
12	21.80	21.63	22.97
13	23.84	21.81	21.34
14	25.29	23.05	25.15
15	28.34	28.31	27.37
16	23.13	22.21	no test
Mean	22.90	22.43	22.98
SD	3.93	3.66	3.48

†Data excluded from analysis because of failed criterion test Pre and +10 wk
‡Datum excluded because no post-tests performed

Work Decrement (%)

HITΔ20

Participant	Pre	+4 wk	+10 wk
1	-8.8%	-6.6%	-6.5%
2	-14.0%	-8.6%	-13.7%
3	-17.7%	-8.9%	-10.7%
4	-11.8%	-8.6%	-11.9%
5	-13.9%	-9.2%	-11.1%
6	-20.0%	-16.2%	-18.4%‖
7	-17.6%‡	no test	no test
8	-8.1%	-9.0%	-8.8%
Mean	-13.5%	-9.6%	-10.4%
SD	4.4%	3.1%	2.5%

HITΔ90

Participant	Pre	+4 wk	+10 wk
9	-12.6%	-11.5%	-11.4%
10	-5.8%	-6.5%	-7.8%
11	-1.8%†	-1.1%†	2.6%†
12	-3.7%	-4.2%	-10.9%
13	-12.0%	-14.7%	-16.6%
14	-17.6%	-6.4%	-11.2%
15	-13.9%	-8.6%	-10.5%
16	-11.4%	-11.9%	no test
Mean	-11.0%	-9.1%	-11.4%
SD	4.8%	3.7%	2.9%

†Data excluded from analysis because of failed criterion test Pre and +10 wk
‡Datum excluded because no post-tests performed
‖Datum excluded because of failure to maintain training diary

Work per Sprint (J)

HITΔ20 (Pre)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
1	5120	4730	4737	4546	4206
2	4342	3859	3548	3566	3350
3	4387	3946	3443	3286	2994
4	5042	4870	4465	4019	3838
5	4729	4149	4019	3708	3747
6	5423	4690	4118	3799	3660
7	6759‡	6033‡	5324‡	5059‡	4663‡
8	5869	5665	5465	5157	4808
Mean	4987	4558	4256	4012	3800
SD	552	632	704	640	586

HITΔ20 (+4 wk)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
1	5337	5233	4847	4870	4649
2	4365	4161	3958	3683	3775
3	4362	4258	4001	3652	3600
4	4875	4552	4304	4362	4177
5	4335	4215	3757	3636	3742
6	5241‖	4706‖	4360‖	3926‖	3715‖
7	no test	no test	no test	no test	no test
8	5323	5288	4961	4509	4136
Mean	4834	4630	4313	4091	3971
SD	475	472	455	491	370

‡Data excluded because no +4 wk test performed

‖Data excluded because of failure to maintain training diary

Work per Sprint (J)

HITΔ90 (Pre)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
9	5141	4882	4395	4098	3947
10	3284	3202	3107	3014	2864
11	3811†	3648†	4078†	3720†	3459†
12	4524	4338	4437	4399	4098
13	5416	4781	4673	4465	4501
14	6138	5492	5192	4551	3919
15	6581	5726	5456	5250	5330
16	5218	4774	4522	4330	4283
Mean	5186	4742	4540	4301	4135
SD	1077	825	749	671	740

HITΔ90 (+4 wk)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
9	5436	5047	4841	4532	4205
10	3413	3196	3129	3185	3038
11	3597†	3841†	3499†	3309†	3546†
12	4518	4242	4398	4251	4221
13	5113	4257	4316	3900	4219
14	4923	4875	4519	4333	4400
15	6195	5659	5638	5555	5266
16	5041	4570	4298	4191	4107
Mean	4948	4549	4448	4278	4208
SD	854	774	748	712	650

†Data excluded from analysis because of failed criterion test Pre

D-3 Graded-Exercise Test

HITΔ20				
	Lactate Threshold (W)		Peak Aerobic Power (W)	
Participant	**Pre**	**+4 wk**	**Pre**	**+4 wk**
1	183	191	278	279
2	211	210	278	300
3	127	167	248	261
4	155	202	247	270
5	182	217	276	280
6	165	181	270	282
7	192	229	290	287
8	160	156	285	280
Mean	172	194	272	280
SD	26	25	16	11
HITΔ90				
	Lactate Threshold (W)		Peak Aerobic Power (W)	
Participant	**Pre**	**+4 wk**	**Pre**	**+4 wk**
9	176	209	285	325
10	147	151	243	264
11	166	165	253	242
12	153	166	249	255
13	221	199	305	300
14	163	164	262	276
15	305	290	407	404
16	180	177	279	283
Mean	189	190	285	294
SD	52	45	53	52

D-4 VO_{2peak} Test

$\dot{V}O_{2peak}$ (mL•min^{-1}•kg^{-1})					
HITΔ20			HITΔ90		
Participant	**Pre**	**+4 wk**	**Participant**	**Pre**	**+4 wk**
1	52.3	54.1	**9**	39.9	42.2
2	54.4	52.8	**10**	47.6	48.4
3	46.4	47.2	**11**	52.1	46.3
4	48.7	50.0	**12**	45.8	50.7
5	58.2	50.6	**13**	51.6	51.4
6	54.8	52.0	**14**	49.6	50.5
7	37.1[†]	no test	**15**	55.1	56.9
8	42.2	44.2	**16**	40.2	41.4
Mean	49.3	50.1	**Mean**	47.7	48.5
SD	7.1	3.4	**SD**	5.5	5.1

[†]Datum excluded because was unable to perform +4 wk test

D-5 Western Blotting

MCT1 abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20							
Participant	**Gel no.**	**Pre**	**+2 wk**	**+2 wk duplicate**	**+4 wk**	**+4 wk duplicate**	**+10 wk**
1	1	1.02	1.10		1.03		1.13
	2	1.30	1.12		1.28		1.33
2	1	0.37	0.86		2.60	1.99	0.42
	2	0.48	0.99		4.32	3.17	0.77
	3	0.30	0.84		1.89		0.48
3	1	1.21	0.72		1.34	1.27	1.43
	2	1.48	0.56		1.72		1.19
4	1	0.28	0.59		0.71	0.65	0.69
	2	0.60	0.95		0.88	0.86	1.09
	3	0.49	0.78		1.09		0.96
5	1	0.70	0.84		4.23	3.76	1.31
	2	0.64	0.56		5.33		1.65
6	1	0.73	0.78		2.29	2.72	
	2	0.44	0.67	0.75	3.22	2.92	no sample
	3	0.29	0.47		1.67		
7	1	0.58	1.05	0.99	0.83	0.82	no sample
	2	0.90	0.87		0.83		
8	1	0.62	1.54		2.31	2.45	0.99
	2	0.66	1.29		1.58	1.61	0.95
	3	0.82	1.81		2.38		1.01
HITΔ90							
Participant	**Gel no.**	**Pre**	**+2 wk**	**+2 wk duplicate**	**+4 wk**	**+4 wk duplicate**	**+10 wk**
9	1	0.65	0.73		0.99	1.19	1.08
	2	0.65	0.67		1.03		0.72
10	1	0.69	1.27		2.11	2.65	2.36
	2	0.67	1.15		1.89	2.47	2.48
	3	0.50	1.33		1.77		1.90
11	1	0.75	0.70		1.64	1.42	0.88
	2	0.55	0.34		1.73		0.32
12	1	0.85	1.00		2.93	3.11	0.53
	2	0.77	1.17		3.31	2.87	0.65
	3	0.89	1.16		5.15		0.92
13	1	0.59	0.67		2.29	2.22	0.93
	2	0.45	0.53		2.14		0.92
14	1	0.43	0.62		0.40	0.51	0.40
	2	0.66	0.74		0.77	0.62	0.70
	3	0.17	0.39		0.24		0.16
15	1	0.79	0.47		1.91	2.25	1.04
	2	0.68	0.24		1.13		0.43
16	1	0.35	1.11		0.55	0.42	
	2	0.78	1.51	1.73	1.14	1.09	no sample
	3	0.61	1.23		0.70		

MCT4 abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	+2 wk	+2 wk duplicate	+4 wk	+4 wk duplicate	+10 wk
1	1	0.84	1.00		0.94	0.82	0.74
	2	1.48	2.01		1.80	1.80	1.00
2	1	0.80	0.88		1.03	1.32	0.85
	2	1.47	1.44		1.49	1.35	1.57
	3	0.98	1.06		1.25	1.00	1.21
3	1	1.21	0.78		1.90	1.93	1.13
	2	1.86	1.11		2.62	7.31	1.37
	3	1.29	1.07		2.40	2.35	1.52
4	1	1.39	1.34		1.47	1.34	1.00
	2	0.73	0.74		1.06	0.99	0.73
	3	0.69	0.72		0.86	0.85	0.71
5	1	0.73	0.84		1.97	2.08	0.88
	2	1.17	1.15		2.20	1.83	1.01
6	1	1.12	1.21	1.38	1.40	1.24	no sample
	3	1.16	1.28	1.29	1.38	1.35	
7	1	0.94	0.95	0.88	1.31	1.45	no sample
8	1	1.29	1.14		1.22	1.28	1.45
	3	0.80	0.81		1.05	1.06	1.05

HITΔ90

Participant	Gel no.	Pre	+2 wk	+2 wk duplicate	+4 wk	+4 wk duplicate	+10 wk
9	1	0.91	1.23		1.16	1.33	0.96
	2	1.00	1.45		1.17	0.89	1.13
10	1	1.08	0.92		1.08	1.32	1.15
	2	2.34	1.38		1.55	1.70	1.42
	3	1.24	1.44		1.44	1.36	1.71
11	1	1.61	1.37		1.54	1.76	1.42
	2	2.15	2.47		2.50	2.29	2.51
12	1	0.78	0.71		0.79	0.74	0.71
	3	0.98	0.96		0.92	0.82	1.00
13	1	1.09	1.20		1.24	1.34	1.20
	2	1.23	1.15		1.28	1.01	1.27
14	1	0.72	1.05		0.83	0.96	0.82
	3	0.87	1.18		1.06	1.05	1.17
15	1	1.42	1.71		1.48	1.70	1.51
	2	1.32	1.53		1.38	1.73	1.17
16	1	0.74	0.61	0.58	0.66	0.67	no sample
	3	0.93	0.81	0.77	0.86	1.02	

Basigin abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
1	1	1.69	1.90	1.62	1.08
	2	1.34	1.72	1.66	1.19
2	1	1.79	1.71	1.04	1.21
	2	0.54	1.02	0.67	0.71
	3	1.65	1.62	0.80	0.99
	4	0.89	1.00	0.84	1.03
3	1	1.41	1.57	1.66	1.10
4	1	1.15	1.06	0.97	1.01
	2	1.09	1.02	1.35	1.02
5	1	0.90	0.75	0.59	0.65
	2	1.26	1.32	0.88	1.22
	3	1.68	1.08	0.40	0.71
6	1	1.47	2.26	0.99	no sample
	2	1.30	1.43	0.84	
7	1	0.99	1.26	1.31	no sample
8	1	1.15	1.22	0.90	1.11

HITΔ90

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
9	1	1.12	1.02	0.96	0.76
	2	1.22	1.45	1.44	0.83
10	1	1.33	1.25	1.18	1.00
11	1	1.46	1.88	1.87	1.90
	2	1.10	1.76	1.35	1.12
12	1	1.19	0.96	1.63	0.93
	2	1.09	0.96	2.87	0.92
13	1	1.02	1.15	0.74	1.04
	2	1.54	1.83	0.84	0.99
	3	0.74	1.17	0.67	1.32
14	1	0.81	1.44	1.07	1.25
	2	0.70	1.08	0.81	0.70
15	1	1.35	1.41	0.95	0.91
	2	1.12	1.64	1.77	1.01
16	1	0.70	1.22	1.13	no sample

NHE1 abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	Pre duplicate	+2 wk	+4 wk	+4 wk duplicate	+10 wk
1	1	0.48		0.66	1.60	1.60	0.61
2	1	0.48		0.76	1.28	1.32	0.94
	2	0.70		0.79	2.23	1.98	0.62
	3	2.38		1.19	2.66	1.57	2.28
3	1	1.32		0.74	2.00	2.17	1.37
	2	0.93		1.14	1.44	1.40	1.58
4	1	0.96		0.86	2.39	2.10	1.61
5	1	0.74	0.87	1.06	2.01	1.57	no sample
6	1	0.83	0.65	0.69	2.58	2.67	no sample
	2	1.08	1.29	1.70	1.39	1.08	
7	1	0.77		0.83	1.22	1.21	0.91
8	1	0.48		0.66	1.60	1.60	0.61

HITΔ90

Participant	Gel no.	Pre	Pre duplicate	+2 wk	+4 wk	+4 wk duplicate	+10 wk
9	1	0.68		0.59	1.08	0.92	0.65
10	1	0.68		1.55	1.18	1.16	1.73
11	1	0.56		1.04	1.60	1.33	1.10
	2	0.60		0.66	1.71	1.35	0.79
12	1	0.82		0.85	1.40	1.53	0.54
	2	0.44		0.65	1.00	0.81	0.39
13	1	0.96		0.84	2.39	1.71	1.26
14	1	0.48		0.60	0.69	0.69	0.65
15	1	1.51		0.92	1.92	2.39	1.43
16	1	0.68	1.01	1.20	1.03	0.75	no sample
	2	0.86	0.86	1.78	1.14	0.94	

NBCe1 abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
1	insufficient muscle sample to prepare HEPES-sucrose homogenate				
2	1	0.95	1.43	15.22[†]	1.65
	2	1.02	1.58	11.56[†]	1.92
3	insufficient muscle sample to prepare HEPES-sucrose homogenate				
4	insufficient muscle sample to prepare HEPES-sucrose homogenate				
5	1	0.79	0.76	4.16	0.76
	2	1.14	1.22	2.44	0.69
6	1	0.51	0.52	0.87	no sample
7	1	0.25	0.42	0.57	no sample
8	1	0.65	0.46	1.27	0.70

HITΔ90

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
9	1	0.81	no sample	0.96	1.19
10	1	0.44	0.38	0.97	0.55
11	1	1.20	0.61	0.74	0.89
12	1	0.47	1.05	1.29	1.12
13	1	0.44	0.60	2.56	1.25
	2	0.57	0.76	1.31	1.22
14	insufficient muscle sample to prepare HEPES-sucrose homogenate				
15	insufficient muscle sample to prepare HEPES-sucrose homogenate				
16	insufficient muscle sample to prepare HEPES-sucrose homogenate				

