Limiting factors to high intensity exercise: the role of intramuscular pH and skeletal muscle buffering.

by

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A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of the University of Salford, Department of Biological Sciences.

July 1990
Statement of responsibility.

I hereby certify that I am responsible for the work submitted in this thesis, that the original work is my own except as specified, and that neither the thesis nor the original work contained therein, has been submitted to this or any other institution for a higher degree.

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University of Salford
1990
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List of abbreviations

ADP  Adenosine diphosphate
AMP  Adenosine monophosphate
ATP  Adenosine triphosphate
ATPase  Adenosine triphosphatase
B  Buffer value
Bvit  "In vitro" buffer value - determined by HCl titration of a muscle homogenate.
Bviv  "In vivo" buffer value - determined by $\Delta\ [\text{La}] / \Delta \text{pH}$
Bvivl  Bviv determined following isometric exercise
BvivD  Bviv determined following dynamic exercise
Ca$^{2+}$  Calcium ion
CAR  Carnosine
C.MPO  Corrected mean power output
C.PPO  Corrected peak power output
C.Tpp  Time to corrected peak power output
Cr  Creatine
CV (%)  Coefficient of variation
dm  Dry mass
END  60% MVC endurance time to fatigue
H$^+$  Hydrogen ion
H$_2$PO$_4$$^-$  Diprotonated phosphate ion
HIE  High intensity exercise
HPO$_4^{2-}$  Monoprotonated phosphate ion
IMP  Inosine monophosphate
IMP (performance)  Impulse: time integral of force production
K$^+$  Potassium ion
Km  Michaelis constant: the concentration of substrate at which a given enzyme yields one half its maximum velocity
La  Lactic acid (or lactate)
mWAnT  Modified Wingate Anaerobic Test
MFA  Mean muscle fibre area (um$^2$)
MVC  Maximum voluntary contraction (N)
Na$^+$  Sodium ion
ns  Non-significant (at the given significance level)
PCr  Phosphocreatine
Pi  Inorganic phosphate
pKa  Acid dissociation constant
REVS  Peak velocity during performance of WAnT
SD     Standard deviation
SR     Sarcoplasmic reticulum
TIME   Test duration during performance of mWAnT
Tn     Troponin
U.MPO  Uncorrected mean power output (W)
U.PPO  Uncorrected peak power output (W)
U.Tpp  Time to uncorrected peak power output (s)
Vmax (muscle contraction)  Maximum contraction velocity
Vmax (enzyme kinetics)    Maximum enzyme activity
WAnT   Wingate Anaerobic Test
WORK   Work done during dynamic HI exercise test (J)
x      Mean value
[S]    Concentration of substance 'S'
Δ      Delta; 'change in'

Conversion factors for different reference bases

1 mmol.kg wet weight⁻¹(ww) = 4.3 mmol.kg dry weight⁻¹
  = 1.3 mmol.l muscle water⁻¹

1 mmol.l muscle water⁻¹ = 1.11 mmol.l intracellular water⁻¹
  = 0.77 mmol.kg wet weight⁻¹ = 0.3 mmol.kg dry weight⁻¹

(from Hultman and Sahlin, 1980.)
Acknowledgements

I would like to express my thanks to a number of individuals from a number of institutions, without whose assistance, completion of this work would not have been possible.

The majority of the research was carried out at Crewe and Alsager College and first and foremost, I express my deep gratitude to my college advisor, Dr. Phil. Jakeman, for his unrelenting support, enthusiasm, guidance and generosity throughout the duration of my studies. Even in the final stages, at a time when his own career had somewhat changed direction (geographically and otherwise), he managed to devote countless hours and immeasurable commitment to ensuring the successful completion of this manuscript.

From Crewe and Alsager College, I am grateful to June Flanigan, for her technical assistance, friendship and encouragement, and to all the science technicians, and library and resources staff, for managing to comply with my many, last-minute requests. My fellow research workers, past and present, I thank for their interaction and companionship; particularly Russell Best for his stimulating statistical discussions and Roger Palfreeman, for his invaluable assistance in the post-training performance assessments. Finally, I am most obliged to the numerous individuals who participated in the research, who through their cheerfulness, hard work and motivation, made extracting their maximal physical efforts, and portions of their quadriceps, an absolute pleasure!

From Manchester University, I am indebted to Dr. Peter Willan for his time, and admirable skills, both clinical and personal, in successfully obtaining a total of 188 muscle biopsy samples. I am also grateful to the technical staff at the Department of Cell and Structural Biology, for their instruction in the techniques of muscle histochemical analysis.

I would like to thank Dr. Roger Harris for his kind invitation to use the facilities at the Animal Health Trust (Newmarket), and Mark Dunnett, for his enlightening introduction to HPLC and assistance in executing the muscle carnosine analyses.

I extend my gratitude to Dr. Rose Baker, at the Computer Advisory Centre, University of Salford, for her patience in expounding the benefits of the "Statistical Package for the Social Sciences", and to Dr. David Davies (Dept. Biological Sciences), for taking on the role of project supervisor at such short notice.

I would like to thank the Sports Council, and the Department of Sport and Human Sciences (Crewe and Alsager College), for their joint funding of this work.

Finally, although by no means least, I am deeply grateful to my mother who has consistently supported and encouraged me in this venture, and above all, to Gordon, whose forbearance has been unyielding.
To my mother and the memory of my father.
Abstract

Within the context of a metabolic model of fatigue, formulated from a review of the literature, a decrease in intramuscular pH was identified as a potential limiting factor to the performance of high intensity exercise (HIE). This suggested a role for skeletal muscle buffering ($B$) in retarding the intramuscular acidosis typically incurred during HIE. Previous studies on human muscle, were largely unable to confirm this role, partly due to the lack of a clearly defined and consistently utilised method of measurement of $B$. The validity of the procedures currently employed in the determination of $B$ was therefore investigated, revealing that many of the individual differences in $B$, previously attributed physiological significance, may have originated from inappropriate methodology.

In a cross-sectional examination of the vastus lateralis muscle of young, active individuals, low correlations ($p<0.05$) between the muscle carnosine concentration ([CAR]) and in the 'in vitro $B$' ($B_{vit}$) ($r=0.30$), and between either [CAR] or $B_{vit}$ and the % type II fibre area were observed. An elevated $B_{vit}$ was shown to be of importance in minimising the decrement in pH during HIE, but did not per se permit the accumulation of a higher muscle lactate concentration, or allow for an enhanced HIE performance; these factors were more dependent on the % type II fibre area. The muscle pH, following dynamic HIE was highly variable between individuals, and showed a significant ($p<0.05$) negative correlation with the % type II fibre area.

16 weeks of isokinetic training of the quadriceps resulted in significant improvements ($p<0.05$) in dynamic HIE performance with no concomitant improvements in [CAR] or $B_{vit}$.

The data suggest that the muscle buffer value per se, is not a major limiting factor to HIE, and that rather, the glycolytic capacity and pH tolerance of the muscle may be the more important factors to consider.
Chapter 1
1. REVIEW OF LITERATURE AND AIMS OF STUDY

1.1 Introduction

The production of force in a muscle represents the culmination of a sequence of preceding physiological events (Fig. 1.1; Edwards, 1983). For a muscle to be able to realize its full potential for force generation, each of these processes must be capable of maximally activating subsequent processes; failure at any level will consequently result in only submaximal activation and fatigue will ensue. Fatigue, is herein defined as "the inability of a physiological process to continue functioning at a particular level and/or the inability of the total organism to maintain a predetermined exercise intensity" (Knuttgen et al, 1983). It is unlikely that fatigue occurs as a result of suppression of any singular process; its multifactorial nature has been acknowledged throughout the many years of its investigation. Moreover, the duration, intensity and type of exercise, the predominantly recruited muscle fibre type, the environmental conditions and the motivation and training status of the individual involved, can all combine to render one or more factors limiting to continued performance.

Nonetheless, this should not preclude detailed examination of single, putative agents of fatigue; indeed, it should provide more of an impetus to identify and examine the relative contribution of each of the proposed factors and establish their specific site(s) of action. Ultimately then, it may become possible to discern the elements of paramount importance in the process of fatigue and as such, develop suitable programmes for adaptation, in an attempt to moderate their incapacitating effects.

The concept of "central failure", involving failure of any of the events preceding depolarisation of the sarcolemma (steps 1 - 3, Fig. 1.1) may certainly occur in some circumstances during voluntary contraction (Edwards, 1983). However, studies using direct stimulation of the muscle, and data from peeled fibre experiments, demonstrate that substantial fatigue can also result from failure of peripheral events in the chain of command (steps 4-9; Fig. 1.1). For the purposes of this review, it is these events, occurring distal to the neuromuscular junction, which will be considered, in an attempt to elucidate some of the possible peripheral (and specifically, metabolic) limitations to high intensity exercise.
Fig. 1.1 Chain of command for muscular contraction

(adapted from Edwards, 1983)
The term "high intensity exercise" will include exercise intensities which fulfil at least two of three criteria, devised to encompass both dynamic and isometric exercise. The criteria stipulate that to be defined as high intensity, the exercise level must 1) lead to fatigue within approximately 1 minute, 2) rely predominantly on the utilisation of anaerobic metabolic pathways for the provision of energy, 3) exceed that of maximal oxygen uptake. Some of the studies cited from the literature employ exercise/electrical stimulation modes which do not necessarily satisfy these criteria, but will be referred to in context if they yield information relevant to the review.

A more detailed description of what is currently known of the mechanisms involved in excitation-contraction coupling and the generation of tension will firstly be given, in order that reference to this sequence of events may later be made in discussing the sites commonly implicated in muscle fatigue.

1.2 Excitation-contraction coupling and the generation of tension

The resting vertebrate skeletal muscle fibre is polarised with a potential difference of approximately -90 mV across the sarcolemma from the myoplasm to the extracellular fluid. The resting membrane potential is maintained by the action of the Na⁺/K⁺ pump, in an energy-dependent process that is controlled by the catalytic activity of the enzyme, Na⁺/K⁺ ATPase (Guyton, 1984). Depolarization of the sarcolemma, by means of a propagated action potential, is the only *in vivo* physiological stimulus for contraction; it leads to activation by the release of Ca²⁺ from the sarcoplasmic reticulum (SR) (Donaldson, 1989) (see schema of Fig. 1.2).

Skeletal muscle sarcoplasmic reticulum is a membranous compartment, closed to the cytosol, that exists in close proximity and parallel to the individual myofibrils. At the level of the A-I region in mammalian muscle, triadic junctions are formed between two lateral sacs of the SR (the terminal cisternae (TC)) and one transverse tubule (TT). Transverse tubules are specialised invaginations of the sarcolemma running perpendicular to the long axis of the muscle fibre.

The action potential is communicated from the sarcolemma via the transverse tubules to the sarcoplasmic reticulum. The precise mode of this communication remains to be elucidated. The transverse tubules and sarcoplasmic reticulum are structurally discontinuous, but they are linked in some manner by dense material
1) Development of action potential at motor end-plate
2) Action potential generated at sarcolemma
3) Coupling of excitation between t-tubule and sarcoplasmic reticulum
4) Ca^{2+} release from sarcoplasmic reticulum
5) Ca^{2+} binding to troponin-C
6) Execution of cross-bridge cycle
7) Ca^{2+} reaccumulation by sarcoplasmic reticulum

Fig. 1.2 Peripheral events in muscle contraction
(Adapted from Green, 1987).
that appears as bridging structures or "feet" (Klug and Tibbits, 1988). There is
evidence that "voltage sensors" which behave as membranous dipoles and appear to
control SR Ca\(^{2+}\) release, are present in the transverse tubule membrane (Peachey et
al, 1983). The density of these dipole molecules is similar to that of binding sites
for dihydropyridines, a class of calcium-antagonist drugs, which immobilise the
dipoles and SR Ca\(^{2+}\) release in parallel (Rios and Brum, 1987). The "feet"
structures have been shown to be the binding site for ryanodine, a plant alkaloid
known to hold the SR Ca\(^{2+}\) channel in an open state (Inui et al, 1987), and the
ryanodine receptors have indeed been shown to be functional Ca\(^{2+}\) channels
(Imagawa et al, 1987). It has been suggested that connection between the dipoles of
the TT voltage sensor and the "feet" of the terminal cisternae, by either electrical,
mechanical, or chemical mechanisms, may be the link between the TT depolarisation
and SR Ca\(^{2+}\) release (Klug and Tibbits, 1988). Current evidence supports a
combination of mechanical and chemical mechanisms of communication, with the
implicated chemical being inositol triphosphate (InsP\(_3\)) (formed by the enzymatically
catalysed decomposition of the membrane phospholipid, phosphatidylinositol-4,5-
bisphosphate) (Donaldson, 1989).

Calcium is released from its binding sites in the lumen of the terminal cisternae
(Klitgaard et al, 1989), approximately 2 ms after propagation of the action potential
down the transverse tubules (Klug and Tibbits, 1988). The release of calcium into
the cytosol increases its intracellular concentration from \(10^{-7}\) M, to values that may
approach \(10^{-5}\) M, upon which calcium binds to troponin-C (TnC). TnC is the Ca\(^{2+}\)-
binding subunit of the troponin molecule (Tn) which also contains the subunits TnT
(the tropomyosin-binding subunit) and TnI (the actomyosin ATP-ase inhibiting
subunit). The Tn molecule is attached to tropomyosin, a coiled helical molecule
spanning seven actin monomers along each of two helical "grooves" of the F-actin
double-strand filament. A popular view concerning the interactions of these proteins
suggests that at low Ca\(^{2+}\) concentrations (< \(10^{-7}\) M) the regulatory proteins of the
thin filament, Tm and Tn, allow the muscle to relax by physically preventing the
interaction between myosin heads and actin (Ebashi, 1980). Following Ca\(^{2+}\)
binding to Tn-C, a conformational change in the thin filament occurs, resulting in
disinhibition of this interaction. The steric blocking of actin-myosin interaction has
long been a working hypothesis in the study of the regulation of skeletal muscle
contraction. However, recent evidence suggests that this may not be the only
mechanism for the regulation of muscle contraction, and that kinetic constraints on actin-myosin interaction influenced by the binding of ATP and the intermediates of ATP hydrolysis may also have a governing effect (El-Saleh et al, 1986). The exact details of regulation remain controversial (Brenner, 1987).

Development of tension occurs upon the cyclic interaction between myosin and actin. The energy for tension development is made available by the actin-activated hydrolysis of Mg²⁺ATP at the myosin head. Studies on the kinetics of the contractile proteins in solution have suggested the following basic cycle for the actomyosin-ATPase in the intact filament array:

\[
\text{A} \rightarrow \text{M.T} \rightarrow \text{AM} \rightarrow \text{AMD} \rightarrow \text{AMD.P}
\]

where A represents actin; M, myosin; T, ATP; D, ADP and P, phosphate (Pate and Cooke, 1989b).

A cross-bridge with bound hydrolysis products is assumed to attach weakly to actin at the beginning of the power stroke (stages 2 to 3). Due to relative sliding between the parallel actin and myosin filaments, the free energy of the attached crossbridge decreases during the powerstroke with the actomyosin bond becoming tighter as the crossbridge continues to produce force (stages 3 to 5). At some point in the powerstroke, the hydrolysis products are sequentially released. At the end of the powerstroke, when continued attachment of the crossbridge would resist useful work, Mg²⁺ATP binding results in cross-bridge detachment (stages 5 to 1) (Pate and Cooke, 1989b).

Relaxation, following the cessation of stimulation, involves reuptake of Ca²⁺ by the sarcoplasmic reticulum by the action of a calcium pump protein present on the SR membrane that is driven by ATP hydrolysis (Ca²⁺ transport ATPase) (see Fig. 1.3) (Klug and Tibbits, 1988). 1 mol of Mg²⁺ATP and 2 mols of Ca²⁺ bind to the appropriate sites of the ATPase on the cytoplasmic side of the SR membrane.
Fig. 1.3  Schematic representation of the mechanism of Ca$^{2+}$ uptake by the sarcoplasmic reticulum
(from Klug and Tibbits, 1988)
Occupation of these sites is followed by the formation of a phosphorylated intermediate of the enzyme, and subsequently ADP is released into the medium. This phosphorylation occurs only in the presence of Ca\(^{2+}\) and is obligatory for the completion of Ca\(^{2+}\) transport. Phosphorylation is followed by a conformational change in the phosphorylated enzyme complex and the ATPase is transported through the membrane. The binding sites are no longer accessible to the extravesicular medium and a dramatic reduction in their affinity for Ca\(^{2+}\) occurs. As such Ca\(^{2+}\) is released into the intravesicular space. Release of Ca\(^{2+}\) is inhibited by increases in intravesicular Ca\(^{2+}\), which inhibit the entire process of Ca\(^{2+}\) transport. Calsequestrin, a low-affinity but high-capacity calcium-binding protein that is localised in the terminal cisternae (Klitgaard et al., 1989), may play a significant role in maintaining the necessary low Ca\(^{2+}\) levels. In isolated SR, the absence of calsequestrin causes inhibition of Ca\(^{2+}\) uptake after only a few cycles of the pump (Klug and Tibbits, 1988).

Another calcium-binding protein, parvalbumin, is believed to be of importance in the process of relaxation. Parvalbumin (PA) is a soluble protein found in high concentration in the myoplasm of many skeletal muscles (Cannell and Allen, 1984). PA contains two high-affinity Ca\(^{2+}\) binding sites which also competitively bind Mg\(^{2+}\) but with a lower affinity (Klug and Tibbits, 1988). It is suggested that PA takes up calcium in parallel with the SR and hence influences the time course of the decline in intracellular [Ca\(^{2+}\)] during relaxation (Pechere et al., 1977). However, its role as a soluble relaxing factor has been questioned on the basis that the time frame of a twitch is insufficient to allow appreciable Ca\(^{2+}\)/Mg\(^{2+}\) exchange on the PA binding site (Mg\(^{2+}\) is preferentially bound during the low [Ca\(^{2+}\)] found in resting muscle) (Robertson et al., 1982). During a tetanic contraction of duration greater than approximately 2s, it is likely that the parvalbumin sites become fully loaded with Ca\(^{2+}\) (Cannell, 1986) and as such, are unable to further bind Ca\(^{2+}\) upon termination of the tetanic contraction. Therefore, PA could not assist as a calcium buffer, during relaxation. Instead, it would tend to delay the decrease in [Ca\(^{2+}\)]\(i\), by offloading its bound calcium into the cytosol. Nonetheless, the PA content of a muscle does appear to be highly correlated to its half-relaxation time (Klug and Tibbits, 1988).
1.3 Potential sites and mechanisms of peripheral fatigue

1.3.1 Energy supply

Within the contraction-relaxation cycle there are at least three major processes which are coupled to the hydrolysis of ATP: operation of the Na\(^+\)/K\(^+\) pump, Ca\(^{2+}\) reuptake into the sarcoplasmic reticulum and cross-bridge cycling. As such, specific ATPases with different levels of catalytic activity have evolved in the sarcolemma (Na\(^+\)/K\(^+\) ATPase), the sarcoplasmic reticulum (Ca\(^{2+}\)-transport ATPase) and the contractile proteins (actomyosin ATPase) (Kushmerick, 1983). It has been reported that functioning of these processes, respectively account for approximately 10\%, 20-35\% and 65-80\% of the ATP consumed during an isometric tetanus (Homsher, 1987).

A loss of functional integrity in any one of these energy-consuming processes could result from 1) a reduction in either the rate, or free energy, of ATP hydrolysis by the specific ATPase due to alterations in its catalytic activity or 2) a decreased ATP supply, further to a disturbance in the flux rate of specific metabolic pathways.

The following section will deal predominantly with the latter factor, in terms of the pathways of ATP synthesis and substrate availability during the performance of high intensity exercise.

Pathways of ATP synthesis: capacity and power of the energy sources

During high intensity exercise an increase in ATP turnover, by as much as 500-600 fold upon resting levels can be observed (Hultman et al, 1987). Due to the limited availability of stored ATP (16-20 mmol.kg dm\(^{-1}\); Saltin and Gollnick, 1983), the rate of utilisation of ATP must be matched by an identical rate of resynthesis, for continuous muscle contraction. This is accomplished primarily by the hydrolysis of phosphocreatine (PCr) and the degradation of glycogen to lactate.

(i) PCr hydrolysis

PCr rephosphorylates ADP to ATP by means of a near-equilibrium reaction catalysed by creatine phosphokinase (CPK) (the Lohmann reaction):

\[
\text{PCr} + \text{ADP} + n\text{H}^+ \rightleftharpoons \text{ATP} + \text{Cr} \quad \text{CPK}
\]
CPK is found at three strategic locations in the muscle cell: tightly bound to the contractile apparatus at the M line, in the outer membrane of the mitochondria and in the cytoplasm (Kushmerick, 1983). The substrate for the reaction, PCr, exists free in solution within the muscle (Meyer et al, 1982), at a concentration of 60-80 mmol. kg dm$^{-1}$ (Saltin and Gollnick, 1983). The $K_m$ of CPK for PCr is 5 mmol.kg dm$^{-1}$ (Hultman et al, 1987). Given a sufficient concentration of substrate, the activity of CPK is higher than ATPase, consistent with its apparent role as a high-energy buffer serving to maintain adequate ATP levels (Kushmerick, 1983). Only when the PCr content decreases to approximately 60% of resting levels, does [ATP] begin to significantly decrease (Hultman et al, 1987). Complete depletion of the PCr store would produce an equimolar concentration of ATP and as such, would generate 60-80 mmol ATP.kg dm$^{-1}$ (Table 1.1). The maximum power of the reaction, has been calculated as 8-9 mmol ATP kg dm$^{-1}$.s$^{-1}$ (Hultman and Sjoholm, 1983); working at this rate the PCr store would theoretically be depleted within 10 s.

(ii) Glycogen degradation and anaerobic glycolysis

Glycogenolysis

Glycogen degradation to glucose-1-phosphate is catalysed by two enzymes, glycogen phosphorylase and debranching enzyme (Chasiotis, 1983). Phosphorylase, the rate-limiting enzyme for glycogenolysis in muscle, exists in two forms, $a$ and $b$ and undergoes reversible enzymatic phosphorylation mediated by a kinase and a phosphatase (Chasiotis, 1983). During intense exercise, a transformation of phosphorylase $b$ to $a$ is observed, in accordance with contraction-induced increases in the myoplasmic calcium concentration. The calcium released is thought to bind to calmodulin (a calcium binding protein having a primary structure sharing a large homology with troponin C) which exists as one of the sixteen subunits of phosphorylase kinase, causing activation of the kinase (Cheung, 1980). However, an increase in the activity of phosphorylase is observed only if glycogen and phosphate ($P_i$), the substrates for the enzyme, are available in adequate amounts. At rest, and even after high intensity exercise, glycogen levels are well in excess of the $K_m$ (2mM) of phosphorylase for this substrate (Chasiotis, 1988). However, the resting concentration of $P_i$ at the active site of the enzyme, is sufficiently low (approximately 3mM; Dawson et al, 1977) to prevent any significant glycogenolytic activity (the $K_m$ of phosphorylase a for $P_i$, at low [AMP], is 26 mmol.l$^{-1}$; Chasiotis, 1988). Only during muscle contraction, when the hydrolysis of
Table 1.1 Capacity and power of anaerobic pathways for ATP resynthesis.

<table>
<thead>
<tr>
<th>Process</th>
<th>Resting muscle substrate conc* (mmol.kg dm⁻¹)</th>
<th>Available Energy** (mmol ATP.kg dm⁻¹)</th>
<th>Max. Power** (mmol ATP.kg dm⁻¹.s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP → ADP + P_i</td>
<td>16-20</td>
<td>8-10⁴</td>
<td>11.2</td>
</tr>
<tr>
<td>PCr + ADP → Cr + ATP</td>
<td>60-80</td>
<td>60-80⁴</td>
<td>8.6</td>
</tr>
<tr>
<td>CHO + ADP → La+ ATP</td>
<td>200-500</td>
<td>150-1110⁴</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* from Saltin and Gollnick, 1983.
** adapted from Sahlin, 1986a.
⁴ assuming the maximum possible depletion of muscle ATP content = 50% (Tesch et al, 1989).
⁵ assuming complete utilisation of the PCr store.
⁶ variable depending on whether the lactate produced is accumulated within, or free to leave, the muscle.
PCr begins to release $P_i$, can the process of glycogenolysis be initiated. Even so, conditions have also been observed whereby extensive transformation of phosphorylase into the $a$ form in the presence of a high $P_i$ concentration, are accompanied by low glycogenolytic activity, implying that additional (unknown) factors are involved in the regulation of glycogen breakdown (Ren and Hultman, 1989).

Phosphorylase activity produces glucose-1-P which must be converted into glucose-6-P in order to enter the pathway of glycolysis. The isomerisation is catalysed by the enzyme phosphoglucomutase in a near equilibrium reaction (Fig. 1.4).

**Glycolysis**
The process of glycolysis involves the degradation of G-6-P (from either glucose or glycogen) to the 3-carbon compound pyruvate (Fig. 1.4). The reactions of glycolysis occur in the cytosolic compartment of the muscle cell, where it is generally considered that the enzymes involved are predominantly free in solution, and that the transfer of intermediates from one enzyme to the next occurs by diffusion (Newsholme and Leech, 1983). ATP generation occurs during the reactions catalysed by phosphoglycerate kinase and pyruvate kinase. NAD$^+$ is reduced to NADH in the glyceraldehyde-3-phosphate dehydrogenase reaction and it is essential that NADH is rapidly reoxidised to NAD$^+$ for the continuation of glycolysis. Under anaerobic conditions, this is achieved by the reduction of pyruvate to lactate, in a reaction catalysed by lactate dehydrogenase.

Lactate production begins early, following the start of intensive muscle contraction (within 2s; Hultman and Sjoholm, 1983) and with time, demonstrates an increasing contribution to the total energy turnover, as PCr levels begin to decline (Spriet et al, 1987a). The amount of ATP that can be produced through lactate formation depends to a large extent on whether the lactate formed is free to leave the muscle, or accumulates within it (indirectly inhibiting its own formation - see later) (Sahlin, 1986a). Utilisation of the whole glycogen store for lactate formation has the capacity to provide in theory, approximately 1 mol ATP.kg dm$^{-1}$ at a maximal power of 5.2 mmol ATP.kg dm$^{-1}$.s$^{-1}$ (Table 1.1).
Fig. 1.4 The metabolic pathway of glycolysis.

(All enzymes can function physiologically in the reverse direction except those marked with an asterisk.)

From Newsholme and Leech, 1983.
1.3.2 Substrate depletion

Adenosine triphosphate (ATP) depletion

Typically, the decline in mean muscle ATP content elicited by high intensity dynamic exercise (Cheetham et al, 1986; Jacobs et al, 1982, 1983; Jones et al, 1985; McCartney et al, 1986; Jansson et al, 1987), isometric exercise (Miller et al, 1987) or in response to electrical stimulation (Chasiotis et al, 1987; Hultman et al, 1987; Spriet et al, 1987a), never exceeds 50% of resting levels in normal muscle. The lack of an entire depletion of whole muscle ATP levels at exhaustion is frequently interpreted as evidence against ATP availability per se, being the limiting factor to continued muscle contraction (Dawson et al, 1978; Sahlin et al, 1981; Miller et al, 1987). However, the possibility that fatigue develops due to a total depletion of ATP in either individual fibres (as observed by Hultman et al, 1987) or within certain compartments of the fibre, cannot be discounted; neither can the notion that the rate of supply of ATP, secondary to a depletion of the substrates involved in ATP resynthesis, may be limiting to the maintenance of a pre-determined exercise intensity.

Phosphocreatine (PCr) depletion

The PCr store is either depleted or reduced to very low concentrations, following high intensity dynamic or isometric exercise to fatigue (Harris et al, 1977; Karlsson et al, 1975; Miller et al, 1987; Tesch et al, 1989b). Many studies have examined the relationship between the decrease in muscle PCr concentration ([PCr]) and the loss of force during fatigue, and the reverse relationship during recovery (Dawson et al, 1978; Norman et al, 1986; Sahlin et al, 1987; Miller et al, 1987, 1988). Sahlin et al (1987) chemically analysed the PCr content of rat soleus muscle at rest and after 1.5 and 7.0 minutes of electrical stimulation (2Hz). Although the authors concluded that force declined in parallel with the decline in [PCr], close examination of their data reveals that the relationship was non-linear, with a greater reduction in [PCr] per unit decrease in tension during the initial stages of stimulation. The limited number of measurements of muscle [PCr] used in plotting the relationship between tension and [PCr] (three, including rest values), may have obscured a more temporally discrete relationship between these two parameters.
Nuclear magnetic resonance (NMR) studies have proven useful in examining the relationship between changes in [PCr] and force production in vivo, since these two variables can be measured simultaneously over the duration of a fatiguing contraction. Dawson et al (1978) examined the change in isometric force developed in amphibian muscle during electrical stimulation, in relation to changes in [PCr]. Whilst [PCr] did decrease in conjunction with a reduction in force, there was not an obligatory proportionality between these two parameters. The data of Miller et al (1987 and 1988), clearly showed a dissociation between changes in [PCr] and maximum voluntary contraction (MVC) of the adductor pollicis muscle in human subjects; the decline in MVC occurred more slowly than the reduction in [PCr]. The non-linear relationship was observed regardless of whether fatigue was induced by a sustained maximal effort for 4 minutes, or a series of intermittent contractions at 75% MVC for 20 minutes (Miller et al, 1988).

During exercise to fatigue, many biochemical changes occur simultaneously, making it difficult to discern the relative importance of each mechanism to the gradual decline in performance. Examination of the process of recovery however, provides a further insight into the potential mechanisms involved in fatigue, since the return to resting levels of the various metabolites produced or depleted during the exercise, generally follow different time-courses. As such, a better discrimination between the effect on performance of individual processes can be obtained. It has been shown that the restoration of maximal force (MVC) and the muscle [PCr], follow an almost identical time course, which is not shared by other metabolites (e.g. H+, P_{i} or H_{2}PO_{4}^-) (Miller et al, 1988; Sahlin and Ren, 1989).

In contrast to the recovery in performance of single maximal contractions, the time-course for recovery of isometric endurance, does not appear to coincide with the restoration of PCr levels (Sahlin and Ren, 1989). As such, it would seem that the concentration of PCr is not the only factor limiting the potential to maintain a fixed work output.

In summary, the reports obtained during fatigue show that the relationship between the decline in [PCr] and the reduction in force/power output is not likely to be a simple one of cause and effect. However the lack of a direct linear relationship between [PCr] and force does not necessarily negate a role for diminishing PCr levels in the development of fatigue, especially when some of the results for recovery are considered. The involvement may be indirect, or may be intrinsically associated with other processes which act in concert to induce a fatigued state (see later).
Glycogen depletion

During high-intensity exercise, the capacity of the glycolytic system is never fully utilised, and glycogen levels of approximately 70-85% of the resting concentration remain in the muscle at the point of fatigue (Boobis et al., 1982, 1983; Cheetham et al., 1986; Spriet et al., 1989). Additionally, lowered resting intramuscular glycogen levels (from approximately 400 to 150 mmol.kg dm⁻¹) do not impair the subsequent performance of high intensity exercise (Symons and Jacobs, 1989). This is not surprising in view of the low $K_m$ of phosphorylase for glycogen (2 mM; Chasiotis, 1988). Thus, the availability of glycogen per se, is unlikely to be a causative agent in the process of fatigue. The effects of product accumulation and inhibition on the rate of glycogenolysis and glycolysis, will be discussed in the following sections.

1.3.3 Accumulation of metabolites

During the performance of high-intensity exercise, many by-products or end-products of anaerobic metabolism accumulate within the muscle (e.g. lactate, hydrogen ions, inorganic phosphate, ADP, IMP). Numerous in vitro and in vivo studies have been carried out focussing on the importance of individual metabolites in the development of fatigue, and whilst isolated studies have produced convincing evidence for the implication of a particular metabolite, the results are not always compatible with other studies, and cannot always account for the decrement in performance observed under slightly differing experimental conditions. It is possible that either a different cause underlies the fatigue experienced during different types of muscle contraction, or that many factors previously ascribed a direct causal role, in fact only contribute indirectly to the development of fatigue.

1.3.3.1 Accumulation of lactate and hydrogen ions

Possibly one of the earliest candidates implicated in muscle fatigue was lactic acid, which can increase 20 - 30 fold during intense dynamic or isometric exercise (Sahlin, 1983). Lactic acid is almost completely dissociated at physiological pH values, producing equimolar concentrations of hydrogen and lactate ions. An increased lactate concentration during exercise, increases the muscle osmolarity and
results in the accumulation of water within the muscle (Bergstrom et al, 1971). Although the subsequent increase in intramuscular pressure may exert a restricting effect on local circulation (Sahlin, 1983), the lactate ion per se, is not known to have any adverse effects on energy metabolism, or on the contractile process (Sahlin, 1978; Chase and Kushmerick, 1988). Whilst a decrease in force/power production frequently correlates well with an increased muscle lactate concentration (Karlsson et al, 1975; Fitts and Holloszy, 1976; Tesch, 1980), it appears that most of the effects are mediated via the concomitant increase in hydrogen ion concentration (or decrease in pH). Lactic acid contributes more than 85% to the hydrogen ions liberated during intensive exercise (Hultman and Sahlin, 1980). Although this typically results in the release of approximately 30 mmol hydrogen ions (H+) per litre muscle water, the muscle buffers will ordinarily sequester all but 0.001% of the H+ ions released (Hultman and Sahlin, 1980 see later), such that the maximum decrease in pH observed, is only 0.6 units (from pH 7.0 to 6.4; Hermansen and Osnes, 1972).

Even the increased H+ levels encountered following fatiguing exercise (4 x 10^-4 mM), represent a minimal absolute concentration relative to other intracellular univalent ions such as K+ (160 mM) or Na+ (13 mM), but the hydrogen ion has such a high reactivity that the consequences of just modest changes in its concentration are far-reaching (Hultman and Sahlin, 1980). An increase in hydrogen ion concentration has been shown to interfere with the processes of energy metabolism, muscle tension development and relaxation.

(i) pH effects on metabolism

pH and the adenine nucleotides
ATP and ADP are composed of different ionic species involving Mg^{2+}, K+ and H+. Both ATP and ADP have pKa values in the physiological range and as such their ionic composition will be influenced by the prevailing pH. In most enzymatic reactions involving ATP and ADP, the active ionic species are the Mg-complexes, the concentration of which slightly decreases during acidosis, with a concomitant increase in the protonated form of ATP (HATP^3-) (Sahlin, 1978). Therefore, whilst ATP may apparently be available in adequate concentrations during the development of fatigue, it may not exist in the most appropriate form for the reactions in which it is involved. This in itself, would be tantamount to a reduction in energy source.
pH effects on glycogenolysis

In vitro studies reveal that acidosis can influence the rate of glycogenolysis in two ways. Firstly, the activity of the active phosphorylase form, as well as the rate of its transformation into the $a$ form, are decreased when pH decreases (Krebs et al., 1964). Secondly, under acidotic conditions, an increase in the concentration of the diprotonated form of phosphate ($H_2PO_4^-$) is observed, at the expense of the monoprotonated form ($HPO_4^{2-}$); $HPO_4^{2-}$ is believed to be the true substrate for phosphorylase (Kasvinski and Meyer, 1977). With decreasing substrate levels ($HPO_4^{2-}$) and a reduction in the activity of the active phosphorylase enzyme itself, the rate of glycogenolysis might be expected to decrease from maximal levels during the development of acidosis and fatigue.

The influence of muscle pH on glycogenolysis has indirectly been examined in many studies carried out in vivo, employing repeated high-intensity cycling or electrical stimulation of the muscle (McCartney et al., 1986; Spriet et al., 1987b; Spriet et al., 1989). In the study of Spriet et al. (1989) a decrease in work done was observed during three successive bouts of 30s maximal isokinetic cycling with intervening rest periods of 4 minutes (bouts 2 and 3 generated 78.5 and 64.5% of the work in the first bout, respectively). Although no values were reported for the extent of glycogenolysis during the first bout of exercise, glycogen utilisation during bout 3 was only 32 % of that observed in bout 2. The reduced glycogenolysis during bout 3 was explained by a down regulation of glycogen phosphorylase activity by increasing hydrogen ion concentration (Spriet et al., 1989). Attractive though this hypothesis is, it implies that a reduced glycogenolytic activity directly causes a reduction in the maximum work able to be performed. The reverse scenario is equally plausible; other factors negatively influencing the ability of the muscle to contract could cause a reduction in the energy requirements and as such, a reduction in the required rate and extent of glycoenolysis.

During short-term, high intensity dynamic and isometric exercise, transformation of phosphorylase $b$ to $a$ is mediated by calcium release (see section 1.3.1) (Chasiotis, 1983). A lowered pH has been shown to inhibit the calcium release channel (ryanodine-receptor) of the sarcoplasmic reticulum (Ma et al., 1988), and a reduced cytosolic calcium concentration, has been strongly associated with the decline in tension generation during the development of fatigue (Westerblad et al., 1990). As such, during repetitive stimulation of the muscle, any reduction in calcium release from the sarcoplasmic reticulum would be expected to result in a simultaneous decrease in both force production and phosphorylase $b$ to $a$.
transformation. An additional effect of increasing H\(^+\) concentration, is a reduction in the affinity of calcium ion binding sites on troponin C (Blanchard et al, 1984, see later); if the same inhibition occurred with the homologous calcium-binding protein, calmodulin, then a reduction in phosphorylase kinase activity would be expected (see section 1.3.1), resulting in a decrease in phosphorylase in the active \(a\) form. Whilst this is only speculative, it would provide a mechanism whereby force production and glycogenolysis were closely matched under increasingly acidotic conditions, and may even account for the decreased fraction of phosphorylase in the \(a\) form, observed (Chasiotis, 1983) towards the end of high intensity exercise.

In summary then, from the experimental evidence available to date, it is difficult to ascertain whether the decrease in muscle glycogenolysis observed under the acidotic conditions created by high intensity exercise, represents a cause, or simply a consequence, of the simultaneously reduced work output. Clearly the two are inextricably linked.

**pH effects on glycolysis**

In 1965, Danforth demonstrated a marked pH dependence of one of the regulatory enzymes in glycolysis, phosphofructokinase (PFK). In vitro studies have since shown that this pH dependence is effective only at inhibitory levels of ATP (approximately 5 mM); the low pH increases the ratio of protonated to unprotonated ionisation groups at the ATP binding sites of PFK, which results in inhibition of the enzyme by facilitating ATP binding and simultaneously reducing the affinity for its substrate, fructose 6-phosphate (Frieden et al, 1976). However caution must be exercised in judging the regulatory significance of in vitro determined activity profiles, since apparently modest changes in assay conditions can dramatically alter the conclusions. Allosteric effector levels can be instrumental in the pH dependence of enzymes. Indeed, Dobson et al (1986) have shown that the presence either in isolation or in combination, of many positive modulators (fructose 2,6-P\(_2\), glucose 1,6-P\(_2\), ADP, AMP, P\(_i\), NH\(_4^+\)) allows PFK to retain significant catalytic function, despite elevated ATP levels and a reduced pH.

This has been verified by in vivo studies, in which diminishing but continued glycolytic activity has been observed in electrically stimulated muscle, during a progressive decline in muscle pH to 6.45 (Spriet et al, 1987b). Nonetheless, once a pH of 6.45 was reached, further stimulation was shown to result in minimal glycolytic activity. It was concluded that pH 6.45 represented a true limiting pH for PFK activity, even with the continued presence of the enzyme's positive modulators.
(Spriet et al, 1987b). In a similar study performed by the same authors, intermittent electrical stimulation of human muscle resulted in a continually declining muscle pH, and a reduction in force output was observed simultaneously with a decrease in ATP turnover rate (Spriet et al, 1987a). As discussed for the process of glycogenolysis, this could reflect either 1) a condition whereby the reduced rate of glycolysis could not support a rate of ATP production commensurate with that required for continued high force production (leading to a necessary reduction in force output), or 2) a situation whereby other processes (see later) interfered with excitation-contraction coupling, thereby reducing the ATP requirements. However, it is likely that the former mechanism is at least partially operative, since in vitro studies (independent of energy demand) have shown that a reduction in PFK activity by at least 40% occurs during a change in pH from 7.2 to 6.8 at 25 °C (equivalent to a pH change of 7.0 to 6.6 at 37 °C) even in the presence of maximal physiological levels of positive modulators (Dobson et al, 1986).

(ii) pH effects on tension generation

In addition to the pH effects on energy metabolism, a decrease in tension generation, occurring as a result of H⁺ inhibition of excitation/contraction coupling and/or actomyosin interaction, has also been proposed as a mechanism of muscle fatigue.

Donaldson and Hermansen (1978) examined the effect of increasing H⁺ levels on force production of rabbit soleus (predominantly slow-twitch) and adductor magnus (fast-twitch) muscles, using the skinned fibre technique. This preparation evaded the dependence on normal excitation/contraction coupling and the influence of intracellular buffering and metabolism, since the calcium, H⁺ and MgATP²⁻ concentrations were determined by the composition of the solution bathing the fibres. Maximum force production was shown to be dependent on the prevailing pH, with the mean maximal tension generated at pH 6.5 (and 1mM Mg²⁺) representing only 88 and 70% of that obtained at pH 7.0, for the soleus and adductor muscles, respectively. Additionally, a differential depressant effect of acidosis on the calcium-sensitivity of the fibres (i.e. force produced at sub-maximal calcium concentrations) was observed, with the fibres from the adductor muscle once again showing a relatively greater sensitivity to pH (Donaldson and Hermansen, 1978). This fibre-type hierarchy in resistance to increasing [H⁺] (fast-twitch fibres less resistant than slow-twitch) has more recently been confirmed,
using skinned skeletal muscle fibres from the rat (Metzger and Moss, 1987) and the rabbit (Chase and Kushmerick, 1988). Since all these experiments were performed on demembranated fibres, activated by exogenous calcium, they collectively imply that the primary site at which protons act is the actomyosin interaction, rather than some step related to activation or excitation-contraction coupling.

Quantitatively similar data to that of Donaldson and Hermansen (1978) has been obtained using skinned fibres from frog semitendinosus muscle, but by employing an extended pH range (7.4 to 6.2) it has been shown that a non-linear relationship between the degree of maximal force depression and pH exists (Fabiato and Fabiato, 1978). On this basis, and in view of the lack of reversal of the pH inhibition by increased, supersaturating calcium levels, the authors concluded that the effect of acidosis on maximum tension was not caused by simple, direct competition between Ca^{2+} and H^+ for a binding site on troponin. They suggested that the lowered pH could decrease the affinity between the subunits of troponin, modify the conformation of tropomyosin, or limit the force developed by the interaction of actin and myosin in the presence of an optimal [Ca^{2+}] (decrease in force developed per cross-bridge or number of cross-bridges formed) (Fabiato and Fabiato, 1978). A variation on this hypothesis, suggested by Metzger and Moss (1987), is that the number of attached cross-bridges remains unchanged at low pH but fewer are in the high force-producing state. No specific details as to how a decrease in pH could elicit these changes, were offered by either group of authors.

Blanchard and Solaro (1981) demonstrated that in spite of the presence of the same Tn-C in three different types of myofibrils (from adult and prenatal dog cardiac muscle and rabbit soleus muscle), the effect of acidic pH on the pCa_{50} (calcium concentration required to cause half maximal activation) of ATPase activity varied considerably. The authors concluded that sites on proteins other than troponin-C that vary among these myofibrils, were most likely titrated with protons in a differing manner during exposure to acidic pH (Blanchard and Solaro, 1981). However, in a later study, the same authors maintained that the H^+ effect on tension generation was at least in part, due to an H^+-induced reduction in the affinity of Ca^{2+}-binding sites on troponin (Blanchard et al, 1984).

Godt (1981) proposed an electrostatic model to account for the H^+-ion induced decrease in calcium sensitivity of the myofibrils. He suggested that the hydrogen ion concentration influenced the degree of dissociation of ionisable groups on the surface of thin filaments, thereby altering their surrounding electrostatic potential. At pH 7.0, the contractile proteins bear a net negative charge and the resultant
electrostatic field should attract cations and repel anions. Lowering the pH would reduce the net negative surface charge and change the electrostatic field such that the concentration of Ca\(^{2+}\) ions in the immediate vicinity of the filaments and near the troponin binding sites, is reduced. Whilst this model could possibly account for the effects of low pH on calcium sensitivity, it appears unable to account for the reduction in maximal tension, since it should be possible to overcome the exclusion of Ca\(^{2+}\) from the myofilament lattice by raising the Ca\(^{2+}\) concentration in the bathing solution (Metzger and Moss, 1987). Yet, even at supersaturating Ca\(^{2+}\) concentrations, maximum tension of skinned fibres is depressed by a decrease in pH (Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978; Metzger and Moss, 1987). Additionally, the theory predicts no effect of pH on Ca\(^{2+}\) binding to pure troponin, since in this situation there would be no influences of thin filament charge. Whilst data on this point are somewhat conflicting, the majority of evidence supports the notion that acidic pH can in fact reduce Ca\(^{2+}\) binding to pure TnC (see Blanchard et al, 1984). Similarly, Godt's theory could not explain the differential pH sensitivity of fast and slow muscles, unless differences in the pKa' of ionisable groups on the thin filaments exist between the fibre types.

In 1972, Nakamaru and Schwartz, in examining the process of sarcoplasmic reticulum (SR) calcium release during excitation-contraction coupling, reported that an abrupt increase in pH from 6.46 to 7.82 could initiate Ca\(^{2+}\) release. Conversely, a sudden reduction in pH over the same range resulted in Ca\(^{2+}\) uptake and binding in the SR. The authors postulated that Ca\(^{2+}\) release may be induced by a rapidly changing gradient of hydrogen ions across the excitable membrane, secondary to a change in the membrane potential, following each excitation event. Although not even particularly relevant to a discussion of the comparatively slow changes in pH elicited through metabolic events in the muscle, this data has frequently been interpreted (and possibly incorrectly) as implying that a decrease in pH limits the calcium released by the SR upon stimulation of the muscle, due to a greater intravesicular binding of calcium (Donaldson and Hermansen, 1978; Kothiyal and Ibramsha, 1986; Miller et al, 1988; Roberts and Smith, 1989). This interpretation suggests that at low pH, Ca\(^{2+}\) is "more tightly bound" in the SR and somehow "resists release" upon suitable stimulation - mechanisms which were neither investigated, implied, nor proposed in the original study.

Fabiato and Fabiato (1978) interpreted the data of Nakamaru and Schwartz (1972) in a different, and possible more correct manner. They adopted the hypothesis that more calcium is likely to be released from a more fully loaded
sarcoplasmic reticulum. Their own studies showed that at physiological calcium concentrations, the pH optimum for calcium loading is between pH 7.0 and 6.6 (Fabiato and Fabiato, 1978). As such, a small decrease in muscle pH (7.0 to 6.6) should in fact result in a greater amount of Ca\(^{2+}\) being released from the SR upon muscle stimulation. This would partially compensate for the decrease in sensitivity of the myofilaments to Ca\(^{2+}\) and so cause an overall minimal disturbance to force generation. The authors concluded that even if a greater degree of acidosis were encountered by the muscle (e.g. to pH 6.2), which could result in a decreased Ca\(^{2+}\) content of the SR, the release of this somewhat diminished amount should still be sufficient to saturate the regulatory proteins (Fabiato and Fabiato, 1978). Then, the only effect observed would be that of the influence of pH on the maximum tension developed by the myofilaments, which is a decrease of just 30 % when the intracellular pH is as low as 6.20 (Fabiato and Fabiato, 1978; Donaldson and Hermansen, 1978; Metzger and Moss, 1987).