[†]Data excluded because sample was degraded on Coomassie stain

CAII abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
1	1	1.11	0.65	0.67	0.71
	2	0.92	1.04	0.84	0.88
2	1	0.45	0.92	1.61	0.67
	2	0.93	1.23	1.49	0.90
3	1	0.84	0.81	1.55	1.96
	2	1.16	0.81	0.94	0.69
4	1	0.53	0.39	0.52	0.39
	2	0.89	0.76	0.88	0.78
5	1	0.46	0.66	2.71	0.76
	2	0.62	0.76	2.16	0.77
6	1	0.70	0.85	0.65	no sample
	2	0.65	0.80	0.81	
7	1	0.59	0.53	0.74	no sample
	2	0.69	0.64	0.78	
8	1	0.50	0.79	0.61	0.55
	2	0.84	0.87	0.92	0.69

HITΔ90

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
9	1	0.62	1.14	1.34	0.78
	2	0.54	0.54	0.35	0.58
10	1	0.83	0.56	1.48	1.61
	2	0.96	0.62	0.91	1.03
11	1	0.80	0.59	0.86	0.71
	2	0.82	0.85	1.00	0.97
12	1	0.55	0.43	1.10	0.41
	2	0.77	0.68	1.44	0.75
13	1	0.44	0.46	1.56	0.53
	2	0.53	0.47	1.39	0.62
14	1	0.51	0.68	0.69	0.55
	2	0.85	1.08	0.95	0.79
15	1	1.01	0.48	1.13	0.54
	2	1.32	0.77	1.89	1.05
16	1	0.93	0.91	0.85	no sample
	2	0.80	0.68	0.70	

CAIII abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
1	1	1.31	1.37	1.52	1.34
2	1	1.23	1.24	2.00	1.63
3	1	0.80	0.79	0.67	0.84
4	1	1.14	1.20	1.46	1.20
5	1	1.60	1.59	1.24	1.19
6	1	1.16	1.23	1.26	no sample
7	1	1.19	1.15	0.94	no sample
8	1	1.69	2.87	1.08	1.75

HITΔ90

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
9	1	0.90	0.86	0.88	0.87
10	1	1.34	1.42	1.10	1.20
11	1	0.87	0.45	1.01	0.97
12	1	0.80	0.96	1.45	1.02
13	1	1.26	1.07	1.50	1.44
14	1	1.18	0.92	1.19	1.07
15	1	1.16	0.95	1.24	1.22
16	1	1.03	0.89	1.00	no sample

CAIV abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
1	1	2.04	1.90	1.75	1.39
2	1	0.97	1.20	1.41	1.64
3	1	0.71	0.50	0.78	0.25
4	1	1.04	0.57	0.43	0.71
5	1	1.32	1.20	sample	
	2	1.12	1.24	degraded	1.05
6	1	1.43	1.25	2.22	no sample
7	1	2.10	1.95	3.10	no sample
8	1	0.79	0.79	0.73	0.78

HITΔ90

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
9	1	0.97	0.92	0.88	1.16
10	1	1.71	1.30	1.79	0.90
11	1	0.75	0.59	0.90	0.73
12	1	0.49	0.45	0.25	0.60
13	1	0.44	0.29	0.70	0.94
14	1	0.69	1.02	1.39	0.68
15	1	0.96	1.01	0.97	0.98
16	1	1.57	0.77	2.27	no sample

CAXIV abundance relative to an internal standard calibration curve and corrected for total protein

HITΔ20

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
1	**1**	1.89	2.54	2.74	1.18
	2	2.74	3.33	4.04	1.76
2	**1**	0.33	1.98	0.81	0.90
	2	0.53	1.84	1.35	0.90
	3	0.30	1.70	1.37	0.91
3	**1**	0.82	2.51	1.68	1.15
	2	0.73	2.50	1.08	0.86
4	**1**	2.63	1.67	1.10	0.19
	2	1.49	1.17	0.93	0.44
	3	1.54	1.01	1.33	0.52
5	**1**	0.69	1.57	1.35	0.57
	2	2.60	2.77		0.72
	3	2.51	1.61		0.85
6	**1**	0.40	0.71	0.45	no sample
	2	0.29	0.59	0.27	
7	**1**	1.59	0.84	1.99	no sample
	2	2.52	1.11	2.46	
8	**1**	0.97	0.87	1.24	0.95
	2	0.99	1.03	0.99	1.18

HITΔ90

Participant	Gel no.	Pre	+2 wk	+4 wk	+10 wk
9	**1**	1.24	2.34	2.67	1.84
	2	1.33	1.94	2.61	1.73
10	**1**	0.72	1.96	1.11	1.90
	2	0.75	1.93	1.94	1.16
11	**1**	1.56	2.35	1.36	1.54
	2	2.00	3.59	1.97	2.90
12	**1**	1.47	1.58	1.77	0.34
	2	0.87	0.95	1.16	0.50
	3	0.99	0.93	1.44	0.52
13	**1**	1.68	1.93	1.86	1.08
	2	0.96	3.44	1.53	0.76
	3	1.06	3.84	2.05	0.72
14	**1**	0.87	3.47	1.76	0.66
	2	2.47	3.03	2.96	1.24
	3	2.83	2.37	2.00	0.92
15	**1**	2.95	2.44		0.67
	2	0.88	3.19	2.95	0.99
	3	0.78	2.15	2.45	0.87
16	**1**	1.60	0.96	2.19	no sample
	2	1.06	0.64	1.51	

D-6 Non-Bicarbonate Muscle Buffer Capacity

$\beta m_{in\ vitro}$ (mmol H^+•kg dm^{-1}•pH^{-1})

HITΔ20					
Participant	**Titration no.**	**Pre**	**+2 wk**	**+4 wk**	**+10 wk**
1	1	139.7	119.3	129.6	137.7
	2	135.4	129.7	142.5	
	3	131.0			
	4	113.2			
2	1	128.7	128.0	100.0	126.5
	2	136.0		112.2	
3	1	167.5	149.3	162.3	138.7
	2	145.9	141.8	137.9	144.0
	3	159.8		147.6	
4	1	115.1	145.1	125.6	117.6
	2	177.8	115.0	158.3	
	3	120.2	138.8	164.8	
	4		159.8		
5	1	144.1	133.2	152.9	
	2	156.5	105.5	142.6	no sample
	3	166.2	134.2	140.4	
6	1	154.9	156.2	140.4	no sample
	2	156.3	148.9		
7	1	189.2	190.3	161.5	160.6
	2	150.5	157.0	181.1	
	3	154.3			
	4	150.5			
8	1	162.4	176.1	151.4	131.1
	2	137.5		135.9	
	3	161.7		130.2	
	4	143.9			
HITΔ90					
Participant	**Titration no.**	**Pre**	**+2 wk**	**+4 wk**	**+10 wk**
9	1	120.8	108.4	120.9	123.4
	2	114.5		118.6	
	3			116.0	
10	1	143.1	138.8	119.9	150.4
	2	123.2	149.5		
11	1	145.0	143.0	129.3	126.2
	2	152.1	139.3	148.6	141.1
	3			149.7	
12	1	136.5	166.3	113.8	126.3
	2	134.9		117.9	
	3	133.1		124.3	
13	1	122.3	173.9	112.8	146.9
	2	124.4		115.7	
14	1	133.8	144.4	154.8	105.4
	2	159.5		147.9	100.1
	3	148.4			
15	1	142.3	152.3	136.4	157.4
	2	161.5	135.6	139.1	136.5
16	1	160.6	138.6	145.6	
	2	143.9		166.4	no sample
	3	155.8			

Appendix E Study 3 (Chapter 5) Results

E-1 Participant Anthropometric Data

Group	Participant	Age (y)	Height (cm)	Mass (kg)	$\dot{V}O_{2peak}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)
	1	28	160.0	63.6	37.3
	2	18	166.0	64.1	44.1
	3	20	165.0	59.4	44.6
Rest-1	**4**	20	162.0	59.5	30.1
	5	24	168.3	68.9	41.6
	6	31	168.0	55.5	46.7
	7	19	160.7	63.5	40.8
Mean		**23**	**164.3**	**62.1**	**40.7**
SD		**5**	**3.4**	**4.3**	**5.6**
	8	28	162.1	60.2	41.8
	9	21	170.8	72.8	31.5
	10	19	169.9	75.6	34.1
Rest-5	**11**	36	166.9	57.9	43.1
	12	20	164.4	72.9	34.6
	13	19	160.7	46.2	43.9
	14	26	160.1	60.9	35.9
Mean		**24**	**165.0**	**63.8**	**37.8**
SD		**6**	**4.3**	**10.6**	**5.0**

E-2 Repeated-Sprint Ability Test

Total Work (kJ)

Rest-1			
Participant	**Pre**	**+4 wk**	**+10 wk**
1	16.67	16.75	16.84
2	13.76	14.60	15.04
3	12.79	12.86	12.66[†]
4	10.66	10.44	9.94
5	16.23	15.31	15.73
6	13.01	13.24	12.30
7	13.69	14.94	13.93
Mean	13.83	14.02	13.96
SD	2.07	2.04	2.51
Rest-5			
Participant	**Pre**	**+4 wk**	**+10 wk**
8	14.56	15.65	15.25
9	16.01	17.41	17.51
10	12.19	13.28	12.95
11	11.66	13.40	13.77
12	11.90	13.94	13.79
13	11.03	13.28	11.97
14	10.92	10.37	9.08
Mean	12.61	13.90	13.47
SD	1.93	2.19	2.63

[†]Datum excluded from analysis because of failed criterion test

Work Decrement (%)

Rest-1			
Participant	**Pre**	**+4 wk**	**+10 wk**
1	−17.3%	−6.4%	−7.0%
2	−7.6%	−8.7%	−4.0%
3	−9.0%	−3.9%	−0.1%[†]
4	−8.8%	−7.1%	−11.9%
5	−12.7%	−18.8%	−15.6%
6	−16.4%	−7.7%	−5.6%
7	−12.7%	−5.8%	−4.6%
Mean	−12.1%	−8.3%	−8.1%
SD	3.8%	4.8%	4.6%
Rest-5			
Participant	**Pre**	**+4 wk**	**+10 wk**
8	−11.2%	−7.6%	−12.2%
9	−19.0%	−13.7%	−14.9%
10	−22.3%	−9.5%	−9.4%
11	−13.4%	−6.5%	−7.5%
12	−19.9%	2.4%	−6.1%
13	−13.7%	−12.4%	−11.8%
14	−28.1%	−25.4%	−32.8%
Mean	−18.2%	−10.4%	−13.5%
SD	5.9%	8.4%	9.0%

[†]Datum excluded from analysis because of failed criterion test

Work per Sprint (J)

Rest-1 (Pre)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
1	4032	3760	3351	2887	2638
2	2977	2706	2744	2645	2686
3	2812	2655	2552	2474	2299
4	2338	2170	2106	2049	1996
5	3718	3438	3177	3010	2885
6	3113	2764	2541	2343	2248
7	3135	2676	2684	2571	2624
Mean	3161	2881	2736	2568	2482
SD	564	536	417	324	309

Rest-1 (+4 wk)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
1	3579	3452	3436	3181	3103
2	3196	2885	2815	2822	2879
3	2677	2635	2624	2455	2465
4	2246	2312	1945	1821	2110
5	3768	3339	2912	2697	2590
6	2867	2727	2568	2453	2621
7	3172	3062	2912	2959	2836
Mean	3072	2916	2745	2627	2658
SD	524	402	452	442	322

Work per Sprint (J)

Rest-5 (Pre)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
8	3277	2989	2975	2666	2649
9	3955	3537	3115	2834	2571
10	3138	2700	2356	2057	1934
11	2694	2583	2282	2103	2000
12	2973	2427	2337	2109	2055
13	2555	2413	2205	1958	1897
14	3038	2288	2068	1801	1723
Mean	3090	2705	2477	2218	2118
SD	456	433	402	381	352

Rest-5 (+4 wk)

Participant	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5
8	3389	3289	3238	2884	2854
9	4032	3740	3434	3264	2936
10	2934	2767	2646	2447	2483
11	2868	2803	2582	2646	2504
12	2723	2632	2896	2909	2777
13	3032	2798	2699	2425	2325
14	2780	2065	1750	1923	1848
Mean	3108	2871	2749	2642	2532
SD	462	525	543	432	374

E-3 Graded-Exercise Test

Rest-1				
	Lactate Threshold (W)		Peak Aerobic Power (W)	
Participant	Pre	+4 wk	Pre	+4 wk
1	104	125	194	214
2	127	125	199	200
3	113	129	194	202
4	93	88	139	130
5	115	119	212	198
6	133	128	196	208
7	90	123	175	198
Mean	111	119	187	193
SD	16	14	24	28
Rest-5				
	Lactate Threshold (W)		Peak Aerobic Power (W)	
Participant	Pre	+4 wk	Pre	+4 wk
8	147	142	201	200
9	93	88	173	172
10	111	108	178	190
11	112	133	195	209
12	132	133	194	194
13	80	76	143	142
14	104	104	154	154
Mean	111	112	177	180
SD	23	25	22	25

E-4 VO_{2peak} Test

$\dot{V}O_{2peak}$ (mL•min^{-1}•kg^{-1})					
Rest-1			Rest-5		
Participant	Pre	+4 wk	Participant	Pre	+4 wk
1	37.3	44.2	**8**	41.8	46.1
2	44.1	46.9	**9**	31.5	33.1
3	44.6	44.8	**10**	34.1	35.2
4	30.1	34.6	**11**	43.1	44.7
5	41.6	38.3	**12**	34.6	36.7
6	46.7	50.0	**13**	43.9	44.2
7	40.8	42.0	**14**	35.9	39.5
Mean	40.7	43.0	**Mean**	37.8	39.9
SD	5.6	5.2	**SD**	5.0	5.1

E-5 Western Blotting

MCT1 abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
1	1	2.58	2.55	1.50
	2	1.55	1.22	1.15
2	1	1.17	0.96	1.22
	2	0.81	0.80	0.80
3	1	1.05	1.30	1.90
	2	0.89	1.62	1.08
4	1	2.08	3.36	3.08
	2	1.02	1.25	1.78
5	1	1.07	1.28	0.81
	2	1.12	1.11	0.78
	3	0.89	0.94	0.64
6	1	1.01	1.05	0.87
	2	0.66	0.97	0.51
	3	0.66	0.59	0.52
7	1	0.60	0.90	1.20
Rest-5				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
8	1	4.08[†]	1.14	1.31
	2	3.78[†]	0.94	1.17
9	1	1.23	1.38	0.84
	2	0.67	0.83	0.72
10	1	1.39	1.84	2.80
	2	0.97	1.11	1.16
11	1	2.20	2.36	3.67
	2	1.00	0.94	1.48
12	1	2.63	2.28	2.14
	2	0.92	1.27	0.80
13	1	0.65	0.82	0.52
14	1	1.02	1.67	1.48
	2	1.21	1.09	1.18

[†]Data excluded from analysis because Pre sample was blood contaminated

MCT4 abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1

Participant	Gel no.	Pre	+4 wk	+10 wk
1	1	1.01	0.94	0.89
	2	0.47	0.46	0.58
2	1	1.07	0.98	0.92
3	1	1.14	1.71	1.10
4	1	0.70	0.75	0.80
5	1	1.74	1.35	1.30
	2	1.12	1.39	0.98
6	1	1.40	1.47	1.42
	2	1.10	1.17	1.23
7	1	1.16	0.80	1.30

Rest-5

Participant	Gel no.	Pre	+4 wk	+10 wk
8	1	1.03	0.92	1.04
	2	0.68	0.56	0.70
9	1	0.74	0.77	0.77
	2	0.64	0.45	0.54
10	1	0.89	1.10	1.16
11	1	0.65	0.77	0.40
12	1	1.02	0.80	0.72
13	1	0.81	1.01	1.07
14	1	0.89	1.15	0.85

Basigin abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1

Participant	Gel no.	Pre	+4 wk	+10 wk
1	1	1.58	1.63	1.48
2	1	0.71	1.06	0.99
3	1	0.75	0.87	0.75
4	1	0.99	0.79	0.82
	2	1.17	1.18	1.10
5	1	0.90	0.89	0.58
	2	1.04	1.17	0.95
6	1	0.71	0.85	0.73
	2	0.97	1.00	1.09
7	1	1.04	0.96	0.84

Rest-5

Participant	Gel no.	Pre	+4 wk	+10 wk
8	1	1.32	1.91	1.41
9	1	1.61	1.25	1.39
10	1	0.61	0.56	0.81
11	1	1.06	1.36	0.90
	2	1.34	1.55	1.36
12	1	0.87	1.11	0.74
	2	1.10	1.25	1.02
13	1	0.86	1.19	1.34
	2	0.97	1.15	1.31
14	1	0.93	0.99	0.86

NHE1 abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1

Participant	Gel no.	Pre	+4 wk	+10 wk
1	1	1.27	1.28	0.99
	2	1.47	1.62	1.22
	3	1.09	1.54	0.68
2	1	0.59	0.61	0.62
	2	0.94	0.83	1.23
3	1	0.74	0.78	0.83
	2	1.23	1.55	1.88
4	1	0.67	0.64	0.75
	2	1.39	1.61	1.67
5	1	1.19	1.38	0.93
	2	0.70	0.61	0.51
6	1	0.96	1.13	0.84
	2	0.40	0.50	0.28
7	1	0.77	1.04	0.80
	2	0.87	0.99	1.34
	3	0.83	0.92	0.61

Rest-5

Participant	Gel no.	Pre	+4 wk	+10 wk
8	1	1.87[†]	0.91	1.21
	2	2.21[†]	0.73	1.55
	3	1.77[†]	0.96	2.78
9	1	0.55	0.99	1.00
	2	0.75	0.83	0.88
	3	0.65	0.65	0.72
10	1	0.67	0.65	0.88
	2	0.85	1.05	1.53
11	1	0.97	1.12	1.73
	3	0.70	0.90	1.33
12	1	0.93	1.42	1.15
	2	0.96	1.35	1.71
13	1	0.84	0.98	1.01
	3	0.49	0.55	0.55
14	1	1.03	0.96	1.40
	2	0.96	0.62	1.14

[†]Data excluded from analysis because Pre sample was blood contaminated

NBCe1 abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
1	1	0.85	1.46	1.15
	2	1.33	1.76	1.19
2	1	0.52	0.61	1.75
	2	0.26	0.31	1.21
3	1	0.84	2.65	1.06
	2	0.40	1.31	0.24
4	1	0.71	0.89	0.78
5	1	1.24	1.42	1.10
6	1	1.23	1.14	0.71
7	1	0.53	0.44	sample degraded
Rest-5				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
8	1	1.98[†]	0.46	0.70
	2	1.52[†]	0.31	0.53
9	1	0.94	1.00	0.65
	2	0.38	0.56	0.32
10	1	0.83	0.99	0.89
	2	0.48	0.49	0.34
11	1	4.54	3.61	no sample
	2	0.83	0.98	no sample
12	1	1.57	2.11	2.27
	2	0.69	0.38	0.52
13	1	1.47	0.62	0.96
14	1	1.82	1.77	1.97

[†]Data excluded from analysis because Pre sample was blood contaminated

CAII abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
1	1	0.21	0.12	0.25
	2	1.54	0.79	0.77
	3	1.41	1.36	0.99
2	1	0.97	0.93	0.87
	2	0.98	0.86	0.86
	3	1.20	1.16	1.14
3	1	0.84	0.95	0.89
	2	0.86	0.93	0.72
	3	1.22	1.22	1.04
4	1	1.38	1.12	1.33
	2	0.92	1.01	1.06
	3	1.06	0.90	0.98
5	1	0.96	0.90	0.88
	2	1.12	1.28	0.90
	3	1.11	0.90	0.80
6	1	0.81	0.80	0.71
	2	1.08	0.98	0.78
	3	0.86	0.82	0.77
7	1	0.90	0.75	0.71
	2	0.76	0.72	0.94
	3	1.23	1.27	1.30
Rest-5				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
8	1	1.00[†]	0.18	0.26
	2	2.49[†]	0.65	0.70
	3	1.89[†]	0.91	1.13
9	1	0.26	0.53	0.21
	2	1.22	1.28	1.21
	3	1.30	1.25	1.16
10	1	0.92	0.89	0.95
	2	0.75	0.65	0.94
	3	1.00	0.91	1.40
11	1	1.33	1.22	1.12
	2	1.88	1.67	1.05
	3	1.44	1.16	1.51
12	1	1.28	1.27	1.10
	2	1.03	0.81	1.05
	3	0.93	0.74	1.01
13	1	0.83	0.72	0.54
	2	1.17	1.14	0.69
	3	1.02	0.80	0.74
14	1	0.67	0.65	0.63
	2	0.85	0.83	0.74
	3	0.89	0.85	0.80

[†]Data excluded from analysis because Pre sample was blood contaminated

CAIII abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
1	1	0.81	0.84	0.72
2	1	1.73	1.50	0.59
3	1	0.64	0.77	1.07
4	1	0.72	0.81	0.91
5	1	0.77	0.60	0.59
6	1	0.70	0.72	0.70
7	1	0.92	1.07	0.91
Rest-5				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
8	1	0.75	0.69	0.97
9	1	0.72	0.63	0.54
10	1	0.86	1.48	2.16
11	1	1.00	0.83	1.26
12	1	1.01	0.97	0.96
13	1	0.49	1.05	1.02
14	1	1.21	1.04	1.02

CAIV abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
1	1	0.91	0.66	0.62
2	1	0.68	0.54	0.48
3	1	0.52	0.46	0.47
4	1	0.76	0.58	0.47
	2	0.33	0.34	0.30
5	1	0.59	0.57	0.53
	2	0.42	0.46	0.33
6	1	0.80	0.81	0.84
	2	0.52	0.34	0.40
7	1	0.66	0.69	0.79
Rest-5				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
8	1	1.29	0.91	0.66
9	1	0.69	0.66	0.59
10	1	0.39	0.15	0.15
11	1	0.40	0.50	sample depleted
12	1	0.57	0.66	0.46
13	1	0.67	0.40	0.43
	2	0.46	0.35	0.57
14	1	0.46	0.71	0.54

CAXIV abundance relative to an internal standard calibration curve and corrected for total protein

Rest-1				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
1	1	0.60	1.46	1.37
	2	0.39	0.83	0.93
	3	0.67	1.13	1.12
2	1	0.65	1.55	1.68
3	1	1.44	2.40	0.54
4	1	1.07	0.94	1.20
	2	1.71	2.49	0.63
5	1	0.90	0.74	0.96
6	1	0.89	2.40	1.68
7	1	0.50	0.59	0.75
Rest-5				
Participant	**Gel no.**	**Pre**	**+4 wk**	**+10 wk**
8	1	1.46	1.33	0.51
	2	0.82	0.20	0.69
	3	1.07	0.49	1.08
9	1	1.50	2.14	2.48
	2	1.13	1.45	1.79
	3	1.64	1.93	1.98
10	1	0.60	1.72	1.18
11	1	1.13	1.71	0.10[†]
	2	1.21	2.17	−0.18[†]
12	1	0.34	1.28	0.12
	2	0.27	1.09	0.12
13	1	1.04	2.08	1.19
14	1	0.66	0.81	0.68

[†]Data excluded from analysis because sample degraded

E-6 Non-Bicarbonate Muscle Buffer Capacity

$\beta m_{in\ vitro}$ (mmol H^+•kg dm^{-1}•pH^{-1})

Participant	Titration no.	Pre	+4 wk	+10 wk
Rest-1				
1	1	132.5	130.7	154.9
	2	143.6	148.4	164.1
2	1	154.5	164.9	140.1
	2	120.2	124.7	163.5
	3	166.9		166.0
3	1	132.6	145.7	145.6
	2	124.8	132.0	
4	1	159.9	182.2	143.9
	2		131.6	164.8
5	1	144.9	157.2	134.4
	2	156.5	137.2	159.8
6	1	153.2	161.6	147.3
	2	166.7	137.4	134.9
7	1	140.0	154.4	124.2
	2	144.9	148.9	134.5
Rest-5				
8	1	140.6	148.7	143.7
	2	146.4	143.9	155.4
9	1	113.6	131.2	148.9
	2	124.6	117.9	120.2
10	1	147.2	160.6	143.3
	2	151.2	149.2	
11	1	120.5	154.7	113.3
	2	139.8	149.9	
12	1	130.0	144.7	113.7
	2	136.6	138.4	140.7
13	1	135.4	131.8	131.7
	2	138.8	127.4	124.2
14	1	142.9	119.4	148.9
	2	134.3	118.3	137.9

$\beta m_{in\ vitro}$ (mmol H^+•kg dm^{-1}•pH^{-1})

Rest-1

Participant	Titration no.	Total βm		Non-protein βm	
		Pre-SIT1	Post-SIT1	Pre-SIT1	Post-SIT1
1	1	132.5	158.3	53.6	59.4
	2	143.6	148.2		61.3
2	1	154.5	138.4	59.4	66.4
	2	120.2		64.2	
	3	166.9			
3	1	132.6	159.6	54.0	75.6
	2	124.8	146.3	67.7	
4	1	159.9	144.0	79.7	61.7
	2		144.7		70.4
5	1	144.9	117.0	60.9	45.8
	2	156.5	129.8	71.3	
6	1	153.2	148.6	71.8	60.9
	2	166.7	157.5	73.4	71.8
7	1	140.0	152.0	72.0	83.7
	2	144.9	160.3	66.9	87.7

Rest-5

Participant	Titration no.	Total βm		Non-protein βm	
		Pre-SIT1	Post-SIT1	Pre-SIT1	Post-SIT1
8	1	140.6	116.2	47.9	54.2
	2	146.4	118.9	53.4	
9	1	113.6	159.2	66.7	67.9
	2	124.6	148.8	54.1	69.3
10	1	147.2	136.7	58.9	57.3
	2	151.2		61.7	
11	1	120.5	119.2	55.7	46.8
	2	139.8		60.9	
12	1	130.0	126.2	55.6	86.2
	2	136.6	140.9		65.5
13	1	135.4	138.4	63.5	83.2
	2	138.8	132.5	68.6	63.0
14	1	142.9	144.4	63.7	64.7
	2	134.3	143.7	75.3	71.8
15	1	136.8	134.3	54.5	61.8
	2	117.7	141.4	53.9	69.7

Appendix F Venous blood pH kinetics following a repeated-sprint ability test

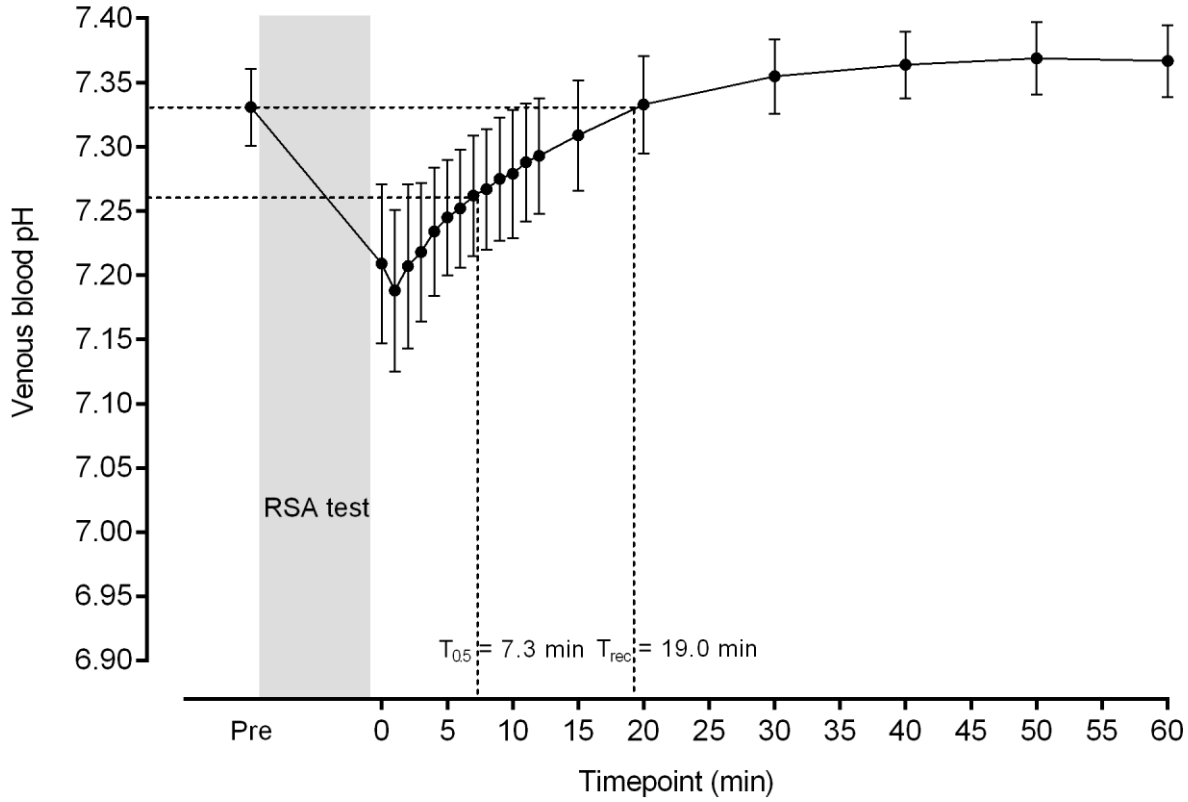


Figure F.1 Venous blood pH kinetics for one hour following a repeated-sprint ability (RSA) test (5 × 6-s sprints, 24 s rest); *n* = 26. Abbreviations: $T_{0.5}$ (mean half-time), T_{rec} (mean time taken for pH to recover to baseline).