Thus, although the exact mechanism is open to dispute, skinned fibre experiments appear to demonstrate that intracellular acidosis is always associated with a reduction in the muscle's ability to generate tension. Whether the reported effects are of sufficient magnitude and physiological relevance to account for the loss in tension observed during the development of fatigue in vivo, is another issue.

One criticism of the skinned fibre preparation, concerns the loss of all soluble constituents from the fibre, resulting in a removal of the potential influence of these substances on the contractile process or on myofilament calcium sensitivity. One such soluble substance is carnosine and it has been shown that this dipeptide strongly facilitates calcium loading of the sarcoplasmic reticulum in skinned fibres (Harrison et al, 1985). As such, its exclusion from the bathing solution creates problems in extrapolating the data to the in vivo situation, where appreciable concentrations of carnosine exist within the muscle (Crush, 1970). Furthermore, differences in the concentration of carnosine between the fibre types (FT > ST; Crush, 1970) may explain some of the observed differences in calcium sensitivity (Harrison et al, 1985).

An alternative approach to the problem is to use whole muscle preparations (or in vivo studies) and observe the relationship between changes in force output and hydrogen ion concentration during muscle contraction to fatigue. Whilst this represents a more physiologically relevant approach, it suffers from the limitation of being unable to isolate the independent variable under examination; a whole plethora of metabolic changes occur concurrently during the development of fatigue.
Therefore, although a cause and effect relationship cannot be confirmed, a high correlation between the two variables of force and pH, would provide at least circumstantial evidence for a role of intracellular acidosis in the development of fatigue.

Dawson et al. (1978) investigated the reduction in tension generated by isolated, anaerobic frog gastrocnemius muscles, stimulated to contract isometrically to fatigue. Phosphorous nuclear magnetic resonance (PNMR) was employed to study the corresponding change in concentration of hydrogen ions and other metabolites. The decrease in force was approximately proportional to the rise in $\text{H}^+$ concentration ([H$^+$]), regardless of the frequency of stimulation (Dawson et al., 1978). Confirmation of the relationship between force and [H$^+$], was provided by the in vivo studies of Miller et al. (1987, 1988). These authors also used PNMR to measure muscle [H$^+$], and force was determined by maximum voluntary contractions (MVC) of the human adductor pollicis muscle. [H$^+$] and force were determined simultaneously during maximal sustained or submaximal intermittent isometric exercise. A significant, linear inverse correlation between MVC and [H$^+$] was observed during both types of exercise ($r = 0.64$ and $0.77$ for sustained and intermittent protocols, respectively) (Miller et al., 1988). These studies were consistent with a role for H$^+$ in fatigue, corroborating the results of the skinned fibre experiments, but were by no means conclusive. Indeed it was observed that the decline in force correlated equally well with the increase in free ADP levels (Dawson et al., 1978) or the increase in diprotonated phosphate (Miller et al., 1988). Additionally, noticeable in the data of Miller et al. (1988) was a lack of any relationship between [H$^+$] and MVC during the later stages of the exercise ([H$^+$] remained constant whilst force continued to decline). Clearly, force production is not related to intramuscular acidosis in a simple fashion, and these experiments alone, are not extensive enough to identify the singular importance of a reduction in muscle pH in the development of fatigue.

The effect of muscle acidosis on peak tension generation was evaluated by Sahlin et al. (1981, 1987), using muscles with varying capacities to utilise glycolysis as a means of energy production. The isolated muscles under examination were the rat extensor digitorum longus (EDL) (a highly glycolytic, fast-twitch muscle) and soleus (a slow-twitch muscle). Additionally, the experiments were carried out on EDL muscle poisoned with iodoacetic acid to inhibit glycolysis. Muscles were electrically stimulated to fatigue, under anaerobic conditions. Peak tension (twitch and tetanic) of unpoisoned EDL and soleus muscles decreased to 50% of initial
values after approximately 3 min stimulation. In unpoisoned EDL, the decline in tension to 30% resting values was approximately proportional to the increase in H\(^+\) concentration (as determined by the homogenate technique). Thereafter, a further decline in tension was associated with no change in the H\(^+\) concentration. Although the pH could not be measured in the soleus, on the basis of the extent of lactate accumulation and PCr hydrolysis it was calculated to remain virtually unchanged during 7 min stimulation. Thus, a similar relative rate of tension decline was observed in both EDL and soleus, but the latter occurred with a minimal change in [H\(^+\)]. In the poisoned EDL, tension declined more rapidly (to 50% within 1.2 min) and with protracted stimulation, developed rigor. pH was unchanged during the contraction period (Sahlin et al, 1981). This data demonstrates that an increase in H\(^+\) concentration is not necessarily a prerequisite for a reduction in tension during the development of muscle fatigue. Studies on individuals with muscle enzyme deficiencies (PFK or myophosphorylase) preventing the use of glycolysis as a means of energy production during exercise, have similarly shown that a reduction in force generation can occur without an accompanying accumulation of lactate or H\(^+\) ions (Cady et al, 1989a). Whether the observed linearity between tension decline and H\(^+\) in the unpoisoned EDL (Sahlin et al, 1981) was simply coincidental, or represented part of an indirect contribution to fatigue, cannot be ascertained from these studies.

Examination of the recovery process following fatiguing contractions is a useful approach in investigating the effects of a high [H\(^+\)] on tension production in whole muscle, since the compounding effects of a continuous high rate of energy turnover are no longer evident. Tension can be determined by the performance of single maximal contractions, measured simultaneously with muscle pH, during their combined return to resting levels. The in vitro studies of Metzger and Fitts (1987) demonstrated that in rat diaphragm muscle, following high and low frequency stimulation to fatigue, the restoration of tension during the first two minutes of recovery was temporally dissociated from that of pH. Tension recovery commenced as soon as stimulation ceased, whilst muscle pH remained stable or continued to decline for a further 2 minutes. Moreover, the influence of pH on tension, was not consistent between the two modes of stimulation; the high-frequency stimulation protocol elicited a greater decline in peak force output but with a higher muscle pH. However, beyond the first 2 min of recovery, pH and force were restored with similar time-courses. Obviously a reduction in muscle pH can only partially explain
the fatigue observed under these circumstances. Other factors clearly have a role to play, and possibly one of varying importance depending on the stimulation protocol.

In vivo studies of the recovery from fatigue, have confirmed this disparity in the restoration of tension and muscle pH (Miller et al, 1988; Sahlin and Ren, 1989). Sahlin and Ren (1989) showed that the maximum voluntary contraction of the quadriceps was approximately 90% restored after 2 min recovery from a fatiguing isometric contraction (66% MVC). On the basis of changes in lactate and PCr, the corresponding intramuscular pH (2 min recovery) was calculated to be approximately pH 6.4-6.5 - not dissimilar from that expected immediately post-exercise. The authors concluded that despite the evidence from skinned fibre preparations, the capacity to generate force is not limited by a high intracellular H+ concentration (Sahlin and Ren, 1989). Considering all the evidence together, such a categorical conclusion may be unjustified, as it implies either an irrelevance, or erroneous interpretation, of the data reported from skinned fibre studies. It would appear more likely, that in debating the contribution of acidosis to the decline in tension, the question is one of degree.

The conclusion from in vitro studies that acidosis causes a decrease in tension is unlikely to be inaccurate, for the conditions under which it was obtained. However the extent of the reduction in force, relative to the degree of acidosis, needs to be carefully examined if such studies are to be extrapolated to in vivo conditions. Similarly, when approving or dismissing the data from in vitro studies, attention must be paid to the method of pH measurement (PMNR, microelectrode or homogenate technique) since this too, could influence the percentage reduction in force expected per unit change in pH. Ignoring the possibly misleading issue of whether the pH effects on tension are statistically significant (and therefore represent "real" effects), and concentrating only on absolute or percentage changes, it can be seen that there is no major disparity between the in vivo and in vitro studies.

At saturating calcium levels (presumably simulating those attained during maximal activation of muscle in vivo), a reduction in muscle pH from 7.0 to 6.6 caused only a 10-15% reduction in the maximal tension of frog semitendinosus muscle (Fabiato and Fabiato, 1978). In the study of Donaldson and Hermansen (1978), the tension generated at pH 6.5 relative to that at pH 7.0 was between 70 and 88%, depending on the predominant fibre type of the muscle. Therefore, in a mixed muscle with an approximately 50:50 ratio of fast to slow twitch fibres, the relative force at pH 6.5, could be expected to be approximately 80% of that at pH 7.0 (i.e. 20% depressed). Although this still represents a greater % depression in
force than that observed (approx. 10%) by Sahlin and Ren (1989) after two minutes of recovery, these authors only estimated the prevailing muscle pH (6.5-6.6) at that time. It is therefore difficult to reconcile the differences observed with any certainty from this study alone.

However, in another study examining recovery from fatiguing isometric exercise (Miller et al, 1988), the precise pH during recovery was measured using P-NMR. After 2 min, muscle pH retained its post-exercise value of 6.4-6.5 and during this time period force was rapidly restored, but only to 80% of pre-fatigue levels. Beyond 2 min, force and muscle pH showed a synchronous rise to resting levels. It is likely then, that the initial rapid recovery from fatigue (from 30 to 80% of maximal force, in 2 min) was elicited by the reversal of some non-H⁺ mediated event, but that this process could only allow a return of force to a level commensurate with the prevailing pH (6.5), leaving the additional 20% of force to be regained upon removal of the excess hydrogen ions. This degree of suppression of maximal force is fully compatible with skinned fibre studies, which have only ever offered the conclusion that an approximately 10-30% reduction in force is observed per 0.5 unit pH decrease (see earlier). None of the in vitro studies reviewed, purport to account for the entire reduction in force encountered during fatiguing exercise (80-90%), by a direct H⁺ effect on the contractile machinery.

This is underscored by the elegant studies of Mainwood et al (1987). An early study by these authors (Mainwood and Renaud, 1985) had demonstrated that isolated frog muscles stimulated to fatigue developed a proton load of 20-25 mmol.kg⁻¹ with a reduction in intracellular pH of 0.6-0.8 pH units. Simultaneously, maximal tetanic force was suppressed by 70-80%. When an even greater decrease in intracellular pH was induced by increasing the CO₂ concentration of the solution bathing the muscle, the decrease in force was less than 40% (Renaud et al, 1986). In order to negate any problems with the specific effect of CO₂/HCO₃⁻ on intracellular events related to contraction (Lea, 1986), the experiments were repeated by generating an intracellular lactacidosis comparable to that observed at fatigue, in unfatigued fibres. This was achieved by adjusting the extracellular [lactate] [H⁺] product so that there was an influx into the muscle giving the same intracellular product at equilibrium (Mainwood et al, 1987). Maximal tetanic tension was subsequently suppressed by just 10-20%.

In summary then, it seems likely that an accumulation of H⁺ ions is not necessarily required, to observe a decrement in maximal force during muscle fatigue, and as such, a direct H⁺-mediated inhibition of the contractile machinery cannot be
held solely responsible for the large reductions in force output observed. However, when present, an intracellular acidosis can be expected to exert an additional negative influence on tension generation (decreasing maximal force by approximately 10-20 % per 0.5 pH unit decrease from resting values) which will accentuate the main, non H⁺-mediated effect.

(iii) pH effects on relaxation rate

The performance of high-intensity dynamic exercise relies on a co-ordinated, rhythmical contraction and relaxation of the muscle. Any delay in the return of a muscle to its pre-contraction state following the generation of tension (i.e an increase in relaxation time), could therefore be expected to result in an impaired performance. Alterations in the time course of relaxation following a twitch or tetanic contraction, have been identified in association with fatigue. Duchateau and Hainaut (1984b) demonstrated that repeated electrical stimulation of human adductor pollicis muscle (1 s isometric tetani at 30 Hz every 2s, for a total of 60 s) resulted in a decrease in the muscle relaxation rate, to just 20% of resting values.

In vitro studies have demonstrated a close relationship between the relaxation rate of different muscles and the rate of calcium (Ca²⁺) uptake by their sarcoplasmic reticulum (SR), suggesting that the removal of calcium from the cytosol is the limiting step in relaxation (Briggs et al., 1977). Similarly, computer simulations of intracellular calcium ion movements have shown that an increase in the rate of calcium uptake by high affinity binding sites on SR can bring about a faster relaxation of the muscle (Kothiyal and Ibramsha, 1986). However, it has also been suggested that the rate of Ca²⁺ release from the myofilaments (Kothiyal and Ibramsha, 1986) and the rate of cross-bridge dissociation (Edwards et al., 1975b) may determine the time course of relaxation. Thus, if H⁺ ions are to mediate an effect on the relaxation process, they must do so through an interference with calcium ion movements (Ca²⁺ release from the myofibrils or re-uptake into the sarcoplasmic reticulum), or the dissociation of myosin cross-bridges upon calcium removal.

Calcium is transferred from the cytosol into the sarcoplasmic reticulum by an active transport process catalysed by the sarcoplasmic reticulum ATPase (see Section 1.2 and Fig. 1.3). In vitro studies of isolated SR have shown that H⁺ competes with Ca²⁺ for binding to the ATPase enzyme; when the H⁺ concentration was
raised, higher Ca\(^{2+}\) concentrations were required to obtain half-maximal SR ATPase activities (Hill and Inesi, 1982). At saturating Ca\(^{2+}\) levels, maximal SR ATPase activity was observed at a pH of 7.2, and decreased as the H\(^{+}\) concentration increased (to pH 6.6) (Inesi and Hill, 1983). This H\(^{+}/Ca^{2+}\) competition was shown to be directed to specific sites on the ATPase whose occupancy by Ca\(^{2+}\) is an absolute requirement for enzyme activation; upon addition of ATP, enzyme phosphorylation could in fact be either prevented or permitted, simply by adjusting the ratio between H\(^{+}\) and Ca\(^{2+}\) concentrations. The same authors also demonstrated that at the end of the enzyme cycle, hydrolytic cleavage of the phosphoenzyme proceeded at a lower rate when the pH was low (pH 6.6). This particular effect would therefore be manifest as a decrease in SR ATP flux (turnover) even in the presence of saturating levels of calcium.

The conclusions of Inesi and Hill (1983) concerning the pH optimum for Ca\(^{2+}\) loading are not altogether compatible with those of Fabiato and Fabiato (1978), who demonstrated that at saturating (physiological) Ca\(^{2+}\) levels, calcium accumulation by the SR was actually increased by acidosis (to pH 6.6). This discrepancy could possibly be explained by the different methodologies employed. In the study of Fabiato and Fabiato (1978), calcium uptake by the SR was only measured indirectly; an estimate of the amount of Ca\(^{2+}\) stored in the SR after modifications of pH, was given by the amplitude of subsequent caffeine-induced contractions. This would also be influenced though, by any processes which interfered with the extent of calcium binding within the SR. The rate of SR ATP flux (Inesi and Hill, 1983) might be expected to give a more accurate representation of the extent of calcium loading under various conditions of acidosis.

Using whole single fibres, Edman and Matiazzi (1981) demonstrated that intracellular acidosis did indeed slow the rate of relaxation. This was confirmed by Allen et al (1989), but these authors reported no effect of pH on the rate of decline of Ca\(^{2+}\) after a tetanus. They concluded that pH caused a moderate slowing of relaxation which was caused by a direct effect of pH on cross-bridge cycling and detachment rates. This is supported by the reduction of the maximal shortening velocity observed in earlier studies (Edman and Matiazzi, 1981; Cooke and Pate, 1985; Johnston and Mutungi, 1987).

It was shown by Dawson et al (1980) that slowing of relaxation in whole muscle preparations was related to many of the biochemical changes occurring during fatigue, one of which was an accumulation of hydrogen ions. However it was impossible to delineate the relative importance of the H\(^{+}\) accumulation, in the
presence of so many other, potentially confounding influences. A more discriminatory approach was taken by Sahlin et al. (1981): the rate of relaxation from a twitch, of isolated iodoacetic acid (IAA) poisoned rat EDL muscle, was compared with that of unpoisoned muscle, during identical stimulation protocols (2 Hz for 8 min) under anaerobic conditions. IAA was added to the muscle to inhibit lactic acid formation and therefore also, H⁺ accumulation. Unpoisoned muscles showed a pronounced slowing of relaxation, in parallel with a decline in tension, whereas twitch relaxation time in IAA poisoned muscles was unchanged even when tension had decreased to 50% of initial values. pH decreased to approximately 6.5 in the unpoisoned muscles, at which point relaxation time had increased more than threefold upon resting values; as expected, no significant alteration in muscle pH was observed in the poisoned muscles. It was suggested that prolongation of relaxation during muscle stimulation was a function of a decreased intracellular pH. In a later study by the same group (Sahlin et al., 1987) a similar stimulation protocol (2 Hz for 7 min) was applied to slow-twitch rat soleus muscle. The unchanged muscle pH (calculated) and relaxation time from a twitch, observed at fatigue, was consistent with the previous hypothesis. However, it was observed that under the same metabolic conditions, relaxation from a tetanus (induced by intermittent stimulation of the muscle at 100 Hz for 2-3 s during the 2 Hz stimulation protocol) was in fact prolonged (about twofold after 7 min stimulation). This confirmed the earlier results of Edwards et al. (1975b) which showed that the relaxation time of mouse soleus after a tetanus, was increased at fatigue. This prolongation of relaxation was observed in the presence of minimal lactate levels and persisted even when glycolysis was completely inhibited after poisoning with iodoacetate (Edwards et al., 1975b).

These results therefore demand qualification of the previous suggestion (Sahlin et al., 1981) to conclude that prolongation of relaxation from a twitch, may be a function of a decreased intracellular pH. Clearly other factors must explain the increase in relaxation time from a tetanus, observed at fatigue. If H⁺/Ca²⁺ competition for binding sites on sarcoplasmic reticulum ATPase (Inesi and Hill, 1983) does in fact occur in whole muscle preparations, then this might possibly explain the discrepancy in twitch and tetanic kinetics at fatigue. The lesser quantity of calcium released from the sarcoplasmic reticulum during a single action potential (Cannell and Allen, 1984), may be forced to compete with H⁺ ions for its reuptake into the SR (and thus result in prolongation of the twitch). The same quantity of H⁺ ions would represent a relatively lower competitive force in the face of the super-
saturating calcium levels released, and therefore available for reuptake, during the high stimulation frequencies required for production of a tetanic contraction. The selective effects of pH, on twitch but not tetanus relaxation time, may be important in the fatigue observed during voluntary muscle contractions. These are accomplished by asynchronous firing and recruitment of motor units, at a frequency which results in an incompletely fused tetanic contraction (MacIntosh et al, 1983).

The possibility still exists however, that neither the twitch or tetanic relaxation rates are directly influenced by the H+ ion concentration; the relationship observed between twitch relaxation and [H+] in unpoisoned EDL muscles (Sahlin et al, 1981) may simply have been coincidental rather than causal. Experiments on patients with myophosphorylase deficiency have indicated that the slowing of relaxation associated with fatigue, can occur in the absence of the formation and accumulation of lactate (Cady et al, 1989b). Additionally, return of the relaxation rate to pre-fatigue levels, has a half-time of about 30s (Edwards et al, 1972b) which is far more rapid than that for lactate removal from the muscle.

1.3.3.2 Accumulation of inorganic phosphate (Pi)

Another metabolite which has more recently been implicated in fatigue is inorganic phosphate (Pi). Free phosphate accumulates in the muscle during the hydrolysis of creatine phosphate and ATP. Some of the released P_i will be utilised in the formation of hexosemonophosphates, but an increase of free P_i, as determined by PNMR, from 2-5 to 15-20 mmol.l^{-1} has been observed following high intensity exercise (Miller et al, 1988). Since P_i is an end-product of ATP hydrolysis it is feasible that an accumulation of P_i could cause product inhibition of any of the ATPase enzymes. This could occur at the site of either myosin-, sarcoplasmic reticulum- or Na^+/K^+-ATPases, each resulting, in different ways, in a detrimental effect on muscle contraction.

Working with skinned fast and slow teleost muscle fibres, Altringham and Johnston (1985) demonstrated that maximum tension was depressed by increasing phosphate concentrations, even in the presence of saturating calcium concentrations. 20 mM phosphate inhibited maximum isometric tension in slow fibres by 34%, but by only 11% in fast fibres. No effect on maximum contraction velocity or ATPase activity was observed. The findings were attributed to a change in cross-bridge kinetics, with no further qualification.
Hibberd et al (1985) measured the tension transients recorded during laser pulse-induced photolysis of "caged" ATP in single skinned rabbit psoas fibres. The fibres were firstly equilibrated in the rigor state, with a photolabile precursor of ATP. In the absence of Ca\(^{2+}\), liberation of ATP caused a rapid drop in tension due to cross-bridge detachment, followed by a transient redevelopment of tension (reattachment of cross-bridges) and then complete relaxation. Equilibration of the fibres with 10 mM P\(_i\) before photolysis, reduced the amplitude of transient redevelopment of tension and markedly increased the rate of final relaxation. When a fibre was first equilibrated with Ca\(^{2+}\), liberation of ATP switched the fibre from rigor into an active contraction. The final steady tension was lower in the presence of 10 mM P\(_i\). Addition of P\(_i\) to a muscle fibre in rigor, did not decrease the tension. The mechanism of P\(_i\) inhibition of tension was explained with reference to a scheme in which the release of P\(_i\) from the actomyosin-products complex occurs at a step which is close to equilibrium and which precedes ADP release (Eisenberg et al, 1980):

\[
\begin{align*}
\text{ATP} & \quad \rightarrow \quad \text{AM} \leftrightarrow \text{AM"ATP} \leftrightarrow \text{AM"ADP,P} \leftrightarrow \text{AM"ADP,P} \leftrightarrow \text{AM"ADP} \leftrightarrow \text{AM} \\
\text{M"ATP} & \leftrightarrow \text{M"ADP,P} \quad \text{P} \quad \text{ADP}
\end{align*}
\]

(A = actin, M = myosin)

Since AM"ADP, AM" and possibly AM"ADP represent the predominant force-generating states of the cross-bridge, the addition of P\(_i\) would reduce the concentration of these complexes and thus decrease net force production (Hibberd et al, 1985). The plausibility of this mechanism as an explanation for the P\(_i\) induced depression of tension was confirmed by the studies of Kentish (1986), Cooke et al (1988) and Pate and Cooke (1989a and 1989b).

Recent PNMR studies indicate that there is a strong correlation between the concentration of the diprotonated form of P\(_i\) ([H\(_2\)PO\(_4^-\)]) and the decline in force in intact skeletal muscle of the frog, during the development of fatigue (Dawson et al, 1986). Nosek et al (1987) further examined these effects using the skinned fibre
preparation on rabbit psoas muscle. The authors firstly reproduced the total \( P_i \) effect on maximum force, observed by previous authors (see above). This was carried out at a fixed pH, at which an increase in total \( [P_i] \) would simply result in greater absolute amounts of both \( [\text{HPO}_4^{2-}] \) and \( [\text{H}_2\text{PO}_4^-] \), but no change in the ratio of each with respect to the other. To examine the possible effect of either of the charged species, it was necessary to change pH as well as \( [P_i] \). Since an alteration of pH can depress maximal force in itself (see section 1.3.3.1), this effect was factored out to examine the additional influence of \( [P_i] \). The strong correlation between total \( [P_i] \) and force no longer existed; at a given concentration of total \( [P_i] \) (30mM), the decline in maximum force varied between 20 and 80% depending on the prevailing pH (7.25-6.00), and thus by inference, on the concentration of \( P_i \) in the form \( \text{H}_2\text{PO}_4^- \). When the data was replotted as force vs. \( [\text{HPO}_4^{2-}] \) or force vs. \( [\text{H}_2\text{PO}_4^-] \), only the latter relationship showed a linearity between the two parameters.

Further support for a role of diprotonated phosphate in declining force generation comes from PNMR studies carried out in vivo. A correlation coefficient of 0.7 was obtained for the relationship between MVC of the adductor pollicis and \( [\text{H}_2\text{PO}_4^-] \) during performance of a 4 min sustained MVC (Miller et al, 1988). Nonetheless, there was considerable spread of individual data points, with a 70% reduction in initial force being associated with \( [\text{H}_2\text{PO}_4^-] \) levels of between 5 and 20 mM. When the relationship was plotted for intermittent exercise, a higher correlation coefficient was obtained (\( r = 0.73 \)), but again, close examination of the individual and mean data points showed that whilst the spread was less variable (thus giving a better correlation coefficient), the later stages of force reduction (from 50 to 25% initial values) were not related in a linear fashion to mean \( [\text{H}_2\text{PO}_4^-] \) levels. Additionally, MVC and \( [\text{H}_2\text{PO}_4^-] \) followed completely different time courses in the first two minutes of recovery from the fatiguing contraction (although after this time, a good correlation between MVC and \( [\text{H}_2\text{PO}_4^-] \) was observed).

Wilson et al (1988) examined the relationship between force and \( [\text{H}_2\text{PO}_4^-] \) during maximal wrist flexion exercise at different contraction durations (1 or 2s per 5s) for a total of 4 min. Testing commenced either from rest or after a 2 min period of submaximal exercise designed to induce pre-test conditions of low muscle pH and high \( [\text{H}_2\text{PO}_4^-] \) concentration. In all subjects, under all conditions, the force developed showed high linear correlations with both pH and \( [\text{H}_2\text{PO}_4^-] \) as measured by PNMR (\( r = -0.90 \pm 0.08 \) and \( -0.89 \pm 0.08 \) respectively). Regardless of the exercise protocol and the metabolic status of the muscle prior to the test, a given % increase in \( [\text{H}_2\text{PO}_4^-] \) was associated with a similar % reduction in force from pre-
test values. This was not true for the relationship between % change in force and pH; following submaximal exercise, any given pH produced a significantly higher % maximal force. This was taken as evidence for an independent \([\text{H}_2\text{PO}_4^-]\) effect on tension generation (i.e. independent of pH per se) and it was concluded that muscle fatigue during short-term exercise is primarily caused by an increase in intramuscular \([\text{H}_2\text{PO}_4^-]\) rather than by a decrease in intramuscular pH (Wilson et al, 1988). However, a later study by the same group, demonstrated that muscle fatigue seen after ramped wrist flexion exercise did not correlate with \([\text{H}_2\text{PO}_4^-]\) (McCully et al, 1989) and the authors concluded that further work was needed, to clarify the role of diprotonated phosphate in fatigue.

It is difficult, during in vivo studies, to identify the singular effects of \([\text{H}_2\text{PO}_4^-]\) as distinct from pH, since the former is to a large extent dependent on the latter, and in most cases these two independent variables (pH and \([\text{H}_2\text{PO}_4^-]\)) are themselves strongly correlated. However, a change in \([\text{H}_2\text{PO}_4^-]\) can occur independently of a change in pH and could in part, explain the reduction in force generated by the repeated contraction of muscle when no change in pH is observed (Sahlin et al, 1981 and 1987). In conditions whereby the muscle is unable to utilise the glycolytic pathway for energy production (as a result of either poisoning, or inherent deficiencies, of key enzymes in glycolysis/glycogenolysis), \([P_i]\) will still continue to increase as PCr is hydrolysed. Although the pH of the muscle remains unchanged, the increase in \([P_i]\) per se will result in an increase in the absolute concentration of \(\text{H}_2\text{PO}_4^-\). Using a pKa2 for phosphoric acid of 6.75 at 37 °C (Miller et al, 1988) it can be calculated that at pH 7.0 approximately 40% of the \([P_i]\) is in the diprotonated form. Thus an increase in \([P_i]\) at fatigue, from 5 to 20 mM at pH 7.0 (e.g. in IAA poisoned muscles; Sahlin et al, 1981) would still represent a 400% increase in the concentration of \(\text{H}_2\text{PO}_4^-\) (from 2 to 8 mM).

Even if \(\text{H}_2\text{PO}_4^-\) accumulation has some effect on force production, it is unlikely to account for the total reduction in tension observed at fatigue. Miller et al (1988) reported a mean \([\text{H}_2\text{PO}_4^-]\) of approximately 13mM at fatigue, at which point MVC was reduced by 70% from resting values. Within 2 minutes force was restored to 80% of its initial value, whilst the \(\text{H}_2\text{PO}_4^-\) concentration remained at approximately 13 mM. It is possible that the remaining 20% depression in force was the result of the 13 mM \(\text{H}_2\text{PO}_4^-\) concentration. These same levels, are associated with a 30 % reduction in maximum force, in skinned fibre preparations (Nosek et al, 1987). When the approximate concentration of \(\text{H}_2\text{PO}_4^-\) is calculated from the pH and total \(P_i\) concentrations used in the skinned fibre studies of Altringham and Johnston
(1985) and Cooke et al (1988), it is found that a concentration of H$_2$PO$_4^-$ of 6-8mM causes a reduction in maximal tension by 20% (Cooke et al., 1988) or 11-34% (depending on the fibre type) (Altringham and Johnston, 1985).

To accord with previous studies on the mechanism of P$_i$ suppression of tension (Hibberd et al., 1985; Kentish, 1986; Cooke et al., 1988; Pate and Cooke, 1989a and 1989b), these findings of the relationship between [H$_2$PO$_4^-$] and force, imply that the P$_i$ that is released during the force-producing step of the cross-bridge cycle is of the form H$_2$PO$_4^-$. Evidence for this, to the author's knowledge, is not as yet forthcoming.

Another potential mechanism for the effect of P$_i$ accumulation on tension generation, is that of a reduction in net free energy available for work, when ATP is hydrolysed. This has been termed the "affinity" for ATP hydrolysis, $A$ (Dawson et al., 1978) and is given by,

$$A = - \left\{ \Delta G^0 + RT \ln \left( \frac{[ADP][P_i]}{[ATP]} \right) \right\}$$

(where $\Delta G^0$ is the standard free energy change, $R$ is the gas constant and $T = $ absolute temperature).

Thus, $A$ decreases as the concentration of P$_i$ or ADP rises. It is therefore possible that the inhibitory effect of P$_i$ on maximal force is merely a reflection of the decrease in the affinity for ATP hydrolysis by myosin ATPase. However, skinned fibre experiments suggest that this is not the case. Kentish (1986) determined the force generated by skinned fibres at a fixed ATP concentration and a constant [ADP] x [P$_i$] product, but with varying ratios of [ADP] to [P$_i$]. In a 1:5 ratio of [ADP] to [P$_i$], force declined by 44% whereas at a ratio of 5:1 (ADP:P$_i$), the reduction was only 13%. Similarly when each was added in isolation (but at the same concentration), only P$_i$ significantly decreased the force generated. These differential effects on force production by identical concentrations of ADP and P$_i$ were confirmed by Cooke et al (1988), and preclude a simple link between tension and the total change in free energy of ATP hydrolysis. Instead, it was proposed that important factors to consider, concerned free energy partitioning among the intermediate states in the actomyosin cycle, and how changes in ligand
concentrations could alter this partitioning and thus influence mechanical properties (Pate and Cooke, 1989b).

It has been suggested that a reduction in the affinity for ATP hydrolysis (A), possibly secondary to an increase in $P_i$, may be responsible for the observed decrease in the muscle relaxation rate during fatigue, and that this effect may be mediated through a lowered rate of ATP-dependent calcium reuptake into the sarcoplasmic reticulum (Dawson et al, 1980). Whilst this theory has not been investigated by direct, independent manipulations of [ADP] and [P_i] and their relative effects on relaxation, it has been studied indirectly in whole muscle preparations. Sahlin et al (1981) showed that electrical stimulation of IAA-poisoned rat EDL muscle resulted in an unchanged twitch relaxation rate, whilst unpoisoned muscle demonstrated a markedly increased relaxation time as fatigue progressed. However, the phosphate potential ([ATP]/[ADP][P_i]) was similarly changed in both poisoned and unpoisoned muscles, and it was therefore considered unlikely that this factor was important in the slowing of relaxation (Sahlin et al, 1981).

In summary then, it is likely that tension generation (and possibly also the relaxation rate) of fatiguing muscles is affected, but not solely determined, by changes in [P_i] or [H_2PO_4^-]. The suppression of tension is most likely a result of P_i binding to a force-generating ADP-bound state of actomyosin, resulting in a reversal of the process in which it is normally released. It is also possible, on theoretical grounds, that an accumulation of P_i would result in a depression of the activity of the Ca-reuptake- and Na^+/K^+-ATPases, though direct experimental evidence is lacking.

1.3.3.3 Accumulation of adenine nucleotides (AMP, ADP) and inosine monophosphate (IMP).

The relative concentrations of ATP, ADP and AMP play a key role in the regulation of energy metabolism, and are determined by the energy state of the cell and the enzyme adenylate kinase (AK). AK catalyses the reaction 2 ADP $\Leftrightarrow$ ATP + AMP and maintains relatively constant ratios between the available adenine nucleotides. During intensive exercise, the rapid rate of ATP hydrolysis results in a tendency towards decreasing ATP and increasing ADP levels (Sahlin et al, 1978b). The reduction in ATP cannot be prevented if the energy systems are unable to rephosphorylate ADP sufficiently rapidly. However the increase in ADP levels can
usually be minimised by the adenylate kinase reaction, and the subsequent deamination of AMP to inosine monophosphate (IMP). IMP formation results from the deamination of AMP through one arm of the purine nucleotide cycle (PNC) (Fig. 1.5).

The activity of the cycle is expected to be low in resting muscle; the resting free AMP concentration ([AMP]) is in the region of 0.2μM (Dawson, 1983) whilst the $K_m$ of AMP deaminase for AMP is reported to be 0.5 - 1.0 mM (Ronca-Testoni et al, 1970; Wheeler et al, 1979). Additionally, AMP deaminase is activated by an environment atypical of resting muscle, including an elevated [ADP] and a low pH (Terjung et al, 1986). Having a relatively high $K_m$ for AMP, AMP deaminase activity is very sensitive to increases in the concentration of AMP. AMP is in equilibrium with ADP through the adenylate kinase reaction, such that the [AMP] free to take part in metabolic reactions, is proportional to $[ADP]^2$ (Dawson, 1983). Therefore as ADP tends to increase due to inadequate rephosphorylation to ATP, AMP will also begin to accumulate, initiating its own deamination to IMP. IMP cannot permeate the cell membrane (Broberg and Sahlin, 1989) but may either be reaminated to AMP, or further broken down to inosine, then hypoxanthine (see Fig 1.5). Whilst either or both of these mechanisms may operate during prolonged, submaximal exercise (Terjung et al, 1986; Broberg and Sahlin, 1989), indirect evidence (a decrease in total adenine nucleotide concentration (TAN), and an increase in NH3, each equal to the increase in IMP; Katz et al, 1986a; Sahlin et al, 1989) suggests that neither appear to be functional during high intensity exercise. As such, AMP entry into the PNC represents a final fate for the nucleotide, which can only be reaminated upon cessation of high intensity exercise (Sahlin et al, 1978b; Katz et al, 1986a).

AMP deamination therefore results in a decrease in TAN (apparent primarily as a decline in ATP concentration), but a relatively well maintained ratio between the nucleotides. The large increase in IMP concentration represents a fairly innocuous metabolic fate for the products of ATP hydrolysis. The observed decline in ATP content following intensive muscle contraction (up to 50%; see earlier) would create an inordinate increase in the free ADP and AMP content of the muscle, if AMP deaminase activity did not reduce the total adenine nucleotide pool (Terjung et al, 1986).

Despite these attempts to minimise the accumulation of ADP, a small net increase is usually observed following intensive exercise (Sahlin, 1986b). Only the [ADP] that exists free in solution is relevant to a discussion of the regulation of energy
Fig. 1.5 Reactions of the purine nucleotide cycle.
metabolism and this represents only a small fraction of the total [ADP]; the remainder is protein-bound, predominantly to actin and myosin (Dawson et al, 1978). However, direct measurement of free [ADP] is not possible. Chemical analysis can only measure total [ADP], as the commonly employed extraction procedures strip ADP from its in vivo binding sites. The protein-bound ADP is inaccessible to phosphorus nuclear magnetic resonance (PNMR), and the free ADP concentration is too low to be detected. The free [ADP] in vivo must therefore be calculated from its equilibrium with ATP, PCr, creatine (Cr) and H+, catalysed by creatine kinase. For frog gastrocnemius muscle, this yields a value of approximately 20 μM at rest (Dawson et al, 1978).

The amount of ADP bound to proteins is unlikely to change following exercise (Sahlin, 1986b), and therefore the observed increase in total [ADP] must reflect an increase in free [ADP]. Confirming this, the change in total [ADP] as measured by chemical analysis of muscle biopsies following fatiguing dynamic exercise (242 μM; Sahlin et al, 1978b) is of the same order of magnitude as the change in free [ADP] calculated from PNMR spectra (250 μM) for fatigued frog muscle (Dawson et al, 1978). However, although the absolute increase of free [ADP] following exercise can be derived indirectly from chemical analysis, the relative increase from resting levels cannot be quantified unless the exact fraction bound to proteins is known with certainty. If in human muscle, as in amphibian muscle, the resting free [ADP] is approximately 20 μM, then the post-exercise value of 242 μM, represents a vast increase (> 10-fold) from resting levels.

Moreover, these levels of free ADP represent only the steady-state concentration since neither the biopsy technique nor PNMR can sample rapidly enough to observe the changes in ADP during the actual contraction. The ADP levels most likely fluctuate with each cross-bridge cycle as ATP is hydrolysed. Using the technique of laser pulse-induced photolysis of a photolabile ATP precursor ("caged" ATP), to increase the ATP concentration surrounding skinned fibres from zero to 5 mM within 1 ms, Ferenczi et al (1984) demonstrated that there was a rapid initial burst of ADP formation amounting to 152 μM which was complete within 50 ms, and had a half-time of less than 20 ms. It has been proposed (Sahlin et al, 1986b) that a reduced rate of ADP rephosphorylation (secondary to PCr depletion and a lowered glycolytic rate) would increase the amplitude and duration of these local ADP transients, over and above the steady-state level, acting to impair the function of the muscle ATPases. Whilst the proposed ADP transients would be undetectable using the commonly employed analytical techniques (chemical analysis of muscle samples
and PNMR), it is suggested that an indication of their presence during contraction is reflected by the accumulation of IMP (which remains in the muscle and can be assessed upon termination of the exercise) (Sahlin, 1986a).

That IMP formation occurs only when the metabolic demand is high, and that it does not necessarily relate to a given steady-state accumulation of ADP or AMP is shown by comparison of the metabolic status of muscle following high intensity exercise with that during long-term ischemia. Exercise to fatigue (isometric or dynamic) results in increased levels of IMP within the muscle, simultaneous with increases in [ADP], [AMP] and [lactate]. 2 hr of ischemia of the same muscle group produces similar levels of ADP and AMP, but no increase in IMP or decrease in TAN occurs (Sahlin, 1986a). This has been interpreted as confirmation that during intensive muscle contraction, ADP/AMP levels increase momentarily above steady-state levels, at which point deamination of AMP to IMP occurs (Sahlin, 1986a).

The frequently reported increase in muscle IMP concentration following intensive dynamic (Sahlin et al, 1978b) or isometric (Katz et al, 1986a) exercise, and the lack of an increase during less metabolically demanding exercise (Sahlin et al, 1978b, 1989) provides evidence that IMP accumulation occurs only when the rate of ATP hydrolysis is high, and cannot be matched by a sufficiently rapid ADP resynthesis (e.g. under conditions of diminishing PCr levels and a reduced glycolytic activity). Further circumstantial evidence for a role of increased [ADP] in fatigue, comes from the findings that individuals with AMPdeaminase deficiency demonstrate a marked high-intensity exercise intolerance (although performance at low levels of exercise remains unaffected) (Fishbein et al, 1978; Fishbein, 1988). The inability of these individuals to deaminate ADP during the increased metabolic stress of high intensity exercise may lead to an inordinate increase in the ADP concentration, which in turn may inhibit further muscle contraction.

An additional extension to this theory would be that the marked reductions in maximal force output observed following muscle contraction to fatigue, would be reversed with a similar time-course to the recovery of [PCr] levels. Although the muscle pH and glycolytic rate may remain depressed during the first few minutes of recovery, the repleted PCr levels should be able to maintain a sufficiently high rate of ADP rephosphorylation to meet the energy requirements of a single maximal voluntary contraction. This has been observed in many studies, whereby the maximal force/peak power is restored to approximately 80% of pre-fatigue values following 2 min recovery (Sargeant and Dolan, 1987; Miller et al, 1988; Hitchcock, 1989; Sahlin and Ren, 1989).
It therefore appears that ADP accumulation may have an important role to play in the process of fatigue. However, to substantiate this theory, it is essential to 1) identify the site(s) at which ADP may exert its inhibitory effect on muscle contraction and 2) confirm that its effect at this site would indeed precipitate the observed changes in the mechanical response (e.g. decreased tension generation or relaxation rates).

Dawson et al (1978) postulated that a dependence of isometric force development on ADP concentration could arise from product inhibition of the contractile system or from interference with activation of contraction. More recent studies however, suggest that an elevation of ADP does not result in a decrease in tension. Until the study of Cooke and Pate (1985), systematic studies of the effects of [ADP] on steady-state contractions of vertebrate muscle had not been carried out, in part, due to the problems involved in rigorously controlling the levels of MgATP and MgADP inside the fibre. The problem was circumvented by Cooke and Pate (1985) by incubating skinned fibres from rabbit psoas muscle in high concentrations of both MgATP and MgADP, and calculating the actual nucleotide concentrations in the fibre using known physical and kinetic parameters. An elevation of MgADP inside the contracting fibre increased the isometric tension (Cooke and Pate, 1985). This effect was consistent with the cross-bridge model described in Section 1.3.3.2, where ADP release occurs at the end of the power-stroke, in that binding of ADP prevents dissociation of the myosin head by ATP, resulting in an increased number of strongly attached cross-bridges (Cooke et al, 1988). Increased levels of ADP also resulted in a decrease in contraction velocity and this was explained by a mechanism whereby heads that are not dissociated by ATP at the end of the power-stroke, are pulled by the relative sliding of the adjacent filaments into configurations in which they produce negative work (Cooke et al, 1988; Pate and Cooke, 1989b). Exact quantitative analysis of these effects is needed to examine whether the changes observed are likely to occur in vivo, during fatiguing contractions.

Both maximum contraction velocity and myosin ATPase activity exhibit classical, Michaelian, saturation behaviour as a function of MgATP concentration, with $K_m$ values of 150 and 20 $\mu$M, respectively (Cooke and Pate, 1985). MgADP acts as a competitive inhibitor of both maximum contraction velocity and of ATPase, with values for $K_i$ (inhibition constant) of 250 $\mu$M and 200 $\mu$M, respectively (Cooke and Pate, 1985). It has been shown that at a point where both tension and contractile velocity have been decreased by a factor of two, the concentration of MgATP remains almost unaltered at 3-5mM and the concentration of free MgADP
has increased from 20 to 200 µM (Dawson et al., 1978, 1980; Cooke and Pate, 1985). Using the given values of $K_i$ (above) it has been calculated that the net result of these changes would be an approximately 5% decrease in contraction velocity and no change in the ATPase activity (due to its much lower $K_m$) (Cooke et al., 1988). Thus the steady-state accumulation of ADP measured at the point of fatigue, appears to have no major role in the inhibition of tension generation or contraction velocity at the site of actomyosin interaction.

Whether or not the proposed ADP transients (Sahlin, 1986a and 1986b) are of sufficient magnitude to exert a more pronounced effect on these processes is at present, unknown. Confirmation of this theory will rely on more accurate estimates of the actual ADP concentration at the site of actin-myosin interaction, during repeated muscle contraction. This may be resolved through further "caged" ATP experimentation accompanied by mathematical modelling/computer simulations of the expected adenine nucleotide changes and mechanical response of the muscle.

Myosin ATPase is not the only ATPase that should be investigated as a potential site of ADP inhibition. It is possible that the observed changes in the mechanical response of muscle during fatigue are elicited through ADP effects on either the SR Ca$^{2+}$-transport ATPase, or the Na$^+/K^+$-ATPase. Unfortunately the kinetics of these two enzymes and their sensitivity to increases in [ADP] have been less well studied in the context of muscle fatigue (to the author's knowledge). A reduced activity of Na$^+/K^+$ ATPase could be implicated in the mechanism of tension decline during fatigue since an inability to maintain high intracellular K$^+$ levels during repeated stimulation of the muscle reduces the membrane potential and subsequently, the Ca$^{2+}$ transient during activation (Vollestad and Sejersted, 1988). The reduction in tension during the development of fatigue through intermittent stimulation has been shown to be strongly correlated with a reduction in intracellular calcium concentration ([Ca$^{2+}$]) (Westerblad et al., 1990). Whilst the accumulation of ADP has not been directly implicated in the failing of the Na$^+/K^+$ antiport mechanism, there certainly appears to be an effect caused by a reduction in the phosphorylation potential, of which the ADP concentration is an integral component (Blum et al., 1988).

An impaired activity of the Ca$^{2+}$ transport-ATPase would not directly affect force generation, though may be fundamental in the observed slowing of relaxation. Occupation of ATP and Ca$^{2+}$ at the appropriate binding sites of Ca$^{2+}$ transport ATPase is followed by the formation of a phosphorylated intermediate of the enzyme (see Section 1.2) and subsequently ADP is released into the cytosol. This stage is
said to be ADP-sensitive as it can be reversed by high levels of ADP (Klug and Tibbits, 1988). Coupled with a slight change in the contraction velocity (mediated by increased ADP at the site of the cross-bridges - see earlier), the aggregate changes in these two factors (contraction velocity and relaxation rate) could be of importance during dynamic exercise. The mechanisms controlling the two may even operate simultaneously, since the kinetics of ADP release (which are strongly related to the maximum contraction velocity) are calcium dependent and quite possibly controlled by the Ca\(^{2+}\) regulatory system (Goldman, 1987).

Thus, whilst the exact location of the proposed ADP inhibition, and even the precise concentration of free ADP likely eliciting this inhibition, have still to be elucidated, considerable and convincing circumstantial evidence exists to suggest that there is a role for transient elevations of ADP, secondary to limiting rates of ATP resynthesis, in the process of fatigue.

1.4 Summary of metabolic events leading to fatigue

Having discussed the various metabolic mechanisms most likely to contribute to a decline in performance during high intensity exercise, a mechanism for the development of fatigue is proposed, incorporating these processes and highlighting their interactive effects (Fig. 1.6).

It is possible to examine whether the proposed model can resolve some of the apparent conflicts surrounding the development of fatigue in metabolically poisoned muscle, or the muscle of individuals with specific enzyme deficiencies (particularly with respect to the involvement of H\(^+\) accumulation). The model predicts two major metabolic influences on muscle contraction; one of a direct H\(^+\) (and/or H\(_2\)PO\(_4^-\)) mediated effect on the contractile apparatus, and one of an imbalance between the rate of ATP supply and utilisation (caused by a depletion of phosphocreatine levels or an H\(^+\)-mediated inhibition of glycolysis), exerting its effect by product (ADP) inhibition of the muscle ATPases. Clearly, muscle with a repressed glycolytic capacity (through metabolic poisoning (MP) or inherent deficiencies of glycogenolytic/glycolytic enzymes (ED)) will only be susceptible to the non-H\(^+\) mediated effects. The fact that these muscles have one less mechanism leading to fatigue, yet demonstrate a marked high intensity exercise intolerance when compared with normal muscle (Edwards et al, 1983; Cady et al, 1989a and 1989b; Sahlin et al, 1981), has previously been viewed as paradoxical (Cady et al, 1989a). Indeed, it
Fig. 1.6 Proposed mechanisms of metabolic fatigue
has led to the proposition that an additional mechanism, which occurs more readily in ED/MP muscle (such as a premature loss of membrane excitability), is responsible for this early onset of fatigue (Cady et al., 1989a).