Appendix G Muscle pH and lactate concentration before and after different modalities of exercise

Reference	Year		Sex	Exercise Bout	Muscle pH		measurement technique	sample	Lactate (dm) mmol.kg dm^{-1}		Lactate (mM) mmol.L muscle water^{-1}	
					pre-ex	post-ex			pre-ex	post-ex	pre-ex	post-ex
Bangsbo	1992	Am J Physiol Regul Integr Comp Physiol, 263, R891-9	M	(EX1) one-leg exercise to exhaustion	7.053	6.725	microelectrode	dry	5.2	97.6	1.6	29.5
Bangsbo	1992	Am J Physiol Regul Integr Comp Physiol, 263, R891-9	M	(EX2) 10 min rest, 7 × 15-s HIT, 2.5-min rest, one-leg exercise to exhaustion	6.851	6.774	microelectrode	dry	56.3	86.0	17.0	26.0
Bangsbo	1993	J Appl Physiol, 74, 2034-9	M	one-legged 28% MVIC	7.050	7.020	^{31}P-MRS	calf	6.1	17.7	1.8	5.3
Bangsbo	1993	J Appl Physiol, 74, 2034-9	M	one-legged 64% MVIC	7.050	7.120	^{31}P-MRS	calf	7.6	34.5	2.3	10.4
Bangsbo	1993	J Appl Physiol, 74, 2034-9	M	one-legged 90% MVIC	7.050	6.940	^{31}P-MRS	calf	12.3	50.1	3.7	15.2
Bangsbo	1993	J Physiol, 462, 115-33	M	(EX1) one-leg exercise to exhaustion	7.040	6.730	microelectrode	dry	4.3	98.9	1.3	29.9
Bangsbo	1993	J Physiol, 462, 115-33	M	(EX2) 10-min rest, 7 × 15-s HIT, 2.5-min rest, one-leg exercise to exhaustion	6.840	6.760	microelectrode	dry	56.3	79.6	17.0	24.1
Bangsbo	1996	J Physiol, 495, 587-96	M	one-leg knee extension to exhaustion	7.170	6.810	microelectrode	dry	6.9	114.0	2.1	34.5
Bangsbo	1996	J Physiol, 495, 587-96	M	one-leg knee extension to exhaustion (preceded by intermittent arm crank)	7.100	6.650	microelectrode	dry	15.9	109.2	4.8	33.0
Belfry	2012	J Appl Physiol, 113, 410-417	M	incremental (ramp) plantar flexion to fatigue	7.020	6.590	^{31}P-MRS	plantar flexors				
Bell	1988	Eur J Appl Physiol Occup Physiol, 58, 158-64	F+M	maximal one-legged cycling × 60 s	6.920	6.590	microelectrode	wet				
Bergman	1999	J Appl Physiol, 87, 1684-1696	M	60-min cycling at 45% VO_{2peak}					24.8	32.6	7.5	9.9
Bergman	1999	J Appl Physiol, 87, 1684-1696	M	60-min cycling at 65% VO_{2peak}					24.8	39.8	7.5	12.0
Bishop	2006	Eur J Appl Physiol, 97, 373-9	F	5 × 6-s repeated sprints	6.990	6.740	microelectrode	dry	7.6	62.7	2.3	19.0
Bishop	2006	Eur J Appl Physiol, 97, 373-9	F	5 × 6-s repeated sprints	6.980	6.780	microelectrode	dry	10.0	60.6	3.0	18.3
Bishop	2008	Am J Physiol Regul Integr Comp Physiol, 295, R1991-8	F	45-s continuous cycling at 190% VO_{2max} (pre-training)	7.108	6.930	microelectrode	dry				
Bishop	2008	Am J Physiol Regul Integr Comp Physiol, 295, R1991-8	F	45-s continuous cycling at 190% VO_{2max} (post-training)	7.108	6.809	microelectrode	dry				
Bishop	2007	J Appl Physiol, 102, 616-21	F	45-s continuous cycling at 200% VO_{2max}	7.110	6.840	microelectrode	dry	6.4	70.0	1.9	21.2
Bishop	2009	Pflügers Arch, 458, 929-36	M	cycling to exhaustion at 120% VO_{2max}	7.000	6.610	microelectrode	dry				
Bishop	2004	Eur J Appl Physiol, 92, 540-7	F	5 × 6-s repeated sprints	7.000	6.760	microelectrode	dry	13.1	61.4	4.0	18.6
Bishop	2004	Med Sci Sports Exerc, 36, 807-13	F	5 × 6-s repeated sprints (BIC)	7.110	6.890	microelectrode	dry	8.0	65.0	2.4	19.7
Bishop	2004	Med Sci Sports Exerc, 36, 807-13	F	5 × 6-s repeated sprints (placebo)	7.120	6.890	microelectrode	dry	5.0	38.0	1.5	11.5
Bishop	2009	Pflügers Arch, 458, 929-36	M	6 × 4-s repeated sprints (post-training)	6.990	6.770	microelectrode	dry				
Bishop	2009	Pflügers Arch, 458, 929-36	M	6 × 4-s repeated sprints (pre-training)	6.980	6.790	microelectrode	dry				
Bogdanis	1996	J Appl Physiol, 80, 876-84.	M	2nd 30-s max sprint after 4-min rest		6.800	microelectrode	dry	72.7	129.3	22.0	39.1
Bogdanis	1996	J Appl Physiol, 80, 876-84.	M	30-s max sprint	7.030	6.690	microelectrode	dry	5.8	108.0	1.8	32.7
Bogdanis	1995	J Physiol, 482 (Pt 2), 467-80	M	30-s max sprint	7.050	6.720	microelectrode	dry	3.8	119.0	1.1	36.0
Bonen	1998	Am J Physiol Endocrinol Metab, 274, E102-7	M	15-min cycling at 30% VO_{2max}					4.0	10.0	1.2	3.0
Bonen	1998	Am J Physiol Endocrinol Metab, 274, E102-7	M	15-min cycling at 60% VO_{2max}					4.0	20.0	1.2	6.0
Bonen	1998	Am J Physiol Endocrinol Metab, 274, E102-7	M	15-min cycling at 75% VO_{2max}					4.0	38.0	1.2	11.5
Bouissou	1989	J Appl Physiol, 67, 1245-9	M	cycling to exhaustion at 375 W or ~125% VO_{2max} (BIC)	n/a	6.500	microelectrode	wet				22.1
Bouissou	1989	J Appl Physiol, 67, 1245-9	M	cycling to exhaustion at 375 W or ~125% VO_{2max} (placebo)	n/a	6.500	microelectrode	wet				41.6
Cady	1989	J Physiol, 418, 311-25	F+M	3 × fatiguing MVICs	7.030	6.510	^{31}P-MRS					
Casey	1996	Am J Physiol Endocrinol Metab, 271, E38-43	M	1 × 30 s sprint					4.4	90.0	1.3	27.2
Casey	1996	Am J Physiol Endocrinol Metab, 271, E38-43	M	2 × 30 s sprints						112.7		34.1
Chasiotis	1982	J Physiol, 335, 197-204	M	66% MVIC to fatigue under occlusion	7.000	6.600	microelectrode	dry	5.8	99.3	1.8	30.0
Cheetham	1986	J Appl Physiol, 61, 54-60	F	30-s sprint	7.050	6.730	pH=−0.00413(lac+pyr)+7.06	dry	2.7	78.0	0.8	23.6

									Lactate (dm)		Lactate (mM)		
Reference	**Year**		**Sex**	**Exercise Bout**	**Muscle pH**		**measurement technique**	**sample**	**mmol.kg dm^{-1}**		**mmol.L muscle water^{-1}**		
					pre-ex	**post-ex**			**pre-ex**	**post-ex**	**pre-ex**	**post-ex**	
Chidnok	2013	J Appl Physiol, 115, 243-250	M	~3 min single-leg MVIC to exhaustion + 10-min recovery	7.000	7.000	^{31}P-MRS	knee extensors					
Chidnok	2013	J Appl Physiol, 115, 243-250	M	~3 min single-leg MVIC to exhaustion + 10-min exercise < critical power	7.000	6.600	^{31}P-MRS	knee extensors					
Chidnok	2013	J Appl Physiol, 115, 243-250	M	~3 min single-leg MVIC to exhaustion + 10-min exercise > critical power	7.000	7.000	^{31}P-MRS	knee extensors					
Churchward-Venne	2010	Eur J Appl Physiol, 108, 1189-1200	M	plantar flexion × 9 min heavy intensity (NH_4Cl)		6.695	^{31}P-MRS	plantar flexors					
Churchward-Venne	2010	Eur J Appl Physiol, 108, 1189-1200	M	plantar flexion × 9 min heavy intensity (placebo)		6.609	^{31}P-MRS	plantar flexors					
Chwablinska	1989	J Appl Physiol, 66, 2710-6	M	GXT at <OBLA stage	7.040	7.011	auto analyser	wet					
Chwablinska	1989	J Appl Physiol, 66, 2710-6	M	GXT at >OBLA stage	7.040	6.990	auto analyser	wet					
Chwablinska	1989	J Appl Physiol, 66, 2710-6	M	GXT at OBLA stage	7.040	6.980	auto analyser	wet					
Conley	1997	Am J Physiol Cell Physiol, 273, C306-15	F+M	electrical stimulation of the forearm	7.080	6.610	^{31}P-MRS	forearm					
Constantin-Teodosiu	1997	Exp Physiol, 82, 593-601	F+M	3 × MVICs of tibialis anterior with occlusion, 20 × 1.6 s stimulations at 50% MVIC	7.070	6.820	pH=−0.00532(lac+pyr)+7.06	tibialis anterior	1.3	47.1	0.4	14.2	
Constantin-Teodosiu	1997	Exp Physiol, 82, 593-601	F+M	3 × MVICs of tibialis anterior with occlusion, 20 × 1.6 s stimulations at 50% MVIC	7.060	6.660	^{31}P-MRS	tibialis anterior					
Costill	1983	Med Sci Sports Exerc, 15, 325-9	M	run to exhaustion at 125% VO_{2max}	7.036	6.856	microelectrode	wet (VL)				14.0	
Costill	1983	Med Sci Sports Exerc, 15, 325-9	M	run to exhaustion at 125% VO_{2max}	7.032	6.880	microelectrode	wet (gastroc)				16.3	
Costill	1984	Int J Sports Med, 5, 228-31	F+M	5 × 1 min cycling (BIC)	7.100	6.860	microelectrode	wet (VL)					
Costill	1984	Int J Sports Med, 5, 228-31	F+M	5 × 1 min cycling (PLA)	7.100	6.720	microelectrode	wet (VL)					
Costill	1982	J Appl Physiol, 53, 1310-3	F+M	n/a	7.050		microelectrode	wet (gastroc)	13.8	58.5	4.2	17.7	
Costill	1983	Med Sci Sports Exerc, 15, 325-9	M	400-m run		6.634	microelectrode	wet (gastroc)				25.6	
Davies	2011	J Appl Physiol, 111, 782-90	M	one-legged knee extension to exhaustion	7.020	6.960	^{31}P-MRS	knee extensors					
Degroot	1993	Muscle Nerve, 16, 91-8	M	4-min maximal isometric plantar flexion	7.090	6.470	^{31}P-MRS	plantar flexors					
Duffield	2009	J Sci Med Sport, 10, 127-134	F	cycle to exhaustion at 85% VO_{2max}	7.100	6.850	microelectrode	dry	7.0	67.3	2.1	20.3	
Edge	2013	Exp Physiol, 98, 481-490	F	45-s continuous cycling at 200% VO_{2peak} (post-training for HIT-1)	7.140	7.010	microelectrode	dry	8.0	54.5	2.4	16.5	
Edge	2013	Exp Physiol, 98, 481-490	F	45-s continuous cycling at 200% VO_{2peak} (pre-training for HIT-1)	7.110	6.870	microelectrode	dry	7.0	69.1	2.1	20.9	
Edge	2013	Exp Physiol, 98, 481-490	F	6 × 2-min HIT at 92% VO_{2peak}, 2:1 work:rest	7.106	6.810	microelectrode	dry	6.6	84.2	2.0	25.5	
Edge	2013	Exp Physiol, 98, 481-490	F	6 × 2-min HIT at 92% VO_{2peak}, 2:3 work:rest	7.101	6.903	microelectrode	dry	7.2	46.9	2.2	14.2	
Edge	2005	Med Sci Sports Exerc, 37, 1975-82	F	5 × 6-s repeated sprints	7.040	6.700	microelectrode	dry	10.4	63.0	3.1	19.0	
Edge	2005	Med Sci Sports Exerc, 37, 1975-82	F	5 × 6-s repeated sprints	7.080	6.740	microelectrode	dry	10.0	56.9	3.0	17.2	
Edge	2005	Med Sci Sports Exerc, 37, 1975-82	F	5 × 6-s repeated sprints (post-training)	7.020	6.710	microelectrode	dry	9.7	70.8	2.9	21.4	
Edge	2005	Med Sci Sports Exerc, 37, 1975-82	F	5 × 6-s repeated sprints (pre-training)	7.050	6.690	microelectrode	dry	8.3	70.8	2.5	21.4	
Edge	2006	J Appl Physiol, 101, 918-25	F	time trial to fatigue at 100% VO_{2max} (BIC)	7.130	6.940	microelectrode	dry	9.4	52.6	2.8	15.9	
Edge	2006	J Appl Physiol, 101, 918-25	F	time trial to fatigue at 100% VO_{2max} (PLA)	7.150	6.890	microelectrode	dry	6.3	47.9	1.9	14.5	
Edge	2006	Med Sci Sports Exerc, 38, 2004-2011	F	60 s at 160% VO_{2peak}	7.022	6.699	microelectrode	dry					
Edge	2006	Med Sci Sports Exerc, 38, 2004-2011	F	60 s at 160% VO_{2peak}	7.022	6.662	microelectrode	dry					
Forbes	2005	J Appl Physiol, 99, 1668-75	M	wrist flexion × 9 min moderate intensity + 9 min heavy intensity (BIC)	7.090	6.610	^{31}P-MRS	forearm					
Forbes	2005	J Appl Physiol, 99, 1668-75	M	wrist flexion × 9 min moderate intensity + 9 min heavy intensity (control)	7.060	6.540	^{31}P-MRS	forearm					
Gaitanos	1993	J Appl Physiol, 75, 712-9	M	1 x 6 s sprint					3.8	28.6	1.1	8.6	
Gaitanos	1993	J Appl Physiol, 75, 712-9	M	10 x 6 s sprints						112.3		34.0	
Gore	2001	Acta Physiol Scand, 173, 275-86	M	2 min at 105% VO_{2peak} (post LHTL)	7.172	6.854	microelectrode	dry	6.0	40.1	1.8	12.1	
Gore	2001	Acta Physiol Scand, 173, 275-86	M	2 min at 105% VO_{2peak} (pre LHTL)	7.148	6.805	microelectrode	dry	5.9	44.5	1.8	13.5	
Graham	1990	Am J Physiol Endocrinol Metab, 259, E170-6	M	leg extension at 139% VO_{2max}	7.131	6.676	microelectrode	dry					