However, using the above model (Fig. 1.6), the paradox can possibly be resolved. If one of the major debilitating processes in the muscle (normal or otherwise), during the performance of high intensity exercise, is an inability to rephosphorylate ADP at the required rate, then the extended performance time of the normal muscle can simply be attributed to the increased capacity provided by the use of anaerobic glycolysis. As such, the benefits so obtained from utilising this metabolic pathway temporarily outweigh the disadvantages of creating an increasingly acidic muscle.

Thus, the same process may underly the fatigue observed in both normal and ED/MP muscle, but it would arise from the differential availability of mechanisms for the provision of ATP. The ED/MP muscle would have to rely predominantly on PCr hydrolysis for ATP resynthesis, and once the PCr stores begin to decline, the muscle would have no other mechanism available to provide ATP at a rate commensurate with that required for continued high intensity exercise; the transient elevations of [ADP] would be increased in amplitude and duration (Sabina et al., 1984; Terjung et al., 1986; Sahlin, 1986a) and the muscle would suffer a premature fatigue. Normal muscle would utilise anaerobic glycolysis to supplement its energy supply, but in doing so, it would in time, be subject to the inhibitory influences of a reduced muscle pH. The low muscle pH would exert its direct inhibitory effects on the process of actomyosin interaction (Fig 1.6) and ultimately, on the glycolytic pathway itself (possibly at the level of PFK), to bring about fatigue through the decreased capacity of glycolysis to rephosphorylate ADP.

1.5 Factors governing the capacity to perform high intensity exercise.

Adhering to the model proposed in Fig. 1.6, a muscle with an enhanced ability to perform high intensity exercise should possess a well-developed capacity for 1) anaerobically regenerating ATP (and/or removing the products of ATP hydrolysis) and 2) negating the inhibitory end-products of anaerobic glycolysis. The former process is determined by substrate stores and the activity of key enzymes.
in glycogenolysis, glycolysis and PCr hydrolysis, and the latter is determined by the ability of the muscle to sequester the protons produced during anaerobic glycolysis (i.e. the muscle buffer value).

1.5.1 ATP resynthesis: metabolic potential of skeletal muscle fibre types.

Human skeletal muscles are composed of muscle fibres possessing different biochemical and contractile properties. The difference in the sensitivity of myosin for retaining or losing ATPase activity after exposure to either high or low pH is a reliable method for the histochemical classification of muscle fibres (Saltin and Gollnick, 1983). This procedure has identified two distinct types of fibre; type I and type II. Type I fibres are predominantly found in slow twitch (ST) muscle (defined on the basis of its contractile properties) and type II fibres, in fast twitch (FT) muscle. As such, in describing fibre types, the terms "type I" and "type II" are often used synonymously with "slow twitch" and "fast twitch", respectively. Justification for this overlap of nomenclature comes from the finding that the ATPase activity of myosin purified from either FT or ST muscle behaves similarly when exposed to acid or alkaline preincubation (Barany and Close, 1971).

From what is currently known of the metabolic capacity of different muscle fibre types, it might be expected that there would be a marked difference in the ability to rapidly resynthesise ATP, between muscles which differ in their fibre type composition and/or state of training.

1.5.1.1 Substrate levels of fibre types and changes with training.

In contrast to earlier studies (Rehunen and Harkonen, 1980) it has recently been observed that single human FT fibres have a significantly higher (10-13%) resting phosphocreatine (PCr) concentration than ST fibres (Edstrom et al, 1982; Tesch et al, 1989b), confirming the data obtained from whole muscles of lower mammals (Edstrom et al, 1982; Meyer et al, 1985; Kelso et al, 1987). As such, individuals with a preponderance of FT fibres possess a greater capacity to generate ATP through PCr hydrolysis. Additionally, the greater quantity of \([P_i]\) released in FT fibres during PCr hydrolysis should provide more of a stimulus for activation of
both glycogenolysis ($P_i$ is a substrate for glycogen phosphorylase) and glycolysis ($P_i$ acts as a positive modulator of PFK activity (Newsholme and Leech, 1983)). Changes in PCr concentration following high intensity strength or sprint training show either no change (Boobis et al, 1983; Boobis, 1987; Nevill et al, 1989) or a significant increase (MacDougall et al, 1977), and may be partially dependent on the duration and type of training.

The muscle glycogen concentration is reported to be similar in FT and ST fibres (Essen et al, 1975), or slightly higher in FT fibres (Saltin and Gollnick, 1983), and may be elevated in mixed muscle samples following a period of strength training (MacDougall et al, 1977). However, considering the possible dietary and activity-induced modifications of the resting muscle glycogen concentration, and its non-limiting nature during high intensity exercise, it is questionable whether the observed increase in concentration would be of any benefit to performance (Gollnick and Bayly, 1986).

1.5.1.2 Enzyme activities of fibre types and changes with training.

Major differences in the activity of the enzymes creatine kinase (CK), adenylate kinase (AK), lactate dehydrogenase (LDH), glycogen phosphorylase (PHOS), phosphofructokinase (PFK), pyruvate kinase (PK) and AMP deaminase exist between the fibre types, with FT fibres demonstrating higher values than ST fibres for each of these enzymes (Costill et al, 1976; Sjodin, 1976; Thorstensson et al, 1977; Saltin and Gollnick, 1983; Terjung et al, 1986; Borges and Essen-Gustavsson, 1989; Tesch et al, 1989a). Accordingly, individuals possessing a preponderance of FT fibres demonstrate a greater capacity to perform high intensity exercise than those with a predominance of ST fibres (Bar-Or et al, 1980; Kaczkowski et al, 1982) and in doing so, show a greater absolute decrease in [PCr] (Tesch et al, 1989b), increase in [lactate] (Sahlin and Henriksson, 1984) and increase in [IMP] (Jansson et al, 1987; Terjung et al, 1986; Sahlin et al, 1989).

The acute muscle metabolite changes induced by typical sprint (Boobis et al, 1983; Cheetham et al, 1986; Jacobs et al, 1987) or strength (Tesch et al, 1986) training sessions indicate that the energy requirements of such, are met primarily through anaerobic glycolysis and utilisation of local ATP and PCr stores. Consequently, it might be expected that prolonged periods of sprint/strength training
would elicit an adaptation of the enzymes involved in these metabolic pathways, allowing for an increased capacity to regenerate ATP during high intensity exercise.

The effects of training on creatine kinase (CK) activity are inconsistent; strength training has been shown to increase (Costill et al, 1979), leave unchanged (Thorstensson et al, 1976; Houston et al, 1983; Apple and Tesch, 1989) or decrease CK activity (Tesch et al 1987), whilst sprint training has shown an increase (Thorstensson et al, 1975) or no effect (Jacobs et al, 1987) on CK activity.

Short periods of strength training (2-4 mo) have been shown to increase the adenylate kinase (AK) activity of mixed muscle samples by 4-20% (Thorstensson et al, 1976; Costill et al, 1979; Komi et al, 1982) whereas six months of strength training resulted in no change in AK activity (Tesch et al, 1987). Individuals who had strength-trained for a period of 4 yrs (bodybuilders, weight-lifters and power-lifters) showed no difference in adenylate kinase (AK) activity of their ST fibres compared with sedentary males, but demonstrated significantly higher AK activities in their FT fibres (x 1.5) (Tesch et al, 1989a). Adenylate kinase is an enzyme which has received comparatively little attention in reviews concerned with the enzymatic adaptations to training. However, in the proposed model for metabolic fatigue (Fig. 1.6) this enzyme could possibly be of prime importance as a "first line of defence" in attempting to reduce the potentially harmful accumulations of ADP. Its two-fold higher activity in FT fibres (Tesch et al, 1989a), coupled with the elevated activity of AMP deaminase in these same fibres (Katz et al, 1986a; Terjung et al, 1986), would provide for an enhanced rate of removal of ADP/AMP, offsetting the inhibitory influences of a transient ADP accumulation. As such, this may allow for a more prolonged and extensive usage of glycolytic pathways for the provision of ATP in FT fibres.

The cross-sectional study of Tesch et al (1989a) demonstrated consistently higher lactate dehydrogenase (LDH) activities in both fast and slow twitch fibres of strength trained individuals, compared with sedentary controls. However, 3 or 6 months strength training was insufficient to elicit comparable changes in LDH, although significant muscle hypertrophy occurred (Houston et al, 1983; Tesch et al, 1987). It was later suggested that the rate of synthesis of contractile proteins was initially faster or just equivalent to synthesis of enzyme proteins, and as such, muscle hypertrophy could result in no change or even a reduction (by "dilution") in enzyme concentrations (Tesch, 1988). Shorter periods of high intensity interval-training (Sjodin, 1976) or strength-training (Costill et al, 1979) similarly, showed no change in LDH activity, or in the distribution of LDH isozymes (Sjodin, 1976).
Phosphofructokinase (PFK) is a non-equilibrium enzyme catalysing an intermediate step in the pathway of glycolysis, with its activity being regulated by various allosteric effectors. It therefore represents one of the flux-generating steps in glycolysis and its maximal activity provides an indication of the maximum flux rate through glycolysis (Newsholme and Leech, 1983). Long-term (8yrs) strength trained muscle showed no significant difference in PFK activity compared with endurance trained muscle (Schantz and Kallman, 1989) and PFK activity either increased (Costill et al, 1979; Jacobs et al, 1987; Troup et al, 1986; Sharp et al, 1986) or showed no change (Houston et al, 1983; Thorstensson et al, 1976) following short-term (7-10 wks) strength or sprint training.

In summary, the data concerning the effects of sprint/strength training on the enzymes involved in ATP resynthesis during high intensity exercise are by no means conclusive. It has been suggested that although some enhancement of glycolytic enzyme activity appears to accompany certain types of sprint/strength training, the quantitative changes are of insufficient magnitude to solely account for the differences demonstrated in performance (Parkhouse and McKenzie, 1984). It has also been questioned whether an increase in enzymes of the glycolytic pathway would be of any benefit to the performance of high intensity exercise, on the basis that the concentration of enzymes for lactate production is greater than that needed to degrade the muscle glycogen to lactate in a very short time, should this system be activated to even half-maximal (a level assumed to be that at which enzymes function in vivo) (Gollnick and Bayly, 1986). Nonetheless, if any of the flux-generating enzymes were adversely effected by changing levels of modulators and other inhibitory agents (such as are PFK and PHOS, by [H+] ), then it is possible that the higher the initial enzyme concentration, the less successful the modulator would be in reducing the overall effectiveness of that enzyme, and the capacity of the metabolic pathway it catalyses.

1.5.2 Skeletal muscle buffer values.

Referring to Fig 1.6, a second mechanism which would improve the ability of a muscle to perform high intensity exercise would be to increase the muscle's capacity to sequester the protons produced during anaerobic glycolysis. This should then a) delay the direct H+-induced impairment of tension generation and relaxation, and 2)
allow for more prolonged usage of glycolysis, consequent to the maintenance of a more optimal pH for PFK activity. Despite the obvious potential of this mode of adaptation, it is not one which has been particularly well exploited, either in the study of limitations to high intensity exercise or in the search for training-induced adaptations.

1.5.2.1 Buffer definitions

Buffering is defined as the transfer of free H+ ions into the non-dissociated state by association with anionic or with non-dissociated bases (or the reverse process upon addition of OH− ions) (Heisler, 1986a):

\[ \text{H}^+ + \text{A}^- \leftrightarrow \text{HA} \]  

(1)

(where \( \text{A}^- \) is a base (proton acceptor) and \( \text{HA} \) is an acid (proton donor) according to the Bronsted definition.

This mechanism reduces changes in pH in the system as compared with the behaviour of pure water or non-buffering electrolyte solutions, by masking active protons (H+ ions) (Heisler, 1986a).

The buffer value of a system is an indication of its effectiveness in minimising the pH change that results from the addition of a standard quantity of strong acid (H+) or strong base (OH−), and has been defined as the concentration of free H+ ions transferred for a given volume into the non-dissociated state per unit change in pH (where pH = - log [H+]; [H+] = hydrogen ion concentration, or more correctly, activity) (Van Slyke, 1922):

\[ B = \frac{\Delta [\text{H}^+]}{\Delta \text{pH}} \text{ or } \frac{\Delta [\text{OH}^-]}{\Delta \text{pH}} \]  

(2)

(typical dimension: mmol.l⁻¹.pH⁻¹) ........(2)

where \( \Delta [\text{H}^+] \) = an increment of strong acid

\( \Delta [\text{OH}^-] \) = an increment of strong base

(Van Slyke, 1922).
The term buffering capacity is frequently used to designate the buffer value. This is misleading, since the buffer capacity \( x \) is defined as the product of buffer value \( B \) and the volume of the system \( V \):

\[
x = B \cdot V \text{ (mmol.pH}^{-1}) \quad \text{(Heisler, 1986a).}
\]

The buffer value of a solution can be determined experimentally by adding measured increments of strong acid \( \Delta [H^+] \) or strong base \( \Delta [OH^-] \) and determining the corresponding changes in pH \( \Delta \text{ pH} \). The slope of the typical sigmoidal titration curve produced (Fig. 1.7) is an expression of the buffer capability of that system. This value will depend on both the total buffer concentration and its efficacy as a buffer within the system.

The association of \( H^+ \) ions with bases upon addition/removal of \( H^+ \) ions depends on the physicochemical properties of the buffer system consisting of weak acid (HA) and conjugate base (A\(^-\)) and can be described quantitatively by the mass-action equation:

\[
Ka' = \frac{[H^+] [A^-]}{[HA]}
\]

where \( Ka' \) = the apparent dissociation constant of the weak acid and \([H^+], [A^-], [HA] \) = concentrations of \( H^+, A^- \) and HA respectively.

Introducing the definitions \( \text{pH} = -\log [H^+] \) and \( \text{pKa}' = -\log Ka' \) yields the Henderson-Hasselbach equation:

\[
\text{pH} = \text{pKa}' + \log \frac{[A^-]}{[HA]} \quad \text{........................(3)}
\]

Buffering in any given system is optimal when equal concentrations of the base and acid forms of the buffer substance exist. At this point \( \text{pH} = \text{pKa}' \) (see equation (3)). Thus, buffers contribute to acid-base regulation in the human body only if their pKa' values are close to physiological pH values. Values of pH minus pKa' = ±1 unit (U) reduce the buffer value to about one third of the peak value (Heisler, 1986a). However if substances with pKa' values ±1U exist in appreciable concentrations, they may make a significant contribution to total buffering.
Fig. 1.7 Theoretical titration curve for a weak acid buffer with a pKa' of 7.0.

(modified from Hultman and Sahlin, 1980)
1.5.2.2 Components of muscle buffering.

With regard to the above discussion of buffering, strictly speaking only those substances within the muscle which display the necessary physicochemical characteristics can be defined as buffer substances. However the importance of maintaining a stable pH within muscle necessitates examination of all systems designed to minimise acute disturbances in acid-base homeostasis, and as such, includes metabolic processes which consume or generate hydrogen ions and the flux of hydrogen ions out of the muscle.

(A) Physicochemical buffers

The quantity of H\textsuperscript{+} ions taken up by a physicochemical buffer, of known concentration and pKa' value, for any given change in pH, can be calculated from reorganisation of the Henderson-Hasselbach equation as follows:

\[ [T] = [A^-] + [HA] \]

where \([T]\) = total concentration of buffer substance as the sum of acid (HA) and base (A\textsuperscript{-}) forms,

therefore \([A^-] = [T] - [HA]\)

Substituting into equation 3 (Henderson-Hasselbach equation),

\[
\text{pH} = \text{pKa}' + \log \left( \frac{[T] - [HA]}{[HA]} \right) \\
\text{..................(4)}
\]

Rearranging the equation,

\[
\text{antilog (pH - pKa')} = \frac{([T] - [HA])}{[HA]} = \frac{[T]}{[HA]} - 1
\]

\[
(1 + \text{antilog (pH - pKa'))} = \frac{[T]}{[HA]}
\]

\[
[HA] = \frac{[T]}{(1 + \text{antilog (pH - pKa'))}} \text{..................(5)}
\]
Knowing $[T]$ (total buffer concentration) and the pKa' for the buffer, $[HA]$ can be calculated at two pH values ($pH_1$ and $pH_2$). The difference in the protonated concentration of buffer ($[HA]$) is then equivalent to the concentration of $H^+$ ions taken up by the buffer substance, during the pH change ($pH_1$ to $pH_2$).

Using this equation, the $H^+$ uptake by the physicochemical buffers can be calculated, during a pH change of 7.1 to 6.5 (= approximate $\Delta pH$ during high intensity exercise; Hultman and Sahlin, 1980).

(i) Phosphate compounds
Of the various phosphate compounds contained within the intracellular fluid, inorganic phosphate ($P_i$) (pKa' = 6.78) makes the most significant contribution to total muscle buffering. Its concentration increases stoichiometrically with the decrease in phosphocreatine during high intensity exercise (although some of the $P_i$ released may be subsequently bound as hexose phosphates; Sahlin et al, 1978b). PNMR studies have demonstrated an increase in $P_i$ from 5 to 20 mmol.l$^{-1}$ muscle water during high intensity exercise (Miller et al, 1988), although enzymatic analysis of muscle biopsy samples yields values of just 12 mmol.l$^{-1}$ post-exercise (Sahlin et al, 1978b). With a concentration of 12-20 mmol.l$^{-1}$ it can therefore be calculated (Eqn 5) that $P_i$ would buffer 4.0 - 6.6 mmol H$^+$.l$^{-1}$ (pH 7.1-6.5).

(ii) Amino acids/peptides/proteins
The basic structure of the amino acid can be represented as:

\[
R-\text{CH-C-O}^- \\
\text{NH}_3^+
\]

where -NH$_3^+$ and -COO$^-$ are known as the $\alpha$-amino and $\alpha$-carboxyl groups respectively.

The identity of the amino acid depends upon the chemical nature of the R group (the side-chain).

All free $\alpha$-amino groups have a pKa' < 3 for the $\alpha$-carboxyl group and a pKa' > 9 for the $\alpha$-amino group, preventing these groups from participating in buffering at physiological pH. The buffering power of amino acids is therefore dependent on the nature of the R group. Only histidine has a pKa' value for its R-group (pKa = 6) resembling the intramuscular pH, but since its free concentration in muscle is low (0.38 mM; Bergstrom et al, 1978) its contribution to total buffering is minimal (0.06 mmolH$^+$.l$^{-1}$.0·6 pH$^{-1}$.).
Peptides and proteins are formed by the condensation of α-amino and α-carboxylic acid groups of adjacent amino acids. When amino acids are incorporated into peptides and proteins the pKa of the R-group changes (Hultman and Sahlin, 1980). The pKa of the imidazole group of histidine increases from 6.0 to 6.8 when incorporated into the dipeptide carnosine. The carnosine concentration of human muscle is reported to be 4-6 mmol.l⁻¹ (Bergstrom et al., 1978; Parkhouse et al., 1983), and it can be calculated that this dipeptide would buffer 1.3-2.0 mmol H⁺.kg dm⁻¹.0-6 pH⁻¹.

The majority of the buffering capability of proteins is due to the imidazole group of histidine residues (pKa 6.0-7.5) and the sulphhydryl group of cysteine residues (pKa 7.0-8.3), with an additional small contribution from terminal α-amino groups (pKa 7.4-8.5) (actual pKa's dependent on microenvironment; Heisler, 1986a). The molar buffer value of the protein cysteine residue is expected to be lower than that of histidine since its pKa is further removed from the physiological pH range. Nonetheless, its concentration in muscle is high (3.1 g.100 g⁻¹ protein (Furst et al., 1970) = 69 mmol.l⁻¹ muscle water) and even using its most unfavourable pKa of 8.3, it can be calculated that protein-incorporated cysteine would buffer 3.0 mmol H⁺.l⁻¹.0-6 pH⁻¹ making it quantitatively, a more important buffer than carnosine. The histidine content of muscle protein is 2.7 g.100 g⁻¹ protein (= 46 mmol.l⁻¹) (Furst et al., 1970) and using a pKa of 6.8 this yields an uptake of approximately 15.3 mmolH⁺.l⁻¹.0-6 pH⁻¹.

(iii) CO₂/bicarbonate system
The intracellular bicarbonate concentration is approximately 10 mmol.l⁻¹ (Sahlin et al., 1977) and with an open circulation, the CO₂/HCO₃⁻ system is capable of buffering an equimolar concentration of H⁺ ions. In reality, a decrease in bicarbonate of approximately 7.2 mmol.l⁻¹ is observed at the end of exhausting exercise (Sahlin et al., 1978a).

\[ \text{H}^+ + \text{HCO}_3^- \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_2\text{O} + \text{CO}_2 \]

Successful operation of this system relies on removal of the CO₂ evolved, by diffusion into the circulation and eventual excretion by the lungs, in order to drive the equilibrium to the right. In this respect, HCO₃⁻ can continue to buffer until its own concentration is depleted or brought into equilibrium with the prevailing CO₂ tension. In a closed system (e.g. occluded blood flow), where the CO₂ produced cannot leave the muscle, the reaction soon comes to equilibrium and the system has
only a minimal capacity to buffer the metabolically produced H⁺ ions (H⁺ uptake calculated as 1.9 mmol.l⁻¹.0·6 pH⁻¹, with a pKa' of 6.1).

(B) Metabolic buffers

(i) PCr hydrolysis

Hydrolysis of phosphocreatine (PCr) during the rephosphorylation of ADP to ATP, results in an uptake of H⁺ ions, with a stoichiometry (nH⁺ = mol H⁺ taken up per mol PCr hydrolysed) dependent upon the prevailing pH. At pH 7.1, nH⁺ = 0.30 and increases linearly to nH⁺ = 0.67 at pH 6.5 (Hultman and Sahlin, 1980). PNMR analysis of whole muscle, places the resting PCr concentration at 30-35 mmol.l⁻¹, reducing to approximately 5 mmol.l⁻¹ following high intensity exercise (Miller et al, 1988). Enzymatic analysis of biopsy samples yields a change in [PCr] of 20mol/l (Sahlin et al, 1978b) and may be a slight underestimate. Thus, PCr hydrolysis, has the capacity to buffer 13-20 mmolH⁺.l⁻¹.0·6 pH⁻¹.

(ii) IMP formation

During high intensity exercise, an increase in inosine monophosphate (IMP) is observed which is quantitatively equivalent to the decrease in ATP. The net reaction is given by: ATP + nH⁺ ——> IMP + 2Pᵢ + NH₄⁺ and the stoichiometrical uptake of hydrogen ions is 0.41 mole per mole IMP formed (Hultman and Sahlin, 1980). With an increase in IMP of 1.1-1.5 mmol.l⁻¹ (Sahlin et al, 1978b; Katz et al, 1986a; Sahlin et al, 1989) this represents an uptake of approximately 0.5-0.6 mmolH⁺. l⁻¹.0·6 pH⁻¹.

(C) Transmembrane flux of H⁺ ions

Elimination of H⁺ ions from the working muscle following a period of high intensity exercise is the only mechanism whereby the acid-basis homeostasis of muscle can be restored - physicochemical buffering represents only a transient mechanism for regulation. However, the existence and extent of H⁺ efflux during the exercise bout remains a subject of some controversy. It is likely that the significance of the H⁺ efflux will depend upon the rate of intracellular H⁺ production, the maximal capacity of the carrier mechanisms on the cell membrane, the degree of perfusion and the buffer value of the extracellular fluid (Mainwood et al, 1972; Heisler, 1986b; Juel, 1988).

Following the introduction of an acid load, isolated superfused muscle preparations (in which the muscle is continuously supplied with fresh perfusate of
normal pH and pCO2) demonstrate unequal efflux rates for H+ and lactate (by a factor of about 12; H+ > La−) (Benade and Heisler, 1978). This was originally interpreted as evidence that H+ ions are transferred across the cell membrane by a separate carrier mechanism, and not as undissociated lactic acid. It is more likely however, that several mechanisms operate during the extrusion of hydrogen ions and lactate from the muscle, only one of which is non-ionic diffusion of lactic acid.

Aicken and Thomas (1977) reported that the predominant process in the pH regulation of resting mouse muscle, was the Na+/H+ exchange system, accounting for 70-80% of the H+ efflux. The remainder was due to anion exchange (HCO3−/Cl−). However in the response to an exercise induced metabolic acid load (causing a decrease in pH of 0.5 units within 2-3 min), anion exchange was not involved in pH recovery (as determined by the inability of the inhibitors SITS and tetrathionate to influence intracellular pH recovery or lactate efflux) (Juel, 1988). Instead, it was concluded that La−/H+ symport and diffusion of undissociated lactic acid produced an equal rate of efflux of H+ and lactate, and that the involvement of the Na+/H+ exchange mechanism allowed for the additional removal of H+ (Juel, 1988).

Potentially, the H+ extrusion rate can exceed that of the lactate ion, an effect examined and observed most frequently in the pH recovery from an acid load, in well perfused preparations. However, during high intensity exercise, the time period for efflux of either ion is relatively slow compared with the rapid rates of formation, and probably shows at least partial saturation kinetics (Jorfeldt et al, 1978; Juel et al, 1988; Juel and Wibrand, 1989). This, coupled with a slow (with respect to the duration of the exercise task) adjustment of the circulation, makes it unlikely that H+ efflux exceeds than of lactate− during high intensity exercise. Indeed, even with relatively high muscle blood flows, similar extrusion rates for lactate and H+ have been observed during maximal cycling exercise (Katz et al, 1985). Certainly, during high intensity isometric exercise, H+ efflux can have no role in buffering as the blood flow to the muscle is restricted by the high intramuscular pressure created during the contraction (Edwards et al, 1972a).

Summary of components of muscle physicochemical and metabolic buffering
The contribution of the various buffering mechanisms to total muscle buffering over the pH range 7.1-6.5 is summarised in Table 1.2. In a closed system, the total buffer value is estimated at 39-49 mmol.l−1.0·6 pH−1 (= 65-82 mmol.l−1.pH−1), increasing in an open system to 44-54 mmol.l−1.0·6 pH−1 (= 73-90 mmol.l−1.pH−1) due to the contribution from bicarbonate.
Table 1.2  Uptake of hydrogen ions by buffering mechanisms during a pH change from 7.1 to 6.5

<table>
<thead>
<tr>
<th>Buffer</th>
<th>H⁺ uptake (%)</th>
<th>Contribution²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mmol.l muscle water⁻¹.0-6 pH⁻¹)</td>
<td></td>
</tr>
<tr>
<td><strong>Physicochemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inorganic phosphate (Pᵢ)</td>
<td>4.0 - 6.0</td>
<td>11.4</td>
</tr>
<tr>
<td>Free histidine</td>
<td>0.06</td>
<td>0.1</td>
</tr>
<tr>
<td>Carnosine</td>
<td>1.3 - 2.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Protein: histidine residues</td>
<td>15.3</td>
<td>34.8</td>
</tr>
<tr>
<td>cysteine residues</td>
<td>3.0</td>
<td>6.8</td>
</tr>
<tr>
<td>HCO₃⁻/CO₂</td>
<td>7.2 (open circulation)</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>1.9 (closed &quot; )</td>
<td></td>
</tr>
<tr>
<td><strong>Metabolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr hydrolysis</td>
<td>13.0-20.0</td>
<td>37.5</td>
</tr>
<tr>
<td>IMP formation</td>
<td>0.5-0.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

²% contribution calculated for a closed system, using mean values of H⁺ uptake for each buffering mechanism.

1.5.2.3 Influence of method of measurement on the muscle buffer value.

One of the most commonly employed methods of measurement of muscle buffering is the direct titration of a homogenate; the muscle is homogenised in an appropriate solution and then titrated with a fixed acid/alkali over a set pH range. By applying equation (2) (section 1.5.2.1), an "in vitro" buffer value for that muscle sample can then be obtained (Bᵥᵢᵣᵣ). Provided the measurement is made in conditions replicating those experienced in vivo (with respect to temperature and ionic strength) the given
value can be expected to approximate to the physicochemical buffering ability of the muscle in vivo.

A modification of this method involves allowing the muscle to "self-titrate" with metabolic acids during a fatiguing exercise bout. The production of lactic acid accounts for more than 85% of the hydrogen ions produced during high intensity exercise (Hultman and Sahlin, 1980). Thus, the resting muscle pH and lactate concentration can be measured, and utilised in conjunction with post-exercise values, to determine the muscle buffer value \( B \) as follows:

\[
B = \frac{\Delta \text{Lactate}}{\Delta \text{pH}} \quad \text{where} \quad \Delta \text{Lactate} = \text{change in [La] pre to post exercise}
\]

\[
\text{and} \quad \Delta \text{pH} = \text{change in pH pre to post exercise}
\]

It has been suggested that this value of \( B \) ("in vivo" buffer value; \( B_{\text{viv}} \)) comprises all components of physicochemical buffering in addition to the contribution from the metabolic buffers and would therefore yield higher values than those determined in vitro (\( B_{\text{vit}} \)) (Hultman and Sahlin, 1980).

### 1.5.2.4 Fibre type differences in the muscle buffer value.

To the author's knowledge, no systematic evaluation of the buffer value of single human fast and slow twitch fibres has been performed. However studies of vertebrate muscles differing in their predominant fibre type or glycolytic potential, have demonstrated that highly glycolytic muscles have a significantly higher buffer value (\( B_{\text{vit}} \)) than more oxidative muscles (Castellini and Somero, 1981; King et al, 1984). Curtin (1987) found a 20% (non-significant) difference in the buffer value (\( B_{\text{vit}} \)) of single frog (Xenopus laevis) type 1 and type 2 fibres (2 > 1). (The nomenclature for these fibre types does not correspond with that devised for mammalian fibres; Xenopus fibres are differentiated on the basis of their fatiguability - type 1 fibres fatigue more rapidly and have a lower oxidative and glycolytic capacity than the fatigue-resistant type 2 fibres). It therefore appears, and quite appropriately, that the higher the glycolytic capacity of a muscle (or muscle fibre), the greater its buffer value.

This conclusion was also inferred from the study of Parkhouse et al (1985) where a significant correlation was observed between the relative proportion of fast twitch fibres in a mixed human muscle sample and its non-protein in vitro buffer
value ($Bviv$). However, no such relationship was observed between the in vivo buffer value ($Bviv$) and % fibre type distribution in the study of Sahlin and Henrikkson (1984).

1.5.2.5 Training effects on the muscle buffer value.

(i) Cross-sectional studies
Two cross-sectional studies have been carried out to date, comparing the buffer value of the quadriceps muscle of trained and untrained individuals (Sahlin and Henriksson, 1984; Parkhouse et al, 1985). Each reported a significantly elevated muscle buffer value in "anaerobically trained" individuals* (18-34% greater than sedentary controls). In the former study, the elevated buffer value was not associated with a significant increase in performance (endurance time at 60% MVC knee extensors) and did not permit the accumulation of a greater concentration of muscle lactate (Sahlin and Henriksson, 1984). The benefit of the reported increase in buffer value therefore remains obscure. In contrast, in the study of Parkhouse et al (1983), a significantly superior high-intensity running performance, and higher post-exercise lactate concentration, were displayed by the trained athletes.

In neither study was the aetiology of the elevated muscle buffer value identified. Using the relevant data given in the study of Parkhouse et al (1983) (i.e. the carnosine concentration, the pKa’ of carnosine and the pH range over which the muscles were titrated), the buffering due to carnosine can be calculated from Equation (5) (Section 1.5.2.2). When this value is then deducted from the total buffer value for each group, it can be observed that the sprinters still demonstrated a 40% greater non-carnosine buffer value than the untrained subjects (36 vs 25 mmol.l$^{-1}$.pH$^{-1}$ respectively). Since the buffer value was determined on deproteinised muscle samples, the only other major mechanism able to influence the buffer value would have been the phosphate concentration. With an 11 mmol.l$^{-1}$ .pH$^{-1}$ difference in non-carnosine buffering, this would require a difference in phosphate levels (or PCr, since this is the predominant source of the phosphate) of

---

* Although the subjects in the study of Sahlin and Henriksson (1984) were highly trained athletes from sports "requiring a high degree of anaerobic energy utilisation" (according to their methods section; pg 332), the reportedly higher % ST fibres, % ST fibre area and mitochondrial enzyme activity, and the lower glycolytic enzyme activity in the muscle of the trained compared with untrained subjects, suggests that their muscles were more typically endurance trained.
approximately 22mmol.l\(^{-1}\)\(^*\). Whilst some difference is expected in the PCR concentration of muscles with differing fibre type distribution, a 20 mmol.l\(^{-1}\) discrepancy has never been observed. No significant differences in either total protein or phosphocreatine concentration were observed between the trained and untrained groups in the study of Sahlin and Henrikkson (1984).

(ii) Longitudinal studies
As with all cross-sectional studies, it is difficult to distinguish whether the observed difference in a given physiological variable is a direct result of long-term training or an inherent factor influencing the athlete's choice of activity. Longitudinal studies must be employed to further investigate the specific effects of training.

A 37\% (range 12-50\%) increase in the in vivo muscle buffer value (\(B_{\text{viv}}\)) was reported for a group of previously untrained subjects who participated in intensive sprint-training, 4 days/wk, for 8 weeks (Sharp et al., 1986). \(B_{\text{viv}}\) was determined following an incremental cycle exercise test (25W increase every min) to exhaustion. Following training the work done during a 45 s high-intensity isokinetic cycling test was increased (28 \%), as was the work time for the incremental exercise test (not quantified). Training induced no change in the extent of PCR utilisation (the only buffering mechanism examined) during performance of the graded exercise test.

A similar training study, demonstrated no significant change (decrease 4.4\%) in HCl titrated muscle samples (\(B_{\text{vit}}\)) following training (Bevan et al., 1985), although muscle carnosine and total protein concentrations increased by 5 and 9 \% respectively (NS). It was suggested that the mechanism responsible for the previously observed changes in \(B_{\text{viv}}\) may reside outside the intracellular environment (i.e. increased or unequal efflux of H\(^+\) or lactate, or active transport of H\(^+\)). Whilst this mechanism could possibly account for the improved performance during the incremental exercise test, it is unlikely to account for the increased work done during the 45 s high-intensity exercise test, for the reasons outlined in Section 1.5.2.1C.

Following 8 wk sprint-training, Nevill et al (1989) observed a non-significant 5 \% increase in \(B_{\text{vit}}\). A concomitant 44\% (NS) increase in \(B_{\text{viv}}\) was observed (determined following 30 s maximal sprinting). The difference was once again speculatated to arise from an increase in the transmembrane flux of H\(^+\) (relative to

\[ * * P_i \cdot nH^+(pH 7.0-6.0) = 0.48. \]  
In titrating from pH 7 to 6, an 11 mmol.l\(^{-1}\).pH\(^{-1}\) difference in B (if due to \(P_i\)) would require \(11/0.48 = 22\)mmol.l\(^{-1}\) difference in \(P_i\).
lactate) during the exercise. However, data from the same study demonstrated no change with training in the mean muscle [lactate] and pH, pre and post-exercise, when the exercise task was a 2 min run at 110% VO\textsubscript{2}max. If training had induced an increase in the muscle's capacity to eliminate H\textsuperscript{+} ions in excess of La anions, (e.g. through an increase in Na\textsuperscript{+}/H\textsuperscript{+} exchange) this would have been expected to assist in effecting a lower H\textsuperscript{+} concentration during the 110% VO\textsubscript{2}max run. It may be argued that before training, the capacity to extrude H\textsuperscript{+} was limiting only during the rapid rates of [H\textsubscript{4}] accumulation of the 30 s sprint, and that at the lower levels and rates of H\textsuperscript{+} production, the maximal capacity of the Na\textsuperscript{+}/H\textsuperscript{+} channels was not exceeded. This is unlikely however, because if the extrusion capacity was not exceeded, then no H\textsuperscript{+} accumulation at all would be expected to occur during the 110% VO\textsubscript{2} max run.

The only study in humans (to the author's knowledge) which reports any significant modification of the in vitro muscle buffer value (B\textsubscript{vit}) is one in which a 2 wk period of altitude training (cross-country skiing) resulted in a 6% (p < 0.05) increase in B\textsubscript{vit} of the gastrocnemius and triceps brachii muscles (Mizuno et al., 1990). It was suggested that hypoxia, rather than the endurance training per se, was responsible for eliciting the increase in B\textsubscript{vit}, with the stimulus for adaptation possible arising as a result of the greater reliance on anaerobic metabolism during the onset of exercise (Mizuno et al., 1990). The increase in B\textsubscript{vit} correlated well with the increase in short-term running performance (time to exhaustion during VO\textsubscript{2}max assessment) (p < 0.05). Of note in this study, were the generally high values of B\textsubscript{vit} (pre-training median values: 298 (range 273-344) and 344 (range 274-438) mmol H\textsuperscript{+}.kg dm\textsuperscript{-1}.pH\textsuperscript{-1} for gastrocnemius and triceps brachii, respectively). As non-bicarbonate buffer values, these are more than double the values expected from the known concentration of the constituents of physicochemical buffering (39.4-43.9 mmol.l\textsuperscript{-1}.pH\textsuperscript{-1} equivalent to 130.1 - 144.9 mmol H\textsuperscript{+}.kg dm\textsuperscript{-1}.pH\textsuperscript{-1}; calculated from Table 1.2, Section 1.5.2.2, assuming 77% muscle water content). Such high values may have been the result of the very dilute nature of the homogenate used (dry muscle: homogenising solution = 1:200 (= approximately 1mg wet muscle.50ul\textsuperscript{-1}). Previous studies have shown that the buffer value of rat EDL muscle increases with increasing dilution from 70 mmol H\textsuperscript{+}.l\textsuperscript{-1}.pH\textsuperscript{-1} at 5mg ww.50 ul\textsuperscript{-1} to 101 mmolH\textsuperscript{+}.l\textsuperscript{-1}.pH\textsuperscript{-1} at 1mg.50 ul\textsuperscript{-1}Nevill et al., 1989). If this represents a methodological error, then it is important to establish whether it influences individual buffer values in an identical fashion (i.e. whether the increase due to dilution causes the same relative increase in all muscle homogenates, or whether it
systematically adds a certain amount to each measured buffer value). Even so, if the relevance of the elevated buffer value is to determine the improved capacity to tolerate the consequences of enhanced metabolic acid production, then true absolute values are essential to quantify these improvements. For example if a muscle generated 80 mmol.kg dm$^{-1}$ lactate, with a buffer value of 150 mmol H$^+$.kg dm$^{-1}$.pH$^{-1}$ then the expected pH change would be 0.533 ($\Delta [La]/B = \Delta pH$). With a 6% higher buffer value of 159 mmol H$^+$.kg dm$^{-1}$.pH$^{-1}$, the same lactate accumulation would produce a pH change of 0.503. The higher buffer value therefore offsets the reduction in pH by 0.030 units. Doing the same calculations with a buffer value of 300 and 318 mmol H$^+$.kg dm$^{-1}$.pH$^{-1}$ (still 6% higher), the change in pH is 0.266 compared with 0.252; the reduction in pH is now offset by only 0.014 units.

Another interesting, unexplained and possibly methodology-related phenomenon regarding the measurement of buffer values, arises from the training study of Nevill et al (1989). The results of their study showed that prior to training, the control group demonstrated a 6% lower in vitro buffer value than the training group (67.6 v 63.8 mmol.l$^{-1}$.pH$^{-1}$ training and control groups, respectively) yet the corresponding in vivo buffer value was 56% higher (87.9 v 138.8 mmol.l$^{-1}$.pH$^{-1}$) training and control groups, respectively). If $B_{vit}$ and $B_{viv}$ are effectively indices of the same physiological entity, then there ought to be some relationship between the two. Clearly, from the above study (Nevill et al, 1989), no such relationship exists. A lack of any relationship between individual values of $B_{viv}$ and $B_{vit}$ is also observed, upon close examination of data reported for equine muscle where $B_{viv}$ was determined either following high-intensity exercise or in post-mortem muscle samples (where the lack of blood flow to the muscle negates a differential H$^+/La^-$ efflux) (Martin, 1989).

The mean buffer value ($B_{viv}; \Delta [La]/\Delta pH$) of 138.8 ± 68.1 mmol.l$^{-1}$.pH$^{-1}$ (=455mmol H$^+$.kg dm$^{-1}$.pH$^{-1}$) in the study of Nevill et al (1989) is also extraordinarily high, and it is difficult to envisage how such a value could be obtained. In Section 1.5.2.2, it was calculated that the buffer value could theoretically reach a maximum of 90 mmol.l$^{-1}$.pH$^{-1}$ (297 mmol H$^+$.kg dm$^{-1}$.pH$^{-1}$) (using maximum reported values for the concentrations of each of the known physicochemical and metabolic buffers, in a fully "open" system). The values of Nevill et al (1989) are 54% greater than this maximum theoretical value. Whilst the differential efflux of lactate/hydrogen ions may serve to slightly elevate the buffer value above that calculated in Table 1.2 (Section 1.5.2.2), it is difficult to
concede that such a disparity (48 mmolH⁺.l⁻¹.pH⁻) could exist. Either additional, unidentified components are contributing to total buffering in that particular subject populace, or a potentially serious error must exist in the use of the $\Delta [\text{La}] / \Delta \text{pH}$ as an indicator of the muscle buffer value. Until such anomalies are investigated, it is impossible to attach any physiological relevance to the parameter "muscle buffer value", as measured in this way.

1.6 AIMS OF THE STUDY

The literature reports a prominent role (both direct and indirect) for intramuscular acidosis in the development of fatigue during intensive muscle contraction. In logical progression, it is expected that the capacity of the muscle to sequester excess hydrogen ions, and thereby delay the point at which pH becomes limiting, would be an important determinant of the muscle's ability to maintain a given high intensity exercise level. Despite this, the experimental evidence collected to date concerning the significance of the muscle buffer value in humans, is by no means conclusive. The controversy is further complicated by the lack of a clearly defined and consistently used method of measurement of muscle buffering, between studies.

The major aims of the following study were therefore:

1) To investigate the potential problems surrounding the current methods of measurement of human skeletal muscle pH and buffer value by the homogenate technique.

2) To compare in the same group of subjects, the buffer values obtained using the "in vitro" method of measurement with those obtained "in vivo" following high intensity isometric and dynamic exercise.

3) To investigate the interrelationships between the muscle buffer value, intramuscular pH, fibre type distribution and high intensity exercise capacity, and examine the effects of high intensity training on each of these parameters.
Chapter 2
2. GENERAL METHODS

The following chapter reports the general methods pertaining to the studies described in Chapters 3 and 4. The specific procedures and protocols for each study reside in the appropriate chapters.

2.1 Performance tests

The studies described in the following chapters, required the implementation of both isometric and dynamic high intensity exercise tests involving substantial usage of the quadriceps femoris muscle group. Isometric performance tests are relatively easy to administer, and interpretation of the data obtained is accordingly, quite uncomplicated. As such, by their simplicity, isometric strength/endurance tests have become well-established (Tornvall, 1963; Edwards et al, 1977; Maughan et al, 1983a, 1983b, Maughan and Nimmo, 1984), and are commonly employed in studies concerned with various aspects of muscle metabolism (Harris et al, 1977; Sahlin et al, 1975; Sahlin, 1978; Sahlin and Henriksson, 1984). Therefore the protocol described in Section 2.1.2, warrants no further justification of its application.

In contrast, dynamic exercise tests are more diverse, and no singular test has the incontrovertible equivalence of the isometric performance tasks. A recent review of "standard anaerobic exercise tests" highlights this diversity (Vandewalle et al, 1987). In recent years, the Wingate Anaerobic Test (WAnT) (Ayalon et al, 1974) has seen increasing use as a laboratory-based, high intensity dynamic exercise test, and several studies have since verified its reliability, validity and sensitivity (for review, see Bar-Or, 1987). The original Wingate Anaerobic Test comprised 30 s maximal cycling against a predetermined resistive load, on a stationary cycle ergometer. The power generated every 5 s was calculated as the product of the average velocity (over 5 s) and the resistive load applied. Peak power output (PPO) was taken as the highest average power over any 5 s period, and mean power output (MPO), the average power sustained throughout the 30 s test period.

Since the introduction of its original prototype, the WAnT has undergone numerous modifications. Some of the most important of these refinements will be described below, in chronological order of their development, with a view to establishing the most appropriate protocol for administration of the test.

i) Automated velocity measurement (by means of photo-electric or electromechanical devices interfaced to microcomputers) has superceded the manual timing of pedal revolutions, improving the accuracy of measurement and allowing for better discrimination between the rapid velocity changes that occur during the test (Lakomy
and Wooton, 1981). More frequent sampling rates, has permitted the identification of higher power outputs than previously reported (Wooton and Williams, 1983; Jakeman and Godfrey, 1984).

ii) Recognition of the importance of the resistive load applied, to the subsequent power generated, has given rise to the concept of load optimisation. The relationship between either mean power output (MPO) or peak power output (PPO) and resistive load is parabolic (although see section (iii)), with the optimal load representing that which corresponds to the apex of the parabola. The precise optimal load shows a) interindividual differences and b) a dependence on the performance parameter being optimised (PPO or MPO) (Evans and Quinney, 1981; Dotan and Bar-Or, 1983; Jakeman and Godfrey, 1984).

iii) More recently, the method of calculation of power output from the WAnT has been subject to criticism (Lakomy, 1985; Coleman et al, 1986). These authors have demonstrated that simply calculating power as the product of resistive load and velocity, with no consideration of the additional work done in accelerating the flywheel, results in an underestimation of the peak power generated during the test. Indeed, the Wingate index of PPO (highest power during any 5 s period) corrected for acceleration was shown to be 20% higher than corresponding uncorrected values (Lakomy, 1986). Additionally, corrected PPO is reported to be less influenced by the resistive load applied, decreasing approximately linearly by 10% over the load range 3.2 - 6.8 J.rev.^-1kg bw^-1 (54-116 g.kg bw^-1), compared with a 32% increase in uncorrected PPO observed over the same range (Lakomy, 1985). MPO appears to be unaffected by applying the correction. Presumably, this is a consequence of the overestimate of work done in the later stages of the test (when kinetic energy stored in the flywheel accounts for some of the mechanical work accomplished (Bassett, 1989)) counterbalancing the underestimate in the initial acceleratory phases, and fortuitously resulting in no difference in corrected and uncorrected values over a 30 s period. Consequently, corrected values of MPO may be different if the test duration is shorter than, or exceeds, 30s.

iv) A further modification, that termination of the test be velocity-dependent rather than time-dependent, has been suggested (Mannion and Jakeman, 1986) on the basis of criticisms arising from proponents of isokinetic cycle power tests (Sargeant et al, 1981; McCartney et al, 1983a). These authors have highlighted that the large changes in velocity occurring during performance of the WAnT, limit the time for which the muscle maintains an optimal velocity of contraction. Indeed, it has been observed that performance of the test against relatively heavy resistive loads frequently results in very low pedal velocities towards the end of the 30 s test period (30-45 rpm; Jakeman and Godfrey (1984)). During this time period, velocity
is far from optimal (Sargeant et al., 1981). Additionally, it is possible that when there is a restriction on the test duration, particularly for more arduous tests, individuals may attempt to start at less than maximal effort with the fear that they might not otherwise complete the task. This would result in an underestimation of PPO and might also compromise the reproducibility of the test.

On the basis of these refinements, it was therefore decided that the most sensitive test of dynamic, high-intensity exercise capacity would be one which:

a) utilises a shorter averaging period than 5 s, for power calculations
b) takes account of the acceleration and deceleration of the flywheel,
c) is employed over a range of resistive loads, and
d) is terminated not upon completion of 30 s work, but upon the inability to exceed a pre-determined velocity.