									Lactate (dm)		Lactate (mM)	
Reference	**Year**		**Sex**	**Exercise Bout**	**Muscle pH**		**measurement technique**	**sample**	**mmol.kg dm^{-1}**		**mmol.L muscle water^{-1}**	
					pre-ex	**post-ex**			**pre-ex**	**post-ex**	**pre-ex**	**post-ex**
Greenhaff	1988	Eur J Appl Physiol Occup Physiol, 57, 531-9	M	3 min at VO_{2max} (high carbohydrate diet)	6.980	6.890	blood gas analyser	wet	5.5	104.4	1.7	31.6
Greenhaff	1988	Eur J Appl Physiol Occup Physiol, 57, 531-9	M	3 min at VO_{2max} (low carbohydrate diet)	6.980	6.760	blood gas analyser	wet	4.4	125.3	1.3	37.9
Gross	2014	Eur J Appl Physiol, 114, 221-34	M	90-s cycling at 110% W_{peak}	7.030	6.770	microelectrode	dry				
Gross	2014	Eur J Appl Physiol, 114, 221-34	M	90-s cycling at 110% W_{peak}	7.070	6.890	microelectrode	dry				
Gunnarsson	2013	Am J Physiol Regul Integr Comp Physiol, 305, R811-21	M	2-min cycling at 90% VO_{2peak} (EX1)	7.130	6.900	microelectrode	dry	2.9	48.8	0.9	14.8
Gunnarsson	2013	Am J Physiol Regul Integr Comp Physiol, 305, R811-21	M	2.5 min after EX1, cycling at 90% VO_{2peak} to fatigue (EX2)		6.950	microelectrode	dry	38.8	58.6	11.7	17.7
Hargreaves	1998	J Appl Physiol, 84, 1687-91	M	2 × 30-s sprints	7.159	6.652	microelectrode	dry	4.8	104.7	1.5	31.7
Harmer	2000	J Appl Physiol, 89, 1793-803	M	sprint to exhaustion at 130% VO_{2max} (post-training)	7.205	6.712	microelectrode	dry	3.8	95.8		
Harmer	2000	J Appl Physiol, 89, 1793-803	M	sprint to exhaustion at 130% VO_{2max} (pre-training)	7.195	6.627	microelectrode	dry	6.2	112.5		
Hermansen	1972	J Appl Physiol, 32, 304-8	F+M	2-min continuous exercise to exhaustion	6.980	6.460	microelectrode	wet				
Hermansen	1972	J Appl Physiol, 32, 304-8	F+M	5 × (40–60 s)	6.920	6.410	microelectrode	wet				
Hogan	1999	J Appl Physiol, 86, 1367-1373	F+M	incremental (ramp) plantar flexion to fatigue	7.030	6.900	^{31}P-MRS	plantar flexors				
Hollidge-Horvat	1999	Am J Physiol Endocrinol Metab, 277, E647-58	M	15 min at 30% VO_{2max}	7.210	7.040	pH=−0.00413(lac+pyr)+7.06	dry		1.7		0.5
Hollidge-Horvat	1999	Am J Physiol Endocrinol Metab, 277, E647-58	M	15 min at 60% VO_{2max}		6.910	pH=−0.00413(lac+pyr)+7.06	dry		5.9		1.8
Hollidge-Horvat	1999	Am J Physiol Endocrinol Metab, 277, E647-58	M	15 min at 75% VO_{2max}		6.820	pH=−0.00413(lac+pyr)+7.06	dry		8.9		2.7
Hollidge-Horvat	2000	Am J Physiol Endocrinol Metab, 278, E316-29	M	15 min at 30% VO_{2max}	7.197	7.036	pH=−0.00413(lac+pyr)+7.06	dry		1.3		0.4
Hollidge-Horvat	2000	Am J Physiol Endocrinol Metab, 278, E316-29	M	15 min at 60% VO_{2max}	7.197	6.947	pH=−0.00413(lac+pyr)+7.06	dry		5.2		1.6
Hollidge-Horvat	2000	Am J Physiol Endocrinol Metab, 278, E316-29	M	15 min at 75% VO_{2max}	7.197	6.795	pH=−0.00413(lac+pyr)+7.06	dry		8.4		2.5
Hood	1988	Am J Physiol Renal Physiol, 255, F479-85	M	2-min exhaustive forearm exercise with occlusion	7.060	6.310	^{31}P-MRS	finger flexor				
Hultman	1985	Clin Sci (Lond), 69, 505-10	F+M	75-s electrical stimulation of quadriceps at 50% MVIC	7.040	6.700	microelectrode	wet				
Hultman	1985	Clin Sci (Lond), 69, 505-10	F+M	75-s electrical stimulation of quadriceps at 50% MVIC (after NH_4Cl supplementation)	6.980	6.540	microelectrode	wet				
Iaia	2011	J Appl Physiol, 110, 1555-1563	M	2-h cycle to exhaustion	7.230	7.180	microelectrode	dry	3.9	9.7	1.2	2.9
Iaia	2011	J Appl Physiol, 110, 1555-1563	M	3-min cycle to exhaustion	7.180	6.760	microelectrode	dry	6.5	82.8	2.0	25.0
Iaia	2011	J Appl Physiol, 110, 1555-1563	M	30-s cycle to exhaustion	7.140	6.900	microelectrode	dry	4.6	59.7	1.4	18.0
Iaia	2008	Am J Physiol Regul Integr Comp Physiol, 294, R966-74	M	130% VO_{2max} run to exhaustion (EX1)	7.215	6.987	microelectrode	dry	8.7	31.4	2.6	9.5
Iaia	2008	Am J Physiol Regul Integr Comp Physiol, 294, R966-74	M	2 min after EX1, 130% VO_{2max} run to exhaustion (EX2)		6.936	microelectrode	dry	29.6	38.1	8.9	11.5
Jones	2007	Am J Physiol Regul Integr Comp Physiol, 293, R392-R401	M	incremental	7.040	6.950	^{31}P-MRS	quadriceps				
Jubrias	2003	J Physiol, 553, 589-99	F+M	7-min constant exercise	7.000	7.000	^{31}P-MRS	leg				
Jubrias	2003	J Physiol, 553, 589-99	F+M	7-min constant exercise	7.050	6.920	^{31}P-MRS	hand				
Jubrias	2003	J Physiol, 553, 589-99	F+M	24-s maximal exercise	7.000	6.880	^{31}P-MRS	leg				
Jubrias	2003	J Physiol, 553, 589-99	F+M	24-s maximal exercise	7.100	6.900	^{31}P-MRS	hand				
Juel	1990	Acta Physiol Scand, 140, 147-59	M	one-legged knee extension to exhaustion	7.140	6.710	microelectrode	dry		120.4		36.4
Juel	2004	Am J Physiol Endocrinol Metab, 286, E245-51	M	one-legged knee extension to exhaustion		6.690	microelectrode	dry		96.5		29.2
Kemp	2001	J Physiol, 535, 901-28	M	3-min ischaemic finger flexion at 7% MVIC	6.999	6.500	^{31}P-MRS	finger flexor				
Kemp	1997	Eur J Appl Physiol Occup Physiol, 76, 462-71	F+M	plantar-flexion to exhaustion	7.025	6.730	^{31}P-MRS	plantar flexors				
Kent-Braun	1993	J Appl Physiol, 75, 573-80	F+M	repeated 4-s contractions at increasing % of MVIC	7.070	6.770	^{31}P-MRS	tibialis anterior				
Kent-Braun	1999	Eur J Appl Physiol Occup Physiol, 80, 57-63	F+M	4-min MVIC	7.010	6.490	^{31}P-MRS	tibialis anterior				
Kowalchuk	1988	J Appl Physiol, 65, 2080-2089	M	rest	6.880		SID	wet				
Kowalchuk	1988	J Appl Physiol, 65, 2080-2089	M	30-s maximal isokinetic cycling +0.5 min		6.480	SID	wet				
Kowalchuk	1988	J Appl Physiol, 65, 2080-2089	M	30-s maximal isokinetic cycling +3.5 min		6.380	SID	wet				
Kowalchuk	1988	J Appl Physiol, 65, 2080-2089	M	30-s maximal isokinetic cycling +9.5 min		6.460	SID	wet				

										Lactate (dm)		Lactate (mM)	
Reference	**Year**		**Sex**	**Exercise Bout**	**Muscle pH**		**measurement technique**	**sample**		**mmol.kg dm^{-1}**		**mmol.L muscle water^{-1}**	
					pre-ex	**post-ex**				**pre-ex**	**post-ex**	**pre-ex**	**post-ex**
Linossier	1997	Eur J Appl Physiol Occup Physiol, 76, 48-54	F+M	120% VO_{2peak} to exhaustion	7.160	6.510	microelectrode	dry		4.5	107.7	1.4	32.6
Mannion	1993	J Appl Physiol, 75, 1412-8	F+M	60% quadriceps MVIC to fatigue	7.190	6.840	microelectrode	dry		6.2	64.7	1.9	19.6
Mannion	1995	Exp Physiol, 80, 89-101	F+M	60% quadriceps MVIC to fatigue	7.170	6.890	microelectrode	dry		6.5	99.6	2.0	30.1
Mannion	1993	J Appl Physiol, 75, 1412-8	F+M	sprint-cycling to fatigue (end @ 0 rpm)	7.200	6.730	microelectrode	dry		6.7	84.6	2.0	25.6
Mannion	1995	Exp Physiol, 80, 89-101	F+M	sprint-cycling to fatigue (end @ 0 rpm)	7.170	6.680	microelectrode	dry		6.5	58.0	2.0	17.5
Mendez-Villanueva	2012	Plos One, 7(12)	M	10 × 6-s sprints	6.950	6.750	microelectrode	dry		12.2	108.4	3.7	32.8
Messonier	2007	J Appl Physiol, 102, 1936-44	F+M	120% VO_{2max} to exhaustion	7.160	6.490	microelectrode	dry		4.5	101.7	1.4	30.7
Miller	1988	J Clin Invest, 81, 1190-6	Not stated	11 × 5-min intermittent contractions (6min^{-1}) @ 75% MVC	7.080	6.550	^{31}P-MRS	adductor pollicis					
Miller	1988	J Clin Invest, 81, 1190-6	Not stated	4-min sustained MVIC	7.080	6.580	^{31}P-MRS	adductor pollicis					
Mohr	2007	Am J Physiol Regul Integr Comp Physiol, 292, R1594-602	M	15 × 6-s sprints		7.060	microelectrode	dry			30.0		9.1
Mohr	2007	Am J Physiol Regul Integr Comp Physiol, 292, R1594-602	M	Yo-Yo IR2	6.940	6.820	microelectrode	dry		4.8	47.4	1.5	14.3
Mohr	2007	Am J Physiol Regul Integr Comp Physiol, 292, R1594-602	M	Yo-Yo IR2	7.020	6.790	microelectrode	dry		5.1	45.3	1.5	13.7
Mohr	2007	Am J Physiol Regul Integr Comp Physiol, 292, R1594-602	M	8 × 30-s sprints		6.980	microelectrode	dry			47.1		14.2
Nakagawa	2002	J Physiol Anthropol Appl Human Sci, 21, 129-31	Not stated	60-s isometric dorsiflexion at 50% MVIC	6.990	6.750	^{31}P-MRS	dorsiflexors					
Nevill	1989	J Appl Physiol, 67, 2376-82	F+M	2-min run at 110% VO_{2max}	7.030	6.920	microelectrode	wet		3.7	28.2	1.1	8.5
Nevill	1989	J Appl Physiol, 67, 2376-82	F+M	30-s treadmill sprint	7.020	6.780	microelectrode	wet		4.1	86.0	1.2	26.0
Perry	2008	Appl Physiol Nutr Metab, 33, 1112-23	F+M	5-min cycling at 90% VO_{2max} (post-training)		6.600	estimated	dry		8.5	98.0	2.6	29.6
Perry	2008	Appl Physiol Nutr Metab, 33, 1112-23	F+M	cycling at 90% VO_{2max} to exhaustion (post-training)	6.950	6.600	estimated	dry		8.5	100.4	2.6	30.4
Perry	2008	Appl Physiol Nutr Metab, 33, 1112-23	F+M	5-min cycling at 90% VO_{2max} (pre-training)		6.520	estimated	dry		6.5	118.8	2.0	35.9
Perry	2008	Appl Physiol Nutr Metab, 33, 1112-23	F+M	cycling at 90% VO_{2max} to exhaustion (pre-training)	6.950	6.490	estimated	dry		6.5	125.4	2.0	37.9
Pesta	2013	Int J Sports Med, 34, 669-675	M	single-leg knee extension at 80% peak power	7.040	7.020	^{31}P-MRS	VL					
Pesta	2013	Int J Sports Med, 34, 669-675	M	single-leg knee extension at 80% peak power (sprinters)	7.030	6.980	^{31}P-MRS	VL					
Pesta	2013	Int J Sports Med, 34, 669-675	M	single-leg knee extension at 80% peak power (untrained)	7.090	6.950	^{31}P-MRS	VL					
Putman	1995	Am J Physiol, 269, E458-68	M	2 × 30-s maximal isokinetic cycling	7.183		pH=−0.00521(lac+pyr)+7.22	dry		6.6	130.8	2.0	39.5
Putman	1995	Am J Physiol, 269, E458-68	M	2 × 30-s maximal isokinetic cycling		6.556	pH=−0.00413(lac+pyr)+7.06	dry					
Raymer	2004	J Appl Physiol, 96, 2050-6	M	wrist flexion to fatigue (control)	7.100	6.350	^{31}P-MRS	forearm					
Raymer	2004	J Appl Physiol, 96, 2050-6	M	wrist flexion to fatigue (BIC)		6.410	^{31}P-MRS	forearm					
Roussel	2003	J Appl Physiol, 94, 1145-52	Not stated	6-min high frequency (0.47 Hz) finger flexion	7.010	6.550	^{31}P-MRS	finger flexor					
Roussel	2003	J Appl Physiol, 94, 1145-52	Not stated	6-min low frequency (0.85 Hz) finger flexion	7.020	6.870	^{31}P-MRS	finger flexor					
Sahlin	1978	J Appl Physiol, 45, 474-80	M	10–11 min exhaustive cycling at W_{peak}	7.000	6.400	H-H eqn	wet					
Sahlin	1976	Pflügers Arch, 367, 143-9	F+M	6–11 min exhaustive cycling at W_{max}	7.080	6.600	microelectrode	wet					
Sahlin	1984	Acta Physiol Scand, 122, 331-9	M	isometric single-leg knee extension at 60% MVIC to fatigue	7.120	6.610	microelectrode	wet					
Sahlin	1984	Acta Physiol Scand, 122, 331-9	M	isometric single-leg knee extension at 60% MVIC to fatigue	7.100	6.800	microelectrode	wet					
Sahlin	1975	Biochem J, 152, 173-80	M	1-min MVIC to fatigue	7.090	6.560	blood gas analyser	wet					
Sahlin	1977	Clin Sci Mol Med, 53, 459-66	F+M	none	6.950		H-H eqn	wet					
Sairyo	2003	Int J Sports Med, 24, 179-82	M	isokinetic wrist flexion at 60% MVC (followed by 10 min active recovery)	7.000	6.390	^{31}P-MRS	forearm					
Sairyo	2003	Int J Sports Med, 24, 179-82	M	isokinetic wrist flexion at 60% MVC (followed by 10 min passive recovery)	7.000	6.380	^{31}P-MRS	forearm					
Sharp	1986	Int J Sports Med, 7, 13-7	M	graded-exercise test	7.100	6.650	unclear	wet		4.3	92.1	1.3	27.8
Sharp	1986	Int J Sports Med, 7, 13-7	M	graded-exercise test	7.100	6.910	unclear	wet		4.3	48.0	1.3	14.5