2.1.1 Dynamic high-intensity exercise test: mWAnT

(i) Modified Wingate Anaerobic Test (mWAnT) protocol

The modified WAnT was performed on a Monark 864 mechanically braked bicycle ergometer (basket weight loading system) which was securely anchored to the ground. The seat height was adjusted for each subject such that there was a slight degree of knee flexion, when the pedal was at bottom dead-centre. The subject's feet were secured to the pedals by means of toe clips, and a belt attached to the rear of the seat was fastened around the subject's waist to ensure a seated position during cycling.

The test was preceded by a warm-up consisting of 5 min submaximal cycling (males 90 W, females 60W) and a 5 s maximal sprint against the load to be employed in the subsequent test. This was followed by 1 min active recovery (backpedalling) then 4 min passive recovery. The test began with the subject pedalling steadily at 90 W (at 60 rpm). After a countdown, the full load was introduced and the subject pedalled with an all-out effort, until instructed to stop. Verbal encouragement was given periodically throughout the test. The test was terminated when the pedal velocity had consistently reduced to less than 60 rpm, after which the load was removed and the subject was encouraged to pedal at will to full recovery.

In the study described in Chapter 3 and Study A of Chapter 4, dynamic exercise performance (mWAnT) was assessed over a range of loads expressed as a ratio to body weight: 0.09-0.14 (males) or 0.08-0.13 (females). In Study B of Chapter 4, the resistive load applied was expressed relative to the maximum voluntary contraction (MVC) of the quadriceps (see Section 2.1.2, this Chapter) (range
employed = 0.09-0.14 MVC). MVC was considered to be a better indicator (than body weight) of the muscle mass involved in the activity; determination of the range according to MVC was therefore expected to yield a more comparable load range for individuals of widely differing body composition (e.g. males v females).

(ii) mWAnT data analysis
Data collection and analysis were slightly different for the various studies reported in this thesis, as a result of improvements made to the velocity sampling technique and modification of the power calculation following the recommendations of Coleman et al (1986) and Lakomy (1986).

(A) For performance tests in Chapter 3 and Study A of Chapter 4:
Flywheel speed was determined by means of a reflecto-optic sensor which discriminated between alternate black and white lines on the rim of the rotating flywheel (Lakomy and Wooton, 1981). The sensor was interfaced to a BBC microcomputer and sampling was carried out at a frequency of 5 Hz. Power was calculated as the load multiplied by the instantaneous velocity (averaged over 1 s periods).

(B) For performance tests in Study B of Chapter 4:
Flywheel speed was determined by means of a photo-optic sensor which discriminated between alternate black and white lines marked on a disc attached to the centre mounting of the flywheel. The sensor was interfaced to a BBC microcomputer and sampling was carried out at a frequency of 25 Hz. Power was calculated taking into account the acceleration/deceleration of the flywheel (averaged over 1 s periods) according to the methods described by Coleman et al (1986).

In both cases, recording of pedal revolutions was initiated approximately 5s prior to the sprint, and sampling continued for a further 55 s. The stored data was then edited, to include only the data representing the work done from the time that the full load was applied, until the pedal revolutions decreased to below 60 rpm. Table 2.1 summarises the parameters derived from the mWAnT performed at each loading ratio. In addition, from the relationship between work done and time of test at each load (Fig. 2.1), the work done over a test lasting 30s was interpolated. This provided a single measure of high intensity dynamic exercise capacity (e.g. for use in correlations with muscle histochemical/biochemical parameters), which was controlled with respect to both duration (30s) and pedal velocity (> 60 rpm).
Fig. 2.1 Relationship between work done and test duration over range of resistive loads, during performance of modified Wingate Anaerobic Test.
Table 2.1. Performance parameters derived from modified WAnT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak power output (PPO)</td>
<td>highest power recorded over 1 sec</td>
<td>Watts</td>
</tr>
<tr>
<td>Mean power output (MPO)</td>
<td>mean power generated whilst pedal revs &gt; 60 rpm</td>
<td>Watts</td>
</tr>
<tr>
<td>Peak velocity (PV)</td>
<td>maximum velocity recorded over 1 sec</td>
<td>rpm</td>
</tr>
<tr>
<td>Duration of test (TIME)</td>
<td>time of velocity-based test</td>
<td>s</td>
</tr>
<tr>
<td>Work done (WORK)</td>
<td>resistive load x flywheel distance moved</td>
<td>kJ</td>
</tr>
</tbody>
</table>

(iii) **Reliability of modified WAnT**

The reliability of measurement of the parameters derived from performance of the modified WAnT, was assessed using 17 subjects (14 male, 3 female). Following one practice attempt, the subjects attended the laboratory on two occasions, to perform a modified WAnT at a loading ratio of 0.09 (load.body weight\(^{-1}\)). Differences between the two test trials, were analysed using a one-way analysis of variance with repeated measures, followed by determination of the intraclass correlation coefficient (reliability coefficient, "R") (Safrit, 1981). No significant difference (p > 0.05) existed between trial (1) and trial (2) for any of the performance parameters, and each demonstrated a reliability coefficient greater than 0.97 (and a CV less than 3.6%) (Table 2.2).
Table 2.2  Test-retest reliability of modified WAnT performance parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>SIG.</th>
<th>R</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>SD</td>
<td>x</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Peak Power Output (W)a</td>
<td>913</td>
<td>173</td>
<td>920</td>
<td>182</td>
<td>n.s.</td>
</tr>
<tr>
<td>Mean Power Output (W)a</td>
<td>626</td>
<td>102</td>
<td>629</td>
<td>100</td>
<td>n.s.</td>
</tr>
<tr>
<td>Work done (kJ)</td>
<td>22.4</td>
<td>4.8</td>
<td>22.2</td>
<td>4.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Test duration (s)</td>
<td>35.4</td>
<td>5.9</td>
<td>35.3</td>
<td>5.0</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* power data analysed according to method A (Section 2.1.1 (ii))
R = reliability coefficient (intraclass correlation coefficient)
CV = coefficient of variation, $\sqrt{\frac{\sum d^2}{2n}} \times \frac{100}{\bar{x}}$ (d = difference between means, n = number of duplicate analyses) (Blomstrand et al, 1984).

(iv) Sensitivity of modified WAnT to training

The sensitivity to training of the modified WAnT was assessed on a group of 10 male subjects (mean ± SD age, body mass and height = 21.0 ± 1.5 yr, 76.3 ± 4.7 kg and 179 ± 5 cm respectively). The subjects were assigned to a training group (n=5) or a control group (n=5). Each subject performed a series of modified WAnTs against a randomly assigned range of 7 resistive loads (0.08 to 0.14 (load:body weight)), during the week before and the week after the training period. Training sessions comprised 6 "all-out" sprints (mWAnTs) on a Monark cycle ergometer, against the subject's own optimal loading ratio (load at which the highest PPO was attained). A 5 min rest period was permitted between consecutive sprints. During a typical training session, both the work done during the final sprint, and its duration, were reduced to approximately 70% of initial values (first sprint). The total work done during one training session (6 sprints) was approximately 85 kJ (1.1 kJ.kg body mass⁻¹). Training was carried out 3 days/week for 6 weeks. Control subjects continued with their usual recreational activities but participated in no specific sprint training.
The training response was analysed using a three-way analysis of variance: group (experimental or control) x training (pre or post) x loading ratio (0.08-0.14), with repeated measures on the latter two factors. Mean values over the total load range, pre and post-training are shown in Table 2.3. Significant increases in both PPO (6% increase) and WORK (20% increase) were observed in the training group (p < 0.05). The mean test duration and MPO increased 14 and 5% respectively in the training group (non-significant compared with control group response).

Table 2.3 Sensitivity of modified Wingate Anaerobic Test to sprint-training.

<table>
<thead>
<tr>
<th>Variable</th>
<th>TRAINING GROUP (n=5)</th>
<th>CONTROL GROUP (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>PPO (W.kg⁻¹)</td>
<td>13.6</td>
<td>14.4 b</td>
</tr>
<tr>
<td>MPO (W.kg⁻¹)</td>
<td>9.5</td>
<td>10.0</td>
</tr>
<tr>
<td>TIME (s)</td>
<td>30.1</td>
<td>34.4</td>
</tr>
<tr>
<td>WORK (J.kg⁻¹)</td>
<td>281</td>
<td>336 b</td>
</tr>
</tbody>
</table>

**a** mean of group mean and SD each test load.

**b** significantly different from pre-training value p < 0.05

The training response in the mean work done during tests with a velocity-dependent cut-off (> 60 rpm), was compared with that achieved using a time-dependent cut-off (30 s) (over a loading ratio range 0.08-0.12). Work done during both the velocity and time-dependent tests showed a significant increase post-training (p < 0.05) but the velocity-dependent test was more sensitive, demonstrating a 17% increase compared with an 8% increase using the 30 s test (Table 2.4).

These results confirm the sensitivity of the modified WAnt to training, in terms of both the PPO and the total work done. Mean power output is given by the total work done / time of test. Since both WORK and TIME were increased following training, this accounts for the lack of a significant change in MPO.
### Table 2.4  Comparison of training response to work done during velocity-dependent (> 60 rpm) and time-dependent (30s) modified Wingate Anaerobic Tests.

<table>
<thead>
<tr>
<th></th>
<th>TRAINING GROUP (n=5)</th>
<th>CONTROL GROUP (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>Velocity-dependent test (&gt; 60 rpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Work Done (J.kg⁻¹)</td>
<td>306ᵃ 55</td>
<td>358ᵇ 72</td>
</tr>
<tr>
<td>Time-dependent test (30s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Work Done (J.kg⁻¹)</td>
<td>281 22</td>
<td>304ᵇ 28</td>
</tr>
</tbody>
</table>

ᵃ significantly different from 30 s test (p < 0.05)
ᵇ significantly different from pre-training value (p < 0.05)

### 2.1.2 Tests of isometric leg strength and isometric endurance

#### (i) Isometric leg strength

Leg strength was determined as the maximum voluntary isometric force generated by the knee extensors, with both hip and knee joints flexed at 90 degrees. The apparatus used was similar to that described by Maughan *et al* (1983b), as illustrated in Fig. 2.2. The back support was adjusted with firm padding such that the knee was positioned at the front edge of the chair and the subject's back was supported in an upright position. The subject was restrained with an adjustable seat belt across the chest and hips which was securely fastened to prevent the tendency of the hip joint to extend when the quadriceps contracted. An inextensible strap (which fastened tightly around the lower leg) was attached to a curved metal plate (moulded to comfortably accommodate the back of the lower leg) which in turn was continuous with a long steel rod screwed into a compression/tension high precision load cell (Showa T500K) (Fig. 2.2). The fixed distance between the leg plate and...
Fig. 2.2 Apparatus used for determination of isometric leg strength (quadriceps maximal voluntary contraction).
the top of the seat (36 cm) maintained a constant lever arm for each subject (regardless of leg length). The output from the load cell was amplified via a high gain strain gauge amplifier (Osca II, Environmental Equipment Ltd.) onto a pen recorder (Phillips).

The linearity of the response of the load cell was confirmed at the start of the study (using a range of forces from 50 to 1000 N) and the calibration was checked weekly, using a 600 N load (accurate to ± 0.5 N), and before each testing session, using a 200 N load.

A 5 min submaximal warm-up on a cycle ergometer (males 90 W, females 60 W) followed by quadriceps stretching exercises, proceeded each testing session. For the assessment of maximal strength, approximately 3-5 trials of maximal voluntary contraction were attempted, with a 15-30 s rest period inbetween trials. Subjects were instructed to remain firmly positioned in the chair and not to attempt to extend the hips during the manoeuvre. They were permitted to grip the sides of the isometric chair to assist in maintaining position. The highest reproducible force output, maintained for 1-2 s, was recorded as the maximal voluntary contraction (MVC) measured in Newtons (N).

(ii) Isometric endurance
Isometric endurance was determined as the time for which 60% maximum voluntary contraction of the quadriceps (see above) could be maintained to fatigue. The force corresponding to 60% MVC was calculated, and marked on the pen recorder which subjects observed constantly throughout the test. A stop-clock was started as soon as the subject reached the target force and stopped when the force declined by > 5% of the target force. More often than not force was maintained steady at 60% MVC then was lost entirely at fatigue; in only a few subjects did the force gradually decline towards the end of the trial. The results were expressed in seconds (endurance time, to the nearest whole second) and also in kN.s (absolute force (60% MVC) multiplied by endurance time = impulse, or work equivalents (Sahlin and Ren, 1989)).

(iii) Reliability of measurement of MVC and isometric endurance
The reliability of measurement of quadriceps MVC and isometric endurance (as described above) was assessed on 42 physically active subjects (22 male, 20 female). The subjects attended the laboratory on three to four occasions for the determination of quadriceps MVC (as described above). The highest value obtained, on each of 3 days, was used in the analysis. Having established the highest
reproducible MVC for each subject, the force corresponding to 60 % MVC was determined. The subjects performed four to five isometric endurance trials (one per day - as described above), and the three best times recorded, were selected for analysis.

The results for each performance parameter were analysed using a one-way analysis of variance with repeated measures, followed by determination of the intraclass reliability coefficient (R) (Safrit, 1981). The results are displayed in Table 2.5. No significant difference (p > 0.05) existed over the three trials for either MVC or 60% MVC, and the reliability coefficients were high (>0.95) for each performance parameter. The MVC tended to be more reproducible, as would be expected for a simpler test of a singular maximal effort.

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Sig.</th>
<th>Reliability coefficient (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVC (N)</td>
<td>(\bar{x}) 547</td>
<td>556</td>
<td>553</td>
<td>n.s.</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>SD 189</td>
<td>201</td>
<td>201</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time at 60 % MVC (s)</td>
<td>(\bar{x}) 48</td>
<td>49</td>
<td>48</td>
<td>n.s.</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>SD 11</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.2 Muscle sampling and analysis

2.2.1 Needle biopsy sampling technique
Muscle samples were obtained from the lateral portion of the quadriceps femoris muscle, employing the needle biopsy technique described by Bergstrom (1962) and Edwards (1971). This portion of the quadriceps is free of major blood vessels and nerves and is therefore a commonly selected site for biopsy. It is utilised extensively during isometric contraction of the knee extensors (Sadamoto et al., 1983) and during cycle ergometry (Ericson et al., 1986; Jorge and Hull, 1986), and thereby serves as an adequate representative of the active muscle mass during these two exercise tasks. All samples were taken by the same clinician (Dr. Peter Willan, Manchester University Medical School) who had considerable experience in the technique.

Muscle biopsy samples were taken with the knee at 90 degrees flexion (see Fig. 2.3). The biopsy site was defined as approximately 10-15 cm proximal to the superior border of the patella. The site was shaved and cleaned with a sterile, alcohol-impregnated swab before local anaesthetic (2-4 ml 1% lignocaine) was infiltrated into the skin and subcutaneous tissue down to the fascia. A 4-6 mm incision was made with a sterile blade, through which the biopsy needle (4.5 mm U.C.H. needle, Shrimpton & Fletcher) was introduced (Fig. 2.3). Once into the belly of the muscle, a small piece of tissue (approximately 55 mg) was guillotined and secured in the barrel of the needle, which was then rapidly withdrawn from the muscle. Repeated muscle samples were obtained through the same initial incision, which was covered with gauze inbetween sampling.

Samples to be used for the measurement of muscle pH, buffer value and metabolite concentration were immediately frozen by plunging the biopsy needle tip into dichlorodifluoromethane ("Arcton 12", ICI) cooled to its melting point (-150 °C) by liquid nitrogen (m.p. -180 °C). Once frozen (approximately 3 minutes), the muscle sample was removed from the needle into a precooled borosilicate vial and stored in liquid nitrogen until further analysis.

2.2.2 Preparation of the muscle for histochemical analysis.
Samples obtained for histochemical analysis were orientated under a dissecting microscope, then mounted in an embedding medium (Tissue-Tek, O.C.T. compound) on a labelled, thin cork disc. The mounted muscle block was carefully dropped into liquid dichlorodifluoromethane (Arcton, ICI) suspended over a flask of
Fig. 2.3 Needle biopsy sampling technique
liquid nitrogen, and snap-frozen. Muscle blocks were stored at -70 degrees C until analysis.

Serial transverse sections, 10 micrometres thick, were cut in a cryostat at -20 °C. A series of six histological and histochemical stains (carried out in duplicate) were applied to the sections:
(1) toluidine blue, (2) haematoxylin and eosin (H & E), (3) nicotinamide adenine dinucleotide tetrazolium reductase (NADH), and (4) (5) & (6) myofibrillar adenosine triphosphatase (ATPase) following pre-incubation at pH 10.2, 4.35 and 4.6. Details of the staining procedures are reported in Appendix 1.

2.2.3 Photomicrography
Monochrome photomicrographs of the whole biopsy cross-section stained with each of the six stains (above), were taken at a magnification of approximately x100, and of the H&E stained section, at a magnification of x500. For each film used, a photomicrograph was taken of a stage graticule (with 10 um gradations) at the appropriate magnification.

2.2.4 Muscle fibre typing.
On the photomicrograph of the H & E stained section (x100) every fibre was numbered using an indelible ink pen (approximately 700 fibres/biopsy). Using both the original microscope slides and the corresponding photomicrographs, each fibre was relocated on the sections stained for NADH and ATPase (following pre-incubation at pH 10.2, 4.35 and 4.6) and assessed for staining intensity. On this basis each fibre was identified as Type I, IIa or IIb (Dubowitz and Brooke, 1973; see Fig 2.4). Occasionally, fibres existed which could not be classified as any of these three types and were simply recorded as "other fibre types"; some could be identified as type IIc fibres (Dubowitz and Brooke, 1973) whilst others more closely fitted the description of a Type IB fibre (Schantz, 1986).

2.2.5 Measurement of muscle fibre area.
Muscle fibre areas were determined by planimetry on photographic enlargements (x 500) of H & E stained sections, using a Grafpad 2+ graphics system (British Micro) interfaced to a BBC microcomputer. Only areas without artefacts, with distinct cell borders and in the middle of the section were measured, following the recommendations of Blomstrand et al (1984). As many fibres as possible were measured, provided that they satisfied these criteria. This resulted in the measurement of approximately 40 % of the fibres in the biopsy cross-section (i.e. approx. 280 fibre areas). Calibration was carried out using the
Fig. 2.4 Fibre type classification:

Serial sections stained
a) with haematoxylin and eosin (H&E)  
b) for NADH  
c) for myofibrillar ATPase (preincubation pH 10.2)  
d) for myofibrillar ATPase (preincubation pH 4.35)  
e) for myofibrillar ATPase (preincubation pH 4.6)
photomicrograph of the stage graticule, and fibre areas were calculated in um². The coefficient of variation for fibre area measurement was 1.0-2.0 %.

2.2.6 Preparation of muscle for measurement of pH, buffer value and metabolite concentrations.

All muscle samples to be used in the analyses of pH, buffer value and metabolite concentration were freeze-dried (over a period of 2-4 h) prior to use. The frozen muscle sample was placed on the weighing pan of a microbalance enclosed in a vacuum head (Robal, C.I. Electronics Ltd.) and housed in a freezer at -30 deg. C (Fig. 2.5). As such, the weight loss of the sample during freeze-drying could be recorded, to determine the % water content and the final dry weight of the muscle. The mean (± SD) water content of resting (n=93), post-isometric (n=75) and post-dynamic (n=18) muscle samples was 77.6 ± 2.0 %, 76.2 ± 1.4% and 76.9 ± 1.5% respectively.

Freeze-dried muscle samples were placed on a glass petri dish under a stereomicroscope and by teasing apart the fibres using watchmaker's forceps, the sample was dissected free of blood and connective tissue. Plastic gloves were worn during the dissection in an attempt to prevent any rise in the humidity in the immediate vicinity of the dried muscle. Although the dissection was a time-consuming procedure, it was considered an imperative treatment prior to analysis, since blood and connective tissue comprised, on average, 22 % of the dry weight (range 1-70%). This has obvious implications for the subsequent metabolite concentrations expressed per mg muscle tissue.

The muscle was redried (to remove any moisture absorbed during the dissection; approximately 8 %), reweighed and then powdered with an agate pestle and mortar. The muscle powder was stored in a borosilicate vial at -30 °C until further analysis.

2.2.7 Extraction procedure for the determination of muscle metabolites

Acid extraction of metabolites was carried out according to the method of Harris et al (1974). 200 ul aliquots of 0.5M perchloric acid containing 1mM ethylene diamine tetraacetic acid disodium salt (EDTA.Na₂) were pipetted into disposable polypropylene vials (Sarstedt) and frozen by placing in an insulated metal block filled with liquid nitrogen. Approximately 2.5 mg powdered muscle (weighed out accurately to 0.01 mg) was added to the frozen perchloric acid. The samples (at < 0 °C) were vortex mixed into an ice slurry, then sequentially agitated for a further 10-12 minutes. Samples were refrozen before adding 50 ul 2.1 M KHCO₃. Samples were spun in a refrigerated centrifuge (IEC Centra 3RS, Damon) at 8 °C and 7000
Fig. 2.5  Apparatus used for freeze-drying muscle samples.

Microbalance enclosed in a vacuum head and housed in a freezer; frozen muscle sample placed on right-hand weighing pan for the recording of water loss during freeze-drying.
rpm for one hour. The lids of the tubes were only lightly screwed on to permit the loss of CO₂ evolved during neutralisation. After equilibrating to room temperature, fixed aliquots of the supernatent were pipetted into separate vials, frozen, and stored at -30 °C until further analysis.

2.2.8 Measurement of muscle carnosine concentration.
Muscle carnosine concentration was determined by high performance liquid chromatography (HPLC) of neutralised perchloric acid extracts (Section 2.2.7), according to the method described by Marlin et al (1989). 20 μl extracts were mixed with 100 μl 0.4 M borate buffer (pH 9.65) in autosampler vials and placed on the carousel of an autosampler (Waters Wisp 712). A 20 μl aliquot of the sample/borate mixture was derivatised with 10 μl O-phthalaldehyde reagent (Pierce Chemical Co.) for 60 seconds in the autosampler syringe before being injected onto the column (150mm x 4.6mm Apex I ODS II, 3μm, Jones Chromatography). The derivatisation time was pre-programmed into the autosampler via the auto-addition accessory.

Amino acids/dipeptides were separated by gradient elution. The gradient was applied to the column over 55 minutes (Fig. 2.6) at a flow rate of 1.2 ml.min⁻¹. Two high pressure pumps (Constametric I and II, LDC Milton Roy), controlled by micro-computer (MP3000 LDC/Milton Roy), were used to provide the gradient. Pump A delivered a solvent comprising 0.1 M sodium acetate buffer (pH 7.2), methanol and tetrahydrofuran in a volume ratio 900:95:5. Pump B delivered methanol. Solvents A and B were filtered (through Millipore filters) and degassed with helium prior to use. Mixing of mobile phases was via a dynamic mixer (LDC Milton Roy). Derivatised amino acids/dipeptides were analysed using a fluorescence detector (Fluoromonitor, LDC Milton Roy) equipped with an excitation monochromator of 365 nm and a broad spectrum emission filter of 418-700 nm. Peaks were integrated by the computer (MP3000 LDC), which provided a printout of the chromatogram (Fig 2.7), and the retention time and peak area for each amino acid/dipeptide.

Carnosine standards (crystalline L-carnosine, Sigma Chemical Co. U.K.) were prepared at concentrations of 60-400 μM. Water used for the preparation of the standards and buffers was glass distilled and deionised through an Elgastat activated charcoal column to remove organics and an Elgastat SC2 ion-exchange column. Linear regression analysis was applied to the plot of carnosine concentration (standards) against peak area, and a coefficient of r = 0.997 was obtained, confirming the linearity of response. The carnosine concentration of each extract was then determined by interpolation from its peak area.
Fig. 2.6 Gradient applied to HPLC column to separate carnosine
Fig. 2.7  HPLC chromatogram of a human muscle extract
The coefficient of variation for the carnosine assay, determined on a 20mM standard solution, taken through the extraction and neutralisation procedure (dilution x 100) to yield a final sample concentration of 200\mu M, was 1.4% (5 samples).

2.2.9 Measurement of metabolite concentrations
Lactate, creatine phosphate, creatine and adenosine triphosphate were determined on neutralised perchloric acid extracts (Section 2.2.7) by fluorimetry (Kontron SMF 25) following the procedures of Lowry and Passoneau (1972). Details of the methods are given in Appendix 2. The actual assays were performed by Dr. Phil Jakeman.

2.2.10 Measurement of muscle homogenate pH
For the measurement of muscle homogenate pH, an aliquot of freeze-dried muscle was weighed out (to an accuracy of 0.01 mg) on a microbalance (Robal, CI Electronics Ltd.) and placed in a 1.5 ml polypropylene vial (Sarstedt) in a cold block at 0°C. The volume of homogenising solution necessary to produce the required final muscle concentration was calculated (variable between experiments; see Chapter 3) and 60% of this volume was pipetted into the vial. The sample was vortex mixed and then homogenised by hand with a tightly fitting PTFE pestle (Wheaton, U.S.A.), for 1 min at 0°C. The excess homogenate remaining on the pestle was rinsed into the sample vial with the remaining 40% of the homogenising solution. The homogenate was centrifuged at 1200 G for approximately 10 s, then briefly vortex mixed before and after equilibration to 37 °C. for 5 min, prior to pH measurement.

All pH measurements were made using an MI-415 microelectrode (Microelectrodes Inc., USA) connected to a Corning 150 pH/ion analyser (Corning, UK). Measurements were made at 37 °C by standing the sample in a temperature controlled block (Fig. 2.8). The pH meter was calibrated prior to each measurement using NBS phosphate buffers (0.0086 M KH\(_2\)PO\(_4\) + 0.029 M Na\(_2\)HPO\(_4\); pH 7.382 and 0.025 M KH\(_2\)PO\(_4\) + 0.025 M Na\(_2\)HPO\(_4\); pH 6.838). The coefficient of variation for the measurement of muscle pH of 10 homogenates from the same muscle block, was 0.2%.

Details of the muscle concentration used in the homogenate, and the composition of the homogenising solution, comprise one of the sub-studies of this thesis and therefore appear in the appropriate section (Chapter 3).
Fig. 2.8 Apparatus used for the determination of muscle homogenate pH
2.2.11 Measurement of in vitro buffer values

Following initial pH measurement (section 2.2.10), muscle homogenates were adjusted to > pH 7.2 with 0.02M sodium hydroxide (NaOH), then titrated by the serial addition of 2 µl aliquots of 0.01M HCl dispensed with a 10 µl S.G.E. glass syringe. The precision and accuracy of the syringe was confirmed by the gravimetric weighing of 10 x 1 µl volumes (x = 0.990 ± 0.016 µl; CV = 1.6 %). Homogenates were briefly vortex mixed following each HCl addition. pH was determined using the "automatic" facility of the pH meter, which ascertained and displayed the stabilised pH value following each acid addition; stabilisation of pH values typically took 10-15 s.

Over the pH range 7.2-6.4, the curve produced by titration of the muscle homogenate (Fig. 2.9) conformed to the middle portion of the classical, sigmoidal shaped buffer curve, typical of all buffer substances. The data from the HCl titration was curve-fitted using a computerised free cubic splines programme, in order to interpolate the volume of acid required to cause a given change in pH. This was considered to yield greater accuracy than interpolating values from a graph fitted by hand. The pH range over which buffering was calculated was 7.1-6.5 (commensurate with the muscle pH values expected pre- and post-high intensity exercise to fatigue (Hultman and Sahlin, 1980)), but values were normalised to the whole pH unit for final expression as mmol H⁺.kg dry muscle mass (dm)⁻¹.unit pH⁻¹.

The slight non-linearity of the slope (Fig. 2.9) (and thus of the buffer value, given by the reciprocal of the gradient at any given pH value) illustrates the importance of stating the pH range over which buffer values are calculated, when expressed per unit pH.

The coefficient of variation for the measurement of buffer value of 10 homogenates made up from the same muscle block, was 2.2 %. 
Fig. 2.9 Typical in vitro titration curve for homogenate of human freeze-dried muscle.

Sample weight = 1.51 mg, homogenised at 30 mg.ml⁻¹
In vitro buffer value = 168 mmol H⁺.kg dm⁻¹.pH⁻¹
Chapter 3
3. METHODOLOGICAL CONSIDERATIONS IN THE MEASUREMENT OF SKELETAL MUSCLE pH AND BUFFER VALUE BY THE HOMOGENATE TECHNIQUE.

Introduction

In order to relate the capacity to perform high intensity exercise to the skeletal muscle buffer value, a reliable and accurate method of measurement of buffer value is required. The measurement of buffer values (B) by either the "in vitro" or "in vivo" method (as described in Chapter 1; Section 1.5.2.3) relies on the accurate measurement of muscle homogenate pH. Procedures routinely used for muscle pH and B determination, involve the prior homogenisation of fresh frozen or freeze-dried muscle in a solution containing 145 mM potassium chloride (KCl), 10 mM sodium chloride (NaCl) and 5 mM iodoacetic acid.

Iodoacetic acid (IAA) is an alkylation agent which reacts with one or more sulphhydryl groups of glyceraldehyde-3-phosphate dehydrogenase, rendering the enzyme inactive and thereby acting as an effective inhibitor to glycolysis. IAA is a weak acid with a pKₐ of < 4.0. From the review in Chapter 1 (Section 1.5.2.1) it would be expected that IAA would have minimal buffering ability within the physiological pH range, and would therefore not influence measurements of muscle pH or B when used in the homogenising cocktail. Indeed, the experimentally determined buffer value of a 5 mM solution of IAA has been shown to be approximately 80 μmol.l⁻¹.pH⁻¹ (Marlin, personal communication) whilst the calculated value, from the Hendersen-Hasselbach equation, yields a value of 68 μmol.l⁻¹.pH⁻¹ over the pH range 7.1-6.5. Although this does represent a typically low B in itself, its importance cannot be overlooked in the muscle homogenate, if relatively low concentrations of muscle are used. With a true muscle B of 160 mmol H⁺.kg⁻¹.pH⁻¹ and an IAA cocktail B of 80 μmol H⁺.l⁻¹.pH⁻¹, the effect of the IAA cocktail on the measured homogenate buffer value can be calculated for different concentrations of muscle (Table 3.1). With decreasing muscle concentration, the buffering due to IAA makes an increasing contribution to the measured muscle buffer value. For example, only above a muscle concentration of 15 g dm⁻¹ does the IAA cocktail contribute less than 5 % to total buffering (Table 3.1).
Nonetheless, the muscle buffer values reported in the literature are frequently
determined using much lower muscle concentrations (e.g. 9.3 g dm.l⁻¹, Nevill et
al, 1989; 5.0 g dm.l⁻¹, Mizuno et al, 1990).

Some of the potential complications arising from this and other chemical
properties of the IAA cocktail solution commonly employed in the measurement of
muscle homogenate pH and B, will be examined in the following experiments.

Table 3.1 Estimated contribution to total buffering from iodoacetic acid cocktail.

<table>
<thead>
<tr>
<th>Muscle conc. (g.l⁻¹)</th>
<th>Bmᵃ</th>
<th>Bcᵇ</th>
<th>Total Bᶜ</th>
<th>Calculated B.kg muscle⁻¹ (mmolH⁺.kg⁻¹.pH⁻¹)</th>
<th>% contribution to calculated B, from cocktail</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>0.2</td>
<td>0.08</td>
<td>0.28</td>
<td>224</td>
<td>29</td>
</tr>
<tr>
<td>2.50</td>
<td>0.4</td>
<td>0.08</td>
<td>0.48</td>
<td>192</td>
<td>17</td>
</tr>
<tr>
<td>5.00</td>
<td>0.8</td>
<td>0.08</td>
<td>0.88</td>
<td>176</td>
<td>9</td>
</tr>
<tr>
<td>10.00</td>
<td>1.6</td>
<td>0.08</td>
<td>1.68</td>
<td>168</td>
<td>5</td>
</tr>
<tr>
<td>15.00</td>
<td>2.4</td>
<td>0.08</td>
<td>2.48</td>
<td>165</td>
<td>3</td>
</tr>
<tr>
<td>20.00</td>
<td>3.2</td>
<td>0.08</td>
<td>3.28</td>
<td>164</td>
<td>2</td>
</tr>
<tr>
<td>30.00</td>
<td>4.8</td>
<td>0.08</td>
<td>4.88</td>
<td>163</td>
<td>2</td>
</tr>
<tr>
<td>50.00</td>
<td>8.0</td>
<td>0.08</td>
<td>8.08</td>
<td>162</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>100.00</td>
<td>16.0</td>
<td>0.08</td>
<td>16.08</td>
<td>161</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>300.00*</td>
<td>48.0</td>
<td>0.08</td>
<td>48.08</td>
<td>160</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

ᵃ Bm = buffering due to muscle (mmolH⁺.pH⁻¹) in a litre of homogenate
(assuming true muscle B = 160 mmolH⁺.kg⁻¹.pH⁻¹)
b Bc = buffering due to IAA cocktail (mmolH⁺.pH⁻¹) in a litre of homogenate
(assuming IAA B = 80 umol.l⁻¹.pH⁻¹)
ᶜ Total B = total buffering (due to muscle + cocktail) (mmolH⁺.pH⁻¹) in a litre of homogenate.
* Muscle concentration in vivo, assuming a 77% water content (Hultman and Sahlin, 1980)
3.1 Methodological considerations in the measurement of muscle homogenate pH

3.1.1 The use of iodoacetic acid within the homogenising cocktail

Methods
The standard IAA cocktail solution used in the following experiments, comprised 5 mM iodoacetic acid (IAA), 10 mM sodium chloride (NaCl) and 145 mM potassium chloride (KCl), titrated to pH 7.0 with 0.1 M bicarbonate-free sodium hydroxide (NaOH). The mixed phosphate buffer (pH 7.06) used in some of the experiments consisted of equal volumes of a 0.086M KH$_2$PO$_4$/0.029M Na$_2$HPO$_4$ buffer (pH 7.382) and a 0.025M KH$_2$PO$_4$/0.025M Na$_2$HPO$_4$ buffer (pH 6.838). All pH measurements were made according to the method described in Chapter 2 (Section 2.2.10).

(i) Stability of IAA cocktail pH over time
The pH of the IAA cocktail was determined at various time intervals over 48 hr. When pH measurements were made over the same day, the sample was maintained at 37°C inbetween measurements; when analysed over consecutive days, it was stored in a refrigerator overnight, then re-equilibrated to 37°C prior to pH measurement.

(ii) Effect of IAA cocktail pH on sample solution pH.
(a) Mixed phosphate buffer (pH 7.06) as sample solution.
The pH and buffer value of 10 μl mixed phosphate buffer (pH 7.06) in 100μl cocktail, is similar to that of a homogenate of resting human muscle at a concentration of 15 g dm$^{-1}$ cocktail$^{-1}$. The phosphate buffer can therefore be used to examine the effects of dilution of a buffer in IAA cocktail, with the advantage that the original pH of the buffer is known. From an original IAA cocktail solution, the pH of which had drifted from 7.0 to pH 6.85 with time (cocktail 1), a volume was withdrawn and readjusted to pH 7.1 with NaOH (cocktail 2). 10 μl of the mixed phosphate buffer (pH 7.06) was pipetted into each of 8 vials. To four of these, 100
ul of cocktail 1 (pH 6.85) was added, and to the other four, 100 ul cocktail 2 (pH 7.1). The samples were vortex mixed, prior to pH measurement.

(b) Acidified mixed phosphate buffer (pH 6.5) as sample solution
In order to mimic the conditions of a post-exercise muscle sample, the mixed phosphate buffer (pH 7.06) was titrated to pH 6.5 with 0.01 M HCl. 10 ul of the acidified buffer solution was pipetted into each of 8 vials containing 100 ul of IAA cocktail solution (pH 7.1). The samples were vortex mixed prior to pH measurement.

(c) Muscle homogenate as sample solution
Two solutions of the IAA cocktail were made up, and each was adjusted to pH 7.0. Portions of 6 freeze-dried human muscle biopsies (Chapter 2) were homogenised at a concentration of 15 mg dw.ml\(^{-1}\) in each of the two IAA cocktail solutions, then measured for pH.

Results

(i) Stability of IAA cocktail pH with time.
Attainment of a stable pH reading for the IAA cocktail required approximately 5 minutes (for a weakly buffered solution, a stable pH is considered to be one which drifts less than 0.1 pH units over a 2 minute period; Bates, 1964). The cocktail pH continually drifted downwards with time, when measured either over the same day or over consecutive days (Table 3.2).

<table>
<thead>
<tr>
<th>IAA cocktail pH</th>
<th>Day (1)</th>
<th>Day (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>7.11</td>
<td>Initial</td>
</tr>
<tr>
<td>+ 40 min.</td>
<td>6.92</td>
<td>+ 3hrs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ 6 hrs</td>
</tr>
</tbody>
</table>

Table 3.2 Change in IAA homogenising cocktail pH with time.
(ii) **Effect of IAA cocktail pH on sample solution pH.**

When a mixed phosphate buffer of known pH (pH 7.06), was diluted in the IAA cocktail solution, the buffer pH decreased (Table 3.3). This occurred (to a lesser extent) even when the cocktail pH was close to that of the buffer solution. The pH of the acidified phosphate buffer was relatively unaffected by dilution in IAA cocktail (Table 3.3).

Muscle homogenate pH was also influenced by the IAA cocktail in which the muscle was homogenised. When portions of the same muscle sample were homogenised in each of two different cocktail solutions (of the same composition and each supposedly adjusted to pH 7 prior to use), different values for homogenate pH were obtained (Table 3.4). As with the mixed buffer solution, this effect was more pronounced in muscle samples with higher pH values. This was not the result of an error in the repeatability of pH measurement per se; using the same cocktail solution to homogenise samples from a given block of muscle, the coefficient of variation (n = 10 trials) for the measurement of homogenate pH was 0.2 %.

**Table 3.3** Effect of IAA cocktail pH on mixed phosphate buffer pH.

<table>
<thead>
<tr>
<th></th>
<th>110μl phosphate buffer</th>
<th>10μl phosphate buffer + 100μl cocktail 1 (pH 6.85)</th>
<th>10μl phosphate buffer + 100μl cocktail 2 (pH 7.10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Final pH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>7.06</td>
<td>6.84</td>
<td>6.96</td>
</tr>
<tr>
<td>SD</td>
<td>0.005</td>
<td>0.006</td>
<td>0.004</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\bar{x})</td>
<td>6.53</td>
<td>--</td>
<td>6.51</td>
</tr>
<tr>
<td>SD</td>
<td>0.006</td>
<td></td>
<td>0.007</td>
</tr>
</tbody>
</table>
Table 3.4  Effect of IAA cocktail on muscle homogenate pH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Muscle homogenate pH TRIAL 1 (IAA cocktail A adjusted to pH 7.0)</th>
<th>Muscle homogenate pH TRIAL 2 (IAA cocktail B adjusted to pH 7.0)</th>
<th>pH unit difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.13</td>
<td>6.98</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>7.04</td>
<td>6.84</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>6.94</td>
<td>6.73</td>
<td>0.21</td>
</tr>
<tr>
<td>4</td>
<td>6.94</td>
<td>6.73</td>
<td>0.21</td>
</tr>
<tr>
<td>5</td>
<td>6.73</td>
<td>6.63</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>6.49</td>
<td>6.46</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Comments

The homogenising solution commonly used in the measurement of muscle homogenate pH consists of 5 mM iodoacetic acid (IAA), 145 mM KCl and 10 mM NaCl. Since this solution has a pH of approximately 2.8, it has been recommended that the cocktail be "adjusted to roughly pH 7.0 with NaOH before use, otherwise the measured pH of the muscle sample will be lowered" (Costill et al, 1982). In practice, this proved difficult to do with any accuracy and the final pH was found to drift appreciably with time, confirming previous findings (Cheetham, 1987). The decrease in cocktail pH with time, possibly occurred as a result of atmospheric CO₂ dissolving in the IAA solution during storage and/or pH measurement. This CO₂ effect is likely to be more noticeable in poorly buffered solutions (Bates, 1964).

Diluting a phosphate buffer in the IAA/NaCl/KCl cocktail solution, not only depressed the measured buffer pH value, but it did so to varying extents depending on the original pH of the cocktail solution. A cocktail solution at one time measured as pH 7.0 but having drifted to pH 6.85 with time, reduced the pH of the phosphate
buffer (pH 7.06) to which it was added, by approximately 0.2 pH units (Table 3.3). The cocktail solution is obviously not acting simply as an inert, diluting medium.

The pH of the iodoacetic acid (IAA) cocktail solution is routinely adjusted to pH 7.0 since this is considered to represent the resting muscle pH. However, in view of the cocktail's ability to influence the pH of a sample, in the fashion illustrated in Tables 3.3 and 3.4, this seems rather like deciding the outcome prior to making the measurement. Moreover, it is difficult to obtain an accurate pH of 7.0 for the cocktail solution, and different solutions which appear to be at pH 7.0 prior to use, can result in homogenates of the same muscle sample demonstrating very different pH values (Table 3.4).

Of note, was the finding that acidified buffer solutions/muscle samples were less affected by dilution in the cocktail solution (Tables 3.3 and 3.4). Whilst the reason for this is unclear, the consequences in terms of the calculation of buffer values, are potentially hazardous. When the method of determination of the in vivo muscle buffer value is considered (ΔLa/ΔpH), it is clear that these non-systematic (with respect to the pH range) manipulations of muscle homogenate pH could significantly affect the final B value calculated. For example, if samples 1 and 6 (Table 3.4) were matching rest and post-exercise muscle samples respectively, with a corresponding change in lactate of 90 mmol.kg⁻¹, then the muscle buffer values, calculated using the pH values determined in cocktail A and cocktail B, would be 140 and 173 mmol H⁺.kg⁻¹.pH⁻¹ respectively; a difference of 24%. There would be little justification for accepting one value in preference to the other.

Some of these problems could possibly be alleviated by ensuring a zero carbon dioxide content of the homogenising cocktail whilst titrating it to pH 7.0 (e.g. by flushing with nitrogen gas then preventing exposure of the solution to air), and by measuring the cocktail pH in this manner prior to each homogenisation. However this would significantly prolong the time necessary to perform each homogenate pH measurement, and the inherent instability and poorly-buffered nature of the IAA cocktail pH may make this a difficult task.
3.1.2 Salt effect on buffer pH

In the preceding experiments (Section 3.1.1) it was shown that even when the pH of the IAA/NaCl/KCl cocktail apparently resembled that of the phosphate buffer to which it was added, the pH of the buffer was still reduced by 0.1 units upon dilution in the cocktail. This suggests that over and above the effect of the original pH of the homogenising solution, is an effect due simply to dilution of the buffer in the cocktail solution. This could possibly be explained by ionic strength effects. Once again, the phosphate buffer represents a useful model for examining these effects, since its exact original pH is known.

Methods

(1) To each of 6 vials containing 1 ml of mixed phosphate buffer (pH 7.06), were added various weights of sodium chloride (NaCl) to make final salt concentrations in the range 0 - 0.16 M. The buffer pH at each NaCl concentration was measured at 37 °C (Chapter 2; Section 2.2.10).

(2) 10 µl mixed phosphate buffer were added to 90 µl of either 0.1, 0.2, 0.3 or 0.4 M KCl/NaCl and the pH measured at 37 °C.

Results

(1) Phosphate buffer pH was a linear function of NaCl concentration (Fig 3.1) over the NaCl range 0-0.2M. Increasing the NaCl concentration within the phosphate buffer, by 0.01 mol.l⁻¹, caused a decrease in the buffer pH value of approximately 0.01 unit (Table 3.5 and Figure 3.1). This value for "the salt effect" is in accordance with values quoted by Bates (1964) for similar phosphate buffers.

(2) Increasing the ionic strength of the solution to which the phosphate buffer was added, resulted in a decrease in measured buffer pH (Table 3.6). Dilution in the solution with an ionic strength of 0.2 M (the approximate ionic strength of the IAA cocktail used in the measurement of muscle homogenate pH), reduced the buffer pH by 0.15 units.
Table 3.5 Effect of salt (NaCl) addition on mixed phosphate buffer pH.

<table>
<thead>
<tr>
<th>Weight NaCl added to 1 ml phosphate buffer (mg)</th>
<th>NaCl conc in buffer solution (mol. l⁻¹)</th>
<th>Buffer pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.000</td>
<td>7.06</td>
</tr>
<tr>
<td>2.25</td>
<td>0.039</td>
<td>7.02</td>
</tr>
<tr>
<td>2.30</td>
<td>0.039</td>
<td>7.02</td>
</tr>
<tr>
<td>4.21</td>
<td>0.072</td>
<td>6.98</td>
</tr>
<tr>
<td>4.53</td>
<td>0.078</td>
<td>6.97</td>
</tr>
<tr>
<td>7.15</td>
<td>0.122</td>
<td>6.92</td>
</tr>
<tr>
<td>9.14</td>
<td>0.156</td>
<td>6.89</td>
</tr>
</tbody>
</table>

Table 3.6 Effect of dilution of mixed phosphate buffer in solutions of increasing ionic strength.

<table>
<thead>
<tr>
<th>Ionic strength of NaCl/KCl (mol. l⁻¹)</th>
<th>10 ul buffer in 100 ul NaCl/KCl pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>---</td>
<td>7.06</td>
</tr>
<tr>
<td>0.1</td>
<td>6.95</td>
</tr>
<tr>
<td>0.1</td>
<td>6.96</td>
</tr>
<tr>
<td>0.2</td>
<td>6.91</td>
</tr>
<tr>
<td>0.2</td>
<td>6.91</td>
</tr>
<tr>
<td>0.3</td>
<td>6.87</td>
</tr>
<tr>
<td>0.3</td>
<td>6.86</td>
</tr>
<tr>
<td>0.4</td>
<td>6.83</td>
</tr>
<tr>
<td>0.4</td>
<td>6.85</td>
</tr>
</tbody>
</table>
Fig. 3.1 Salt effect on phosphate buffer pH.
Comments
When neutral salt (NaCl or KCl) was added directly to the mixed phosphate buffer, or added by means of diluting the buffer in solutions of increasing ionic strength, a reduction in the measured pH of the buffer was observed. The two experimental manipulations are not directly comparable, because complicated within the results of the second experiment, are the changes in pH occurring as a result of dilution per se. The dilution effect can be regarded as opposite in sign to the salt effect (for buffer solutions near the neutral pH) (Bates, 1964). Thus in experiment 2, the magnitude of the difference between the decrease in buffer pH due to the increased ionic strength, and the increase in pH due to dilution, will determine the overall pH change. This explains why diluting the buffer in a solution of ionic strength 0.2 M produces less of a reduction in pH than directly adding 0.2 M NaCl to that same buffer (Tables 3.5 and 3.6). These results have obvious implications for the measurement of muscle pH by the homogenate technique (dilution and homogenisation in a solution of ionic strength 0.16 M).

Both the salt effect and the dilution effect can be explained by changes in the activity coefficients of the buffer species (where the activity coefficient for a species is given by the ratio of its activity to its concentration) (Bates, 1964). When assessing the behaviour of a buffer which exists in a relatively dilute electrolyte solution (< 0.0001 M; Morris, 1974), the terms activity and concentration can be used synonymously, as the activity coefficients of the buffer species can be assumed to approach 1. However, when the electrolyte solution increases in concentration, this same assumption cannot be made and activities rather than concentrations must be considered.

The effect on pH of adding neutral salt to a buffer solution can be examined from a consideration of the buffer equilibria. For a buffer solution composed of an uncharged weak acid HA and its conjugate base A\(^{-}\),

\[
-\log (H^+) = -\log K_a + \log \frac{(A^-)}{(HA)}
\]

where

(A\(^{-}\)), (HA) and (H\(^{+}\)) = activities of A\(^{-}\), HA and H\(^{+}\) respectively, and

K\(_a\) = thermodynamic acid dissociation constant

The activity of a substance = concentration x activity coefficient (γ).

Thus, \log (activity) = \log (concentration) + \log (activity coefficient).
So,

\[
pH = pK_a + \log \frac{[A^-]}{[HA]} + \log \frac{yA^-}{yHA}
\]

where \(pH = -\log (H^+)\), \(pK_a = -\log K_a\)

\([A^-], [HA] = \text{concentration of A}^- \text{ and HA respectively, and}\)

\(yA^-, yHA = \text{activity coefficients of A}^- \text{ and HA respectively.}\)

The primary effect of salt addition is to lower the activity coefficients of the ions. The activity coefficients of uncharged species are affected much less strongly (Bates, 1964). Thus, for a weak acid buffer, increasing the salt concentration would result in a reduction in pH (as \(yA^-\) decreases). Diluting the buffer would result in an increase in activity coefficients, thus causing an elevation in measured pH. This explains the decrease in pH observed when the salt concentration was increased within the mixed phosphate buffer.

The direction and magnitude of change in pH with salt addition or dilution depends on the type of buffer being examined (weak acid-salt buffer mixture or weak base-salt buffer mixture), its concentration and its additional constituents (e.g. salts, strong acids) (Bates, 1964).

Muscle is a complex substance in terms of its array of differing buffers, salt concentration and strong acids (especially when considering post-exercise samples), and predictions of the direction and extent of pH changes with the further addition of salt (e.g. from the homogenising cocktail) cannot readily be made, based on chemical theory. Additionally, examination of the salt effect on muscle pH by experimentation is somewhat more difficult than for a phosphate buffer, since the true, original muscle pH is unknown prior to making the experimental modifications. However, the measured homogenate muscle pH will undoubtedly be influenced by these factors, and it is essential that they are taken into consideration when attempting to create the ideal homogenising solution.

Although the reasons for adding 0.145 M KCl and 0.01 M NaCl to the homogenising cocktail have not, to the author's knowledge, been published, it is reasonable to assume that the intention was to simulate the intracellular ionic conditions observed in vivo. During in vitro experiments performed on whole muscles or single fibres, perfusion/incubation of the muscle with a solution of identical ionic composition to that observed in vivo, is an appropriate and indeed
essential manipulation, to prevent the osmotic movement of substances across the muscle membrane. In a muscle homogenate, this is no longer a necessary requirement, and may even be detrimental to the subsequent measurement of pH. The muscle sample, prior to homogenisation, represents a substance with a fixed concentration of buffers and salt (ions). Once homogenised and diluted in the 0.16M cocktail solution, the homogenate contains a now much lower buffer concentration, but the same high ion concentration. Thus the ratio of salt to buffer concentration is largely elevated. This does not simulate in vivo conditions.

Conclusion
In view of the inherent problems of using a weak buffer/salt solution (iodoacetic acid in KCl/NaCl) for the measurement of muscle homogenate pH, it is suggested that an alternative homogenising solution be sought, that can inhibit glycolysis, but with minimal disturbance to the measured homogenate pH through ionic strength effects or through a contribution to the total buffer value of the homogenate.

3.1.3 The use of fluoride in the homogenising cocktail
Fluoride is a general enzyme inhibitor and is known to specifically inhibit glycolysis (Fig. 1.4) at the level of phosphoglyceromutase, which catalyses the isomerisation of 3-phosphoglycerate to 2-phosphoglycerate (Sarstedt, U.K.). When sodium fluoride (NaF) dissociates in solution, neither of the ions formed (Na+, F−) interact with the ions within water and as such, the concentration of H+ and OH− ions remains equivalent. In this respect, NaF is acid-base neutral. The actual pH of a cocktail solution containing NaF will be governed solely by the dissociation constant of water (Kw) which in turn, depends on the actual salt concentration and temperature of the solution. Therefore, if NaF were used as the inhibitor within the homogenising cocktail in place of iodoacetic acid, the only factor liable to influence the muscle homogenate pH would be that of the total salt concentration (i.e. the concentration of NaF itself and any other ions added to the cocktail). Its potential for use in this regard will be examined in the following section, along with determination of the mimimum concentration required for the inhibition of glycolysis.
Methods

Portions from a number of freeze-dried muscle biopsy samples (Chapter 2; Section 2.2.1) were pooled to provide adequate material for the analyses performed on human muscle. Freeze-dried horse gluteal muscle was kindly provided by Dr. R. Harris (Animal Health Trust, Newmarket). Muscle homogenate pH and lactate concentration ([La]) were measured according to the methods described in Chapter 2 (Sections 2.2.9 and 2.2.10).

(i) Changes in muscle homogenate pH/[La] with time, using distilled water, NaF or IAA/NaCl/KCl cocktail as the homogenising medium

2 mg aliquots of a) resting and b) post-exercise, freeze-dried human muscle were homogenised at a concentration of 20 mg.ml\(^{-1}\), in either:

1) distilled water,
2) IAA cocktail (145 mM KCl, 10 mM NaCl, 5 mM iodoacetic acid - adjusted to pH 7 with NaOH), or
3) NaF (160 mM).

20 μl aliquots of the muscle homogenate (containing the equivalent of 0.4 mg muscle) were withdrawn, pipetted into perchloric acid and analysed for [La] after the following treatments:

A) homogenisation at 0 °C for 1 min
B) homogenisation at 0 °C for 1 min + 2 min. at 37 °C
C) homogenisation at 0 °C for 1 min + 5 min. at 37 °C
D) homogenisation at 0 °C for 1 min + 19 min. at 37 °C

In addition, portions of the same freeze-dried muscle mixes were directly extracted in perchloric acid (HClO\(_4\)) and measured for [La], in order to obtain a control value prior to homogenisation.

On conclusion of each series of experiments (A to D) the pH of the remaining homogenate was measured. (Due to the limited amount of human muscle available, each series of experiments was carried out once only (apart from (1) (distilled water).)
(ii) **Minimal fluoride concentration required to inhibit glycolysis in the muscle homogenate**

2 mg portions of freeze-dried horse gluteal muscle were homogenised at a concentration of 20 mg.ml⁻¹, in 5, 10, 15 or 20 mmol.l NaF⁻¹. Fixed aliquots of the homogenate were withdrawn from the sample, and analysed for [La] following:

A) homogenisation at 0 °C for 1 minute

B) homogenisation at 0 °C for 1 minute + 19 min. at 37 °C.

The pH of the remaining homogenate was measured on conclusion of the series of experiments.

**Results**

(i) **Changes in muscle homogenate pH/\[Lactate\] with time, using distilled water, NaF or IAA/NaCl/KCl cocktail as the homogenising medium**

Muscle lactate concentrations at various times after homogenisation in different solutions are shown in Table 3.7.

There was a slight increase in muscle lactate concentration, above control values, following homogenisation in any of the homogenising solutions. This can probably be attributed to the metabolism of residual glycolytic intermediates existing in the muscle beyond the fluoride/IAA block. There appeared to be no difference between the 3 homogenising solutions, in the concentration of muscle [La] following homogenisation and equilibration at 37 °C, or with time.

Resting muscle homogenate pH was higher (x = 7.19) when distilled water was used as the homogenising solution, as compared with either NaF (homogenate pH = 7.05) or the IAA cocktail (pH 6.94). Post-exercise muscle homogenate pH values showed changes of the same magnitude and in the same direction, with the different homogenising solutions.

(ii) **Minimal fluoride concentration required to inhibit glycolysis in the muscle homogenate**

In the horse gluteal muscle, following incubation at 37 °C, substantially more lactate was produced in the muscle homogenised in distilled water than in the homogenates containing any amount of NaF (Table 3.8). Of the samples containing the inhibitor, there appeared to be slightly more lactate produced (upon incubation) in the muscle homogenised in 5 mmol.l NaF⁻¹, which also recorded the lowest pH value. Tentatively then, an NaF concentration of 10 mmol.l appears to be sufficient to
inhibit any extensive glycolytic activity within a skeletal muscle homogenate, made up at a concentration of 20 mg dry muscle.ml\(^{-1}\).

Table 3.7 Muscle lactate concentration with time after homogenisation in distilled water, NaF or IAA cocktail.

<table>
<thead>
<tr>
<th>HOMOGENISING SOLUTION</th>
<th>HUMAN REST</th>
<th>HUMAN POST-EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactate (mmol.kg(^{-1}))</td>
<td>pH</td>
</tr>
<tr>
<td>No homogenisation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CONTROL) (n=3)</td>
<td>x</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.4</td>
</tr>
<tr>
<td>DIST.H2O</td>
<td>1(^{a})</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>7.18</td>
<td>7.20(^{b})</td>
</tr>
<tr>
<td>NaF (160mM)</td>
<td>1(^{a})</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>7.05</td>
<td></td>
</tr>
<tr>
<td>IODOACETATE/ KCl/NaCl</td>
<td>1(^{a})</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>6.94</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) 1 = immediately after muscle homogenised for 1 min. at 0 °C.
2 = muscle homog. for 1 min. at 0 °C + 2 min. in water bath 37 °C
3 = muscle homog. for 1 min. at 0 °C + 5 min. in water bath 37 °C
4 = muscle homog. for 1 min. at 0 °C + 19 min in water bath 37 °C

\(^{b}\) experiment repeated twice with resting muscle samples in distilled water
Table 3.8 Minimal NaF concentration required to inhibit glycolysis

<table>
<thead>
<tr>
<th>NaF conc. (mmol.l⁻¹)</th>
<th>Lactate mmol.kg dw⁻¹</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
</tr>
<tr>
<td>No homogenisation</td>
<td>16.3 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>24.1</td>
<td>82.6</td>
</tr>
<tr>
<td>5</td>
<td>18.9</td>
<td>24.4</td>
</tr>
<tr>
<td>10</td>
<td>19.1</td>
<td>22.3</td>
</tr>
<tr>
<td>15</td>
<td>18.5</td>
<td>21.7</td>
</tr>
<tr>
<td>20</td>
<td>19.1</td>
<td>21.6</td>
</tr>
</tbody>
</table>

a (1) = immediately post-homogenisation at 0 °C (1 min.)
(2) = homogenisation + 19 min. at 37 °C

Comments
In both human vastus lateralis and horse gluteal muscle, sodium fluoride (NaF) successfully inhibited glycolysis, as determined by the absence of any notable lactate accumulation during a 20 minute incubation period at 37 °C. The minimum concentration of NaF required to inhibit glycolysis in equine muscle, was tentatively defined as 10-15 mmol.l⁻¹.

Of interest, was the finding that freeze-dried human muscle could be homogenised in distilled water with no greater accumulation of lactate than observed in muscle homogenates containing a glycolytic inhibitor. This is in contrast both to previous reports for fresh, frozen muscle samples (Hermansen and Osnes, 1972), and to the results of the present study for freeze-dried equine muscle, which continuously accumulated lactate following homogenisation in distilled water. The
only difference immediately observable between the treatment of the freeze-dried human and equine muscle, was the greater time spent dissecting the human sample free of blood and connective tissue. It has previously been demonstrated that the activity of glycolytic enzymes in freeze-dried muscle samples, declines rapidly in room air (Henriksson and Reitman, 1976). However, when the experiments were repeated following exposure of the horse muscle to room air for 72 hours, appreciable quantities of lactate were still produced when the muscle was homogenised in distilled water and incubated at 37 °C (results not shown). Again, minimal amounts of lactate were accumulated in the human muscle under the same conditions. Thus, it is unlikely that these findings can be explained by a lack of glycolytic enzyme activity within the freeze-dried human muscle samples. Indeed, when the activity of one of the glycolytic enzymes (lactate dehydrogenase, LDH) was assayed in freeze-dried human muscle (according to the method of Bergmeyer, 1974), it was demonstrated that considerable LDH activity was retained in the muscle (results not shown). Even with a reduced glycolytic enzyme activity following freeze-drying and dissection, given the time period of incubation of the muscle homogenate (20 minutes), some accumulation of lactate would still be expected, when the muscle was homogenised in distilled water. The reasons for these findings therefore remain obscure.

In human muscle homogenates, there was no appreciable difference in lactate levels following homogenisation in each of the solutions used, yet the pH values of the corresponding homogenates were different. The greater the ionic strength of the homogenising solution, the lower the measured pH. Thus, with respect to the pH response to salt addition, human muscle behaves in a similar manner to a standard phosphate buffer (increased salt concentration relative to buffer concentration, causes decreased buffer pH - Section 3.1.2). Although the NaF solution and IAA cocktail were of the same ionic strength, the homogenate pH measured in the latter was even lower. This effect can most likely be attributed to the iodoacetic acid per se (see Section 3.1.1).

In conclusion then, it is tempting to speculate that freeze-dried human muscle samples can be homogenised in distilled water for the measurement of homogenate pH. However, the addition of a low concentration of NaF is recommended (approximately 10 mmol.l⁻¹), since this has little effect on the ionic strength of the final homogenate, but as a cautionary measure, gives added protection against any possible glycolytic activity within the muscle.
3.2 Methodological considerations in the measurement of the in vitro muscle buffer value \((B_{vit})\)

Many of the properties of the homogenising solution which influence the measurement of muscle homogenate pH will undoubtedly also affect the muscle buffer value (determined by fixed acid titration), since the effects are predominantly the result of an alteration in buffer equilibrium reactions. The following experiments sought to investigate the factors influencing in vitro \((B_{vit})\) determinations of the muscle buffer value.

**Methods**

All the following experiments were performed on freeze-dried horse gluteal muscle (due to its greater availability), obtained courtesy of Dr. R. Harris (Animal Health Trust, Newmarket). The in vitro buffer value \((B_{vit})\) was determined according to the methods described in Chapter 2 (Section 2.2.11).

(i) Effect of muscle concentration on in vitro muscle buffer values.

Portions from a block of freeze-dried muscle were homogenised at concentrations of 5, 10, 15, 25 and 30 mg.ml\(^{-1}\) in 0.075M NaF/0.075M KCl, and at 5 and 20 mg.ml\(^{-1}\) in 0.01M NaF (the weight of muscle was adjusted in each case such that the final volume of homogenate remained constant at each dilution). The homogenates were then titrated for the determination of \(B_{vit}\). Differences between \(B_{vit}\) determined at each muscle concentration in the NaF/KCl solution, were analysed using a one-way analysis of variance. Where significant F values were found, a Tukey test was used to locate the significant mean differences. Significance was accepted at the 5 % level.

(ii) Effect of ionic strength of the homogenising solution on in vitro muscle buffer values.

Portions from a block of freeze-dried horse gluteal muscle were homogenised at a concentration of 10 mg.ml\(^{-1}\) in solutions of ionic strength 0.05, 0.30 and 0.50 M (NaF:KCl; 1:1). The homogenates were then titrated for determination of \(B_{vit}\).
Results

(i) Effect of muscle concentration on \textit{in vitro} muscle buffer values.

The effect of muscle concentration on $B_{vit}$ is shown in Table 3.9 and Fig 3.2. Data from a similar study reported in the literature, carried out on rat EDL homogenised in an iodoacetate cocktail (Cheetham, 1987), are also included for comparison.

Using the NaF/KCl homogenising solution (ionic strength = 0.15M), the measured muscle buffer value (mmol.kg dm$^{-1}$pH$^{-1}$) decreased with increasing concentration of muscle in the homogenate. The decrease was non-significant ($p > 0.05$) above a muscle concentration of 15 mg.ml$^{-1}$. When the data of muscle concentration versus mean buffer value was entered into a computerised curve-fitting programme, the data was best described by the equation:

$$y = 181.4 + \frac{498.2}{x} \quad (r = 0.99)$$

where $x$ = muscle concentration (mg.ml$^{-1}$), $y$ = muscle buffer value (mmol.kg dm$^{-1}$pH$^{-1}$)

Using the equation to extrapolate to a muscle concentration of 300 mg.ml$^{-1}$ (the "concentration" \textit{in situ}, assuming a 77% muscle water content), the corresponding muscle buffer value would be 183 mmol H$^+$.kg$^{-1}$pH$^{-1}$; only 5% less than the value measured at the highest muscle concentration used in the present experiments (30 mg dm.ml$^{-1}$). Using this value (183 mmol H$^+$.kg$^{-1}$pH$^{-1}$) in conjunction with the difference in mean values required to achieve significance (determined from the ANOVA and Tukey test), there was no significant difference between the buffer value estimated for the \textit{in situ} muscle concentration and that determined in homogenates made up at a muscle concentration of 20 mg dm.ml$^{-1}$ or greater.

With 0.010 M NaF as the homogenising medium, the increase in $B_{vit}$ with decreasing muscle concentration was less marked (Fig. 3.2); the muscle buffer value determined at 5 mg.ml$^{-1}$ was only 19% higher than that determined at 20 mg.ml$^{-1}$, compared with the 36% higher value obtained at 5 mg.ml$^{-1}$ using the NaF/KCl solution (of a higher ionic strength; $I = 0.15$ M).
Fig. 3.2 Effect of muscle concentration (in homogenate) on the *in vitro* buffer value (*Bvit*).

(Standard deviations omitted for clarity; see Table 3.9.)
Table 3.9  Effect of muscle concentration (in homogenate) on *in vitro* buffer values.

<table>
<thead>
<tr>
<th>Muscle concentration (mg dm.ml homog sol⁻¹)</th>
<th>Muscle buffer value (mmol H⁺.kg dm⁻¹.pH⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HORSE GLUTEAL MUSCLE</td>
</tr>
<tr>
<td></td>
<td>RAT EDL a</td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>n = 5*</td>
<td>5</td>
</tr>
<tr>
<td>n = 4</td>
<td>10</td>
</tr>
<tr>
<td>n = 3</td>
<td>15</td>
</tr>
<tr>
<td>n = 3</td>
<td>25</td>
</tr>
<tr>
<td>n = 2</td>
<td>30</td>
</tr>
</tbody>
</table>

* number of observations (present study).

a data from Cheetham (1987), converted from mmol H⁺.l⁻¹.pH⁻¹ to mmol H⁺.kg dm⁻¹.pH⁻¹ by multiplying values x 3.3 (Hultman and Sahlin, 1980). Conditions of measurement: wet muscle homogenised in 145mM KCl, 10mM NaCl and 5mM IAA.

b muscle concentration = 17 mg.ml⁻¹

c significantly different (p < 0.05) from Bvit at 30 mg.ml⁻¹

(ii) Effect of ionic strength of the homogenising solution on *in vitro* muscle buffer values.

Increasing the ionic strength of the homogenising solution, resulted in an increase in Bvit (Table 3.10 and Fig. 3.3). There was a minimal difference (6%) between the mean muscle buffer value obtained after homogenisation in solutions of either 0.05M or 0.3M ionic strength, but each was approximately 45% lower than the corresponding values using the I = 0.5 M solution.

Figure 3.4 shows typical buffer curves derived from individual titrations of portions of the same muscle, homogenised at 10 mg.ml⁻¹ in solutions of ionic
**Fig. 3.3** Effect of ionic strength of the homogenising solution on the *in vitro* buffer value (*Bvit*).

(Standard deviations omitted for clarity; see Table 3.10.)
Fig. 3.4 Individual titration curves for muscle samples homogenised in solutions of varying ionic strength.
strength 0.05, 0.3 or 0.5 M. It can be seen that the greater buffer value of the 0.5 M homogenate, arises from its improved buffering ability over the pH range 6.9 - 6.5. Above pH 6.9, each homogenate appeared to display similar gradients for their titration curves and thus, similar buffer values.

Table 3.10 Effect of ionic strength of homogenising solution on in vitro buffer values.

<table>
<thead>
<tr>
<th>Ionic strength of homogenising solution (M)</th>
<th>Muscle buffer value (mmol H⁺.kg dm⁻¹.pH⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>n = 3</td>
<td>0.05</td>
</tr>
<tr>
<td>n = 2</td>
<td>0.30</td>
</tr>
<tr>
<td>n = 3</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Comments
The experiments carried out to examine the effects of (i) muscle concentration and (ii) ionic strength of the homogenising solution, on the muscle buffer value, cannot be quantitatively compared as they were carried out on two separate biopsy samples (possibly with true differences in their buffer values). However, it would appear that the greater the ratio of salt:buffer concentration, the higher the measured muscle buffer value. Considering experiment (i), increasing the concentration of muscle in the homogenate, is analogous to increasing the buffer concentration. Since the salt concentration of the homogenising solution remains constant (0.15 M), the salt:buffer concentration ratio decreases with increasing muscle concentrations. This will be partially offset by the salt (ion) contribution from the muscle itself (calculated as approximately 0.5 mmol associated with 1 g dry muscle - Lindinger, personal communication), but even at a muscle concentration of 30 g.l⁻¹ this only adds 0.015 mol salt.l⁻¹ to the homogenate.
Thus the salt:buffer ratio is given by:

\[
\frac{[\text{Salt}]_{\text{homog. sol}} + (\text{muscle concn x salt g muscle}^{-1})}{(\text{muscle concn x buffer value g muscle}^{-1})}
\]

Where:

- \([\text{Salt}]_{\text{homog. sol}} = \) salt concentration in the homogenising solution (mol.\text{l}^{-1})
- \(\text{muscle concn} = \) muscle concentration in the homogenate (g.\text{l}^{-1})
- \(\text{salt g muscle}^{-1} = \) moles of salt per g of dry tissue
- \(\text{buffer value g muscle}^{-1} = \) true buffer value, mmol H\(^+\) g dry muscle\(^{-1}\).\text{pH}^{-1}

This equation would explain why there was less of a difference in buffer value with changing muscle concentration when the muscle was homogenised in a solution of much lower ionic strength (0.010 M NaF) (Fig. 3.2). In this instance, the muscle salt (or ion concentration) represents a relatively greater proportion of the total salt concentration in the homogenate, and thus reduces the salt:buffer ratio differences with changing muscle concentration.

The equation would also explain the results of experiment (ii), where increasing the ionic strength of the homogenising solution (from 0.05 M to 0.5 M) at the same muscle concentration (10 mg.m\text{l}^{-1}), resulted in an increase in measured buffer values (Table 3.10; Fig. 3.3).

When muscle is homogenised in an iodoacetic acid cocktail (with the same ionic strength as the 0.15 M NaF solution), a qualitatively similar relationship between the measured buffer value per mg muscle and homogenate muscle concentration, is obtained (increased buffering with decreased muscle concentration) (Marlin, 1989; Cheetham, 1987). In the study of Cheetham (1987) rat extensor digitorum longus (EDL) muscle was used, which is expected to have a much lower buffer value than horse gluteal muscle (Bate Smith, 1938; Cheetham, 1987). However, this was not the case; the buffer values for rat EDL were some 14-25 % greater than those obtained for horse gluteal muscle in the present study.

Part of this discrepancy may be accounted for by bicarbonate buffering, which is presumably available in fresh-frozen wet muscle samples, but is lost from freeze-dried muscle samples (Harris \textit{et al}, 1989). It is possible that the buffering from iodoacetic acid itself, contributing a relatively greater amount with decreasing muscle concentrations (Table 3.1), could account for the remaining difference.

The comments following the experiments of Section 3.1.2 described how increasing the salt concentration decreased, through interionic effects, the activity
coefficients of the ionic species of the buffer solution. This same mechanism could possibly also explain the variations in the measured buffer value observed with changing salt concentrations.

The thermodynamic acid dissociation constant for a substance, $K_a$, has a truly constant value at a given temperature, which is calculable from the *activities* of the components of the dissociation reaction at equilibrium. However, *apparent* values of $K_a$ ($K_a'$), determined from the *concentrations* of the components at equilibrium, will be affected by the ionic strength of the solution. In the dissociation of a hypothetical weak acid HA:

$$\text{HA} \rightleftharpoons \text{H}^+ + \text{A}^-,$$

the law of mass action states that,

$$K_a = \frac{(\text{H}^+)(\text{A}^-)}{[\text{HA}]}$$

where $K_a = \text{thermodynamic} \text{ dissociation constant, and}$

$$\frac{\text{(H}^+)}{(\text{A}^-)} \text{ and } \frac{[\text{HA}]}{[\text{HA}]} = \text{activities of H}^+, \text{A}^- \text{ and HA respectively.}$$

Since the activity of a substance = concentration $\times$ activity coefficient,

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} x \frac{y \text{H}^+ x y \text{A}^-}{y \text{HA}}$$

where $[\text{H}^+], [\text{A}^-]$ and $[\text{HA}] = \text{concentrations of H}^+, \text{A}^- \text{ and HA and,}$

$y \text{H}^+, y \text{A}^- \text{ and } y \text{HA} = \text{activity coefficients of H}^+, \text{A}^- \text{ and HA.}$

Since $K_a' = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$ where $K_a' = \text{apparent} \text{ dissociation constant}$

then 

$$K_a = K_a' x \frac{(y\text{H}^+ x y\text{A}^-)}{y \text{HA}}$$

(adapted from Morris, 1974).

Because the thermodynamic $K_a$ remains constant under all conditions, if $y\text{H}^+$ and $y\text{A}^-$ decrease more so than HA (as they do in a solution of high ionic strength; Bates, 1964), then $K_a'$ must increase (Morris, 1974). If $K_a'$ increases, then $\text{pK}_a'$ (apparent $\text{pK}_a$) decreases (since $\text{pK}_a' = -\log K_a'$).

With regard to muscle homogenates, this could affect the buffer value in three ways:

1) A substance operates optimally as a buffer when its $\text{pK}_a'$ is numerically similar to the pH of the solution within which it exists (see Chapter 1). There are likely to be potential buffer species existing in appreciable quantities within muscle,
which ordinarily, would contribute only minimally to buffering in the pH range 7.1 to 6.5, because their apparent dissociation constants are > 7.1 (e.g. the terminal amino groups of amino acids, peptides and proteins (pKₐ' 7.4 - 8.5)). If however, the muscle was homogenised in a solution of high ionic strength, the pKₐ' values of these species would be reduced, and they might then be able to make a significant contribution to the overall buffer value recorded over the pH range 7.1 - 6.5. Obviously, at the other end of the continuum, it is possible that buffers which originally had pKₐ' values suitable for buffering over this pH range, may lose or decrease their buffering ability upon homogenisation in a strong salt solution, as a consequence of a now, much lowered pKₐ' value. It is therefore the concentration of potential buffers with pKₐ' values just above, just below and within the range 7.1 - 6.5, that will determine the overall muscle buffer value in solutions of varying salt concentration.

2) The concentration of hydrogen ions required to cause a given change in pH, increases at low pH values, simply by virtue of the logarithmic expression of pH. For example, if a quantity of hydrogen ions were added to a litre of buffer solution, pH 7.1, which was capable of buffering all but 46 nmol of these hydrogen ions, the excess 46 nmol.l⁻¹ H⁺ ions would cause a pH decrease of 0.2 units to pH 6.9. However, the same excess of 46 nmol.l⁻¹ H⁺ would cause a litre of solution originally at pH 6.8 to decrease by only 0.11 units, to pH 6.69. The second solution would therefore appear to be a better buffer (based on pH changes), even though the absolute change of hydrogen ions was the same. Thus a buffer substance appears to be able to withstand the addition of relatively more hydrogen ions for a given change in pH, when it operates over a lower pH range. Since adding salt to a buffer solution decreases the pKₐ' value, this permits the buffer to work optimally over a lower pH range and thus demonstrate an apparently greater buffer value, as determined by ∆ [H⁺]/ ∆ pH.

3) Not only will the buffer ions' activity coefficients be reduced by increasing the ionic strength of the solution in which they exist, but so too will those of the dissociated ions of the hydrochloric acid against which the muscle homogenate is titrated. Thus, the activity of the hydrogen ions released from dissociated HCl will be much lower than the added concentration (when added into a strong salt solution) and will therefore represent less of an acid load for the buffers to sequester. Since the buffer's titration curve is determined from the concentration of HCl added (and pH is measured from the activity of the free hydrogen ions) this will produce falsely elevated muscle buffer values.
Conclusion
Some of the foregoing experimental manipulations are clearly non-physiological (e.g. ionic strengths of 0.5 M), but they do serve to illustrate the effect of these physicochemical processes on measured buffer values, and thereby highlight the fact that measurements of muscle buffering cannot be made without a regard for, and adherance to, the principles of basic chemistry. They also serve to assist in arriving at optimal conditions/procedures for the measurement of muscle pH and buffer values, through examination of their proximity to "the ideal".

If indeed, the salt:buffer concentration is of ultimate importance in the subsequent measurement of pH or buffer values, then the closest approximation to in vivo conditions, is given by homogenisation of the muscle in as dilute a solution as possible (with respect to its salt concentration, since the muscle retains its own original salt concentration), and by using muscle concentrations as great as available muscle and methodology will allow. Sodium fluoride is recommended for inclusion within the homogenising solution, since it is required in a relatively low concentration to successfully inhibit glycolysis, and existing as a neutral salt, it is unlikely to adversely affect the acid-base conditions of the muscle itself.
3.3 Comparison of *in vivo* and *in vitro* buffer values (*B*) in human muscle

An index of the muscle buffer value can be obtained either by measuring the change in lactate concentration relative to the change in muscle pH during a single bout of exercise ("*in vivo*" buffering; \(B_{viv}\)), or by titrating the muscle homogenate with a fixed acid ("*in vitro*" buffering; \(B_{vit}\)) (see Chapter 1). In theory, it is expected that \(B_{viv}\) would yield higher values than \(B_{vit}\), since the former not only determines the physicochemical buffering, but it also includes the contribution from the metabolic consumption of hydrogen ions, and possibly, the unequal efflux of H\(^+\) ions (with respect to La\(^-\)) from the muscle (Hultman and Sahlin, 1980).

The following study was designed to examine whether the current methods of measurement are indeed accurate enough to confirm the theoretically expected differences in \(B_{vit}\) and \(B_{viv}\), and to arrive at an estimate of the respective components of total buffering encompassed by each method of measurement.

**Methods**

**Subjects.** Eighteen young, active male (n=12) and female (n=6) volunteers agreed to participate in the study. Each was informed of the purpose, and potential risks of the study before their written voluntary consent was obtained. The mean ± SD age, height and body mass of the subjects was 22.4 ± 3.3 yrs, 178.1 ± 6.7 cm, and 75.9 ± 7.3 kg respectively for the males and 23.5 ± 4.8 yrs, 167.2 ± 8.3 cm and 66.3 ± 7.4 kg respectively for the females.

**Exercise tasks.** For the determination of in vivo buffer values (\(\Delta \text{La}/\Delta \text{pH}\)), two different modes of high intensity exercise were employed:

1) **dynamic exercise** - performance of a modified Wingate Anaerobic Test at the optimal load for peak power production (Chapter 2; Section 2.1.1), extended in duration such that termination of the test coincided with the inability to continue cycling, and

2) **isometric exercise** - maintenance of 60% of the maximum voluntary contraction of the quadriceps to fatigue (Chapter 2; Section 2.1.2).

Habituation to the given exercise task was carried out in the week preceding the biopsy trials, but the subjects refrained from any intensive exercise on, or 24 hours before, the experimental day. In vivo buffer values were determined for each subject following both dynamic (\(B_{vivD}\)) and isometric (\(B_{vivI}\)) exercise to
fatigue (crossover design). Each trial was separated by a period of approximately two months.

**Muscle sampling.** Muscle samples were obtained from the lateral portion of the quadriceps femoris muscle, pre- and post-exercise, using the needle biopsy technique (Chapter 2; Section 2.2.1). Biopsy samples were snap-frozen, freeze-dried and dissected free of blood and connective tissue (Chapter 2; Section 2.2.6).

**Analytical methods.** Resting and post-exercise muscle lactate ([La]), phosphocreatine ([PCr]), creatine ([Cr]) and adenosine triphosphate ([ATP]) concentration, homogenate pH, and $Bv_{vit}$ were determined according to the methods described in Chapter 2 (Section 2.2). The pH of all muscle samples was originally measured on homogenates made up at a concentration of 15mg dm.ml$^{-1}$ in an iodoacetic acid (IAA) cocktail solution (Section 3.1.1). In view of the problems associated with use of the IAA cocktail solution (highlighted earlier in this chapter), the measurements were repeated at a muscle concentration of 30 mg dm.ml$^{-1}$ using 0.010 M sodium fluoride (NaF) as the homogenising solution. Only values obtained using the latter method are reported in this section; the mean pH values using the IAA cocktail solution are reported in Appendix 3. Insufficient muscle was available to repeat the measurements on all samples, accounting for the lower subject numbers in some of the results tables.

**Statistics** Results are expressed as arithmetic mean values, plus or minus one standard deviation. The significance of differences between mean values was examined using a one-way analysis of variance or a paired t-test.

Linear regression analysis was applied to the pH and [La] data for the group, and the mean in vivo $B$ was given by the reciprocal of the gradient (Sahlin, 1978). This was expected to give a better representation of $Bv_{vit}$ than calculation of the mean from individual values of $\Delta [La]/\Delta pH$. Taking individual values of $\Delta La$ and $\Delta pH$ is analogous to constructing a titration curve with a single pair of values at the start and finish of the titration, and this places complete reliance on the accuracy of the two single points in the curve; only slight inaccuracies in the determination of the pH value or the [La] of single samples are needed to induce large errors in individual, and therefore in group mean, values of $Bv_{vit}$. The importance of freezing the resting muscle sample within 2s of excision for pH, but not [La] determination (Costill et al, 1982) highlights one such potential inaccuracy. The large coefficients of variation (SD/mean) reported in previous studies for the measurement of group mean $Bv_{vit}$ from individual values of $\Delta [La]/\Delta pH$ (CV = 31% (vs 10% for $Bv_{vit}$) (Marlin, 1989) and 28-49 % (vs 6-10% for $Bv_{vit}$) (Nevill...
et al, 1989)) indicate that this may not be the most appropriate treatment of the data. It is expected that analysis of the group data by calculation of the slope of the [La] vs pH relationship for all values, may diminish the influence of these potential errors, and give a more accurate measure of mean Bviv for the group.

Analysis of covariance (Snedecor and Cochran, 1980) was used to evaluate the significance of differences between the pH/La relationship obtained during isometric and dynamic exercise, in terms of the slope and intercept derived from the linear regression analyses.

Significance was accepted at the 5 % level (actual significance level ("p" value) given where available).

Results

The performance results obtained after habituation, and on the day of the biopsy are shown in Table 3.11. Biopsy sampling had no significant effect on performance in the subsequent isometric or dynamic exercise tasks (p > 0.05). Both forms of exercise resulted in fatigue within a mean time period of less than 60 s.

The mean resting and post exercise (dynamic (D) and isometric (I)) values of [La], [PCr], [TOT Cr], [ATP] and homogenate pH are shown in Table 3.12 (insufficient muscle was available to obtain a measurement for each variable on every muscle sample - the appropriate "n" values are reported at the head of each column). All values for [PCr] and [ATP] tended to be slightly low; a finding observed previously when a similarly extended period of time was taken in dissecting the freeze-dried muscle samples prior to analysis (see Methods, Chapter 2) (Tesch et al, 1989b). However, this has been shown not to influence the relative decrease in [PCr] or [ATP] (pre to post-exercise) (Tesch et al, 1989b).

No significant differences were observed between pre-isometric and pre-dynamic-exercise values of pH, [La], [PCr], [ATP] or [TOT Cr] (pH p=0.118; [La] p=0.270; [PCr] p=0.231; [ATP] p=0.052; [TOT Cr] p=0.150). A significant increase in [La], and decrease in [PCr] and homogenate pH were observed following both isometric exercise (p=0.0000, p=0.0000 and p=0.0026 respectively) and dynamic exercise (p=0.0001, p=0.0000 and p=0.0002 respectively). A significant decrease in [ATP] was observed post-dynamic (p=0.018) but not post-isometric exercise (p=0.259). [TOT Cr] was unchanged pre to post isometric (p=0.496) or dynamic exercise (p=0.399). The Δ [La] (rest to post-exercise), Δ pH and Δ ATP were significantly greater following dynamic
than isometric exercise (p=0.002, p=0.005 and p=0.036 respectively). \( \Delta [PCr] \) was not significantly different between the two exercise modes (p=0.263).

**Table 3.11** Performance data post-habituation and on day of biopsy (n = 18).

<table>
<thead>
<tr>
<th></th>
<th>Post-habituation</th>
<th>Biopsy Trial</th>
<th>Significance (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ISOMETRIC:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impulse (Work equivalents(^a)) (kN.s)</td>
<td>22.1 ± 7.3</td>
<td>22.3 ± 8.0</td>
<td>0.444</td>
</tr>
<tr>
<td>Endurance at 60% MVC(^b) (s)</td>
<td>58.5 ± 11.9</td>
<td>59.0 ± 11.5</td>
<td>0.441</td>
</tr>
<tr>
<td><strong>DYNAMIC:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work done whilst rpm &gt; 60 (^c) (kJ)</td>
<td>20.0 ± 5.2</td>
<td>20.5 ± 5.6</td>
<td>0.282</td>
</tr>
<tr>
<td>Time whilst rpm &gt; 60</td>
<td>25.7 ± 6.1</td>
<td>26.6 ± 3.9</td>
<td>0.199</td>
</tr>
<tr>
<td>Time to fatigue (s)</td>
<td>---</td>
<td>44.5 ± 6.7</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± standard deviation
\(^a\) from Sahlin and Ren, 1989.
\(^b\) MVC = maximum voluntary contraction
\(^c\) see Chapter 2

Table 3.13 shows the results of the regression analyses of muscle homogenate pH on [La], for dynamic and isometric exercise. There was no significant difference between either the slope (p=0.182) or the elevation (p=0.338) of the regression lines following each type of exercise. The values of \( Bviv \) (reciprocal of the slope) were 217 and 168 mmol H\(^+\).kg dm\(^{-1}\).pH\(^{-1}\) for isometric (\( BvivI \)) and dynamic (\( BvivD \)) exercise respectively. When the results from an
Table 3.12  Muscle lactate ([La]) and phophocreatine ([PCr]) concentrations and muscle homogenate pH in samples obtained at rest and following either isometric or dynamic exercise to fatigue

<table>
<thead>
<tr>
<th></th>
<th>REST</th>
<th>POST-DYNAMIC EXERCISE</th>
<th>REST</th>
<th>POST-ISOMETRIC EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate muscle pH</td>
<td>7.20 ± 0.08</td>
<td>6.73 ± 0.15</td>
<td>7.17 ± 0.09</td>
<td>6.94 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
</tr>
<tr>
<td>[La] mmol.kg dm(^{-1})</td>
<td>6.7 ± 2.1</td>
<td>84.6 ± 25.4</td>
<td>5.0 ± 2.3</td>
<td>49.0 ± 19.5</td>
</tr>
<tr>
<td></td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
</tr>
<tr>
<td>[PCr] mmol.kg dm(^{-1})</td>
<td>53.4 ± 13.3</td>
<td>14.1 ± 10.5</td>
<td>57.4 ± 6.8</td>
<td>21.5 ± 14.9</td>
</tr>
<tr>
<td></td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 10</td>
</tr>
<tr>
<td>[TOT Cr] mmol.kg dm(^{-1})</td>
<td>120.9 ± 7.3</td>
<td>121.5 ± 15.7</td>
<td>115.8 ± 9.6</td>
<td>115.8 ± 10.1</td>
</tr>
<tr>
<td></td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 10</td>
</tr>
<tr>
<td>[ATP] mmol.kg dm(^{-1})</td>
<td>18.2 ± 2.4</td>
<td>14.7 ± 3.2</td>
<td>20.4 ± 2.9</td>
<td>19.8 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 8</td>
<td>n = 10</td>
</tr>
</tbody>
</table>

\(a\) significant difference pre v post-exercise (p < 0.05; see text for actual "p" values)

\(b\) Pre to post exercise difference = significantly different dynamic vs isometric exercise (p < 0.05; see text)
additional 27 samples taken using the same protocol pre and post-isometric exercise (see Chapter 4; pre-training samples) were included in the analysis, the reciprocal of the slope gave a $B_{viv}$ of 170 mmol H⁺.kg⁻¹.pH⁻¹ (n=35) (and the additional samples improved the correlation coefficient between pH and [La] from 0.78 to 0.87).

The mean $B_{viv}$ calculated from individual values of $\Delta [La]/\Delta pH$ are included in Table 3.13 and confirm the inappropriateness of this method of analysis; following isometric exercise, the group mean $\Delta [La]/\Delta pH$ was 21% (n=8) to 28% (n=35) higher than the corresponding value determined from regression analysis of pH on [La], and the coefficients of variation (SD/mean) for the group $B_{viv}$ determined in this manner were 89% (n=8) and 68% (n=35).

Table 3.13  Comparison of regression equations for pH v. [La] following isometric or dynamic exercise to fatigue

<table>
<thead>
<tr>
<th></th>
<th>DYNAMIC (n=8)</th>
<th>ISOMETRIC (n=8)</th>
<th>ISOMETRIC (n=35)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient</td>
<td>0.97</td>
<td>0.78</td>
<td>0.87</td>
</tr>
<tr>
<td>V (%)</td>
<td>1.0</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Elevation (intercept)</td>
<td>7.24</td>
<td>7.18</td>
<td>7.23</td>
</tr>
<tr>
<td>Slope</td>
<td>0.00594</td>
<td>0.00460</td>
<td>0.00589</td>
</tr>
<tr>
<td>Reciprocal of slope</td>
<td>168</td>
<td>217</td>
<td>170</td>
</tr>
<tr>
<td>($B_{viv}$; mmol H⁺.kg⁻¹.pH⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\bar{x} \pm SD B_{viv}$ from individual values of $\Delta [La]/\Delta pH$</td>
<td>172 ± 39</td>
<td>278 ± 248</td>
<td>206 ± 141</td>
</tr>
</tbody>
</table>

V = coefficient of variation of points about the regression line (%)
* includes 27 samples from the study described in Chapter 4 (see text).
Since no significant difference was observed between $B_{viv}$I and $B_{viv}$D, all values of muscle pH and [La] (regardless of exercise mode employed) were analysed collectively, for comparison with the in vitro $B$ values determined by HCl titration. Muscle [La] and homogenate pH values pre- and post-exercise are shown in Table 3.14 and Fig. 3.5. Mean $B_{viv}$ (reciprocal of slope of pH v. [La]) was approximately 8-14% greater than the corresponding $B_{vit}$ (181 v. 159 mmol H$^+$.kg$^{-1}$.pH$^{-1}$ (n=16) or 169 vs 156 mmol H$^+$.kg$^{-1}$.pH$^{-1}$ (n=43); Table 3.15).

**Discussion**

$B_{viv}$I (isometric) compared with $B_{viv}$D (dynamic)

An index of the in vivo muscle buffer value can theoretically be obtained from the linear relationship between muscle lactate concentration ([La]) and pH, following any exercise task which results in the accumulation of appreciable amounts of lactate (provided that any lactate lost from the muscle is accompanied by an equivalent amount of hydrogen ions). However, the exact mechanisms which contribute to total buffering, and therefore the final value obtained, can be expected to depend on the actual exercise mode employed and particularly, on the extent of perfusion of the muscle during exercise.

In the present study, no significant difference was observed between the in vivo buffer value determined following high intensity isometric ($B_{viv}$I) or dynamic ($B_{viv}$D) exercise to fatigue (170 vs 168 mmol H$^+$.kg$^{-1}$.pH$^{-1}$ respectively). Sahlin (1978) made a similar comparison of $B_{viv}$I and $B_{viv}$D, but whilst the isometric exercise (68% quadriceps MVC to fatigue) was similar to that employed in the present study, the dynamic exercise comprised cycling at 60 rpm for 5-11 minutes at 50-75% Wmax or for 6 min at Wmax (where Wmax = the work load required to cause exhaustion within 6 min). Buffer values derived from the [La]/pH relationship were 57 and 73 mmol.l$^{-1}$.pH$^{-1}$ for isometric and dynamic exercise respectively (Sahlin, 1978) (equivalent to 188 and 241 mmol.kg dm$^{-1}$.pH$^{-1}$, with a 77% muscle water content). During dynamic exercise, the muscle was considered to operate as an open system and was therefore able to utilise the bicarbonate buffering system. The closed system created by an isometric contraction maintained at 68% MVC (Edwards et al., 1972a) would negate the involvement of bicarbonate buffering during this type of exercise. It was on this basis that the difference in $B_{viv}$ for the two modes of exercise was explained (Sahlin, 1978).
Table 3.14  Muscle lactate and homogenate pH in muscle samples obtained at rest and following exercise to fatigue (both isometric and dynamic) (n = 16).

<table>
<thead>
<tr>
<th>Muscles</th>
<th>[La] (mmol.kg dm(^{-1}))</th>
<th>Muscle homogenate pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>REST</td>
<td>(\bar{x}) 5.9 (6.3)*</td>
<td>7.18 (7.20)</td>
</tr>
<tr>
<td></td>
<td>SD 2.3 (2.8)</td>
<td>0.08 (0.10)</td>
</tr>
<tr>
<td>POST-EXERCISE</td>
<td>(\bar{x}) 66.8 (68.5)</td>
<td>6.84 (6.82)</td>
</tr>
<tr>
<td></td>
<td>SD 28.5 (20.7)</td>
<td>0.17 (0.14)</td>
</tr>
</tbody>
</table>

* values in brackets, n = 43 (includes data from 27 samples from the study described in Chapter 4 (pre-training samples)).

Table 3.15  In vivo (dynamic+isometric) vs. in vitro muscle buffer values.

<table>
<thead>
<tr>
<th>In vivo buffer value</th>
<th>In vitro buffer value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient ((r))</td>
<td>V* (%)</td>
</tr>
<tr>
<td>n=16</td>
<td>0.92</td>
</tr>
<tr>
<td>n=43*</td>
<td>0.90</td>
</tr>
</tbody>
</table>

*a V = coefficient of variation of points about the regression line (%)  
* including 27 samples from study in Chapter 4 (pre-training samples).
Muscle homogenate pH

\[ y = 7.231 - 0.0059x \quad R = 0.90 \]

Fig. 3.5 Muscle lactate concentration ([La]) vs homogenate pH (resting and post-exercise samples).
In the present study, the muscle samples were freeze-dried prior to pH analysis. As such, only the non-bicarbonate buffering was measured by the Δ [La]/Δ pH relationship (Harris et al., 1989 - and see next section). Therefore, even if bicarbonate buffering was involved during the high intensity dynamic exercise test, this would not have been measured in the subsequent analysis of $B_{viv}$. Without the differential involvement of bicarbonate buffering, $B_{vivI}$ and $B_{vivD}$ would not be expected to differ to any great extent.

$B_{viv}$ compared with $B_{vit}$

According to the theory, the buffer value determined "in vivo" (lactic acid titration; $B_{viv}$) ought to be higher than that determined "in vitro" (HCl titration; $B_{vit}$) by an amount equivalent to the metabolic buffering (approximately 30-40% see Table 1.2, Chapter 1). In practice (in the present study) a minimal, 8-14% difference was observed between the two. This suggests that the current methods of measurement may not be accurate enough to reflect the differences dictated by the theory; examination of the components expected to contribute to the measured values of $B_{vit}$ and $B_{viv}$, may explain why.