									Lactate (dm)		Lactate (mM)	
Reference	**Year**		**Sex**	**Exercise Bout**	**Muscle pH**		**measurement technique**	**sample**	**mmol.kg dm^{-1}**		**mmol.L muscle water^{-1}**	
					pre-ex	**post-ex**			**pre-ex**	**post-ex**	**pre-ex**	**post-ex**
Sinoway	1992	J Clin Invest, 89, 1875-1884	Not stated	2-min handgrip at 30% MVIC		6.990	^{31}P-MRS	forearm				
Sinoway	1992	J Clin Invest, 89, 1875-1884	Not stated	2-min handgrip at 30% MVIC		6.890	^{31}P-MRS	forearm				
Sjogaard	1985	Am J Physiol Regul Integr Comp Physiol, 248, R190-6	M	single-leg knee extension at 100% leg VO_{2max} to exhaustion	7.170	6.800	microelectrode	wet				
Spencer	2008	Eur J Appl Physiol, 103, 545-552	M	6 × 4-s sprints, 24-s 20 W active recovery	7.020	6.870	microelectrode	dry				
Spencer	2008	Eur J Appl Physiol, 103, 545-552	M	6 × 4-s sprints, 24-s 60 W active recovery	7.020	6.810	microelectrode	dry				
Spriet	1986	J Appl Physiol, 61, 1949-54	F+M	intermittent electrical stimulation at 1.6 s × 52, 1.6 s rest (w/o phosphagen hydrolysis)	7.116	6.554	microelectrode	wet				
Spriet	1986	J Appl Physiol, 61, 1949-54	F+M	intermittent electrical stimulation at 1.6 s × 52, 1.6 s rest (with phosphagen hydrolysis)	7.108	6.587	microelectrode	wet				
Spriet	1987	J Appl Physiol, 62, 616-21	M	intermittent electrical stimulation with occluded circulation at 1.6 s × 52, 1.6 s rest	7.000	6.430	microelectrode	wet				
Spriet	1989	J Appl Physiol, 66, 8-13	F+M	3 × 30 s maximal isokinetic cycling	n/a	6.500	microelectrode	wet				
Stephens	2002	Med Sci Sports Exerc, 34, 614-21	M	30 min at 77% VO_{2peak} (BIC)	7.250	7.090	microelectrode	dry	5.4	43.0	1.6	13.0
Stephens	2002	Med Sci Sports Exerc, 34, 614-21	M	30 min at 77% VO_{2peak} (placebo)	7.180	7.030	microelectrode	dry	4.8	40.0	1.5	12.1
Stepto	2001	Med Sci Sport Exer, 33, 303-310	M	8 × 5 min at 83% W_{peak}, 60 s rest	7.090	7.010	blood gas	dry	6.2	32.7	1.9	9.9
Sullivan	1994	J Appl Physiol, 77, 2194-200	M	isometric single-leg knee extension at 30-60% MVIC to fatigue	7.140	6.770	microelectrode	dry				
Sullivan	1994	J Appl Physiol, 77, 2194-200	M	isometric single-leg knee extension at 30-60% MVIC to fatigue	7.140	6.820	^{31}P-MRS	knee extensors				
Trump	1996	J Appl Physiol, 80, 1574-80	M	3 × 30-s maximal isokinetic cycling (3rd bout occluded), 4-min rest		6.542	microelectrode	dry				
Trump	1996	J Appl Physiol, 80, 1574-80	M	3 × 30-s maximal isokinetic cycling, 4-min rest		6.664	microelectrode	dry				
Trump	1996	J Appl Physiol, 80, 1574-80	M	no exercise		6.550	microelectrode	wet				
Trump	1996	J Appl Physiol, 80, 1574-80	M	no exercise		6.520	microelectrode	dry				
Vanhatalo	2010	Exp Physiol, 95, 528-540	M	single-leg knee extension to predicted 10 min exhaustion		6.700	^{31}P-MRS	knee extensors				
Vanhatalo	2010	Exp Physiol, 95, 528-540	M	single-leg knee extension to predicted 2 min exhaustion		6.630	^{31}P-MRS	knee extensors				
Wilson	1988	J Appl Physiol, 64, 2333-9	M	4 min × 1-s wrist flexion, 4-s rest (24 deg•s^{-1})	6.990	6.170	^{31}P-MRS	forearm				
Wilson	1988	J Appl Physiol, 64, 2333-9	M	4 min × 2-s wrist flexion, 3-s rest (12 deg•s^{-1})	7.000	5.860	^{31}P-MRS	forearm				
Yoshida	1993	Eur J Appl Physiol Occup Physiol, 66, 494-9	M	4 × 2-min hip flexion (suggested to be Type I Fibres)		6.600	^{31}P-MRS	biceps femoris				
Yoshida	1993	Eur J Appl Physiol Occup Physiol, 66, 494-9	M	4 × 2-min hip flexion (suggested to be Type II Fibres)		6.200	^{31}P-MRS	biceps femoris				
Zoladz	2010	J Physiol Sci, 60, 331-341	M	single-leg plantar flexion at 50% MVIC to fatigue (participants with [PCR] overshoot)	7.040	6.570	^{31}P-MRS	calf				
Zoladz	2010	J Physiol Sci, 60, 331-341	M	single-leg plantar flexion at 50% MVIC to fatigue (participants without [PCR] overshoot)	7.070	6.780	^{31}P-MRS	calf				
				Overall Mean (SD)	7.07 (0.08)	6.73 (0.20)			7.1 (4.0)	65.4 (34.7)	2.2 (1.2)	19.5 (10.3)
				Mean of wet muscle (SD)	7.04 (0.07)	6.66 (0.19)						
				Mean of dry muscle (SD)	7.09 (0.09)	6.79 (0.15)						
				Mean of ^{31}P-MRS (SD)	7.04 (0.04)	6.69 (0.26)						

Table G.1 Muscle pH and lactate concentration before and after different modalities of exercise. Techniques for measuring muscle pH are listed for wet or freeze-dried muscle. Lactate concentrations are given per dry mass of muscle, as is typically reported, and are converted to mass per Litre of muscle water using a conversion factor of 0.3 (229), to allow comparison with cell culture studies. Abbreviations: BIC (sodium bicarbonate supplementation), dm (dry mass, mmol•kg dm^{-1}), F (female), gastroc (gastrocnemius), GXT (graded-exercise test), H-H (Henderson–Hasselbalch equation), HIT (high-intensity interval training), Hz (Hertz), lac (lactate), LHTL (live high train low), M (male), mM (millmolar), MVIC (maximum voluntary isometric contraction), n/a (not available), NH_4Cl (ammonium chloride), OBLA (onset of blood lactate accumulation), PCr (phosphocreatine), ^{31}P-MRS (phosphorous-31 magnetic resonance spectroscopy), post-ex (post-exercise), pre-ex (pre-exercise), pyr (pyruvate), SID (strong ion difference), SD (standard deviation), VL (*vastus lateralis*), $VO_{2max/peak}$ (maximal/peak oxygen uptake), W (watt), $W_{max/peak}$ (maximal/peak aerobic power), w/o (without), Yo-Yo IR2 (Yo-Yo intermittent recovery test 2)

Appendix H Training-induced changes in muscle buffer capacity

									$\beta m_{in\ vitro}$		$\beta m_{in\ vivo}$	
First Author	**Year**	**Reference**	**Training Type**	**Sex**	**Training modality**	**Rest (s)**	**d• wk^{-1}**	**Duration**	**Pre**	**Post**	**Pre**	**Post**
Baguet	2011	Eur J Appl Physiol, 111, 2571-80	SIT-mixed diet	MF	1–2 × 30 s sprint cycling or 1–2 200 m run	20 min	2–3	5 wk	106.2	102.7		
			SIT-vegetarian	MF	1–2 × 30 s sprint cycling or 1–2 200 m run				93.3	97.1		
Bell	1988	Eur J Appl Physiol Occup Physiol, 58, 158-64	one-legged SIT	MF	15–20 × 20 s at 150% leg VO_{2max}	60 s	4	7 wk	214.6	248.5		
			control leg	MF	no training					223.2		
Bishop	2008	Am J Physiol Regul Integr Comp Physiol, 295, R1991-8	HIT	F	6–12 × 120 s at 100% VO_{2max}	60 s	3	5 wk	145.7	129.6		
Bishop	2009	Pflügers Arch, 458, 929-36	HIT	M	5–8 × 120 s at 100% VO_{2max}	120 s	3	5 wk	139.0	152.0		
Christensen	2000	ECSS Jyväskylä, Finland	HIT	M	Maintained same distance, increased intensity by 30%			4 wk	210.0	211.0		
			HIT	M	Maintained same distance, increased intensity by 60%			4 wk	239.0	297.0		
			Control	M	Maintained normal training intensity				247.0	259.0		
Edge	2006a	PhD thesis	HIT-1	F	6–10 × 120 s at 91–110% VO_{2peak}	60 s	3	5 wk	143.2	127.6		
			HIT-3	F	6–10 × 120 s at 91–110% VO_{2peak}	180 s		5 wk	130.2	137.7		
Edge	2006b	Eur J Appl Physiol, 96, 97-105	HIT	F	2–10 × 120 s at 120-140% LT	60 s	3	5 wk	123.0	153.0		
			Continuous	F	work-matched continuous at 80-90% LT			5 wk	130.0	133.0		
Edge	2006c	J Appl Physiol, 101, 918-25	HIT-bicarb	F	6–12 × 120 s at 91–110% VO_{2peak}	60 s	3	8 wk	145.0	172.6		
			HIT-placebo	F	6–12 × 120 s at 91–110% VO_{2peak}				155.0	169.0		
Edge	2006e	Med Sci Sports Exerc, 38, 2004-2011	Resistance	F	2–5 sets × 15–20 reps resistance training	20 s	n/a	5 wk	132.0	135.0		
			Control	F	no training				128.0	120.0		
Gibala	2006	J Physiol, 575, 901-11	HIT	M	4–6 × 30-s sprints		3	2 wk	170.0	182.9		
			Continuous	M	90–120 min continuous at 65% VO_{2peak}			2 wk	170.0	177.1		
Gross	2013	Eur J Appl Physiol, 114, 221-34	HIT-β-alanine	M	4 × 240 s at 90-95% HR_{max}	180 s	9 sessions	11 d	180.0	184.0		
			HIT-placebo	M	4 × 240 s at 90-95% HR_{max}	180 s	9 sessions	11 d	184.0	186.0		
Gunnarsson	2013	Am J Physiol Regul Integr Comp Physiol, 305, R811-21	Mixed HIT	n/a	10–12 × 30s at 85–95% PPO, 4–5 × 3–4 min at 90–95% W_{peak}	270 s	2–3	7 wk			195.0	227.0
Harmer	2000	Appl Physiol, 89, 1793-803	SIT	M	4–10 × 30-s sprints	180–240 s	3	7 wk	156.6	157.0	171.1	185.5
Iaia	2008	Am J Physiol Regul Integr Comp Physiol, 294, R966-74	HIT	M	4–12 × 30-s at 95% V_{max}	180 s	3–4	4 wk	184.0	178.0	96.5	88.1
Mannion	1994	Eur J Appl Physiol Occup Physiol, 68, 356-61	Fast isokinetic	MF	25 × 6 knee extensions at 4.19 rad•s^{-1}	30 s	3	16 wk	158.8	160.4		
			Slow isokinetic	MF	15 × 5 knee extensions at 1.05 rad•s^{-1}	40 s	3	16 wk	154.4	164.7		
			Control	MF	no training				159.8	157.3		
Nevill	1989	J Appl Physiol, 67, 2376-82	HIT	MF	2 d of 2 × 30-s, 1 d of 6–10 × 6-s, 1 d of 2–5 × 2 min	300–600 s	3–4	7 wk	223.1	235.0	290.1	418.1
			Control	MF					210.5	214.2	458.0	373.9
Pilegaard	1999	Am J Physiol Endocrinol Metab, 276, E255-61	One leg knee extension	M	3–5 sets of 2 × 30-s and 3 × 60-s knee extensions	120 s	3–5	8 wk	197.4			
			control leg	M	no training				193.1			
Sharp	1986	Int J Sports Med, 7, 13-7	HIT	M	8 × 30 s	240 s	4	8 wk			147.4	201.4
Weston	1997	Eur J Appl Physiol Occup Physiol, 75, 7-13	HIT	M	6–8 × 5 min W_{peak}	60 s	6 total	4 wk	206.6	240.4		
								Mean	167.6	176.0	226.4	249.0
								SD	39.0	48.6	130.3	124.0

Table H.1 Training induced changes in muscle buffer capacity measured by titration ($\beta m_{in\ vitro}$) or by $\Delta[La^-]/\Delta pH$ ($\beta m_{in\ vivo}$). Abbreviations: bicarb (sodium bicarbonate supplementation), F (female), HIT (high-intensity interval training), HR_{max} (maximum heart rate), LT (lactate threshold), M (male), PPO (peak power output), reps (repetitions), SD (standard deviation), SIT (sprint-interval training), V_{max} (maximum speed), $VO_{2max/peak}$ (maximal/peak oxygen uptake), W_{peak} (peak aerobic power)

Appendix I Comparison of muscle buffer capacity measured in cross-sectional and longitudinal studies