Bicarbonate buffering. All measurements of pH and $B_{vit}$ were made on freeze-dried muscle samples. When the partial pressure of carbon dioxide is zero in the gas above a solution containing any dissolved carbon dioxide, bicarbonate or carbonic acid, all the equilibrium reactions relating these substances are driven towards gaseous CO2 production and these "CO2 containing" species disappear (Stewart, 1981). Thus, when a muscle sample is freeze-dried, all the bicarbonate should be removed from the sample as CO2, which can be expected to result in an upward bias in the measured pH, according the the equilibrium reaction:

$$H^+ + HCO_3^- \rightarrow H_2O + CO_2$$

Indeed, it has been shown that the homogenate pH values of freeze-dried muscle samples are higher than those of wet, frozen samples from the same muscle block (Harris et al., 1989). Thus, titration of a freeze-dried muscle homogenate (by either in vivo or in vitro methods) will give a measure of only the non-bicarbonate buffering. The observed differences between $B_{viv}$ and $B_{vit}$ cannot therefore be explained by the selective involvement of bicarbonate buffering.
**Physicochemical buffering.** The physicochemical buffers (inorganic phosphate and protein/dipeptide histidine and cysteine residues) are expected to contribute to the same extent to \( Bviv \) and \( Bvit \) (assuming conditions of temperature and ionic strength create identical pK\(_a\)' values for these buffers in vitro - see Section 3.2). This will be true, however, only if the amount of these buffer species remains constant during each method of measurement. During homogenisation of the muscle, and incubation at 37 °C for the measurement of pH and \( Bvit \), phosphocreatine (PCr) and adenosine triphosphate (ATP) are almost completely hydrolysed (> 95%) with the consequent accumulation of creatine (Cr), inorganic phosphate (P\(_i\)) and inosine monophosphate (IMP) (Spriet et al., 1986; Marlin, 1989; Thomson and Seto, 1989). However, in the present study, following isometric and dynamic exercise respectively, PCr was only 63 and 75 % depleted, and ATP 3 and 20 % depleted. Thus a far greater quantity of inorganic phosphate would have been available to contribute to physicochemical buffering *in vitro* than was released for use in buffering during exercise (and therefore in the measurement of \( Bviv \)). It is difficult to attribute an exact numerical value to this component, since the amount of phosphate that exists in a free state is uncertain (Sahlin et al., 1978b). However, at this point in the analysis, it is likely that the physicochemical buffering *in vitro* would have been greater than that *in vivo*, due to the higher concentration of P\(_i\) within the homogenate.

**Metabolic buffering.** During exercise, the hydrolysis of phosphocreatine takes up hydrogen ions with a stoichiometry dependent on the prevailing pH. AMP deamination to IMP also takes up a small quantity of hydrogen ions (Hultman and Sahlin, 1980). These mechanisms may influence the homogenate pH (see below) but neither can be expected to contribute to buffering *in vitro*. Thus, \( Bviv \) now has an additional dimension due to metabolic buffering, which is variable, in relation to the extent of PCr hydrolysis during the exercise task.

**Effect of PCr hydrolysis on muscle homogenate pH.** When the pH of an exercising muscle is analysed by nuclear magnetic resonance spectroscopy, a transient alkalinisation of the muscle is observed at the start of contraction (Meyer, 1984). This is attributed to the breakdown of PCr which occurs prior to substantial lactate formation. It is therefore reasonable to assume that at least some increase in measured pH would be observed in a muscle in which total PCr hydrolysis had occurred in the presence of a glycolytic inhibitor e.g. in a muscle homogenate prepared for pH/\( Bvit \) measurement. Indeed, the buffer value of the muscle would
have to be extraordinarily high for no change in pH to be evidenced. However, experimental determinations of the effect of phosphagen hydrolysis on pH measurement have produced conflicting results; Sprriet et al (1986) report no effect, whilst more recently, a significant increase in measured homogenate pH, upon PCr hydrolysis, has been observed (Thomson and Seto, 1989).

Assuming no other alterations to the hydrogen ion concentration, and using a buffer value for muscle of 159 mmol.kg^-1.pH^-1, the expected change in pH upon complete hydrolysis of various original concentrations of PCr can be calculated, by rearranging the equation for the calculation of a buffer value:

\[
\frac{\Delta H^+}{B} = \Delta pH
\]

(where \(\Delta H^+\) is given by \(\Delta \text{PCr} \times n \text{H}^+\) and \(n \text{H}^+\) = the stoichiometric uptake of hydrogen ions per mmol PCr hydrolysed)

(Sahlin et al, 1987)

Values for the PCr content of muscle at rest, and following either isometric or dynamic exercise to fatigue (from Table 3.12) are shown in Table 3.16, along with the expected change in homogenate pH caused by complete loss of this PCr. The higher the original concentration of PCr, the greater the change in pH upon its complete hydrolysis. The effect would be further exacerbated by the breakdown in the homogenate of ATP to IMP, the net reaction of which consumes hydrogen ions (Hultman and Sahlin, 1980). These effects per se, should be of no consequence to \(Bv_{ir}\); titration of the muscle homogenate may start from a higher initial pH but the buffer value is still only calculated over the same, fixed pH range (7.1 - 6.5). They will however, affect \(Bv_{iv}\), since the elevation of pH due to PCr hydrolysis and IMP formation (upon homogenisation and incubation), will be greater in resting, than in post-exercise muscle samples (Table 3.16). As such, the change in homogenate pH pre to post-exercise will appear to be greater. For a given accumulation of lactate, a greater \(\Delta pH\) produces a lower \(Bv_{iv}\). This therefore makes a "negative" contribution to \(Bv_{iv}\) (i.e. the true in vivo buffer value is underestimated).

Production of additional metabolic acids. In the present study, \(Bv_{iv}\) was determined from the relationship between [La] and muscle homogenate pH. Lactic acid is not the only metabolic acid produced during high intensity exercise, and although it
accounts for 85-95% of the total hydrogen ions liberated (Hultman and Sahlin, 1980), the additional 5-15% H+ ions produced but not entered into the equation \( \Delta [H^+] / \Delta pH \) mean that the true in vivo buffer value is further underestimated.

**Table 3.16** Calculated effect of PCr hydrolysis on measured muscle homogenate pH.

<table>
<thead>
<tr>
<th>Original conc. of PCr (mmol.kg(^{-1}))(^a)</th>
<th>pH (^b)</th>
<th>nH(^+)(^c)</th>
<th>( \Delta H^+)(^d)</th>
<th>( \Delta pH ) through complete loss of PCr during homogenisation (^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R) 55.5</td>
<td>7.18</td>
<td>0.30</td>
<td>16.7</td>
<td>0.11</td>
</tr>
<tr>
<td>(I) 21.5</td>
<td>6.94</td>
<td>0.42</td>
<td>9.0</td>
<td>0.06</td>
</tr>
<tr>
<td>(D) 14.1</td>
<td>6.73</td>
<td>0.54</td>
<td>7.6</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\(^a\) Values taken from Table 3.12; R=rest, I=post-isometric, D=post-dynamic exercise

\(^b\) measured homogenate pH in corresponding samples - used for estimating nH+

\(^c\) from Hultman and Sahlin (Fig. 5), 1980.

\(^d\) \( \Delta H^+ = PCr \times nH^+ \)

\(^e\) \( \Delta (H^+/B) \) assuming an *in vitro* buffer value of 159 mmolH+.kg\(^{-1}\).pH\(^{-1}\)

**Transmembrane flux of H^+** The differential transmembrane flux of H+/lactate ions across the muscle membrane is frequently cited as an additional mechanism which could result in greater values of \( B_{viv} \) than \( B_{vir} \). If the H+ efflux is greater than that of lactate, then the H+ load incurred during exercise is overestimated, when taken as being equivalent to the final lactate concentration.

As discussed earlier (Chapter 1), whilst it is clear that transport mechanisms exist which could elicit differential extrusion rates for H+ / La (Juel, 1988), the bulk of available evidence does not favour unequal elimination rates during exercise. It has been suggested that the major determinant of the parity in efflux rates for these two ions is the rate of blood flow through the muscle (the higher the blood flow the greater the extrusion of H+ relative to La+) (Heisler, 1986b). The study of Sahlin *et*
al (1978c) indicated that during dynamic exercise (at 60 rpm) leading to fatigue within 6 min, lactate and hydrogen ions were released from the muscle at approximately the same rate. A similar conclusion was reached by Katz et al (1985) in examining repeated 1 minute bouts of cycling at VO2max. The intensity and mode of exercise employed by Sahlin et al (1978c) is likely to elicit near maximal perfusion rates for two-legged exercise (Bonde-Peterson et al, 1975). If unequal efflux rates were not observed during this type of exercise, it is unlikely that dynamic exercise of a higher intensity (involving more forceful and rapid contractions of the muscle) and a shorter duration (minimising the time available for adjustment of the circulation) would be able to induce even comparable, let alone greater, perfusion rates. As such, the elimination of H+ and La- can be assumed to be equivalent during the high intensity dynamic exercise performed in the present study.

Furthermore, during isometric exercise (60 % MVC) the muscle undoubtedly exists as a closed system (Edwards et al, 1972a) and therefore the possibility of an unequal H+/La efflux (or indeed any significant efflux, of either ion) can be disregarded. Since in the present study, no significant difference was observed between Bviv following either dynamic or isometric exercise, this confirms that a differential loss of H+ and La- was unlikely to have occurred during the dynamic exercise task. The differential efflux of H+/La- cannot therefore, account for any differences between Bviv and Bvit.

Table 3.17 summarises the components expected (in theory) to contribute to Bvit and Bviv, and highlights the influence of other factors which are likely to affect the measured values of Bvit and Bviv. It becomes clear that when the additional positive and negative factors contributing to the measured buffer value are considered, it could be possible for Bvit and Bviv to arrive at a similar value. Only if Bviv was measured in vivo in the true sense of the word (i.e. if determinations of the metabolic acid concentration and pH were made on the intact muscle in situ), would it be expected to be significantly higher than Bvit, and only then, could the difference be attributed in the main, to metabolic buffering.

Thus, whenever Bviv is calculated from the relationship between [La] and pH (as analysed from muscle biopsy samples), it can be expected to yield similar values to Bvit, and any observed discrepancies cannot simply be attributed to the differential involvement of a single mechanism (e.g. metabolic buffering). In this respect, it is difficult to envisage the 30-118% higher Bviv than Bvit reported in
Table 3.17 Expected and actual components contributing to the measurement of *in vitro* (Bvit) and *in vivo* (Bviv) muscle buffer values.

<table>
<thead>
<tr>
<th>METHOD OF MEASUREMENT</th>
<th>THEORETICAL COMPONENTS CONTRIBUTING TO THE MUSCLE BUFFER VALUE</th>
<th>ADDITIONAL FACTORS CONTRIBUTING TO THE MEASURED MUSCLE BUFFER VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bvit</em> = $\Delta [H^+] / \Delta pH$</td>
<td>1) Proteins, dipeptides, phosphate, (resting concentrations), bicarbonate.</td>
<td>1) No bicarbonate (-)</td>
</tr>
<tr>
<td>Titrant = hydrochloric acid</td>
<td></td>
<td>2) Phosphate from breakdown of &gt; 95% PCr / all ATP in homogenate (+) <em>a</em></td>
</tr>
<tr>
<td><em>Bviv</em> = $\Delta [H^+] / \Delta pH$</td>
<td>1) Proteins, dipeptides, phosphate (exercise concentrations), bicarbonate.</td>
<td>1) No bicarbonate (-)</td>
</tr>
<tr>
<td>Titrant = lactic acid</td>
<td>2) Metabolic buffering (PCr hydrolysis, IMP formation).</td>
<td>2) Upward bias of homogenate pH due to PCr hydrolysis and IMP formation - to a greater extent in resting than post-exercise samples (i.e. overestimate of $\Delta pH$) (-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) Non-inclusion of other metabolic acids in addition to La (i.e. underestimate of $\Delta H^+$) (-)</td>
</tr>
</tbody>
</table>

(+)= additional positive contribution to the measured buffer value  
(-)= additional negative contribution to the measured buffer value

*a* Spriet *et al*, 1986; Marlin, 1989.
the study of Nevill et al (1989) and the 44-66% higher $B_{viv}$ reported by Marlin (1989), representing anything other than error in the use of individual values of $\Delta [La] / \Delta pH$.

**Problems concerning the measurement of $B_{viv}$: possible non-linearity of pH/La relationship.**

Of interest, is the finding that the titration curve produced during $B_{vit}$ determination was curvilinear (Chapter 2; Fig. 2.9), yet that obtained "in vivo", using lactic acid (La) as the titrant, is purportedly linear.

During exercise, the decrease in the phosphocreatine (PCr) concentration of the muscle is curvilinearly related to the increase in muscle lactate concentration (Harris et al, 1977). The stoichiometry of hydrogen ion uptake during PCr hydrolysis varies with pH, demonstrating an almost linear relationship over the pH range 7.1-6.5 (Hultman and Sahlin, 1980). As such, at higher lactate concentrations, PCr hydrolysis takes up relatively fewer H+ ions per unit increase in lactate.

Thus, metabolic buffering contributes more to $B_{viv}$ in the initial stages of lactate production than later on in the exercise task. The effect this has on the overall La/pH relationship, can be assessed using the values for, and relationship between, lactate and PCr given by Harris et al (1977), as follows.

Assuming that:

1) a given accumulation of lactate represents an equivalent release of H+  
2) PCr hydrolysis takes up H+ with a stoichiometry related to the prevailing pH,

then the net hydrogen ion release can be calculated as:

$$\text{net } \Delta H^+ = \Delta \text{ La} - (\Delta \text{ PCr} \times nH^+)$$

(Sahlin et al, 1987)

(where $nH^+$ = the stoichiometrical uptake of H+ ions per mol PCr hydrolysed and varies linearly from 0.39 at pH 7.0 to 0.73 at pH 6.4; Hultman and Sahlin, 1980).
Knowing the net $\Delta \text{H}^+$, and the non-PCr buffer value of the muscle ($B$), the expected change in pH with increasing lactate concentration can be calculated as:

$$\Delta \text{H}^+ = \Delta \text{pH} \quad \frac{1}{B}$$

(Sahlin et al, 1987)

The results of this analysis are shown in Table 3.18, and the corresponding graph of pH versus lactate (using the calculated pH values) is shown in Fig. 3.6.

It can be seen that the data for pH versus [La] (Fig. 3.6) would conform well to a linear function with a high correlation coefficient. However, calculation of the gradients ($\Delta [\text{La}] / \Delta \text{pH}$) over the lactate ranges 1.9-30.2, 1.9-67.9, 1.9-105.7 mmol.kg$^{-1}$ produces buffer values of 250, 190 and 178 mmol.kg$^{-1}$.pH$^{-1}$ unit respectively. Only very slight deviations from linearity are therefore needed to substantially alter the calculated buffer value (by as much as 30-40%).

If PCr hydrolysis (which occurs during homogenisation and incubation of the muscle) does in fact cause an increase in muscle homogenate pH (Thomson and Seto, 1989), then this will serve to elevate the resting muscle homogenate pH more so than the post-exercise homogenate pH (as described in the previous section) and may restore some linearity to the relationship. However, this must be recognised as a methodological manipulation, with the true relationship between [La] and pH remaining non-linear. This may have important consequences when muscle pH is calculated from [La] using established regression equations of muscle pH vs. lactate concentration (e.g. Boobis, 1987) using equations from Sahlin, 1978).

**Conclusion**

Interpretation of the muscle buffer value, as determined from a biopsy sample, is not as straightforward as in theory, it may appear. It is important to establish the components of buffering being measured by either "in vivo" or "in vitro" techniques, particularly when explanations are sought for differences observed between athletic groups, or following periods of training. The methodology must be carefully examined to ensure that it is not this, rather than any physiological mechanism, that explains the observed changes.

It is unlikely that any differences observed in the measurements $Bviv$ and $Bvit$ can be simply attributed to metabolic buffering (or indeed to any singular
Table 3.18 Calculated pH change from lactate accumulation and PCr hydrolysis during isometric exercise.

<table>
<thead>
<tr>
<th>Approx. pH</th>
<th>[La] \text{b} mmol.kg$^{-1}$</th>
<th>[PCr] \text{b} mmol.kg$^{-1}$</th>
<th>nH$^+$ from PCr hydrolysis$^c$</th>
<th>H$^+$ from La formation</th>
<th>H$^+$ uptake from PCr hydrolysis</th>
<th>NET Δ H$^+$</th>
<th>NON-PCr$^d$ BUFFERING (mmolH$^+$ .kg$^{-1}$.pH$^{-1}$)</th>
<th>CALCULATED Δ pH</th>
<th>Final pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.05</td>
<td>1.9</td>
<td>80.5</td>
<td>0.36</td>
<td>9.4</td>
<td>7.5</td>
<td>1.9</td>
<td>81</td>
<td>0.023</td>
<td>7.050</td>
</tr>
<tr>
<td>7.0</td>
<td>11.3</td>
<td>61.3</td>
<td>0.39</td>
<td>28.3</td>
<td>19.0</td>
<td>9.3</td>
<td>83</td>
<td>0.112</td>
<td>6.938</td>
</tr>
<tr>
<td>6.9</td>
<td>30.2</td>
<td>38.4</td>
<td>0.45</td>
<td>47.2</td>
<td>27.0</td>
<td>20.2</td>
<td>91</td>
<td>0.222</td>
<td>6.828</td>
</tr>
<tr>
<td>6.8</td>
<td>49.1</td>
<td>26.5</td>
<td>0.50</td>
<td>66.0</td>
<td>34.3</td>
<td>31.7</td>
<td>91</td>
<td>0.348</td>
<td>6.702</td>
</tr>
<tr>
<td>6.7</td>
<td>67.9</td>
<td>19.2</td>
<td>0.56</td>
<td>84.9</td>
<td>40.4</td>
<td>44.5</td>
<td>93</td>
<td>0.478</td>
<td>6.572</td>
</tr>
<tr>
<td>6.6</td>
<td>86.8</td>
<td>14.2</td>
<td>0.61</td>
<td>103.8</td>
<td>46.8</td>
<td>57.0</td>
<td>98</td>
<td>0.582</td>
<td>6.468</td>
</tr>
<tr>
<td>6.5</td>
<td>105.7</td>
<td>10.7</td>
<td>0.67</td>
<td>122.6</td>
<td>52.9</td>
<td>69.7</td>
<td>105</td>
<td>0.663</td>
<td>6.387</td>
</tr>
<tr>
<td>6.4</td>
<td>124.5</td>
<td>8.1</td>
<td>0.73</td>
<td>122.6</td>
<td>52.9</td>
<td>69.7</td>
<td>105</td>
<td>0.663</td>
<td>6.387</td>
</tr>
</tbody>
</table>

$^a$ from relationship between La and pH during isometric exercise (Sahlin, 1978) - used in estimating nH$^+$

$^b$ from Harris et al (1977)

$^c$ from Hultman and Sahlin (1980)

$^d$ adjusted for change over the given pH range (see Fig. 2.9 (Methods)) - from n=10 in vitro titrations - present study.
Fig. 3.6  Calculated *in vivo* titration curve (from data in Table 3.18).
mechanism). The large differences previously observed (Marlin, 1989; Nevill et al., 1989), may have arisen from the potentially considerable error in the measurement of $B_{viv}$; the use of just two pairs of data points in the construction of a titration curve (which is effectively what is being done when individual values of $\Delta [La]/ \Delta \text{pH}$ are used) is not recommended. The group mean $B_{viv}$ (if required) is better described by regression analysis of all individual values of pH and [La]. However, even this expression of $B_{viv}$ is not without problems, and therefore, measurements of the skeletal muscle buffer value, and comparisons between groups or individuals, are probably best made using in vitro titrations. It is unlikely that any component of buffering will be seriously overlooked in doing this; even though $B_{vit}$ cannot measure the metabolic buffering, it is likely that an indirect measure of the quantity of PCr in the muscle will be given by the additional physicochemical buffering acquired by $P_i$ release, upon PCr hydrolysis during homogenisation.
Chapter 4
4. INTER-RELATIONSHIPS BETWEEN, AND THE EFFECTS OF TRAINING ON, HIGH INTENSITY EXERCISE PERFORMANCE, FIBRE TYPE DISTRIBUTION AND SKELETAL MUSCLE BUFFERING.

4.1 Introduction

Having established the most appropriate method for the measurement of skeletal muscle buffering, the following studies sought to investigate the role of intramuscular pH and buffering, as limiting factors to the performance of high intensity exercise. The studies comprise a cross-sectional analysis of the inter-relationships between fibre type distribution, muscle buffer value and performance, and an evaluation of the trainability of both performance capacity and the skeletal muscle buffer value. Previous studies addressing this issue have been few, and have often been confounded by inconsistent and possibly inappropriate methodology. Additionally, the use of small subject populaces of diverse groups of athletes (Parkhouse and McKenzie, 1985) have made difficult the distinction between the training response per se, and genetic endowment.

4.2 Methods and procedures

A total of 51 young, active male (n=31) and female (n=20) volunteers agreed to participate in the studies. Each was informed of the purpose and potential risks of the studies before their written voluntary consent was obtained. The mean ± SD age, height and body mass of the subjects was 22.9 ± 5.2 yr, 174.8 ± 8.9 cm and 73.6 ± 11.7 kg respectively. Two studies were carried out, the experimental designs for which are shown in Table 4.1.

Study A involved 18 subjects (12 male, 6 female) in a crossover design and sought to examine the inter-relationships between high intensity exercise capacity (isometric and dynamic), fibre type distribution and muscle buffering (see Table 4.1).

Study B included 33 subjects (19 male, 14 female) and sought to determine the effects of training on high intensity dynamic exercise capacity (see Table 4.1).
Table 4.1  Experimental design and variables measured: Study A and Study B

STUDY A (crossover design)

Performance tests
MVC knee extensors
60% MVC endurance time
Impulse (60 % MVC force x endurance time)
Series of mWAnTs (load (N) = 0.08-0.12 (q) and 0.09-0.13 (d) x body weight (N))

Biopsy analyses
TRIAL (1)a 1) RESTING: fibre area, % fibre type, % fibre area 
2) RESTING: homogenate pHb , Bvitb , [carnosine], [lactate]
3) POST-DYNAMIC EXERCISE (modified WAnT to exhaustion): homogenate pH, Bvit, [lactate]

TRIAL (2)a 1) RESTING: fibre area, % fibre type, % fibre area
2) RESTING: homogenate pHb , Bvitb , [carnosine], [lactate]
3) POST-ISOMETRIC EXERCISE (60% MVC to fatigues): homogenate pHb, Bvitb, [lactate]

STUDY B (training study)

Pre-training:
Performance tests
MVC knee extensors
60% MVC endurance time
Impulse (60 % MVC force x endurance time)
Series of mWAnTs (load (N) = 0.09-0.13 x MVC (N))

Biopsy analyses c
1) **
2) RESTING: homogenate pHb, Bvitb, [carnosine], [lactate]
3) POST-ISOMETRIC EXERCISE (60% MVC): homogenate pHb, Bvitb, [lactate]

Training: 3 days/wk for 16 weeks (48 sessions) high intensity isokinetic knee extensions at 60 or 240 degrees. s \(-1\) (see Table 4.2)

Post-training:
Performance tests

} as for pre-training

Biopsy analyses

a trial (1) and trial (2) were separated by a period of approximately 2 months
b measurements of pH/Bvit made on homogenates of 30mg dm.ml 0.01M NaF

8 subjects in Study B did not have muscle biopsy samples taken.

** a resting muscle sample was taken for histochemical analysis, but the data has not been processed for inclusion within this thesis.
All performance tests and muscle biopsy sampling/analyses were carried out as described in Chapter 2.

**Training (Study B)** In Chapter 2, the sensitivity of the modified WAnT to training was established. However, both training and assessment were carried out on the same ergometer, and as such, it is possible that the improvements in performance were elicited not through metabolic changes within the muscle, but rather, as a result of an improved co-ordination and skill in the execution of that particular movement pattern. Therefore in *Study B*, the training manoeuvre was dissociated from the assessment task (modified WAnT) by training one of the major muscle groups involved in cycling (the quadriceps) with repeated maximal isokinetic leg extensions. Training was carried out on an Orthotron KT2 isokinetic leg extension machine (Cybex (Lumex U.S.A.)). This mode of training provides more work per repetition than conventional isotonic resistance machines, in that every contraction of the quadriceps is a maximal effort throughout the full range of motion. Also, improvements in strength are automatically adjusted for, satisfying the principles of overload without the need for continual assessment. The simplicity of the movement itself confers an additional advantage over more traditional training methods, in that complex lifting techniques need not be acquired before a maximal stimulus can be presented to the working muscle (see Rutherford and Jones, 1986). The training was designed to be of a high intensity, with a high work:relief ratio, in order to stimulate substantial usage of anaerobic metabolism (see schedule in Table 4.2). Subjects were randomly assigned to one of three groups to follow the schedules outlined in Table 4.2.

Training sessions were carried out in pairs, under supervision, and individuals were instructed to make a maximal effort throughout the full range of motion during each repetition. Visual feedback was given as the peak torque generated during knee extension (the actuator was locked off for knee flexion such that the knee was returned to 90 deg. flexion with no opposing resistance). Training records were maintained by each subject, documenting that they had adhered to the set training programme.

**Statistical analyses** Results are expressed as arithmetic mean values plus or minus one standard deviation. The coefficient of variation (CV) for a single group of values is given by the standard deviation/mean x 100 %. The CV for duplicate measurements from a group of subjects, is given by CV = √(d²/n) x 100/mean, where d=the difference between duplicate measures and n=the number of duplicate measures (Blomstrand *et al*, 1984). Analysis of variance with repeated measures,
Table 4.2  Training schedules: *Study B.*

<table>
<thead>
<tr>
<th>GROUP (total n=33)</th>
<th>TRAINING SCHEDULE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (n=8)</td>
<td>No training</td>
</tr>
<tr>
<td>TRAINING:</td>
<td></td>
</tr>
<tr>
<td>LEG EXTENSIONS</td>
<td></td>
</tr>
<tr>
<td>AT 60 deg.s(^{-1})</td>
<td>5 sets of: 15 maximal repetitions (25-30 s) right leg, 40 s rest (both legs), 15 maximal repetitions (25-30 s) left leg, 40 s rest (both legs) 3 days/week for 16 weeks (^b) (48 sessions)</td>
</tr>
<tr>
<td>(n=12) (^a)</td>
<td></td>
</tr>
<tr>
<td>TRAINING:</td>
<td></td>
</tr>
<tr>
<td>LEG EXTENSIONS</td>
<td>6 sets of: 25 maximal repetitions (20-25 s) right leg, 30 s rest (both legs), 25 maximal repetitions (20-25 s) left leg, 30 s rest (both legs) 3 days/week for 16 weeks (^b) (48 sessions)</td>
</tr>
<tr>
<td>AT 240 deg.s(^{-1})</td>
<td></td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) two subjects from the 60 deg.s\(^{-1}\) training group ceased training after 1-2 weeks and were transferred into the control group.

\(^b\) the 16 week training period was unavoidably interrupted by two 3 wk periods of student vacations. These occurred after 2 wk and after 12 wk of training (i.e. 2 wk training - 3 wk off - 10 wk training - 3 wk off - 4 wk training - assessment).

\(^c\) The total number of repetitions/session performed by the 240 deg.s\(^{-1}\) group was twice that of the 60 deg.s\(^{-1}\) group, in order to maintain the same total work output for each group (Rosier et al, 1986).
followed by determination of the intraclass correlation coefficient, was used to
determine the reliability of measure in duplicate samples (Study A) (Safrit, 1981).
The difference between two variables was analysed using a t-test for independent
samples (with or without equal variances) or paired samples, as appropriate. The
relationship between two variables was examined by simple linear regression and/or
determination of the Pearson product-moment correlation coefficient. When the
relationship between two variables was influenced by a third variable, the partial
correlation coefficient was determined. Multiple regression analysis was employed
to determine the relationship between a single dependent variable and > 1
independent variable. Predictions from regression equations were made only when a
significant relationship between the variables was demonstrated; predicted values are
given as ± 1 SE of the mean. The data from the training study (Study B) was
analysed using multivariate analysis of variance (SPSSx Release 3.0) (training
group (control, 240 deg.s\(^{-1}\) and 60 deg.s\(^{-1}\)) x trial (pre and post-training) with
repeated measures on the latter factor). The MANOVA of the training study data for
the dynamic exercise tests (modified Wingate Anaerobic Tests) included trend
analysis of the relationship between the criterion performance measure (e.g. PPO,
MPO, WORK etc.) and the test load.

Although 18 subjects completed Study A, the total number of data points
("n" value) for some of the biopsy sample analyses is not always 18. This was the
result of some subjects failing to provide a suitable or large enough sample for all of
the analyses, or in the case of muscle pH and Bvit, insufficient muscle being
available to repeat the measurements using 0.01M NaF as the homogenising solution
(see methods section of Chapter 3). In presenting the results, if "n" equals anything
less than all available samples (i.e in the case of anomalous, rather than missing data
points), this will always be specified in the text. Part of the test battery employed
in Study A was repeated in Study B (i.e. biopsy sample at rest and post-
isometric exercise to fatigue). Therefore, where appropriate, data from both
studies (Study A plus pre-training values from Study B) will be reported
collectively, to increase the total sample number and thus, the accuracy of the
results.

Statistical significance was accepted at the 5 % level (although the actual
significance (p value) is given where available).
4.3 Results

4.3.1 Performance
The mean values for performance in the isometric (ISOM) and dynamic (DYN) exercise tests are shown in Table 4.3. Male subjects demonstrated significantly higher values than females for maximum voluntary contraction (MVC), impulse, work done and peak power output (p < 0.05). There was no significant difference between males and females, in the time for which the force corresponding to 60% MVC could be maintained to fatigue (p=0.290).

Table 4.3 Male, female and group performance results (Study A and pre-training values Study B).

<table>
<thead>
<tr>
<th></th>
<th>MALES (M) (n=31)</th>
<th>FEMALES (F) (n=20)</th>
<th>ALL Ss (M+F) (n=51)</th>
<th>Sig. of M vs F difference (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ISOMETRIC TEST</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MVC (N)</td>
<td>x 711 SD 146</td>
<td>428 69</td>
<td>603 185</td>
<td>0.0000</td>
</tr>
<tr>
<td>60% MVC Endurance (s)</td>
<td>x 56.7 SD 12.1</td>
<td>59.8 14.7</td>
<td>57.9 13.1</td>
<td>0.2090</td>
</tr>
<tr>
<td>Impulse (kN.s)</td>
<td>x 24.1 SD 6.8</td>
<td>15.2 4.1</td>
<td>20.7 7.3</td>
<td>0.0000</td>
</tr>
<tr>
<td><strong>DYNAMIC TEST</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Work done (30s and &gt; 60 rpm) (kJ)</td>
<td>x 21.8 SD 2.7</td>
<td>13.5 2.1</td>
<td>18.7 4.7</td>
<td>0.0000</td>
</tr>
<tr>
<td>Peak power output (W)*</td>
<td>x 1120 SD 163</td>
<td>667 82</td>
<td>947 261</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

* determined according to method (A) of Chapter 2, Section 2.1.1
4.3.2 Muscle histochemistry (Study A only).

Fibre type composition
The three main groups of muscle fibre types (types I, IIa and IIb) comprised on average, 99% (95-100%) of the fibres present in the biopsy samples. A rather wide range of fibre type distributions was observed for the group of subjects; range type I = 27-68% (group CV 21%), type IIa = 26-55% (group CV 18%), type IIb = 0-27% (group CV 76%). There was no significant difference (p > 0.05) between duplicate samples (taken two months apart) for the distribution of any of the fibre types (Table 4.4). A rather high coefficient of variation (CV) was observed for the distribution of type IIb fibres in duplicate samples (Table 4.4), although the reliability coefficient (0.81) demonstrated that a high (or low) value in trial 1 was associated with a similarly high (or low) value in trial 2. Male and female subjects did not differ in their mean fibre type distribution (Table 4.5).

Table 4.4  Muscle fibre type distribution in duplicate samples (Study A).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>R a</th>
<th>CV b</th>
<th>Signif of 1 v 2 diff. (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(%)</td>
</tr>
<tr>
<td>% Type I</td>
<td>14*</td>
<td>49.3</td>
<td>10.8</td>
<td>52.4</td>
<td>9.4</td>
<td>0.84</td>
</tr>
<tr>
<td>% Type IIa</td>
<td>14</td>
<td>38.2</td>
<td>8.0</td>
<td>37.1</td>
<td>5.6</td>
<td>0.64</td>
</tr>
<tr>
<td>% Type IIb</td>
<td>14</td>
<td>11.1</td>
<td>7.7</td>
<td>8.7</td>
<td>6.6</td>
<td>0.81</td>
</tr>
<tr>
<td>% Type IIa+b</td>
<td>14</td>
<td>49.3</td>
<td>10.9</td>
<td>45.8</td>
<td>8.7</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* Two subjects had no repeated sample and two had a total fibre number <200 for the first biopsy, which was considered insufficient for the accurate determination of fibre type distribution (Dubowitz and Brooke, 1973).

a R = Reliability coefficient (intraclass correlation coefficient).
b CV = coefficient of variation for duplicate samples.
Table 4.5  Muscle fibre type distribution in males vs females (using samples from both trial 1 and trial 2; Study A).

<table>
<thead>
<tr>
<th></th>
<th>Males (M)</th>
<th>Females (F)</th>
<th>Signif of M/F diff (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n a</td>
<td>x</td>
<td>SD</td>
</tr>
<tr>
<td>% Type I</td>
<td>20</td>
<td>52.1</td>
<td>9.2</td>
</tr>
<tr>
<td>% Type IIa</td>
<td>20</td>
<td>38.7</td>
<td>6.5</td>
</tr>
<tr>
<td>% Type IIb</td>
<td>20</td>
<td>8.0</td>
<td>6.9</td>
</tr>
<tr>
<td>% Type IIa+b</td>
<td>20</td>
<td>46.7</td>
<td>9.4</td>
</tr>
</tbody>
</table>

* a n refers to the number of biopsy samples rather than the number of subjects (i.e. includes two biopsies from some subjects, one from others).

Fibre area

A mean of 232 ± 118 fibres from the total biopsy cross-section were measured for area (= approximately 36 % of the total section). For individual muscle samples, the coefficient of variation (SD/mean x 100%) for the area of each fibre type within the section ranged from 10 to 37 %. This highlights the difficulty of randomly selecting a given number of fibres for area measurement, which are considered to be "representative of the entire section" (Viitasalo et al, 1981; Froese and Houston, 1985; Tesch and Karlsson, 1985). For the group of subjects the coefficient of variation (group SD/mean) for the area each of the three fibre types was 24, 30 and 34 % for type I, IIa and IIb fibres respectively.

There was no significant difference (p > 0.05) between duplicate samples for each of the fibre type's mean area, or the mean fibre area (mean of all fibre types) (Table 4.6). The areas of type I, type IIa and type IIb fibres, and the mean fibre area, were significantly larger in males than females (p < 0.05; Table 4.7). Male subjects consistently demonstrated a greater mean type IIa than type I fibre area (mean I : IIa area ratio = 1 : 1.31). The reverse was true for females, with the majority of samples having a mean type I area greater than or equal to the mean type IIa area (mean 1 : IIa area ratio = 1 : 0.97) (Table 4.7).
### Table 4.6  Muscle fibre area in duplicate samples (Study A).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
<th></th>
<th>R a</th>
<th>CV b (%)</th>
<th>Signif of 1v2 diff. (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (um²)</td>
<td>12c</td>
<td>4116</td>
<td>810</td>
<td>3816</td>
<td>858</td>
<td>0.73</td>
<td>13.2</td>
<td>0.193</td>
</tr>
<tr>
<td>Type IIa (um²)</td>
<td>12</td>
<td>4876</td>
<td>1441</td>
<td>4483</td>
<td>1537</td>
<td>0.91</td>
<td>13.8</td>
<td>0.120</td>
</tr>
<tr>
<td>Type IIb (um²)</td>
<td>9d</td>
<td>3558</td>
<td>1205</td>
<td>3302</td>
<td>1452</td>
<td>0.90</td>
<td>15.1</td>
<td>0.215</td>
</tr>
<tr>
<td>Mean fibre area (um²)e</td>
<td>12</td>
<td>4441</td>
<td>1085</td>
<td>4031</td>
<td>1050</td>
<td>0.86</td>
<td>12.6</td>
<td>0.063</td>
</tr>
</tbody>
</table>

a R = Reliability coefficient (intraclass correlation coefficient).
b CV = coefficient of variation for duplicate samples
c Two subjects had no repeated sample and 4 samples from trial 2 demonstrated artefacts such as fibres visibly shrunken away from each other on the section, and were therefore excluded from the analysis.
d Not all samples had sufficient type IIb fibres for use in area determination.
e calculated as ((%type I x area I)+(%type IIa x area IIa)+(%type IIb x area IIb))/100

**Percentage fibre area**

There was no significant difference (p > 0.05) between duplicate samples, in the relative area occupied by each of the fibre types (Table 4.8). However, the coefficient of variation (CV) was high for the relative area occupied by type IIb fibres (Table 4.8). This was the result of the high CV for the distribution of type IIb fibres (see Table 4.4 and its associated text). Male subjects had a significantly greater % IIa fibre area than females (p < 0.05), and this occurred at the expense of the area occupied by both the type I and type IIb fibres (no significant male/female difference in % type I or % type IIb area; p > 0.05) (Table 4.9). When type IIa and IIb fibres were considered together (= % type IIa+b area), there was no significant difference between the sexes (Table 4.9).
Table 4.7  Muscle fibre area in males vs females (using samples from both trial 1 and trial 2; Study A).

<table>
<thead>
<tr>
<th></th>
<th>Males (M) (n=19)</th>
<th>Females (F) (n=11)</th>
<th>F fibre size as % of M</th>
<th>Signif of M/F diff. (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x (\text{SD})</td>
<td>x (\text{SD})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I (um²)</td>
<td>4286 (934)</td>
<td>3674 (680)</td>
<td>86</td>
<td>0.0327</td>
</tr>
<tr>
<td>Type IIA (um²)</td>
<td>5610 (1168)</td>
<td>3544 (740)</td>
<td>63</td>
<td>0.0000</td>
</tr>
<tr>
<td>Type IIB (um²)</td>
<td>4603 (1076)</td>
<td>2722 (759)</td>
<td>59</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean fibre area (um²)</td>
<td>4836 (974)</td>
<td>3539 (652)</td>
<td>73</td>
<td>0.0004</td>
</tr>
<tr>
<td>Type I:IIA:IIB area ratio</td>
<td>1 : 1.31 : 1.07</td>
<td>1 : 0.96 : 0.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) n refers to the number of biopsy samples rather than the number of subjects (i.e. includes two biopsies from some subjects, one from others).

Table 4.8  Percentage fibre type area in duplicate samples (Study A).

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>R (\text{a})</th>
<th>CV (\text{b})</th>
<th>Signif of I vs II diff (\text{p value})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x (\text{SD})</td>
<td>x (\text{SD})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% area type I</td>
<td>14</td>
<td>47.1 (11.7)</td>
<td>50.1 (11.7)</td>
<td>0.84</td>
<td>12.7</td>
<td>0.109</td>
</tr>
<tr>
<td>% area type IIA</td>
<td>14</td>
<td>42.2 (9.7)</td>
<td>41.0 (8.1)</td>
<td>0.74</td>
<td>13.7</td>
<td>0.292</td>
</tr>
<tr>
<td>% area type IIB</td>
<td>14</td>
<td>9.4 (6.3)</td>
<td>7.8 (6.5)</td>
<td>0.79</td>
<td>43.6</td>
<td>0.136</td>
</tr>
<tr>
<td>% area type IIA+IIB</td>
<td>14</td>
<td>51.6 (11.8)</td>
<td>48.8 (11.6)</td>
<td>0.82</td>
<td>12.9</td>
<td>0.129</td>
</tr>
</tbody>
</table>

\(a\) R = reliability coefficient (intraclass correlation coefficient)

\(b\) CV = coefficient of variation

\(c\) two subjects had no repeated sample and two samples had total fibre number < 200 (insufficient for accurate representation of % type distribution (Blomstrand et al, 1984)). Samples with fibre shrinkage were included in the analysis, since the relative sizes of the fibre types were considered not to be influenced by this artifact.
Table 4.9  Percentage fibre type area in males vs females (samples from both trial 1 and trial 2; Study A).

<table>
<thead>
<tr>
<th></th>
<th>Males (M)</th>
<th></th>
<th>Females (F)</th>
<th></th>
<th>Signif of MvF diff (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>x</td>
<td>SD</td>
<td>n</td>
<td>x</td>
</tr>
<tr>
<td>% area type I</td>
<td>20</td>
<td>46.5</td>
<td>9.5</td>
<td>12</td>
<td>50.6</td>
</tr>
<tr>
<td>% area type IIa</td>
<td>20</td>
<td>44.8</td>
<td>7.0</td>
<td>12</td>
<td>39.0</td>
</tr>
<tr>
<td>% area type IIb</td>
<td>20</td>
<td>7.6</td>
<td>6.4</td>
<td>12</td>
<td>9.3</td>
</tr>
<tr>
<td>% area type IIa+b</td>
<td>20</td>
<td>52.4</td>
<td>9.7</td>
<td>12</td>
<td>48.3</td>
</tr>
</tbody>
</table>

* n refers to the number of biopsy samples rather than the number of subjects (i.e. includes two biopsies from some subjects, one from others).

Mean results from 2 biopsy samples
Since no significant difference was observed between duplicate biopsy samples for % fibre type, fibre type area or % fibre area, the results from the two samples were pooled for use in some of the later analyses. In each instance the data from the two biopsies was combined as if obtained from one larger biopsy (rather than simply taking the mean value of the two biopsies) as follows:

**e.g. for type I fibres**

\[
\% \text{ type I} = \frac{\text{no. type I (B1)} + \text{no. type I (B2)}}{\text{Total no. fibres (B1+B2)}} \times 100
\]

(Where no. = number, B1 and B2 = biopsy 1 and 2, respectively)

\[
\% \text{ type I area} = \frac{(\text{no. I x area I})_{(B1)} + (\text{no. I x area I})_{(B2)}}{\text{Total fibre area}} \times 100
\]
where \( \text{area I} = \text{mean type I area} \),

and total fibre area =

\[
\left[ \frac{(\text{no. I} \times \text{area I}) \text{ (B1)} + (\text{no. I} \times \text{area I}) \text{ (B2)} + (\text{no. IIa} \times \text{area IIa}) \text{ (B1)} + (\text{no. IIa} \times \text{area IIa}) \text{ (B2)} + (\text{no. IIb} \times \text{area IIb}) \text{ (B1)} + (\text{no. IIb} \times \text{area IIb}) \text{ (B2)} + (\text{no. OT} \times \text{area OT}) \text{ (B1)} + (\text{no. OT} \times \text{area OT}) \text{ (B2)}]}{\text{total number type I fibres (B1+B2)}} \right]
\]

\( \text{(OT = other fibre types (IIc or IB - see Methods, Chapter 2))} \)

\[
\text{mean area type I} = \frac{(\text{no. I} \times \text{area I}) \text{ (B1)} + (\text{no. I} \times \text{area I}) \text{ (B2)}}{\text{total number type I fibres (B1+B2)}}
\]

\[
\text{mean fibre area (all types)} = \frac{\text{total fibre area (B1+B2)}}{\text{total no. fibres (B1+B2)}}
\]

The group values (mean, SD and range) for fibre type distribution, fibre area and % fibre area, from the pooled data of two biopsies (where available) are shown in Table 4.10.
Table 4.10  Summary of % fibre type, fibre area and % fibre area for all subjects  (n = 18;  Study A  - combined data from two samples where available).

<table>
<thead>
<tr>
<th>Fibre type distribution</th>
<th>Mean</th>
<th>SD</th>
<th>CV (%)(^a)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>% type I fibres</td>
<td>51.2</td>
<td>9.4</td>
<td>18.3</td>
<td>33.9 - 66.5</td>
</tr>
<tr>
<td>% type IIa fibres</td>
<td>38.6</td>
<td>6.6</td>
<td>17.1</td>
<td>25.5 - 51.9</td>
</tr>
<tr>
<td>% type IIb fibres</td>
<td>9.0</td>
<td>7.1</td>
<td>79.0</td>
<td>0 - 26.5</td>
</tr>
<tr>
<td>% type IIa+b fibres</td>
<td>47.6</td>
<td>9.8</td>
<td>20.5</td>
<td>30.8 - 66.0</td>
</tr>
<tr>
<td>% other types</td>
<td>1.2</td>
<td>1.1</td>
<td>92.0</td>
<td>0 - 3.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fibre areas (um(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean type I area</td>
</tr>
<tr>
<td>Mean type IIa area</td>
</tr>
<tr>
<td>Mean type IIb area (^b)</td>
</tr>
<tr>
<td>Mean area other types  (^c)</td>
</tr>
<tr>
<td>Mean type IIa+b area</td>
</tr>
<tr>
<td>Mean fibre area (all fibres)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Fibre type area</th>
</tr>
</thead>
<tbody>
<tr>
<td>% type I fibre area</td>
</tr>
<tr>
<td>% type IIa fibre area</td>
</tr>
<tr>
<td>% type IIb fibre area</td>
</tr>
<tr>
<td>% type IIa+b fibre area</td>
</tr>
<tr>
<td>% other types area</td>
</tr>
</tbody>
</table>

\(^a\)CV = SD/mean x 100 %

\(^b\) n = 16 no IIb areas available for 2 subjects

\(^c\) n = 14 no other fibre areas available for 4 subjects
4.3.3 Muscle carnosine concentration

The mean (±SD) muscle carnosine concentration ([CAR]) was 20.4 ± 4.8 mmol.kg dm⁻¹ (range 7.2 - 30.7 mmol.kg dm⁻¹; group CV= 23.5%) (n=65, all samples from Study A and pre-training samples from Study B). Male subjects demonstrated a significantly higher (21%) muscle carnosine concentration than female subjects (21.7 ± 4.4 mmol.kg dm⁻¹ (n=43) vs 17.9 ± 4.6 mmol.kg dm⁻¹ (n=22) respectively, p = 0.001). There was no significant difference (p > 0.05) in [CAR] between duplicate muscle samples (from Study A; Table 4.11).

Table 4.11 Muscle carnosine concentration and buffer value of duplicate muscle samples (Study A)

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>R (%)</th>
<th>CV (%)</th>
<th>Signif of 1 v 2 diff. (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle carnosine</td>
<td>x 21.5</td>
<td>22.0</td>
<td>0.75</td>
<td>13.4 d</td>
<td>0.403</td>
</tr>
<tr>
<td>concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mmol.kg dm⁻¹</td>
<td>SD 4.0</td>
<td>5.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=17)</td>
<td>Range 14.2 - 27.8</td>
<td>11.9 - 30.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle buffer</td>
<td>x 157</td>
<td>160</td>
<td>0.68</td>
<td>8.2</td>
<td>0.453</td>
</tr>
<tr>
<td>value</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol H⁺.kg⁻¹.pH⁻¹)</td>
<td>SD 19</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=22)</td>
<td>Range 128 - 190</td>
<td>110 - 193</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a R = reliability coefficient (intraclass correlation coefficient)
b CV = coefficient of variation of duplicate samples.
c one subject had no repeated sample
d the coefficient of variation was almost halved (to 7.6%) by exclusion of the anomalous results of one subject; 16.5 vs 30.7 mmol.kg dm⁻¹, trial 1 and 2 respectively.
e Reliability measured on matching (i.e. trial 1 v. trial 2) rest and matching (trial 1 v trial 2) post-exercise samples.
4.3.4 Muscle buffer value (Bvit)
The mean (±SD) muscle buffer value (Bvit) was 157± 19 mmol H⁺.kg dm⁻¹.pH⁻¹ (range 110 - 195 mmol H⁺.kg dm⁻¹.pH⁻¹; group CV=12.1%) (n=57; all available samples from Study A and pre-training samples from Study B). Male subjects demonstrated a significantly greater buffer value than females (160 ± 17 (n=37) vs 151 ± 20 (n=20) mmol H⁺.kg dm⁻¹.pH⁻¹ respectively; p = 0.031). However, the difference between the sexes for the non-carnosine buffer value*, just failed to reach significance (149 ± 17 vs 141 ± 20 mmol H⁺.kg dm⁻¹.pH⁻¹, males and females respectively; p=0.064). HCl titration of post-exercise muscle samples generated approximately 5% higher buffer values than the corresponding Bvit of resting samples (165 ± 14 vs 157 ± 19 mmolH⁺.kg dm⁻¹.pH⁻¹ respectively, p=0.016, n = 50). There was no significant difference (p > 0.05) in the mean buffer value of duplicate muscle samples taken two months apart (Study A ; Table 4.11).

4.3.5 Muscle lactate and homogenate pH
The mean muscle lactate concentration ([La]) and muscle homogenate pH at rest, post-dynamic and post-isometric exercise to fatigue are shown in Table 4.12. Muscle [La] post-dynamic-exercise was significantly higher, and muscle pH significantly lower, than the respective values post-isometric exercise (p < 0.05). A large range existed for the individual [La] and pH values at fatigue, both within and between exercise modes (Table 4.12). No significant difference (p > 0.05) was observed between males and females for the [La] or pH at fatigue, following either exercise mode, although there was a tendency for males to generate higher lactate concentrations with higher post-exercise pH values, post-dynamic exercise (Table 4.13).

4.3.6 Interrelationships between carnosine concentration, muscle buffer value and fibre type distribution.
The correlations involving fibre type distribution, include data from Study A only (no histochemical data available from Study B). Owing to the relatively high coefficient of variation obtained for duplicate determinations of the % type IIb fibres (Tables 4.4 and 4.8), all correlations were performed using the distribution of type II fibres without any sub-classification into type IIa and IIb (referred to as % (or % area) type IIa+b).

* The expected buffering due to carnosine, for each individual's muscle carnosine concentration, was calculated according to Equation 5, Chapter 1, Section 1.5.2.2. This value was then deducted from the total buffer value for each muscle sample.
Table 4.12  Group mean muscle lactate and homogenate pH at rest, post-dynamic and post-isometric exercise.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Post Dynamic Exercise (DYN)</th>
<th>Post Isometric Exercise (ISOM)</th>
<th>Signif of difference DYN v ISOM (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle homogenate pH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54</td>
<td>11</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>7.19</td>
<td>6.73</td>
<td>6.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0057</td>
</tr>
<tr>
<td>SD</td>
<td>0.10</td>
<td>0.15</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6.95-7.39</td>
<td>6.53-6.96</td>
<td>6.60-7.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Muscle lactate concentration (mmol.kg dm&lt;sup&gt;-1&lt;/sup&gt;)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64</td>
<td>15</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>6.4</td>
<td>94.3</td>
<td>65.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0012</td>
</tr>
<tr>
<td>SD</td>
<td>3.0</td>
<td>29.4</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.8-14.0</td>
<td>49.3-136.6</td>
<td>32.7-95.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> rest and post-isometric exercise pH and [La] values include all available data points from *Study A* and pre-training values from *Study B*. (No post-dynamic exercise biopsy taken in *Study B*).

<sup>b</sup> significantly different from post-dynamic exercise (p < 0.05).

<sup>c</sup> 6 samples had post isometric exercise [La] and pH values atypical of muscles exercised to fatigue ($\bar{x}$ [La] = 14.9 ± 2.6 mmol.kg dm<sup>-1</sup> and $\bar{x}$ pH = 7.21 ± 0.05). These were not considered to be representative of previously active muscle and were omitted from the analysis (see comments of Appendix 5).
Table 4.13  Male vs female muscle lactate concentration and homogenate pH post-dynamic and post-isometric exercise to fatigue.

<table>
<thead>
<tr>
<th></th>
<th>Male (M)</th>
<th>Female (F)</th>
<th>Signif. of M v F difference (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(\bar{x})</td>
<td>SD</td>
</tr>
<tr>
<td>POST-ISOMETRIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homogenate pH</td>
<td>24</td>
<td>6.83</td>
<td>0.13</td>
</tr>
<tr>
<td>Lactate (mmol.kg(^{-1}))</td>
<td>24</td>
<td>64.8</td>
<td>17.6</td>
</tr>
<tr>
<td>POST-DYNAMIC</td>
<td>7</td>
<td>6.75</td>
<td>0.18</td>
</tr>
<tr>
<td>Homogenate pH</td>
<td>10</td>
<td>97.3</td>
<td>33.4</td>
</tr>
<tr>
<td>Lactate (mmol.kg(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Muscle carnosine concentration and muscle buffer value

There was a low, but significant positive relationship between the muscle buffer value (B) and carnosine concentration ([CAR]) (\(r=0.30\), \(p=0.0106\), \(n=56\); Fig. 4.1). The regression equation describing the relationship was:
\[
B (\text{mmol H}^+ . \text{kg}^{-1} . \text{pH}^{-1}) = 133.7 + 1.171 \times [\text{CAR}] (\text{mmol.kg}^{-1}) \quad (\text{SEE} = 18.0) \quad (R.Eq^n1)
\]
Carnosine accounted for only 9% of the variance in B (\(R^2=0.09\)). According to the regression equation, a unit (mmol.kg\(^{-1}\)) increase in carnosine would result in a 1.17 mmolH\(^+\).kg\(^{-1}\).pH\(^{-1}\) increase in the buffer value.

Carnosine concentration and fibre type distribution

There was a low, but significant positive relationship between [CAR] and the % area occupied by type II(a+b) fibres (\(n=33\): \(r=0.32\), \(p=0.034\); Fig. 4.2), described by the regression equation:
\[
[\text{CAR}] (\text{mmol.kg}^{-1}) = 15.39 + 0.121 \times \% \text{area IIa+b} \quad (\text{SEE} = 4.4) \quad (R.Eq^n2)
\]
[CAR] did not correlate significantly with the % (by number) type II(a+b) fibres (\(n=33\), \(r=0.19\), \(p=0.150\) respectively).

Muscle buffer value and fibre type distribution

The muscle buffer value demonstrated a low, but significant positive correlation with the relative area occupied by type II(a+b) fibres (\(n=26\), \(r=0.36\), \(p=0.033\); Fig. 4.3);
\[
B (\text{mmol H}^+ . \text{kg}^{-1} . \text{pH}^{-1}) = 126.7 + 0.581 \times \% \text{area IIa+} \quad (\text{SEE} = 18.7) \quad (R.Eq^n3)
\]
No significant relationship was observed between B and the % (by number) type II(a+b) fibres (\(n=26\), \(r=0.21\), \(p=0.152\)).
Fig. 4.1 Relationship between muscle carnosine concentration and muscle buffer value.
Fig. 4.2 Relationship between percentage type IIa+b fibre area and muscle carnosine concentration.
Fig. 4.3 Relationship between percentage type IIa+b fibre area and the muscle buffer value.
4.3.7 Interrelationships between muscle lactate production, muscle pH, fibre type distribution and muscle buffer value (Bvit).

Muscle lactate concentration and fibre type distribution
The muscle lactate concentration ([La]) post-dynamic (DYN) and post-isometric (ISOM) exercise was significantly correlated with the % area occupied by type Ila+b fibres (DYN: n=13, r=0.60, p=0.014 and ISOM: n=11, r=0.79, p=0.002; Figs. 4.4a and b). The regression equations of [La] on % type Ila+b area for DYN and ISOM exercise were:

\[ [\text{La}]_{\text{DYN}} \text{ (mmol.kg}^{-1}\text{)} = 31.17 + 1.382 \% \text{ area Ila+b (SEE = 23.0)} \] .......(R.Eq n4)

\[ [\text{La}]_{\text{ISOM}} \text{ (mmol.kg}^{-1}\text{)} = 5.77 + 1.054 \% \text{ area Ila+b (SEE = 9.9)} \] .......(R.Eq n5)

The equations predict that a muscle with 100 % type Ila+b fibres would generate a [La] DYN and [La] ISOM of 169.4 ± 23.0 and 111.2 ± 9.9 mol.kg dm\(^{-1}\) respectively. Similarly, a muscle with 0 % type Ila+b fibres (i.e. 100% type I and "other fibre types") would have a [La] DYN and [La] ISOM of 31.2 ±23.0 and 5.8 ± 9.9 mol.kg dm\(^{-1}\) respectively.

The relationships between [La] DYN or [La] ISOM and % (by number) type Ila+b fibres were less strong (compared with [La] vs % type area), but still statistically significant (DYN: n=13, r=0.51, p=0.038 and ISOM: n=11, r=0.72, p=0.061).

Muscle homogenate pH and fibre type distribution
The final muscle pH post DYN exercise was negatively correlated with the relative area occupied by type Ila+b fibres (n=9, r=-0.59, p=0.046; Fig. 4.5). The relationship was described by the regression equation:

\[ \text{pH}_{\text{DYN}} = 7.030 - 0.00669 \% \text{ area Ila+b (SEE = 0.109)} \] ...........(R.Eq n6)

It can be estimated that a muscle composed entirely of type Ila+b fibres would demonstrate a post-exercise (DYN) pH of 6.361 ± 0.109, and one with 100% type I and "other fibre types", a pH of 7.03 ± 0.109.

A non-significant negative relationship was observed between pH post isometric exercise and % type Ila+b fibre area (n=11, r=-0.245, p=0.236).

Muscle lactate concentration, muscle pH and buffer value
Multiple regression analysis revealed that the Δ muscle pH pre to post exercise was significantly related to the combined effects of the Δ [La] and the muscle buffer value, following either dynamic (n=8, r= 0.81, p=0.042) or isometric (n=33,
Fig. 4.4 Relationship between percentage type IIa+b fibre area and muscle lactate concentration following (a) dynamic and (b) isometric exercise to fatigue.
Fig. 4.5 Relationship between percentage type IIa+b fibre area and muscle homogenate pH following dynamic exercise to fatigue.
r=0.66, p < 0.0003) exercise. The regression equations describing the relationships were:

\[ \Delta \text{pH}_\text{DYN} = 0.351 + 0.0047 \Delta [\text{La}] - 0.0015 B \text{ (SEE = 0.114)} \] (R.Eq\textsuperscript{n7})

\[ \Delta \text{pH}_\text{ISOM} = 0.286 + 0.0064 \Delta [\text{La}] - 0.0020 B \text{ (SEE = 0.123)} \] (R.Eq\textsuperscript{n8})

Thus, for a given \( \Delta [\text{La}] \), a unit (mmol\textsuperscript{+}.kg\textsuperscript{-1}.pH\textsuperscript{-1}) increase in buffer value effects an upward shift in the muscle pH of approximately 0.002 units.

In both of these equations, the regression coefficients for \( \Delta [\text{La}] \) (0.0047 and 0.0064) are approximately 3 times the corresponding values for \( B \) (0.0015 and 0.0020). Thus, to maintain the same \( \Delta \text{pH} \) with an increased \( \Delta [\text{La}] \), the absolute increase in the buffer value (in mmol.kg\textsuperscript{-1}.pH\textsuperscript{-1}) must be approximately threefold the absolute increase in the \( \Delta [\text{La}] \) (in mmol.kg\textsuperscript{-1}). For example, for a muscle to accumulate a 10 mmol.kg\textsuperscript{-1} greater \( \Delta [\text{La}] \), the buffer value would have to increase by 30 mmol\textsuperscript{+}.kg\textsuperscript{-1}.pH\textsuperscript{-1} for there to be no further change in the \( \Delta \text{pH} \), pre to post exercise.

An elevated buffer value was not associated with an increased ability to generate a high [La] during dynamic or isometric exercise: \( B_{vit} \) vs \( \Delta [\text{La}]_\text{DYN} \), \( r=-0.17, \) p=0.278 (n=14) and \( B_{vit} \) vs \( \Delta [\text{La}]_\text{ISOM} \), \( r=-0.05, \) p=0.383 (n=34). The muscle buffer value did not correlate significantly with the \( \Delta \text{pH}_\text{DYN} \) (n=9, \( r=-0.34, \) p=0.188) or the \( \Delta \text{pH}_\text{ISOM} \) (n=33, \( r=-0.25, \) p=0.077), but there was a tendency for a higher buffer value to be associated with a lesser \( \Delta \text{pH} \) pre to post exercise.

4.3.8 Relationships between performance and muscle histochemical and biochemical parameters

Performance in many of the exercise tests described in the present study is likely to be influenced by the size of the active muscle mass. It is impossible therefore, to relate performance to muscle fibre type distribution, buffer value etc., if the performance data is not "normalised" (i.e. expressed in a manner which reflects the size of the active muscle mass for each individual). A commonly used measurement for providing an indication of the active muscle mass is the muscle cross-sectional area (Maughan et al, 1983a, 1983b). The quadriceps is one of the major muscle groups utilised during cycling and leg extensions (Forge et al, 1986; Ericson et al, 1986); as such, the cross-sectional area of the quadriceps is a useful indicator of the active muscle mass involved in these activities.

It has been suggested that the major determinant of a muscle's total cross-sectional area is the mean area of the individual fibres comprising that muscle.
Haggmark et al. (1978; Schantz et al., 1981, 1983; MacDougall et al., 1984). Haggmark et al. (1978) demonstrated a high positive correlation between mean fibre area (determined from a biopsy sample from the vastus lateralis) and total cross-sectional area (by CT scan) of either the vastus lateralis (r=0.91) or the total thigh muscle (r=0.84). Using different muscle groups, other investigators have confirmed this relationship (triceps surae, r = 0.75 - Alway et al., 1989; biceps brachii, r = 0.71 - McDougall et al., 1984).

In the absence of the required instrumentation to determine whole muscle cross-sectional area, the performance data was normalised to the mean fibre area, as determined from the analysis of muscle biopsy samples. Confirmation of the validity of this measure as an indicator of the quadriceps CSA, is given by the significant positive correlation between maximum voluntary contraction (MVC) of the quadriceps and the mean fibre area (r=0.52, p = 0.011; Fig. 4.6). Exclusion of one outlying point (circled in Fig. 4.6) improved the linear fit of the data, to yield a correlation coefficient of r=0.66 (p = 0.002). These coefficients are similar to those reported by Borges and Essen-Gustavsson (1989) for the same correlation (males r=0.56, females r=0.74; p<0.05) and within the same range as those reported between total quadriceps CSA and MVC, using a similar subject populace (Maughan et al., 1983a, 1983b; Maughan and Nimmo, 1984: r = 0.51, 0.59, 0.70).

The following results are from the data of Study A only (no mean fibre area data available from Study B).

Males and females did not differ significantly in their normalised values for maximum voluntary contraction (MVC), impulse (IMP), and work done (WORK) (p > 0.05; Table 4.14). Peak power output/MFA was significantly higher in male than female subjects (p=0.047). One female subject had consistently low values for all normalised performance parameters (possibly the result of an erroneous value for mean fibre area, which was approximately 35 % higher than the mean MFA of all the other female subjects, and was obtained from one sample only). When the data from this subject was omitted from the analyses, there was no significant difference between males and females for any of the performance variables (presented in italics in Table 4.14). As such, all correlations between performance and muscle histochemistry/biochemistry were carried out on the data of male and female subjects collectively (but omitting the anomalous results of the aforementioned female).
Fig. 4.6 Relationship between mean fibre area and maximal voluntary contraction of the quadriceps (MVC).
 Male and female normalised performance results (normalised to mean fibre area; MFAa).

<table>
<thead>
<tr>
<th></th>
<th>MVC(N)/MFAb</th>
<th>IMP(Nxs)/MFA</th>
<th>PPO(W)/MFA</th>
<th>WORK(J)/MFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALE (M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12) SD</td>
<td>0.156</td>
<td>5.78</td>
<td>0.253</td>
<td>4.86</td>
</tr>
<tr>
<td>Range</td>
<td>0.042</td>
<td>1.52</td>
<td>0.058</td>
<td>1.11</td>
</tr>
<tr>
<td>Sig. of M/F diff</td>
<td>0.096-0.251</td>
<td>3.92-8.45</td>
<td>0.174-0.382</td>
<td>3.60-7.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEMALE (F)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=6) SD</td>
<td>0.125(0.129)</td>
<td>5.01(5.44)</td>
<td>0.205(0.219)</td>
<td>4.29(4.63)</td>
</tr>
<tr>
<td>Range</td>
<td>0.029(0.030)</td>
<td>1.55(1.27)</td>
<td>0.047(0.033)</td>
<td>1.05(0.74)</td>
</tr>
<tr>
<td>Sig. of M/F diff</td>
<td>0.102-0.173</td>
<td>2.87-5.65</td>
<td>0.130-0.258</td>
<td>2.63-5.65</td>
</tr>
<tr>
<td>(p value)</td>
<td>0.060(0.109)</td>
<td>0.167(0.336)</td>
<td>0.047d(0.125)</td>
<td>0.156(0.335)</td>
</tr>
</tbody>
</table>

a mean fibre area (MFA) = mean of 2 biopsy samples where available
b all performance data is expressed merely as a ratio of the performance value (in its appropriate units) to the mean fibre area. This avoids the interpretation that a given mean fibre area per se, is capable of generating a certain amount of force, power etc.
c values in brackets, in italics, are the results for females excluding one subject with anomalous results (see text).
d significant difference males v females (p < 0.05).

Interrelationships between performance variables
The correlation matrix involving all the performance measures is shown in Table 4.15 (all mass-dependent performance measures have been normalised to mean fibre area (MFA)). Peak power output (PPO/MFA), work done (WORK/MFA) and impulse (IMP/MFA) were each highly correlated with isometric leg strength (MVC/MFA) (p < 0.05). There was a significant, positive correlation between the two measures of dynamic high intensity exercise capacity (PPO/MFA and WORK/MFA; r=0.95) which was retained (partial r = 0.87) even when the common effects of MVC/MFA were removed. PPO/MFA and WORK/MFA each showed a significant positive correlation with IMP/MFA (r=0.53, p=0.013 and r=0.53, p=0.014 respectively). However, when the influence of MVC/MFA was
Table 4.15  Correlation matrix of performance variables, fibre type distribution and muscle buffer value.

<table>
<thead>
<tr>
<th></th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PPO/MFA$^a$</td>
<td>0.95</td>
<td>0.84</td>
<td>-0.42</td>
<td>0.53</td>
<td>0.57</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>p=0.000</td>
<td>p=0.000</td>
<td>p=0.027</td>
<td>p=0.013</td>
<td>p=0.008</td>
<td>p=0.074</td>
</tr>
<tr>
<td>2. W0RK/MFA</td>
<td>0.78</td>
<td>-0.39</td>
<td>0.53</td>
<td>0.48</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.0002</td>
<td>p=0.059</td>
<td>p=0.014</td>
<td>p=0.025</td>
<td>p=0.095</td>
<td></td>
</tr>
<tr>
<td>3. MVC/MFA</td>
<td>-0.42</td>
<td>0.76</td>
<td>0.33</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.044</td>
<td>p=0.000</td>
<td>p=0.09</td>
<td>p=0.165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. 60%MVC TIME</td>
<td>0.26</td>
<td>-0.30</td>
<td>-0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.154</td>
<td>p=0.112</td>
<td>p=0.229</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. IMPULSE/MFA</td>
<td>0.25</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.166</td>
<td>p=0.123</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. % TYPE II AREA$^b$</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p=0.047</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$MFA = mean fibre area

$^b$mean values from 2 samples where available.

N=17: The normalised performance values from one subject were omitted from the analyses, because they consistently gave anomalous values which seriously influenced the general relationships (see results section of male/female performance differences (Table 4.13)).
eliminated, the correlation between PPO/MFA or WORK/MFA and IMP/MFA became negative and non-significant (r=-0.31 and r=-0.15, respectively). PPO/MFA and WORK/MFA, each correlated negatively with 60% MVC endurance time (END) (r=-0.42, p=0.027 and r=-0.39, p=0.059 respectively). Thus, performance in the dynamic and isometric high intensity exercise tests was effectively inversely related.

Relationships between fibre type distribution and performance. PPO/MFA and WORK/MFA were each significantly related to the % type Iia+b area (r=0.57, p=0.008 and r=0.48, p=0.025 respectively; Figs. 4.7a and 4.7b), as described by the following regression equations:

\[
PPO(W)/MFA = 0.0939 + 0.003 \% \text {area type Ila+b} \quad \text{(SEE} = 0.045) \quad \text{... (R.Eq n9)}
\]

\[
WORK(kJ)/MFA = 2.48 + 0.05 \% \text {area type Ila+b} \quad \text{(SEE} = 0.903) \quad \text{... (R.Eq n10)}
\]

The equations predict that a muscle composed of 100% type Iia+b fibres would generate a PPO(W)/MFA of 0.394 ± 0.045 and a WORK(J)/MFA of 7.48 ± 0.903. A muscle with 0% type Iia+b fibres would produce a PPO/MFA of 0.094 ± 0.045 and a WORK/MFA of 2.48 ± 0.903. The hypothetical 100 % type Iia+b muscle would therefore generate approximately 4 times as much power and perform 3 times as much work as one composed entirely of type I + "other fibre types".

MVC/MFA and IMP/MFA demonstrated positive but non-significant relationships with the % area occupied by type Iia+b fibres (r=0.33, p=0.09 and r=0.25, p=0.166 respectively). END showed a negative but non-significant relationship with % type Iia+b area (r=-0.30, p=0.112).

Relationship between muscle buffer value and performance. The buffer value (Bvit) accounted for only 12 % of the variance in WORK/MFA, the relationship between the two reaching significance only at the 10% level (r=0.34, p=0.095):

\[
WORK(J)/MFA=2.16 + 0.02 \text{Bvit(mmol H+.kg^{-1}.pH^{-1})} \quad \text{(SEE}=0.71)\text{... (R.Eq n11)}
\]

From the equation, it can be seen that a unit increase in Bvit is associated with a 0.02 unit (J/MFA) increase in WORK/MFA (or, a unit increase in WORK/MFA requires a 50 mmolH+.kg^{-1}.pH^{-1} increase in Bvit).

IMP/MFA and 60% MVC endurance time (END) were not significantly correlated with the muscle buffer value (r=0.31, p=0.124 and r=-0.20, p=0.229 respectively).
Fig. 4.7 Relationship between percentage type IIa+b fibre area and (a) peak power output (b) work done

(performance measures = in ratio with mean fibre area).
Relationship between performance and post-exercise muscle pH and lactate concentration ([La])

WORK/MFA showed a significant positive correlation with the muscle lactate concentration post dynamic exercise (n=14, r=0.54, p=0.023) and a non-significant negative correlation with the post-exercise muscle pH (n=10, r=-0.44, p=0.102) (Figs. 4.8a and 4.8b). The WORK/MFA accomplished during the dynamic exercise test was also positively correlated with the ∆ [La] during performance of the isometric exercise test (n=12, r=0.48, p=0.057).

IMP/MFA showed no direct relationship with the post isometric exercise lactate concentration ([La]) (n=12, r=-0.05, p=0.436), but showed a significant positive correlation with the post-exercise muscle pH (n=12, r=0.54, p=0.034). A significant relationship was observed between impulse/MFA and the ratio of impulse/MFA to lactate accumulated (IMP/MFA ÷ mmol[La].kg⁻¹) (n=12, r=0.77, p=0.002; Fig. 4.9). The regression equation describing the relationship was:

\[
\text{IMP/MFA} = 2.2 + 32.04 \left( \frac{\text{IMP/MFA}}{\text{mmol[La].kg}^{-1}} \right) \quad (\text{SEE}=7.71) \quad \text{(R.Eqn12)}
\]

60% MVC endurance time (END) showed no significant correlation with the post-exercise lactate concentration ([La]) (n=12, r=-0.27, p=0.198) or the post-exercise muscle pH (n=12, r=0.063, p=0.420), although a significant positive relationship was observed between END and the ratio of END to lactate accumulated (END ÷ mmol[La].kg⁻¹) (n=12, r=0.76, p=0.001; Fig. 4.10), described by the regression equation:

\[
\text{END(60\% \text{ MVC})} = 36.5 + 24.2 \left( \frac{\text{END}}{\text{mmol.kg[La]^{-1}}} \right) \quad (\text{SEE}=7.71) \quad \text{(R.Eqn13)}
\]

4.3.9 Training effects on performance

Isometric exercise tests

The effects of 16 weeks isokinetic training of the knee extensors, on performance in the isometric exercise test, are shown in Table 4.16. A significant difference was found between pre and post training values of MVC for all three groups together (p=0.022), but the group x training interaction was non-significant (p=0.167). Thus, although MVC was increased on average by 8.1% (range -9.0% to 23.9%) in the two training groups, the improvements were not significant compared with the control group response (mean increase 3.6%, range 0 to 14.7%). Male and female subjects (both training groups) demonstrated a similar percentage increase in MVC (6% and 8% respectively). There was no significant training response in 60% MVC endurance time or impulse (Table 4.16).
Fig. 4.8 Relationship between work done and post-exercise (a) muscle lactate concentration (b) homogenate pH.

(work done = in ratio with mean fibre area (MFA))
Fig. 4.9 Relationship between impulse/mean fibre area (MFA) and the ratio of impulse/MFA to lactate accumulated during isometric exercise.
Endurance (s) at 60% MVC

Fig. 4.10 Relationship between 60% MVC endurance time (END) and the ratio of END to muscle lactic acid accumulated during isometric exercise.
Table 4.16 Training effects on isometric performance parameters.

<table>
<thead>
<tr>
<th></th>
<th>MVC (N)</th>
<th>60 % MVC (s)</th>
<th>IMPULSE (kNxs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE POST</td>
<td>PRE POST</td>
<td>PRE POST</td>
</tr>
<tr>
<td>CONTROL (n=10)</td>
<td>x 550 570</td>
<td>58.8 53.7</td>
<td>18.9 17.4</td>
</tr>
<tr>
<td></td>
<td>SD 276 265</td>
<td>8.6 8.6</td>
<td>8.3 5.6</td>
</tr>
<tr>
<td>TRAINING AT 240 deg.s⁻¹ (n=13)</td>
<td>x 583 643</td>
<td>50.0 46.4</td>
<td>16.9 17.6</td>
</tr>
<tr>
<td></td>
<td>SD 166 184</td>
<td>15.7 10.5</td>
<td>5.7 5.5</td>
</tr>
<tr>
<td>TRAINING AT 60 deg.s⁻¹ (n=10)</td>
<td>x 598 631</td>
<td>57.8 57.4</td>
<td>19.9 21.2</td>
</tr>
<tr>
<td></td>
<td>SD 181 150</td>
<td>15.0 13.5</td>
<td>6.0 5.5</td>
</tr>
<tr>
<td>BOTH TRAINING GROUPS</td>
<td>x 590 638</td>
<td>53.5 51.4</td>
<td>18.2 19.2</td>
</tr>
<tr>
<td></td>
<td>SD 169 166</td>
<td>15.5 12.9</td>
<td>5.9 5.7</td>
</tr>
</tbody>
</table>

Significance of group x training interaction (p value) 0.167 0.467 0.122

* one control subject experienced pain in generating an MVC in the 'post' assessments - 60 % MVC load was therefore set from the 'pre' value of MVC, which was assumed, in line with the rest of the group, not to have changed significantly, pre to post.

Dynamic exercise tests
The data from the modified Wingate Anaerobic Tests was analysed both with and without corrections for the acceleration/deceleration of the flywheel (see Chapter 2 (Methods); Section 2.1.1). Comparison of performance values calculated using each method of analysis is given below, prior to presentation of the training results.
Corrected vs uncorrected measures of power

Trend analysis of the relationships between the performance criteria and the test load (all subjects pre- and post-training) showed that over the load range under examination (0.09-0.14 x MVC), time of test (TIME), work done (WORK), peak velocity (REVS), mean power output (uncorrected; U.MPO and corrected; C.MPO) and time to peak power (uncorrected; U.Tpp and corrected; C.Tpp) were a linear function of the resistive load (Appendix 4). Uncorrected and corrected peak power output (U.PPO and C.PPO) were better described as a quadratic function of the resistive load (Appendix 4).

Over the entire load range, the average C.PPO was 17% greater than the average U.PPO (p=0.0000, Table 4.17). Similarly, the maximum C.PPO (i.e. maximum value attained across the load range) was significantly greater (18%) than the maximum U.PPO (p=0.0006). There was a significant positive correlation between C.PPO and U.PPO, expressed either as average, or maximum values (r=0.97, p=0.0000 and r=0.93, p=0.0000, respectively). Time to corrected peak power (C.Tpp) was on average, 2.4 s shorter than time to uncorrected peak power (U.Tpp); U.PPO occurred after approximately 3 s, whereas C.PPO was reached within less than 1 s (see Table 4.18). Corrected MPO over the load range 0.09-0.14% MVC was similar to uncorrected MPO (corrected values approximately 1-2% lower than uncorrected) (see Table 4.18).

### Table 4.17 Comparison of corrected (C.PPO) and uncorrected (U.PPO) peak power output (normalised to body mass).

<table>
<thead>
<tr>
<th></th>
<th>Average value over range 0.09-0.14 x MVC</th>
<th>Highest value over range 0.09-0.14 x MVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORRECTED PPO (W.kg bm⁻¹)</td>
<td>x</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.3</td>
</tr>
<tr>
<td>UNCORRECTED PPO (W.kg bm⁻¹)</td>
<td>x</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.4</td>
</tr>
<tr>
<td>Significance of U.PPO vs C.PPO difference (p value)</td>
<td>0.0000</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Pre-training values; n=32 (one subject performed no dynamic exercise tests).
Training response
The effects of training on dynamic high intensity exercise capacity are shown in Table 4.18, where the values for each performance parameter represent the average over the entire load range (0.09-0.14 x MVC). The statistical analysis was carried out by means of contrasts between:

(a) the "no-training" response of the control group and the training response of the two training groups together, and

(b) the training response of the two training groups (60 and 240 deg.s\(^{-1}\)). However, the training response was not significantly different between the two training groups for any of the performance variables (p > 0.05). Therefore, the significance of the training response (last column, Table 4.18) is reported as for contrast (a) (above).

There was a significant difference (pre to post-training) in TIME when all three groups were considered together (p=0.02), but the difference between the response of the control group (mean increase=1\%) and the training groups (mean increase=6-8\%) was not significant (p=0.157; Table 4.18).

The training response in the variables U.PPO (and therefore REVS), C.PPO and WORK was significantly different between the training and control groups (p < 0.05) (mean increase in U.PPO and C.PPO pre to post-training (training groups) = 5-8\%, mean increase in WORK = 8-9\%) (Table 4.18). There was no significant training response in U.MPO, C.MPO, U.Tpp and C.Tpp (Table 4.18).

Changes with training, in the relationship between each of the performance parameters and the resistive load applied, were analysed by means of contrasts between the linear or quadratic trends. There was no significant difference between the control group and training groups for any of these relationships. Thus the mean training-induced increases in performance, were consistent across the load range.

Training effects on relationship between MVC and PPO
Training groups
Pre-training (both training groups), there was a significant positive relationship between MVC and C.PPO (average C.PPO over the load range) (r=0.86, p=0.0000; Fig. 4.11). The correlation between MVC and average work done over the load range, just failed to reach significance (r=0.34, p=0.061). Analysis of covariance
Fig. 4.11 Relationship between maximum voluntary contraction of the quadriceps (MVC) and corrected peak power output (C.PPO).

(C.PPO = average over the resistive load range.)
demonstrated no significant difference with training, in either the slope or the elevation of the linear regression of C.PPO on MVC (p > 0.05):

**PRE-TRAINING:**

\[ x \text{ C.PPO (W)} = 211.2 + 1.326 \text{ MVC (N)} \ (r=0.86; \text{ SEE } = 116.5) \ldots \text{(R.Eqn 14)} \]

**POST-TRAINING:**

\[ x \text{ C.PPO (W)} = 211.6 + 1.344 \text{ MVC (N)} \ (r=0.82; \text{ SEE } = 148.9) \ldots \text{(R.Eqn 15)} \]

However, from the pre-training regression equation, of C.PPO on MVC (regression coefficient = 1.326), an increase in MVC of 48 N (the post-training increase; Table 4.16) would be expected to cause an increase in mean C.PPO of 64 W. The actual mean increase observed was 71 W, which is 11% greater than predicted.

**Control group**

Again, a significant positive relationship between MVC and \( x \text{C.PPO} \) was observed (r=0.96, p=0.0000), with no significant difference pre and post, in either the slope or the elevation of the linear regression equation (p > 0.05):

**PRE-"NO-TRAINING"**

\[ x \text{ C.PPO (W)} = 264.1 + 1.208 \text{ MVC (N)} \ (r=0.96; \text{ SEE } = 105.2) \ldots \text{(R.Eqn 16)} \]

**POST-"NO-TRAINING":**

\[ x \text{ C.PPO (W)} = 198.3 + 1.255 \text{ MVC (N)} \ (r=0.97; \text{ SEE } = 90.4) \ldots \text{(R.Eqn 17)} \]

The mean change in MVC (pre to post) in the control group was 20 N (Table 4.16). With a regression coefficient of 1.208 (see above equation), this should have been associated with an increase in mean C.PPO of 24 W; the observed change was a 13 W decrease.

**4.3.10 Training effects on the *in vitro* muscle buffer value (Bvit) and muscle carnosine concentration**

No significant training effects on the muscle carnosine concentration ([CAR]) or the in vitro muscle buffer value (Bvit) were observed (p > 0.05; Table 4.19), although [CAR] and Bvit were increased by 11% and 6% respectively (post-training) in the 60 deg.s\(^{-1}\) group.
Table 4.18  Effects of training on performance of modified Wingate Anaerobic Tests.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n=6)</th>
<th>TRAINING GROUPS</th>
<th>Signifb of training response (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre Post</td>
<td>Pre Post</td>
<td></td>
</tr>
<tr>
<td>Time of test (s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>x SD</td>
<td>Pre Post</td>
<td></td>
</tr>
<tr>
<td>Uncorrected Peak power (W.kg bm⁻¹)</td>
<td>31.7 5.4</td>
<td>29.5 7.7</td>
<td>31.4 9.5</td>
</tr>
<tr>
<td>Corrected Peak power (W.kg bm⁻¹)</td>
<td>31.4 5.2</td>
<td>7.7 8.4</td>
<td>9.5 9.9</td>
</tr>
<tr>
<td>Uncorrected Mean power (W.kg bm⁻¹)</td>
<td>11.55 1.69</td>
<td>11.39 1.64</td>
<td>12.17 2.66</td>
</tr>
<tr>
<td>Corrected Mean power (W.kg bm⁻¹)</td>
<td>11.46 1.72</td>
<td>14.26 1.79</td>
<td>15.27 3.09</td>
</tr>
<tr>
<td>Uncorrected Work done (J.kg bm⁻¹)</td>
<td>6.74 1.02</td>
<td>8.19 1.21</td>
<td>8.72 1.78</td>
</tr>
<tr>
<td>Corrected Work done (J.kg bm⁻¹)</td>
<td>6.67 2.04</td>
<td>8.37 1.37</td>
<td>8.61 1.76</td>
</tr>
<tr>
<td>Work done (J.kg bm⁻¹)</td>
<td>209 50</td>
<td>233 48</td>
<td>267 93</td>
</tr>
<tr>
<td>Time to uncorrected peak power (s)</td>
<td>6.59 1.00</td>
<td>8.07 1.18</td>
<td>8.61 1.76</td>
</tr>
<tr>
<td>Time to corrected peak power (s)</td>
<td>6.59 1.00</td>
<td>8.28 1.34</td>
<td>8.61 1.76</td>
</tr>
<tr>
<td>Peak revs (rpm)</td>
<td>209 50</td>
<td>233 48</td>
<td>267 93</td>
</tr>
</tbody>
</table>

Values are mean of the group mean and SD at each resistive load.  
[a] not all subjects successfully completed the post-training tests over the full load range.  
[b] = significance of training response of both training groups, as compared with control group response.
Table 4.19 Effects of training on the *in vitro* muscle buffer value and muscle carnosine concentration.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n=10)</th>
<th>TRAINING GROUPS</th>
<th>Signif(^{b}) of training response (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>240 deg.s(^{-1})</td>
<td>60 deg.s(^{-1}) (n=7)</td>
</tr>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
</tr>
<tr>
<td>Carnosine concentration (mmol.kg dm(^{-1}))</td>
<td>$\bar{x}$</td>
<td>17.6</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>4.3</td>
<td>4.9</td>
</tr>
<tr>
<td>Muscle buffer value (mmol H(^+). kg dm(^{-1}).pH(^{-1}))</td>
<td>$\bar{x}$</td>
<td>159.8</td>
<td>157.3</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>22.4</td>
<td>18.8</td>
</tr>
</tbody>
</table>

\(^{a}\) n=7 for carnosine concentration - insufficient muscle was available to determine [CAR] in the post-training sample from one subject.

\(^{b}\) = significance of group x training interaction.

4.3.11 Training effects on muscle lactate concentration and muscle homogenate pH post isometric exercise

In the 240 deg.s\(^{-1}\) training group, two subjects pre-training, and two subjects post-training, demonstrated values for pH and lactate concentration ([La]) atypical of muscles exercised to fatigue ([La] 7.5-14.4 mmol.kg dm\(^{-1}\); pH 7.09-7.23). Similarly, in the 60 deg.s\(^{-1}\) training group, one subject pre-training, and two subjects post-training, demonstrated atypical post-exercise [La] and pH values ([La] 8.1-12.3 mmol.kg dm\(^{-1}\); pH 7.11-7.17). The preceding isometric performance was not however, significantly different from that recorded post-habituation and this was therefore believed to be an error in the sampling technique (see Appendix 5). These erroneous [La] and pH values were not considered suitable for inclusion within the analysis of the group training responses, and therefore the numbers for each of the two training groups (matched pairs pre and post training) decreased to n=4. (All data including these anomalous values are shown in Appendix 5.)

The pH and [La] values at rest and post-isometric exercise (60 % MVC to fatigue), pre and post-training are shown in Table 4.20. Both training groups demonstrated a tendency (n.s.) to generate higher lactate concentrations and lower
pH values post-training (although inferences drawn from such a small subject populace must necessarily be guarded).

Table 4.20  Muscle lactate concentration ([La]) and homogenate pH at rest and post isometric exercise, pre and post training.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n=10)</th>
<th>240 DEG/s (n=4/8)a</th>
<th>60 DEG/s (n=4/7)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>LACTATE CONCENTRATION ([La]) (mmol.kg dm⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>4.7 ± 1.8</td>
<td>6.0 ± 2.2</td>
<td>7.7 ± 4.7</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>68.0 ± 14.6</td>
<td>67.4 ± 21.0</td>
<td>65.4 ± 25.7</td>
</tr>
<tr>
<td>POST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>8.5 ± 4.6</td>
<td>5.8 ± 2.7</td>
<td>5.3 ± 2.1</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>56.1 ± 27.2</td>
<td>76.3 ± 18.3</td>
<td>74.4 ± 11.9</td>
</tr>
<tr>
<td>MUSCLE HOMOGENATE pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>7.22 ± 0.12</td>
<td>7.16 ± 0.11</td>
<td>7.20 ± 0.13</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>6.82 ± 0.09</td>
<td>6.91 ± 0.11</td>
<td>6.84 ± 0.22</td>
</tr>
<tr>
<td>POST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>7.20 ± 0.15</td>
<td>7.15 ± 0.11</td>
<td>7.21 ± 0.15</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>6.86 ± 0.20</td>
<td>6.85 ± 0.15</td>
<td>6.79 ± 0.09</td>
</tr>
</tbody>
</table>

a 4/8 and 4/7 = 4 out of 8 samples and 4 out of 7 samples, respectively; data points were excluded, which were not considered to be representative of muscles exercised to fatigue (see text and Appendix 5).

4.3.12 Training effects on the *in vivo* buffer value (*B*viv )

When *B*viv was determined from regression analysis of the relationship between [La] and pH, no significant training response (p > 0.05) in the elevation or slope of the relationship was observed, for any of the groups (Table 4.21). However, Table 4.21 highlights some important findings (which confirm the recommendations of Chapter 3) concerning the various methods of analysis of the data.

In the control group there was little difference in either the *in vitro* (*B*vit) or *in vivo* buffer value (*B*viv) pre to post "no training". *B*vit and *B*viv gave similar values, regardless of whether the latter was measured as the slope of the regression of individual values of [La] and pH, or as the mean of individual values of Δ [La]/ Δ pH.
Table 4.21 Comparison of *in vivo* vs *in vitro* buffer values, pre and post-training.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL (n=10)</th>
<th>240 deg.s(^{-1}) (n=4)</th>
<th>60 deg.s(^{-1}) (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vitro buffer value</strong> (mmolH(^+).kg dm(^{-1}).pH(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PRE</strong></td>
<td>160 ± 22</td>
<td>158 ± 19</td>
<td>145 ± 19</td>
</tr>
<tr>
<td><strong>POST</strong></td>
<td>157 ± 19</td>
<td>168 ± 19</td>
<td>165 ± 7</td>
</tr>
<tr>
<td><strong>In vivo buffer value(^a)</strong> (mmolH(^+).kg dm(^{-1}).pH(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(A) <strong>Mean</strong> ((\Delta [La]/\Delta pH))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PRE</strong></td>
<td>161 ± 29</td>
<td>314 ± 153</td>
<td>159 ± 40</td>
</tr>
<tr>
<td><strong>POST</strong></td>
<td>146 ± 37</td>
<td>236 ± 46</td>
<td>196 ± 99</td>
</tr>
<tr>
<td>(B) <strong>Slope from regression of [La] vs pH(^b)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PRE</strong></td>
<td>163 ((r=-0.90))</td>
<td>301 ((r=-0.72))</td>
<td>148 ((r=-0.94))</td>
</tr>
<tr>
<td><strong>POST</strong></td>
<td>148 ((r=-0.87))</td>
<td>257 ((r=-0.77))</td>
<td>164 ((r=-0.92))</td>
</tr>
</tbody>
</table>

\(^a\) In vivo buffer value calculated as:
A) The mean of individual values of \(\Delta [La]/\Delta pH\)
B) The inverse of the slope from all individual data pairs of [La] and pH (value in italics in brackets = correlation coefficient for the relationship between [La] and pH)
\(^b\) no significant difference pre vs post training, in the elevation or slope of the relationship between [La] and pH (p > 0.05).

The 240 deg.s\(^{-1}\) training group demonstrated a 6% increase in \(B_{vit}\) and a 15-24% decrease in the calculated \(B_{viv}\), post-training. The values for \(B_{viv}\) (either pre or post-training) were 40-90% greater than the corresponding values for \(B_{vit}\). The lower correlation coefficients for the regression analyses of [La] vs pH, and the rather high standard deviation for the individual values of \(\Delta [La]/\Delta pH\) pre-training, point to an error in the determination of \(B_{viv}\) (by either of these methods) for this group, and emphasise the potential misinterpretation of such data.

The 60 deg.s\(^{-1}\) group showed a similar increase post-training in both \(B_{vit}\) (14% increase) and \(B_{viv}\) determined from regression analysis of [La] vs pH (11% increase). However, the mean of individual values of \(\Delta [La]/\Delta pH\) was much higher, as was the standard deviation associated with this measure. Again, this is indicative of an error in this mode of analysis of the muscle buffer value.

Thus, for small subject populations, the use of individual values of \(\Delta [La]/\Delta pH\) as a measure of \(B_{viv}\) appears to be inaccurate, and determination of \(B_{viv}\) by regression analysis of [La] on pH is justified only if a high correlation coefficient is obtained for the relationship. In this respect, the non-significant changes in \(B_{vit}\) are probably most representative of the response of the buffer value to training.
Discussion

The efficacy of the biopsy sample: variability in muscle fibre type and fibre area.

In the present study, needle biopsy samples were employed in the evaluation of the histochemical and biochemical properties of the vastus lateralis muscle, and this muscle in turn, was assumed to represent the respective properties of the quadriceps femoris muscle group. The ability of a biopsy sample to accurately reflect the properties of the entire muscle, and the variation observed between multiple samples, have long been the subject of inquiry. From the duplicate samples obtained in the present study, an examination of this variation was made, which can be compared with that observed in previous studies reported in the literature.

Following established guidelines for the determination of fibre type distribution (Dubowitz and Brookes, 1973) and the measurement of fibre area (Blomstrand et al, 1984), good reliability of measurement was obtained for these two parameters, as evidenced by the lack of any significant difference between, and the high reliability coefficients for, values obtained from the duplicate samples. The coefficients of variation (duplicate samples) for the area of type I, IIa, IIb and mean fibre area - 13.2, 13.8, 15.1 and 12.6 % respectively - compare favourably with those reported in the literature:

<table>
<thead>
<tr>
<th>CV from duplicate/multiple samples</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(fibre area) (%)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Tesch, 1980</td>
</tr>
<tr>
<td>10 (type I)</td>
<td>Blomstrand et al, 1984</td>
</tr>
<tr>
<td>15 (type IIa/IIb)</td>
<td>Blomstrand et al, 1984</td>
</tr>
<tr>
<td>17</td>
<td>Mahon et al, 1984 a</td>
</tr>
<tr>
<td>13</td>
<td>Simoneau and Bouchard, 1989</td>
</tr>
<tr>
<td>15-20</td>
<td>Mahon et al, 1984 a</td>
</tr>
<tr>
<td>10-20</td>
<td>Lexell and Taylor, 1989 a</td>
</tr>
</tbody>
</table>

* from review of previous studies cited in the literature

No significant difference (p > 0.05) was observed between the duplicate samples for the relative distribution of the fibre types, or for their relative occupation of the total muscle fibre area (% fibre area). The coefficients of variation (CV) (duplicate samples) for the frequency of occurrence of type I and type IIa fibres (10.5 and 13.1 % respectively) and the relative area occupied by type I and type IIa fibres (12.7 and 13.7 % respectively) were of the same order as those reported in the literature:
The standard deviation (duplicate samples) for % type IIb fibres was similar to that for the % type I and % type IIa (approximately 4%), but expressed as a percent of the mean type IIb occurrence (9.9%), generated a much higher CV (41.1%) than for the other two fibre types. Nonetheless, no significant difference was observed between duplicate samples (p > 0.05), and the reliability coefficient of 0.81 indicated that individuals maintained their "position" within the group, in terms of their % type IIb fibres in the duplicate samples. A particularly elevated CV for the % type IIb fibres (for the same reason), was also reported by Simoneau and Bouchard (1989).

The present data therefore appear to be subject to the same variability as observed in previous studies, where duplicate or multiple sites from the entire muscle were sampled. From the analysis of whole muscle cross-sections, it has been shown that this variation emanates from the non-random distribution of fibre types/areas throughout the muscle (Elder et al., 1982; Lexell et al., 1983, 1985; Lexell and Taylor, 1989. These authors report marked differences in both fibre distribution and fibre area with depth of sampling, with a tendency for a higher proportion of type I fibres, and larger fibre areas (both types) with increasing depth. As such, it has been suggested that attention to, and greater consistency in, the depth of sampling may assist in improving the representative nature of the biopsy sample (Saltin and Gollnick, 1983). However, in practice, attempting to sample at a specific depth into the muscle which is consistent for all individuals alike, is almost impracticable, due to the large interindividual differences in the amounts of both subcutaneous tissue and muscle, and the limited amount of time available for accurate location of 'the site', once into the belly of the muscle. It has also been suggested that an improved accuracy of measure would be furnished by obtaining two (Blomstrand et al., 1984), three (Lexell et al., 1985), or three to five (Elder et al., 1982) samples from the whole muscle, and at varying depths. Obviously, the
feasibility of this approach will depend in part, on the logistics of the study, and the emphasis to be placed on the interpretation of individual results. Saltin and Gollnick (1983), in reviewing this problem, concluded that for most cases the mean value for a group of subjects closely reflects the true value for a given muscle. The non-significant differences between the mean values from duplicate samples in the present study, would appear to support this conclusion.