					$\beta m_{in\ vitro}$		$\beta m_{in\ vivo}$		%
First Author	**Year**	**Reference**	**Intervention / Participants**	**Sex**	**Pre**	**Post**	**Pre**	**Post**	**diff**
Baguet	2011	Eur J Appl Physiol, 111, 2571-80	SIT-mixed diet	MF	106	103			
			SIT-vegetarian	MF	93	97			
Bangsbo	1993	Int J Sports Med, 14, 207-213	Elite Team Sport (VL)	M	219				
			Elite Runners (gastrocnemius)	M	219				
			Elite Sprint Cyclists (gastrocnemius)	M	213				
Basset	2014	Int J Sports Med, 35, 1084-1089	Elite skiiers/skaters (VL)	MF	187				
Bell	1988	Eur J Appl Physiol Occup Physiol, 58, 158-64	SIT	MF	215	249			
			Control	MF		223			
Bishop	2004a	Med Sci Sports Exerc, 36, 807-13	Active (placebo)	F	180		145		-19%
			Active (bicarbonate)	F	180		260		44%
Bishop	2004b	Eur J Appl Physiol, 92, 540-7	Untrained	F	153		191		25%
Bishop	2006	Eur J Appl Physiol, 97, 373-9	Team Sport (trained)	F	148		238		61%
			Team Sport (untrained)	F	148		230		56%
Bishop	2007	J Appl Physiol, 102, 616-21	Team Sport	F	135				
Bishop	2008	Am J Physiol Regul Integr Comp Physiol, 295, R1991-8	HIT	F	146	130			
Bishop	2009	Pflügers Arch, 458, 929-36	Team Sport	M	139	152			
Bishop	2009	Pflügers Arch, 458, 929-36	Endurance	M	146				
Christensen	2000	ECSS Jyväskylä, Finland	HIT	M	210	211			
			HIT	M	239	297			
			Control	M	247	259			
Clark	2004	J Appl Physiol, 96, 517-25	LHTL-continuous	M	145	148			
			LHTL-intermittent	M	141	146			
			Control	M	150	151			
Duffield	2007	J Sci Med Sport, 10, 127-134	Active	F	170				
Edge	2006a	PhD thesis	HIT-1	F	143	128			
			HIT-3	F	130	138			
Edge	2006b	Eur J Appl Physiol, 96, 97-105	HIT	F	123	153			
			Continuous	F	130	133			
Edge	2006c	J Appl Physiol, 101, 918-25	HIT-bicarbonate	F	145	173			
			HIT-placebo	F	155	169			
Edge	2006d	Eur J Appl Physiol, 96, 225-34	Team Sport	F	181				
			Endurance	F	148				
			Untrained	F	122				
Edge	2006e	Med Sci Sports Exerc, 38, 2004-2011	Resistance	F	132	135			
			Control	F	128	120			
Gibala	2006	J Physiol, 575, 901-11	HIT	M	170	183			
			Continuous	M	170	177			
Gore	2001	Acta Physiol Scand, 173, 275-86	LHTL	M	140	165	106	105	-25%
			Control	M	145	146	115	137	-20%
Gross	2013	Eur J Appl Physiol, 114, 221-34	HIT β-alanine	M	180	184			
			HIT placebo	M	184	186			
Gunnarsson	2013	Am J Physiol Regul Integr Comp Physiol, 305, R811-21	Mixed HIT	n/a			195	227	
Harmer	2000	Appl Physiol, 89, 1793-803	SIT	M	157	157	171	186	9%
Harris	1990	Comp Biochem Physiol A Comp Physiol, 97, 249-51	Unknown	M	133				
Harris	1998	J Sport Sci, 16, 639-643	Type I fibres	M	129				
			Type II fibres	M	136				
Hultman	1985	Clin Sci (Lond), 69, 505-10	Placebo	MF			226		
			Ammonium chloride	MF			180		
Iaia	2008	Am J Physiol Regul Integr Comp Physiol, 294, R966-74	HIT	M	184	178	97	88	-48%
Juel	1990	Acta Physiol Scand, 140, 147-59	Healthy	M	211		268		27%
Kemp	2001	J Physiol, 535, 901-28	^{31}P-MRS	M			172		
Linossier	1997	Eur J Appl Physiol Occup Physiol, 76, 48-54	Placebo	MF	142				
			Sodium citrate	MF	144				
Mannion	1993	J Appl Physiol, 75, 1412-8	Active	MF	157		168		7%
			Active	MF	156		170		9%
Mannion	1994	Eur J Appl Physiol Occup Physiol, 68, 356-61	Fast isokinetic	MF	159	160			
			Slow isokinetic	MF	154	165			
			Control	MF	160	157			
Mannion	1995	Exp Physiol, 80, 89-101	Active	MF	159		184		16%
Mizuno	1990	J Appl Physiol, 68, 496-502	LHTH (gastrocnemius)	M	298	316			
			LHTH (triceps)	M	344	365			
Nevill	1989	J Appl Physiol, 67, 2376-82	HIT	MF	223	235	290	418	30%
			Control	MF	211	214	458	374	118%
Nordsborg	2012	J Appl Physiol, 112, 2027-36	LHTL	MF	140	140			
			Placebo		145	140			
Pilegaard	1999	Am J Physiol Endocrinol Metab, 276, E255-61	One leg knee extension	M	197				
			Control leg	M	193				

					$\beta m_{in\ vitro}$		$\beta m_{in\ vivo}$		%
First Author	**Year**	**Reference**	**Intervention / Participants**	**Sex**	**Pre**	**Post**	**Pre**	**Post**	**diff**
Sahlin	1976	Pflügers Arch, 367, 143-9	Active	MF			241		
Sahlin	1984	Acta Physiol Scand, 122, 331-9	Elite Team Sport	M			194		
			Sedentary	M			164		
Saltin	1995	Scand J Med Sci Sports, 5, 222-230	LHTH Scandinavian	MF	342	358			
			LLTL Scandinavian	MF	346	344			
			LHTH Kenyan	MF	292				
Sharp	1986	Int J Sports Med, 7, 13-7	HIT	M			147	201	
			Endurance	M			156		
Spriet	1987	J Appl Physiol, 62, 616-21	Active	M			256		
Weston	1997	Eur J Appl Physiol Occup Physiol, 75, 7-13	HIT	M	207	240			
			Mean		175	188	201	217	19%
			SD		54	68	74	121	41%

Table I.1 Muscle buffer capacity in cross-sectional and longitudinal studies measured by titration ($\beta m_{in\ vitro}$) or by $\Delta[La^-]/\Delta pH$ ($\beta m_{in\ vivo}$). Where both techniques have been performed in the same study, the difference between the two techniques is given in the rightmost column (% diff). Abbreviations: F (female), HIT (high-intensity interval training), LHTH (live high train high), LHTL (live high train low), LLTL (live low train low), M (male), ^{31}P-MRS (phosphorous-31 magnetic resonance spectroscopy), SD (standard deviation), SIT (sprint-interval training), VL (*vastus lateralis*)

Appendix J Rate of evaporative water loss during muscle pH measurement

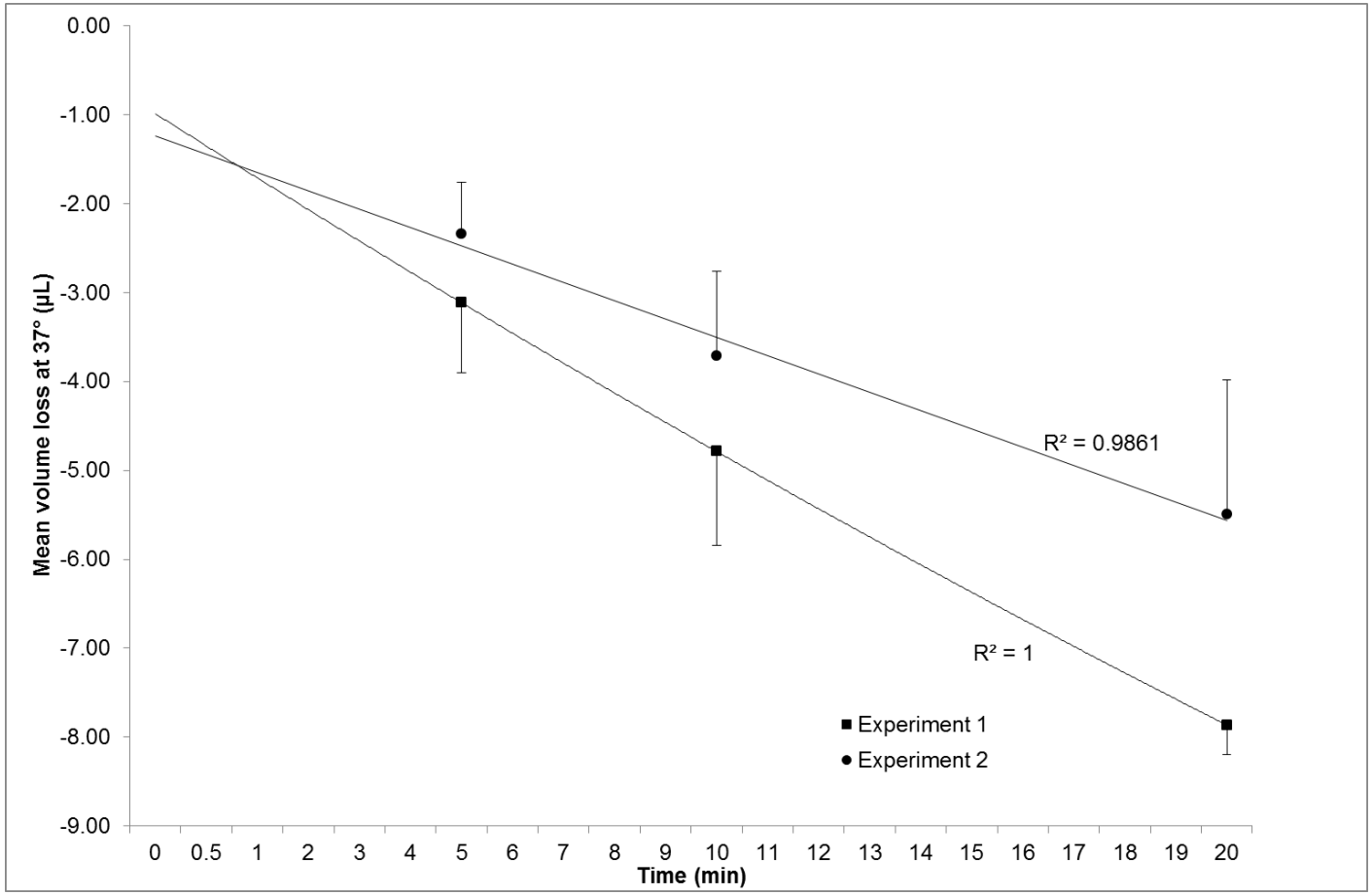


Figure J.1 Mean (SD) volume of water loss in muscle homogenates maintained at 37°C in a water bath during pH measurement. Time is from the first insertion of the microelectrode, after allowing 5 min for the samples to equilibrate to temperature. Two experiments were conducted using 4 or 5 aliquots prepared from a single muscle sample. Samples were weighed after 5, 10, and 20 minutes.

Appendix K Cumulative error in $\beta m_{in\ vitro}$ calculation resulting from error in muscle dry mass measurement

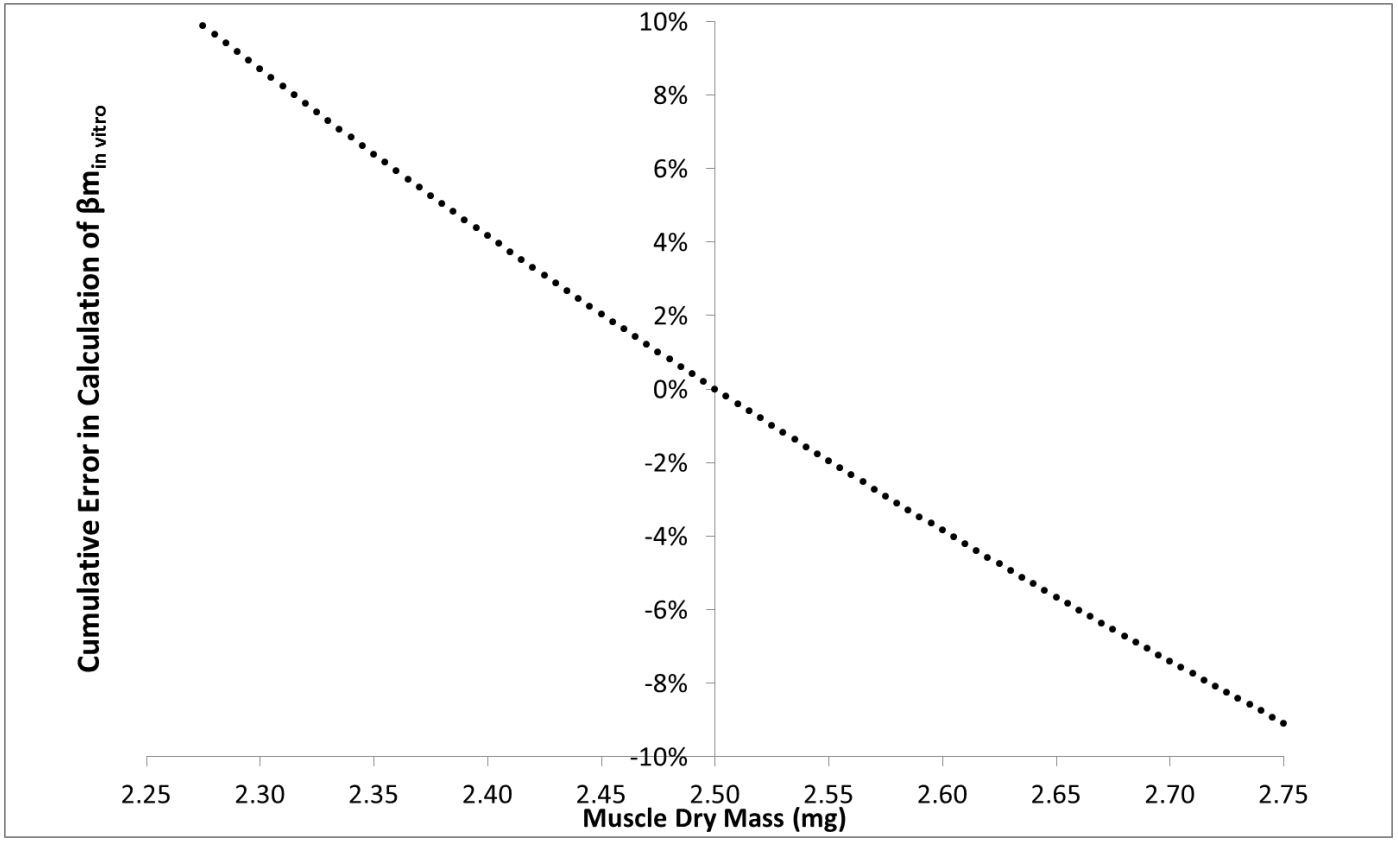


Figure K.1 Cumulative error in βm calculation owing to error in muscle mass measurement, using 2.50 mg as the reference mass. For example, incorrectly recording the mass of a 2.50 mg sample as 2.55 mg results in a 2% underestimation of $\beta m_{in\ vitro}$.

Appendix L Protein Sequence Alignments

L-1 Amino acid sequence alignments of NBCe1-B and NBCe2-C

Example sequence alignment of the two electrogenic NBC isoforms, NBCe1 (SLC4A4) and NBCe2 (SLC4A5), performed using EMBOSS Needle software (385). This process was used to illustrate percentage identity and similarity between the NCBT isoforms for Table 2.1 of the Literature Review. These scores are highlighted below in yellow. Gene names are abbreviated: S4A4 (SLC4A4), S4A5 (SLC4A5).

```
########################################
# Program: needle
# Rundate: Tue 21 Apr 2015 09:12:22
# Commandline: needle
#    -auto
#    -stdout
#    -asequence emboss_needle-I20150421-091220-0049-86899706-
oy.asequence
#    -bsequence emboss_needle-I20150421-091220-0049-86899706-
oy.bsequence
#    -datafile EBLOSUM62
#    -gapopen 10.0
#    -gapextend 0.5
#    -endopen 10.0
#    -endextend 0.5
#    -aformat3 pair
#    -sprotein1
#    -sprotein2
# Align_format: pair
# Report_file: stdout
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: S4A4_HUMAN
# 2: S4A5_HUMAN
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1160
# Identity:      680/1160 (58.6%)
# Similarity:    838/1160 (72.2%)
# Gaps:          120/1160 (10.3%)
# Score: 3439.5
#
#
#=======================================

S4A4_HUMAN         1 ---MEDEAVLDRGASFLKHV------CDEEEVEGHHTIYIGVHVPKSYRR     41
                       .|::|    |...|.|.      .|::|..   .|:||:.|| :|.:
S4A5_HUMAN         1 MKVKEEKA----GVGKLDHTNHRRRFPDQKECP---PIHIGLPVP-TYPQ     42

S4A4_HUMAN        42 RRRHKRKTGHKEKKEK-------ERISENYSDKSDIENADESSSSILKPL     84
                     |:..::  ||....:|       ::.:.:.....::.:||..::...
S4A5_HUMAN        43 RKTDQK--GHLSGLQKVHWGLRPDQPQQELTGPGSGASSQDSSMDLISRT     90

S4A4_HUMAN        85 ISPAAERIRFILGEEDDSPAPPQLFTELDELLAVDGQEMEWKETARWIKF    134
                     .|||||:::.|||||::| .|.||||:| .|..||.:|||||:||||||
S4A5_HUMAN        91 RSPAAEQLQDILGEEDEAP-NPTLFTEMD-TLQHDGDQMEWKESARWIKF    138
```

```
S4A4_HUMAN      135 EEKVEQGGERWSKPHVATLSLHSLFELRTCMEKGSIMLDREASSLPQLVE   184
                    |||||:||||||||||:||||||||||||||::.|:::||.::.||||:::
S4A5_HUMAN      139 EEKVEEGGERWSKPHVSTLSLHSLFELRTCLQTGTVLLDLDSGSLPQIID   188

S4A4_HUMAN      185 MIVDHQIETGLLKPELKDKVTYTLLRKHRHQTKKSNLRSLADIGKTVSSA   234
                    .::::.|||.|||:|||:::|:|.|||:|||||||...||||||||:||:.
S4A5_HUMAN      189 DVIEKQIEDGLLRPELRERVSYVLLRRHRHQTKKPIHRSLADIGKSVSTT   238

S4A4_HUMAN      235 SRMFTNPDNGSPAMTHR----------------NLTSSSLNDISDKPEKD   268
                    :|.........|::.|.                :..|.|:||||..|..|
S4A5_HUMAN      239 NRSPARSPGAGPSLHHSTEDLRMRQSANYGRLCHAQSRSMNDISLTPNTD   288

S4A4_HUMAN      269 QLKNKFMKKLPRDAEASNVLVGEVDFLDTPFIAFVRLQQAVMLGALTEVP   318
                    |.|||||||:|:|:|||||||||||||.||||||||.|:.|||.:||||
S4A5_HUMAN      289 QRKNKFMKKIPKDSEASNVLVGEVDFLDQPFIAFVRLIQSAMLGGVTEVP   338

S4A4_HUMAN      319 VPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKAKDRHDLIA   368
                    ||||||||||||.|:||||:||||||||||.|::|.|:||||::|.||||
S4A5_HUMAN      339 VPTRFLFILLGPSGRAKSYNEIGRAIATLMVDDLFSDVAYKARNREDLIA   388

S4A4_HUMAN      369 GIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMNGD   418
                    ||||||||||||||||||.|||||||.:|||:||||:::|..|..||||.
S4A5_HUMAN      389 GIDEFLDEVIVLPPGEWDPNIRIEPPKKVPSADKRKSVFSLAELGQMNGS   438

S4A4_HUMAN      419 T-----PHDGGHGGGGHGDC---------------------------EEL   436
                    .     ...||:||||.|..                           |||
S4A5_HUMAN      439 VGGGGGAPGGGNGGGGGGGSGGGAGSGGAGGTSSGDDGEMPAMHEIGEEL   488

S4A4_HUMAN      437 QRTGRFCGGLIKDIKRKAPFFASDFYDALNIQALSAILFIYLATVTNAIT   486
                    ..||||.|||..|||||.|:|.|||||..:||::||||||||..:|||||
S4A5_HUMAN      489 IWTGRFFGGLCLDIKRKLPWFPSDFYDGFHIQSISAILFIYLGCITNAIT   538

S4A4_HUMAN      487 FGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAGQPLTILSSTGPVLVFE   536
                    ||||||||||.|||:||||||||::|::||||:||||.|||||||:|:||
S4A5_HUMAN      539 FGGLLGDATDNYQGVMESFLGTAMAGSLFCLFSGQPLIILSSTGPILIFE   588

S4A4_HUMAN      537 RLLFNFSKDNNFDYLEFRLWIGLWSAFLCLILVATDASFLVQYFTRFTEE   586
                    :|||:|||.|..||:||||||||.||..||||||||||:::|.||||||
S4A5_HUMAN      589 KLLFDFSKGNGLDYMEFRLWIGLHSAVQCLILVATDASFIIKYITRFTEE   638

S4A4_HUMAN      587 GFSSLISFIFIYDAFKKMIKLADYYPINSNFKVGYNTLFSCTCVPPDPAN   636
                    |||:||||||||||.||||....|||||.:|||..:.|.:.|.||.||..|
S4A5_HUMAN      639 GFSTLISFIFIYDAIKKMIGAFKYYPINMDFKPNFITTYKCECVAPDTVN   688

S4A4_HUMAN      637 ISISN-DTTLAPEYLPTMSSTDMYHNTTFDWAFLSKKECSKYGGNLVGNN   685
                    .::.| ...|||:  ...|..::.:.|..||:.||||||..|||.|:|||:
S4A5_HUMAN      689 TTVFNASAPLAPD--TNASLYNLLNLTALDWSLLSKKECLSYGGRLLGNS   736

S4A4_HUMAN      686 CNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFPTTARKLISDFAIILS   735
                    |.|:||:.|||||||.|||:.::.||||||.|.||||..|.|::||:|:.|
S4A5_HUMAN      737 CKFIPDLALMSFILFFGTYSMTLTLKKFKFSRYFPTKVRALVADFSIVFS   786

S4A4_HUMAN      736 ILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWVCLAAA   785
                    ||:||.|||..|::|||||.|||..|||.|:||||||.|||:||||||..|:.
S4A5_HUMAN      787 ILMFCGIDACFGLETPKLHVPSVIKPTRPDRGWFVAPFGKNPWWVYPASI   836

S4A4_HUMAN      786 IPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVICSL   835
                    :|||||||||||||||||||||:||||.||||||||||.|||.:||.
S4A5_HUMAN      837 LPALLVTILIFMDQQITAVIVNRKENKLKKAAGYHLDLFWVGILMALCSF   886

S4A4_HUMAN      836 MALPWYVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVFI   885
                    |.||||||||||||||||||||||||||||:||||||||||.:|||
S4A5_HUMAN      887 MGLPWYVAATVISIAHIDSLKMETETSAPGEQPQFLGVREQRVTGIIVFI   936

S4A4_HUMAN      886 LTGLSVFMAPILKFIPMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKH   935
                    |||:|||:|||||.||:||||||||||||||||:||.:|.||.|||.||
S4A5_HUMAN      937 LTGISVFLAPILKCIPLPVLYGVFLYMGVASLNGIQFWERCKLFLMPAKH   986

S4A4_HUMAN      936 QPDFIYLRHVPLRRVHLFTFLQVLCLALLWILKSTVAAIIFPVMILALVA   985
```

```
                    |||..:||||||||:||||.:|:||||:||||||||||||||||||.|:.
S4A5_HUMAN       987 QPDHAFLRHVPLRRIHLFTLVQILCLAVLWILKSTVAAIIFPVMILGLII   1036

S4A4_HUMAN       986 VRKGMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKKKKKKGSLDSDNDDSD   1035
                     ||:.:|::|||||||:::|:::|||:  |||.:||:|:|||:      ..|
S4A5_HUMAN      1037 VRRLLDFIFSQHDLAWIDNILPEKE--KKETDKKRKRKKGA------HED   1078

S4A4_HUMAN      1036 CPYSEKVPS---IKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC---   1079
                     |....:.|.   |||||:.::..|                 :....|
S4A5_HUMAN      1079 CDEEPQFPPPSVIKIPMESVQSDP-----------------QNGIHCIAR   1111

S4A4_HUMAN      1080 ----------   1079

S4A5_HUMAN      1112 KRSSSWSYSL   1121


#---------------------------------------
#---------------------------------------
```

L-2 Sequence alignment of the NBCe1-A 54aa epitope with other putative human muscle sodium-coupled bicarbonate transporters

Sequence analysed using Jalview 2.8.2b1 (488)

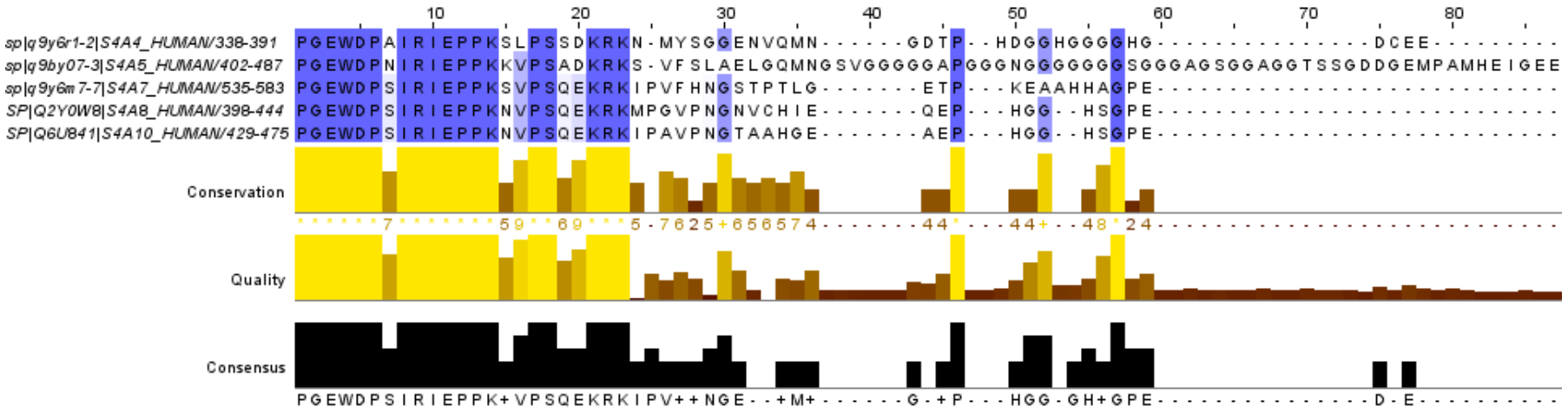


Figure L.1 Sequence alignment of the electrogenic sodium/bicarbonate cotransporter NBCe1-A (isoform 1, splice variant A) with each of the human muscle sodium-coupled bicarbonate transporters (NCBTs) for which transcriptional evidence exists. Alignment is performed for the NBCe1-A 54 amino acid (aa, 338–391) N-terminus epitope against which the Merck antibody (AB3212) – used to identify NBC(e1) in human skeletal muscle to date (129, 252, 271) – was raised. The NBCe1-A (isoform 2) splice variant was the first electrogenic NBC isoform cloned (395), and is predominantly found in the kidney, whereas NBCe1-B (isoform 1) is the probable skeletal muscle variant. Nevertheless, the 54aa epitope is shared completely among all five NBCe1 splice variants (360). The sequence similarity (blue) illustrates the isoform non-specificity of this antibody. The gene names are abbreviated, e.g., S4A4 = SLC4A4. Corresponding protein names: S4A4 (NBCe1-A), S4A5 (NBCe2-C), S4A7 (NBCn1-D), S4A8 (NDCBE-A), S4A10 (NBCn2-A).

Appendix M Buffering capacity of carnosine calculated from the Henderson–Hasselbalch equation

Physicochemical buffers comprise a weak acid (HA) and its conjugate base (A^-). Knowing the total muscle content ([A] = [HA] + [A^-]) and the pK_a of a substance, its relative contribution to total physicochemical buffering can be estimated from the Henderson–Hasselbalch equation (H–H). This method was used to produce the graphs in section 2.7 of Chapter 2.

H–H equation: $pH = pK_a + \log \frac{[A^-]}{[HA]}$

Using carnosine as an example, with a pK_a of 6.83, and assuming a carnosine content [A] of 25 mmol•kg dm^{-1} (typical reported values for active men range from 20–30 mmol•kg dm^{-1}):

For buffering between pH 7.1 and 6.5:

1) Solving H–H at pH 7.1: $7.1 = 6.83 - \log \frac{25 - [HA]}{[HA]}$

$$\therefore 10^{(7.1-6.83)} = \frac{25}{[HA]} - 1$$

$$\therefore [HA] = \frac{25}{1+10^{0.27}} = 8.7 \text{ mmol•kg } dm^{-1}$$

2) Similarly, solving H–H at pH 6.5: [HA] = 17.0 mmol•kg dm^{-1}

$\therefore$ 17.0 – 8.7 = 8.3 mmol•kg dm^{-1} (change in amount of acidic form of carnosine)

i.e., $\beta m_{carnosine}$ = 8.3 mmol H^+•kg dm^{-1} between pH 7.1 and 6.5, for a total carnosine concentration of 25 mmol•kg dm^{-1}

Assuming total physicochemical βm = 140 mmol H^+•kg dm^{-1}

$\therefore$ $\beta m_{carnosine}$ = 5.9% of βm_{total}