The CVs for duplicate samples (intra-individual variation), for all parameters derived from the biopsy sample, were on average, only half the corresponding values for the group mean CVs (inter-individual variation):

<table>
<thead>
<tr>
<th></th>
<th>Intra-individual variation (mean CV(%) duplicate samples)</th>
<th>Inter-individual variation (mean CV(%) group results*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% fibre type</td>
<td>11.4</td>
<td>19.4</td>
</tr>
<tr>
<td>fibre area</td>
<td>12.9</td>
<td>22.8</td>
</tr>
<tr>
<td>% fibre type area</td>
<td>12.8</td>
<td>21.9</td>
</tr>
</tbody>
</table>

CVs = mean of the CV for type I fibres and for type Ila+b fibres.
* SD/mean x 100% for group results.

Therefore, in accordance with previous studies (Mahon et al, 1984; Simoneau and Bouchard, 1989) it appears that the greatest component of the variation is between individuals, and cannot be ascribed only to variation in tissue sampling and measurement errors (as was inferred by Lexell and Taylor, 1989).

**Male/female differences in muscle histochemistry**

The distribution (by number) of the three fibre types (type I, Ila and Iib) did not differ significantly between the sexes, although male subjects demonstrated a slightly higher percentage of type I fibres. However, the type Ila : type I fibre area ratio was much greater in males (1.31 : 1) than females (0.96 : 1), which resulted in a significant difference between the sexes, for the relative area occupied by type Ila fibres. The mean fibre areas for male subjects compared favourably with those presented by Mahon et al (1984), who summarised the data of 245 young, healthy male subjects from 20 different studies (see Table 4.22). Fewer studies have been carried out on females, although the tendency for the type I fibre area to be slightly greater than, or similar to, the type II fibre area was consistent with previous
findings (Gregor et al., 1979; Saltin and Gollnick, 1983; Schantz et al., 1983; Simoneau and Bouchard, 1989).

Table 4.22 Comparison of male mean fibre areas (present study) with values cited in the literature.

<table>
<thead>
<tr>
<th>FIBRE AREA (um²)</th>
<th>Type I</th>
<th>Type II A</th>
<th>Type II B</th>
<th>Mean fibre area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahon et al. (1984)a</td>
<td>4531</td>
<td>5343</td>
<td>4634</td>
<td>4968</td>
</tr>
<tr>
<td>Present study</td>
<td>4286</td>
<td>5610</td>
<td>4603</td>
<td>4836</td>
</tr>
</tbody>
</table>

* summarised from 20 different studies, see Table 5 of Mahon et al., 1984.

Intra- and inter-individual variation in skeletal muscle buffer values and carnosine concentration.

If the fibre type distribution and the fibre area are expected (or observed) to vary between randomly obtained samples from a given muscle (Elder et al., 1982; Lexell et al., 1983, 1985; Lexell and Taylor, 1989), then so too may the corresponding biochemical properties. Two such parameters, the muscle buffer value and the muscle carnosine concentration, were examined in this respect, from a group of subjects' duplicate muscle samples. No significant difference (p > 0.05) in either the in vitro buffer value \((B_{vit})\) or the carnosine concentration \([\text{CAR}]\) was observed between the 2 samples, and the coefficients of variation (duplicate samples) were 8.2% \((B_{vit})\) and 13.4% \([\text{CAR}]\). When the anomalous result of one subject was removed from the analysis for \([\text{CAR}]\), the CV was reduced to 7.6%.

To the author's knowledge, no such comparable analyses have previously been performed to investigate the intraindividual variability of \(B_{vit}\) and \([\text{CAR}]\) in human muscle. In a study of the gluteal medius muscle from untrained thoroughbred horses, CVs of 9.9%, 12.2% and 9.0% were obtained for the \(B_{vit}\) determined at multiple biopsy sites from three horses respectively (calculated from the author's raw data, where the CV for each horse = SD/mean x 100 % of 6-10 samples obtained at varying depths and lengths along the muscle) (McCUTCHEON et al., 1987).

The relative consistency of measurements of \(B_{vit}\) and \([\text{CAR}]\) from repeated samples,
tends to suggest that these parameters are quite uniform throughout the muscle and that a single sample is capable of providing an adequate reflection of the whole muscle $Bvit/[\text{CAR}]$ (although this would need to be confirmed by the analysis of whole muscle cross-sections, in a similar manner to the analyses of the variability of fibre type distribution/area performed by Lexell et al, 1983, 1985; Lexell and Taylor, 1989). The results also imply that if $Bvit$ and $[\text{CAR}]$ vary between the fibre types, they do not do so markedly (see later).

Comparison of the mean $Bvit$ determined in the present study with values reported in the literature is difficult, due to the widely differing methodologies employed (see Chapter 3). The mean $\pm$ SD value for the entire subject populace ($157 \pm 19 \text{ mmol H}^+\cdot\text{kg}^{-1}\cdot\text{pH}^{-1}$; $n=57$) was at the low end of the rather wide range of values observed in previous studies (Table 4.23).

The range of buffer values obtained in the present study was from 110-195 mmol H$^+\cdot\text{kg}^{-1}\cdot\text{pH}^{-1}$ and the coefficient of variation for the group (12.1%) was comparable with that reported in previous studies (3-23 % - see Table 4.23). However, this variation was approximately half the value obtained for the group variability in fibre type distribution (group CV=19-23%; see earlier), suggesting that less intersubject variability is associated with the muscle $Bvit$ than with the fibre type distribution. Again, this tends to suggest that either there is a large overlap in the buffer value of different fibre types, or that all fibre types have similar buffer values.

Can the range in individual buffer values be accounted for by the known interindividual variability in the components expected to contribute to the measured muscle buffer value? For freeze-dried-muscle homogenates these components have been identified as : a) phosphate b) carnosine and c) histidine and cysteine residues of proteins. One way of answering this question is to examine the range of values for the individual constituents of total buffering, and calculate the expected difference in buffering from the highest and lowest values in that range (using Equation 5, in Chapter 1(Section 1.5.2.2)). Unfortunately, all the individual components of buffering were not analysed in the present study, and the literature generally reports only mean $\pm$ SD values, without the accompanying range. Therefore some approximations must be made. An estimation of the range of values for a given variable can be obtained from a knowledge of the sample size, mean and SD of a set of values (assuming they are normally or near-normally distributed) (Sokal and Rohlf, 1981) (see calculations for estimated muscle protein ranges in Appendix 6).

The total phosphate ([P$_i$]) available to contribute to the measurement of homogenate $Bvit$ will be equal to the free phosphate concentration plus the
Table 4.23  *In vitro* buffer value of human muscle (summary from literature).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>n</th>
<th>In vitro buffer value (Bvit) (mmol H⁺.kg⁻¹.pH⁻¹)ᵃ</th>
<th>CV (%) (group SD/mean x 100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkhouse <em>et al</em> (1985)</td>
<td>Untrained males  5</td>
<td>91 ± 21 (228)ᵇ</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sprinters males  5</td>
<td>129 ± 24 (322)ᵇ</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rowers males     5</td>
<td>136 ± 31 (340)ᵇ</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marathoners males 5</td>
<td>90 ± 19 (225)ᵇ</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Bevan <em>et al</em> (1985)</td>
<td>Untrained males  6</td>
<td>176 ± 6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Untrained male/female 8</td>
<td>211 ± 17</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Marlin (1989)</td>
<td>Not specified  7</td>
<td>142 ± 22 (110-177)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Mizuno <em>et al</em> (1990)</td>
<td>Trained (X-country skiers) males 10</td>
<td>298ᶜ</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>344ᵈ (274-438)</td>
<td>--</td>
</tr>
<tr>
<td>Present study</td>
<td>Active male/female 57</td>
<td>157 ± 19 (110-195)</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± SD (with ranges (where available) in italics underneath mean values) from the vastus lateralis, unless otherwise specified.

ᵃ Values recalculated where necessary, to mmol H⁺.kg⁻¹.pH⁻¹ (assuming a 77% muscle water content; Hultman and Sahlin, 1980).

ᵇ *Bvit* measured on deproteinised muscle. Values in brackets are estimated values assuming that muscle protein accounts for approximately 60% of physicochemical buffering; Hultman and Sahlin, 1980.

c from gastrocnemius, median values

d from triceps brachii, median values
phosphate released from the hydrolysis of PCr and ATP upon homogenisation of the muscle. An exact value for the range in this total muscle [P_i] is difficult to define: whilst the resting muscle PCr content ([PCr]) has been shown to vary from 57 to 94 (range=37) mmol.kg dm^{-1} (calculated from Harris et al, 1974) or from 40 to 80 (range=40) mmol.kg dm^{-1} (present study), the hydrolysis of these varying amounts of PCr may not equate to a similar range in values of homogenate [P_i]; differences in muscle PCr may only reflect individual variations in the [PCr]/free [P_i] ratio. Indeed, studies on whole muscles (from cats) have shown that whilst the PCr content of the biceps was 39 mmol.kg dm^{-1} greater than the soleus, this was predominantly at the expense of a lower free P_i concentration (15 mmol.kg dm^{-1} lower than the soleus) (Meyer et al, 1982). Thus the homogenate P_i made available from the breakdown of PCr plus the pool of free P_i may or may not vary between individuals, and cannot at present be ascertained. The resting human muscle ATP concentration has been reported to vary from 18 to 30 (range=12) mmol.kg dm^{-1} (calculated from Harris et al, 1974) and from 15 to 26 (range=11) mmol.kg dm^{-1} (present study). The net reaction for the breakdown of ATP to IMP within the homogenate (Spriet et al, 1986; Marlin, 1989) results in the stoichiometric release of 2 mmol P_i (Hultman and Sahlin, 1980). Thus, a range of [ATP] of 11 mmol.kg dm^{-1} can be expected to be associated with a range of [P_i] (within the homogenate) of 2x11=22 mmol.kg dm^{-1} which would account for a difference in BvIt of 12.1 mmol H^+ .kg^{-1}.pH^{-1} (see Table 4.24).

The range in muscle carnosine concentration in the present study was 23.5 mmol.kg dm^{-1} (7.2 to 30.7 mmol.kg dm^{-1}), which was comparable with the range calculated from the data of Bergstrom et al, 1978 (calculated range=23.4 mmol.kg dm^{-1}), and would account for a difference in BvIt of 12.9 mmol H^+ .kg^{-1}.pH^{-1} (see Table 4.24).

The concentration of the major intracellular physicochemical buffer, protein, was not determined in the present study, and no range of values for this component could be located in the literature. Sahlin and Henriksson (1984) and Bevan et al (1985), report mean and SD values for the muscle protein content (of untrained individuals) of 167±21 g.kg ww^{-1} and 165±10 g.kg ww^{-1} respectively. Since these were the protein contents of wet muscle, it is possible that part of the variation may have been in the individual muscle water contents. If this were so, the protein content per kg dry muscle (N.B. BvIt determined on dry muscle) may be more, or less, variable. However, from the present study, it appears that the % muscle water content varies only minimally between individuals (resting muscle water content (n=93) = 77.6±2.0; CV=2.6% (see Chapter 2, Section 2.2.6)), suggesting that the protein contents reported by these authors reflect true inter-subject variability. In support
of this, Kelso et al (1987) have observed similar means and standard deviations for the protein content of various animal muscles, the CVs for which (group SD/mean x 100%) were relatively unchanged when the % water content of individual samples was accounted for. From the mean, SD and sample number reported in these studies, the expected range of muscle protein contents can be estimated (see Appendix 6). If the histidine/cysteine residues represent a constant proportion of the total protein amino acids, then the range in these residues is expected to be similar to that for the total protein. However, there is also the possibility that the histidine/cysteine residues are distributed differentially between the various muscle proteins (e.g. mitochondrial, enzyme, contractile protein). As such, individual differences in the volume density of the major protein-containing compartments of muscle (Howald, 1985), could effect equivalent differences in the total histidine/cysteine content of a muscle. Unfortunately, at present, this cannot be quantified, and therefore, for the following calculations, the histidine/cysteine residues will be assumed to represent a constant proportion of the total protein. From the average calculated range in total muscle protein content (from Sahlin and Henriksson, 1984; Bevan et al, 1985; Kelso et al, 1987) it can be estimated that the range in histidine and cysteine concentrations would be 34.0 and 50.4 mmol.kg dm\(^{-1}\) respectively (Table 4.24). A range of 34.0 mmol.kg dm\(^{-1}\) in the histidine concentration would equate to a difference in \(B_{vit}\) of 18.7 mmol H\(^{+}\).kg\(^{-1}\).pH\(^{-1}\). Depending on the pKa' used for protein cysteine residues (reported to vary from pKa'=7.0-8.3 depending on the microenvironment; Heisler, 1986a) a range for the muscle cysteine concentration of 50.4 mmol.kg dm\(^{-1}\) would effect a difference in \(B_{vit}\) of between 3.7 and 26.7 mmol H\(^{+}\).kg\(^{-1}\).pH\(^{-1}\) (Table 4.24).

Having estimated (or measured) the range in individual values for the major components of buffering, the expected range in total \(B_{vit}\) can be calculated (Table 4.24). Depending on the pKa' used for protein cysteine residues, a range in the total buffer value of between 47.4 and 70.4 mmol H\(^{+}\).kg\(^{-1}\).pH\(^{-1}\) can be accounted for (Table 4.24) (assuming that a muscle with the lowest concentration of one buffering constituent, also demonstrates the lowest value for all other components). The actual range observed in the present study was 85 mmol H\(^{+}\).kg\(^{-1}\).pH\(^{-1}\) (110-195 mmol H\(^{+}\).kg\(^{-1}\).pH\(^{-1}\)), with 92 % of individuals having a buffer value between 125 and 188 mmol H\(^{+}\).kg\(^{-1}\).pH\(^{-1}\) (range = 63 mmol H\(^{+}\).kg\(^{-1}\).pH\(^{-1}\)). This latter figure, is just within the maximum calculated range of values. However, the measured values will also include some error in the titration (CV for repeated determinations on the same muscle block = 2.2 %), and some additional error if any blood or other extramuscular material remained in the sample before titration (buffer value of
dried blood dissected from 5 freeze-dried samples = 136 ± 2.9 mmol H+·kg⁻¹·pH⁻¹). Whilst every effort was made to remove the blood from the muscle sample, in very contaminated samples, it was impossible to remove it all. Additionally, the ranges calculated in Table 4.24, take no account of any other buffers (e.g. terminal amino groups of proteins (pKa 7.4-8.5), which may make a further (unknown)

Table 4.24 Expected range of buffer values calculated from ranges in concentration of individual buffering components.

<table>
<thead>
<tr>
<th></th>
<th>nH⁺ a</th>
<th>Observed range in conc of buffering component (mmol·kg⁻¹·dm⁻¹)</th>
<th>Range in buffer value expected from range in buffer conc (range value x nH⁺) (mmol H⁺·kg⁻¹·pH⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pᵢ from breakdown of PCr in homogenate, and free Pᵢ</td>
<td>0.55</td>
<td>unknown</td>
<td>unknown</td>
</tr>
<tr>
<td>Pᵢ from breakdown of ATP in homogenate</td>
<td>0.55</td>
<td>21.2 b</td>
<td>11.7</td>
</tr>
<tr>
<td>Carnosine</td>
<td>0.55</td>
<td>23.5 c</td>
<td>12.9</td>
</tr>
<tr>
<td>Protein:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>0.55</td>
<td>34.3 d</td>
<td>18.9</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.073 (pKa'=8.3)</td>
<td>50.4 d</td>
<td>3.7</td>
</tr>
<tr>
<td>0.53 (pKa'=7.0)</td>
<td>50.4</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td>0.236 (pKa'=7.7)</td>
<td>50.4</td>
<td>11.9</td>
<td></td>
</tr>
</tbody>
</table>

a nH⁺ = calculated from Henderson-Hasselbach equation (see equation 5 Section 1.5.2.2.1), over the pH range 7.1 - 6.5, then extrapolated to the whole pH unit. pKa' values used are 6.78 (Pᵢ) and 6.8 (carnosine and protein histidine residues) (Hultman and Sahlin, 1980). The pKa' for protein cysteine residues is reported to vary from 7.0 to 8.3 depending on the microenvironment (Heisler, 1986a). Values are therefore calculated for pKa's of 7.0, 8.3 and 7.7 (mid-way between 7.0 and 8.3).

b from range of resting [ATP] found in present study = 10.6 (15.3 to 25.9) mmol·kg dm⁻¹; from n=21). The net reaction for the hydrolysis of ATP to IMP (in the muscle homogenate), results in the accumulation of 2 mmol·kg dm⁻¹ Pᵢ per mmol·kg dm⁻¹ ATP hydrolysed (Hultman and Sahlin, 1980).

c from range of resting [CAR] found in present study (7.2-30.7;from n=65)

d range of protein contents calculated from Sahlin and Henriksson, 1984; Bevan et al, 1985; Kelso et al, 1987 (see Appendix 6 for details of calculated values).
variable contribution to total buffering. It is therefore likely that the range of values reported in the present study (which is similar to the range calculated for the data of Nevill et al (1989) and observed by Marlin (1989) (see Table 4.23)), represents a true range for the given muscle samples. It would be difficult however, to attempt to account for the much wider range of buffer values ($B_{vit}$) reported by Mizuno et al (1990) for the triceps brachii; pre-training, range = 164 (274-438) mmol H$^{+}$.kg$^{-1}$.pH$^{-1}$; post-training, range = 250 (300-550) mmol H$^{+}$.kg$^{-1}$.pH$^{-1}$).

**Relationship between $B_{vit}$ and fibre type distribution**

It has been suggested, on the basis of animal studies, that the buffer value of a muscle is highly correlated with its glycolytic capacity, and that these two metabolic properties possibly coadapt (Crush, 1970; Castellini and Somero, 1981; Hochacka, 1990). In view of the expectedly higher glycolytic capacity of type II fibres (Essen et al, 1975), it was surprising that in the present study, only a relatively low (though significant) correlation was observed between the % type II fibre area and either the muscle buffer value ($r=0.36$, $p=0.03$) or the carnosine concentration ($r=0.32$, $p=0.03$) (% type II area accounted for 10-13% of the variance in buffer value/carnosine concentration). The regression coefficient (Sokal and Rohlf, 1981) for the relationship between the muscle buffer value and % type II area was 0.58 (i.e. a unit increase in the % type II area was associated with an increase in the buffer value by 0.58 mmol H$^{+}$.kg$^{-1}$.pH$^{-1}$). The difference between the highest and lowest value for the % type II area in the present study was approximately 36% (Table 4.10); using the regression coefficient, this would result in a difference in buffer value of 21.1 mmol H$^{+}$.kg$^{-1}$.pH$^{-1}$, which is just less than twice the standard deviation for duplicate measurements of buffer value from the same group of subjects (i.e. the total measurement and sampling error; 12.1 mmol H$^{+}$.kg$^{-1}$.pH$^{-1}$). A previous examination of the relationship between the muscle buffer value and % type II fibres in humans (athletes) revealed a slightly higher (but less significant) correlation ($r=0.45$, $p=0.09$), and a regression coefficient of 0.189 (McKenzie et al, 1983). The buffer value in the latter study was determined on wet, deproteinised muscle; conversion to dry muscle (assuming 77% muscle water content) gives a regression coefficient of 0.81 (i.e. an increase of 1% in % type II distribution would cause an increase in the non-protein buffering of 0.81 mmol H$^{+}$.kg$^{-1}$.pH$^{-1}$), which is not dissimilar from the value obtained in the present study.
It is therefore tempting to speculate that the fibre type distribution plays only a minimal part in determining the muscle buffer value in humans, and that the range in buffer values observed (see earlier) does not arise in the main, from systematic differences in fibre type distribution. In a previous study, on equine muscle, the buffer value was shown to be highly correlated with the % type II area (Harris, personal communication). However, when the contribution from carnosine buffering was excluded, the strong correlation between muscle buffering and % type II fibre area was no longer evident (the carnosine concentration ([CAR]) of type II fibres was calculated to be five-fold the value for type I fibres). The mean muscle carnosine concentration reported in the present study (20.4 mmol.kg dm\(^{-1}\)), which was comparable with values cited in the literature for normal human muscle (9.5-25.7 mmol.kg dm\(^{-1}\); Bergstrom et al, 1978; Bevan et al, 1985; Parkhouse et al, 1985), was much lower than the [CAR] of equine muscle (109 ± 15 mmol.kg dm\(^{-1}\); Marlin et al, 1989). Furthermore, extrapolation from the regression equation of [CAR] on % type II area (R.Eq2, results section) revealed that the [CAR] of human type II fibres was just twice the value for type I fibres (25.7 and 15.4 mmol.kg dm\(^{-1}\), respectively). Thus, if the major factor influencing the relationship between the fibre type distribution and the buffer value in other species is the carnosine concentration, then it is unsurprising that in human muscle (with its relatively low [CAR] and lesser differential between the fibre types) only a low correlation was observed. Indeed, the % type II area correlated similarly with muscle buffering determined either with (r=0.36, p=0.03) or without (r=0.40, p=0.02) the contribution from carnosine buffering. Fibre type specific factors which may contribute in part to the non-carnosine buffering, include differences in the total phosphate or protein concentrations; type II fibres typically have a higher [PCr] (approximately 13 %) (Tesch et al, 1989b) and higher [ATP] (approximately 8 %) (Jansson et al, 1987) than type I fibres, and a lower concentration of non-protein constituents (similar glycogen concentration, but approximately 64% lower [triglyceride] (Essen et al, 1975)).

Despite the relatively low correlation between the % type II area and the human muscle buffer value, when subject populates are divided into subgroups with varying fibre type distributions, it is possible to demonstrate that a significantly higher mean buffer value is associated with groups of individuals with a predominance of type II fibres. For example, in the present study, male subjects had a greater % type II area and a higher mean muscle buffer value (and carnosine concentration) than female subjects, and in the study of Parkhouse et al (1983), 800m runners and rowers had a higher % type II fibres and a higher buffer value than marathon runners.
The role of the muscle buffer value during high intensity exercise.

It has been suggested that the extent of the decrease in muscle pH during high intensity exercise is dependent upon the amount of H+ ions released (mainly from lactic acid) and on the muscle buffer value (Sahlin and Henriksson, 1984). Multiple regression analysis of Δ muscle homogenate pH (ΔpH, rest to post-exercise) on Δ lactate concentration (Δ [La]) and buffer value (Bvit) (R.Eqn 7 & 8, results section), revealed that for any given lactate concentration, a unit increase in the buffer value effected an upward shift in the post-exercise muscle pH by approximately 0.002 pH units. For example, with a Δ [La] of 60 mmol.kg dm⁻¹, the Δ pH in a muscle with a buffer value of 110 mmol H⁺.kg⁻¹.pH⁻¹ would be 0.45 pH units; the corresponding Δ pH with a buffer value of 195 mmol H⁺.kg⁻¹.pH⁻¹ would be 0.28 pH units (110 and 195 mmol H⁺.kg⁻¹.pH⁻¹ represent the lowest and highest buffer values obtained in the present study, 60 mmol.kg dm⁻¹ represents the mean Δ [La] following isometric exercise). Therefore, it appears that muscle buffering does indeed moderate the change in muscle pH encountered during high intensity exercise. An extension to the theory proposed by Sahlin and Henriksson (1984), was that an elevated buffer value should allow the muscle to accumulate more lactic acid before reaching a limiting pH, and thereby enhance the ability for anaerobic energy production, and consequently, the ability to perform high intensity exercise. However, the results of the present study raise several objections to this extension of the theory.

Firstly, a rather wide range of individual post-exercise pH values was observed (6.53 to 6.96 (mean 6.73) and 6.60 to 7.12 (mean 6.84) following dynamic and isometric exercise respectively). This appears to contradict the concept of a "critical pH", which once reached, prevents continued muscle activity (usually cited as being approximately pH 6.4-6.5, from the original work of Hermansen and Osnes (1972)). Instead, this suggests either no role for pH as a limiting factor to high intensity exercise, or the existence of inter-individual differences in the pH sensitivity of the various processes involved in muscle contraction (possibly educed by different isoforms of the contractile and enzyme proteins). Previous studies using skinned fibres, have demonstrated a differential sensitivity to acidosis of the force-generating apparatus of various muscle fibre types, with slow-twitch (type I) fibres consistently demonstrating a greater resilience to H⁺-mediated contractile dysfunction than fast-twitch (type II) fibres (Donaldson and Hermansen, 1978; Metzger and Moss, 1987; Chase and Kushmerick, 1988). However, in this respect, another conflict arises: in the present study, individuals with a greater relative type II fibre area fatigued at a lower pH (see R.Eqⁿ 6, results section). The
post-exercise (dynamic) pH values of type I and type II fibres were estimated to be 7.03 and 6.36 respectively (determined by extrapolation from the regression equation of post-exercise pH on % type II area). These values compare favourably with the values determined by P-NMR spectroscopy of human gastrocnemius muscle, whereby two compartments (believed to represent type I and type II fibres, and identified by a prominent split in the Pi resonance during exercise to exhaustion) demonstrated a pH at exhaustion of 6.92 and 6.23 respectively (Achten et al, 1990).

If fatigue occurred simply as a result of direct H+ inhibition of the contractile machinery, and if type II fibres are indeed more sensitive to this type of H+-mediated inhibition, then the pH at fatigue would be expected to be higher (not lower) in type II than type I fibres (i.e. type II fibres would be incapacitated by a relatively lower H+ concentration (higher pH)). Clearly then, the situation does not appear to be one of simple, direct H+-mediated inhibition of contraction. This is not to deny that a gradual, direct H+ effect could be operating during the development of fatigue, but rather, that it is unlikely to be the factor causing the muscle to finally cease functioning. Similarly, this does not negate an indirect role for pH as a limiting factor to high-intensity exercise (see later).

A second objection to the theory regarding the proposed benefits of an enhanced muscle buffer value, is that an elevated buffer value was not associated with an increased ability to generate lactate during either high-intensity dynamic or isometric exercise. Close examination of data previously reported in the literature indicates that this is not a finding unique to the present study. Sharp et al (1986) demonstrated that whilst the muscle buffer value of endurance trained individuals was 5% (ns) higher than that of untrained subjects, the lactate accumulated at exhaustion by the untrained group was almost 100% (44 mmol.kg dm⁻¹) higher than the endurance trained. Similarly, Sahlin and Henriksson (1984) recorded an 18% higher buffer value in their trained subjects (compared with moderately active controls), but a 30% lower post isometric exercise lactate concentration. Following a 6 wk period of high intensity exercise training (in rats), Troup et al (1986) observed a 24% greater muscle buffer value in trained than control animals, but the trained animals accumulated significantly less lactate following electrical stimulation of the muscle to fatigue. Therefore, although the buffer value does appear to moderate the reduction in pH for a given lactate concentration, it does not per se, permit the accumulation of greater quantities of lactate, or allow for the performance of significantly more work during high intensity exercise. From the results of the present study, the more important factor appears to be simply the inherent glycolytic capacity of the the muscle; the post-exercise lactate concentration (dynamic or isometric), and the work done during the dynamic exercise test, were each highly
correlated with the % type II fibre area. This accords with the study of Sahlin and Henriksson (1984), whereby the trained subjects with their lower % type II fibre area, despite having a higher buffer value, generated less lactate.

In summary then, the muscle buffer value appears to be of only limited importance during high intensity exercise, and acts simply by permitting the muscle to moderate the extent of the acidosis typically incurred. It does not, in itself, allow for the accumulation of greater quantities of lactate or for the performance of more work, since this depends predominantly on the muscle's inherent glycolytic capacity. Unlike other species, within human muscle, the % type II area and the muscle buffer value do not appear to be tightly co-regulated, in that rather a wide range of fibre type distributions is associated with only minimal differences in the buffer value.

Fibre type distribution and high intensity exercise performance

That type II fibres are better equipped, physiologically and metabolically, for performing maximal contractions at high velocities is by no means a new finding; it is a concept which is suggested both in theory, from a knowledge of the contraction kinetics and the metabolic profile of type II fibres (see Saltin and Gollnick, 1983), and in practise, from the significant relationships observed between the percentage of type II fibres and dynamic high intensity exercise performance (Thorstensson, 1976; Bar-Or et al, 1980; Tesch, 1980). In the present study, it was estimated that during a high intensity dynamic exercise test, the peak power output of a muscle with 100 % type II fibres would be 4 times that of a muscle with 100 % type I + other fibre types (performance measures normalised to mean fibre area) (R.Eq 9, results section). The same disparity in peak power output between fast and slow twitch fibres (FT PPO 4 x ST) was observed by Faulkner et al (1986) in working with bundles of fibre segments from human skeletal muscles. The fibre-specific differences in PPO arise from the differences in maximum contraction velocity and accordingly, the velocity at varying proportions of maximum force, since the maximum force is believed not to vary between the fibre types (Maughan and Nimmo, 1984; Rall, 1985; Faulkner et al, 1986).

The significant correlation of % type II area with work done during the dynamic exercise test (r=0.48) was slightly lower than with the PPO (r=0.57), confirming previous findings (r=0.42 and r=0.60; % II area v work done and % II area v PPO, respectively (Bar-Or et al, 1980)). Extrapolation from the relationship between the % type II area and work done, revealed that a hypothetical muscle composed of 100% type II fibres would generate 3 times as much work as one consisting entirely of
type I (plus 'other fibre types') (R.Eqn 10, results section). This implies that the type I fibres are able to contribute more to the total work done than to the peak power output. This is in accordance with the suggestions of Faulkner et al (1986) that in a mixed muscle, slow twitch fibres contribute only minimally to the maximal power output attained at high velocities, but make an increasing contribution as the velocity declines (in the dynamic test used in the present study, the peak velocity achieved declined constantly throughout the duration of the test). This is not to say that type I fibres are less active during the initial stages of the test; the involvement of type I fibres would be obligatory, according to the size principle of recruitment (Green, 1986). The importance of a high proportion of type II fibres in maintaining a series of rapid and forceful contractions is expected to depend on the duration of the test employed. It has been noted in a previous study, that whilst the peak torque (or peak power output) was highly correlated with the % type II fibre area, the total work done during a series of repeated leg extensions was not related to fibre type distribution (Tesch, 1980). This was attributed to the greater relative decline in torque with repeated contractions, demonstrated by individuals with a higher % type II fibre area. The total test time was approximately 2 min and comprised the completion of 100 leg extensions. After the first minute of the test, individuals with a high proportion of type II fibres had completed considerably more work than their counterparts with a high proportion of type I fibres. During the latter part of the test, the reverse was true, and indeed only a minimal reduction in peak torque was observed over the final 50 contractions in the group with a high % type I fibres. Thus, towards the end of such a task, aerobic energy sources are likely to make an increasing contribution to its execution. Clearly the longer such a test is allowed to continue, the lower the work intensity becomes, and the less important becomes the relative abundance of type II fibres.

Fibre type distribution and 'metabolic' pH tolerance.

Earlier in the discussion, it was noted that during high-intensity dynamic exercise, individuals with a high % type II fibre area tended to fatigue with a higher muscle lactate concentration and a correspondingly lower muscle pH than individuals with a higher % type I fibre area. Similarly, these factors (high [La], low pH) were associated with a higher work output during the dynamic exercise test. This was unlikely to be the result of non-involvement of the type I fibres, since the test was a maximal effort continued to complete exhaustion. Instead it tends to suggest that more work can be done by individuals with a preponderance of type II fibres, as a result of their being able to tolerate a lower muscle pH. Saltin (1990) speculated that a possible limitation to the performance of high intensity exercise may be the pH
tolerance of the muscle. He hypothesised that the result of a suitable adaptation, in the direction of a reduced sensitivity to pH, would be that a given decrease in pH would cause less impairment of metabolism and force generation, and suggested that muscles adapted in this manner, would demonstrate a lower pH at exhaustion. Since type II fibres can, ostensibly, be considered to be "adapted" for the performance of high intensity exercise, this proposition (Saltin, 1990) may indeed be correct. How then, might an improved pH-tolerance be educed?

As mentioned earlier, with regard to the pH sensitivity of the contractile apparatus (determined on skinned fibres), the evidence does not support the notion of a decreased sensitivity in muscles composed of predominantly fast twitch fibres (at pH 6.5 the depression of the maximal shortening velocity is similar in fibres from fast and slow muscles (Metzger and Moss, 1987) and the maximal force is depressed 5-15% more in fast compared with slow muscles (Donaldson, 1983; Metzger and Moss, 1987). The reasons for this differential sensitivity are unknown, although so too, are the mechanisms whereby H+ accumulation (in any muscle type) affects tension generation or the shortening velocity - unlike the effects of ADP and Pi, the effects of H+ could not be reproduced by a model describing cross-bridge kinetics (Pate and Cooke, 1985). However, it is distinctly possible that the different myosin (or other contractile protein) isosymes are implicated; indeed, the very method for the histochemical classification of the fibre types relies on their differential myofibrillar ATPase sensitivities to extremes of pH.

In Chapter 1, it was suggested that an additional and possibly more important site of H+-mediated inhibition was at the level of the key enzymes involved in glycogenolysis (phosphorylase) and glycolysis (phosphofructokinase; PFK). On this basis, two mechanisms are proposed which could allow for an increased metabolic pH tolerance of predominantly fast-twitch muscles. The first possibility is that a true differential metabolic sensitivity to pH might exist between the fibre types, manifested by the existence of different isosymes of the key enzymes i.e. for any given pH, the percentage decrease in activity would be less for a fast- than a slow-twitch muscle. This could easily be resolved by well-controlled, in vitro enzyme-kinetic studies, but to the author's knowledge, no such information is presently available (although the data of Spriet (1989) suggests differences in the direction of a decreased pH sensitivity of more glycolytic muscles). A second possibility, is that fast twitch muscles demonstrate an apparent decrease in pH sensitivity, owing to their intrinsically higher glycolytic enzyme activity i.e. the relative decrease in activity would be the same for fast- and slow-twitch muscles but the absolute activity at any pH would be higher in fast-twitch muscles. As such, for any given pH, glycolysis would be maintained at a greater absolute rate in a muscle.
with a higher initial glycolytic enzyme activity, and that same muscle would give the appearance of being more pH tolerant than a muscle with a lower intrinsic enzyme activity.

Furthermore, if an improved pH tolerance is demonstrated by highly glycolytic muscles, such that they can attain a lower pH during high intensity exercise, a further benefit may be bestowed upon them. The glycolytic enzymes are known to be localised not only within the soluble compartment of the cytoplasm, but also bound, most probably in association with the structural proteins of the 1-band filaments (actin) (Michaelidis et al., 1989). It has been shown in various vertebrate species, that metabolic situations which lead to an increased glycolytic flux, result in an increase in the proportion of glycolytic enzymes (including PFK) associated with the particulate fraction of the cell (Clarke et al., 1980; Walsh et al., 1981). Moreover, the greater the concentration of lactate accumulated (and the lower the pH), the greater the ratio of bound:unbound enzyme (Walsh et al., 1981). Binding of these enzymes has been shown to result in significant changes in their kinetic and control parameters. Binding of PFK to F-actin increases the apparent affinity of the enzyme for fructose-6-phosphate (Liou and Anderson, 1980). Additionally a desensitisation to allosteric control (ATP inhibition and ATP inhibition at low pH) has been reported for rabbit muscle PFK bound to human erythrocyte membranes (of which a major component is actin) (Karadsheh and Uyeda, 1977) or bound to F-actin/reconstituted thin filaments (Liou and Anderson, 1980). It has been suggested that localisation of the glycolytic enzymes, such that the energy source is at the point where it is most needed (the contractile proteins), is likely to enhance the efficiency of ATP delivery and utilisation in the system (Luther and Lee, 1986). If these same phenomena can be demonstrated in human muscle, and in vivo, then this would represent an effective method of metabolic control, serving to assist in the maintenance of the glycolytic rate in acidotic, intensely contracting muscle. In this sense, a reduction in pH would effectively moderate its own inhibitory effects on the glycolytic rate (PFK activity). However, a point may eventually be reached, whereby the action of all positive modulators of PFK activity can no longer override the pH inhibition and the glycolytic rate must decrease.

**The applicability of the proposed metabolic model of fatigue**

A model was constructed in Chapter 1 (Fig. 1.6), describing a potential metabolic basis for fatigue. A major feature of this model, was the inability to rephosphorylate ADP at the required rate, secondary to a pH-induced inhibition of the glycolytic enzymes. It has been suggested that in this situation, otherwise transient accumulations of ADP, become amplified and extended in duration, and this has
been tentatively linked with the development of fatigue (Sahlin, 1986a). This model, in conjunction with the proposed apparent decrease in pH sensitivity displayed by type II fibres, could well account for the findings of the present study. During high intensity exercise, once the phosphocreatine store begins to deplete, the utilisation of anaerobic glycolysis for the rephosphorylation of ADP becomes all-important as a mechanism by which to energise continued muscle contraction and to prevent the untoward accumulation of ADP. As the glycolytic enzyme activity becomes reduced, by an increasing hydrogen ion concentration, a point is eventually reached whereby ADP cannot be rephosphorylated at the required rate, and the muscle must cease contracting. For individuals with a predominance of type I fibres, starting with a lower glycolytic enzyme activity, a given accumulation of hydrogen ions would result in less absolute PFK activity than in muscles with a preponderance of type II fibres; the point at which ADP begins to accumulate would therefore occur earlier and at a relatively higher muscle pH. Since type I fibres also display lower adenylate kinase (Tesch et al., 1989a) and AMP deaminase activities (Terjung et al., 1986), the momentary accumulations of ADP would also be less effectively dealt with.

In the present study, following isometric exercise to fatigue, the muscle lactate concentration was lower and the muscle pH higher than the corresponding values observed post-dynamic exercise. This finding can also be accommodated within the context of the proposed model. The dynamic exercise test constituted a maximal all-out effort against a fixed load, during which the pedal velocity (and therefore the work done) gradually declined throughout the duration of the test, until complete exhaustion occurred. It can be expected that as the glycolytic rate declined (due to the partial inhibition of PFK by accumulating H+ ions), the reduced ability to rephosphorylate ADP would necessitate a reduction in the ATP turnover rate and thus, in the work output. Although the work done would be diminishing, the continued activity would correspond with a continued (albeit at a declining rate) lactate production and the establishment of an ever-declining pH. In contrast, during the isometric exercise test, the opportunity to reduce the "work output" was not available - the test demanded that a fixed tension was maintained to fatigue. In this instance, the increasingly ineffective glycolytic activity could not be dealt with by a reduction in energy demand (by decreasing the intensity of contraction) and it is suggested that the "crisis" point, whereby ADP rephosphorylation cannot match its rate of production (i.e. the rate of ATP utilisation), would therefore be manifested earlier, resulting in a fatigued muscle with a relatively lower lactate accumulation.
The isometric endurance test - a test of high intensity exercise capacity?

High intensity exercise tests are usually considered to reflect the ability of the muscle to derive its energy from anaerobic sources. As such, a higher "score" in the performance test is usually associated with high post-exercise lactate concentrations and accordingly, is achieved by individuals with a predominance of type II fibres. Whilst this was the case for the dynamic exercise test, the performance outcome in the isometric test (time to fatigue) did not show significant correlations with % type II fibre area or post-exercise lactate concentration, and the tendency was indeed for a better performance to be associated with a lower % type II area and a lower post-exercise lactate concentration (r=-0.30 and -0.27 respectively). Consequently, the finding that performance in the dynamic test (PPO or WORK) was inversely related to performance in the isometric test (endurance time) (Table 4.14), was unsurprising. (Expression of the isometric performance measure as impulse (60% MVC force x endurance time) did not alter the significance of the relationships with % type II area or post-exercise lactate (p > 0.05).) Sahlin and Henriksson (1984) report similar findings for the relationship between the % type II area and the isometric endurance time; a sub-group of trained individuals with an 8% (ns) lower area of the muscle occupied by type II fibres demonstrated a 13% (ns) longer 60% MVC endurance time and a 30% (p < 0.01) lower post-exercise lactate concentration than a sub-group of untrained individuals. A significant positive correlation between the % type I fibres and the isometric endurance time at 50% MVC has been reported by Hulten et al (1975) although Litchfield et al (1984) report no significant correlation between fibre type distribution and isometric endurance at either 80%, 50% or 20% MVC.

If fatigue does indeed occur via the mechanisms outlined in the previous sections, it appears something of a paradox that individuals with a predominance of type II fibres were only equally (if not less) able, during isometric exercise, to maintain a given relative force level compared with individuals with a preponderance of type I fibres. Interesting in this respect, was the finding that a prolonged endurance time (or greater impulse (force x endurance time)) was not associated with a higher post-exercise muscle lactate concentration. Since it is well-established that at a tension of 60% MVC the blood flow to the muscle is effectively occluded (Edwards et al, 1972a; Sadamoto et al, 1983) it is unlikely that the contraction could have been energised by anything other than anaerobic mechanisms (apart from the relatively minimal contribution from oxidative phosphorylation using the oxygen bound to myoglobin: 4-15 mmol ATP.kg dm⁻¹,
Edwards *et al*, 1975b; Bangsbo *et al*, 1990; Spriet, 1989). This therefore tends to suggest that individuals with a prolonged isometric endurance time (and generally, a higher proportion of type I fibres), also demonstrated an improved economy of contraction. The economy of tension maintenance is given by the ratio of the time integral of force production per cross-sectional area of the muscle to the amount of chemical energy used per kg muscle during the contraction (Rall, 1985). For the purposes of the present study, the impulse/MFA (mean fibre area) can be considered to represent the time integral of force per CSA. Although the total ATP utilisation was not calculated in the present study, it can be expected that the glycolytic pathway would be the most important source of ATP during this type of muscle contraction (Spriet, 1989). Thus, the amount of lactate kg\(^{-1}\) accumulated by the muscle during the exercise task should give a reasonable indication of the amount of chemical energy utilisation (unless PCr utilisation was exceptionally variable between individual muscles, which is unlikely (Spriet, 1989)). As such, the ratio of the impulse/MFA to the amount of lactate accumulated (mmol kg\(^{-1}\)) can be used to estimate the economy of contraction. When the data was analysed in this manner, a higher impulse and longer 60% MVC time were indeed observed in individuals with an apparently higher economy (r=0.773, p=0.002 and r=0.766, p=0.001 respectively; Fig. 4.9 and 4.10). Additionally, the 60 % MVC time per mmol kg dm\(^{-1}\) lactate accumulated (though not impulse/MFA per mmol [La].kg dm\(^{-1}\)) was correlated with the relative area occupied by type I fibres (r=0.602, p=0.024).

The economy of force maintenance depends (inversely) on the specific ATPase rate and the SR Ca\(^{2+}\)-ATPase rate (Rall, 1985), both of which are approximately three-fold higher in fast- than slow-twitch resting muscle (Barany and Close, 1971; Ruegg, 1987; Rall, 1985). As such, economy is generally much higher in slow- than fast-twitch muscle during fatiguing isometric contractions (Crow and Kushmerick, 1982; Rall, 1985; Petrofsky, 1986). The economy of contraction is not invariable (see Edwards *et al*, 1975a) and in fast-twitch muscle, increases during the course of a maintained contraction such that it becomes approximately 2 rather than 3 times less than in slow-twitch muscle (Crow and Kushmerick, 1983). This increase in economy in fast-twitch muscles is apparently due to a decrease in cross-bridge cycling rate (due to a decrease in actomyosin ATPase rate; Vmax upon release of tension is similarly reduced) (Crow and Kushmerick, 1983; Rall, 1985), but may also be associated with a prolonged relaxation time (Edwards *et al*, 1975c; Spriet, 1989).

With respect to these observations, it is therefore unsurprising that a prolonged performance in the isometric test was not associated with a high relative area of the muscle occupied by type II fibres, or with performance in the dynamic exercise test
Indeed, it is theoretically impossible to have a high economy of force maintenance and also a high rate of work production: a high power output is dependent on high actomyosin- and SR Ca2+-ATPase rates, whereas an enhanced economy of force maintenance depends on low rates of these enzymes (Rall, 1985).

These findings can neither lend support to, nor refute the proposed model of metabolic fatigue (see earlier) since the fibre types within the mixed muscle are most likely demonstrating different economies: whilst a predominance of type II fibres would be expected to support the given intensity of contraction for a more prolonged period of time (according to the model), this is offset by the fact that type I fibres would be demonstrating a relatively lower rate of ATP utilisation, delaying the point at which ATP utilisation cannot be matched by production.

The isometric exercise test resulted in relatively large accumulations of lactate within the muscle, and to a greater extent in individuals with a high relative area of the muscle occupied by type II fibres. Therefore, this test does appear to successfully challenge the metabolic anaerobic potential, and the maintenance of 60% MVC to fatigue remains a useful vehicle for investigating the metabolic response to high intensity exercise. However, since the performance criteria were not related to the degree of anaerobic energy utilisation, these indices per se (impulse and endurance time) cannot be used to assess anaerobic performance capacity, in the context of standard anaerobic performance tests (such as described by Vandewalle et al, 1987). The isometric endurance test, if anything of a "performance test", is more a measure of fatiguability, in which a high score indicates a lower fatiguability.

Effects of high intensity exercise training
A 16 week period of high-intensity exercise training (repeated maximal isokinetic leg extensions) resulted in an 8.1 % increase in the maximal strength of the muscle (MVC). However, the response of the training groups was not significantly greater than that of the controls (increase 3.6 %). Previous training studies (of 12-24 wks duration) in which the training movement (isotonic, concentric) was dissociated from the assessment task (isometric), have demonstrated improvements in quadriceps MVC of 13-15 % (Rutherford and Jones, 1986; Hakkinen and Komi, 1986). However, in the latter two studies, no control group (non-training) was examined; the importance of including a control group in training experiments, particularly when dealing with active individuals, has been emphasised previously (Dons et al, 1979). In the studies of Rutherford and Jones (1986) and Hakkinen and Komi (1986), the training schedules placed a greater emphasis on the development of absolute strength, as evidenced by the fewer repetitions per set (6 and 1-10
respectively), and the heavy loads employed per repetition (80% 1RM and 70-120% 1RM respectively). The present study was not specifically directed towards the development of absolute strength and although a maximal effort was made during each leg extension, the peak force (or torque) which could be generated was in part, limited by the pre-set velocity, and declined throughout the training session. Maximum torque generated at 60 deg.s⁻¹ and 240 s⁻¹ is typically (in non-fatigued muscle) 70-80% and 50-60% respectively, of maximum isometric torque (Caizzo et al, 1981). If the major determinant of the adaptive response in terms of strength is the tension generated by the muscle during training (Goldberg et al, 1975; McDonagh and Davies, 1984) then this may explain the lower increases in MVC observed in the present study. Indeed, McDonagh and Davies (1984) have suggested that at loads less than 66% of maximum, no increase in MVC is observed, even if up to 150 contractions per day are used (present study 75-150 reps/session). Shorter training studies (6-7 wks) have reported no effect of dynamic strength training on isometric strength, although strength measured in the specific training mode (isokinetic or isotonic) increased significantly (Dons et al, 1979; Kancheisa and Miyashita, 1983) - the neurogenic component of the adaptive response in short-duration training studies, is well-established (McDonagh and Davies, 1984; Enoka, 1988).

In addition to the moderate increases in MVC, a 5-8% increase in peak power output was observed pre- to post-training, and was significantly different from the control group response (1% decrease). This is in contrast to the study of Rutherford et al (1986), where, even though greater increases in MVC were observed, their was no significant increase in the PPO developed during an isokinetic cycle test. Although the latter study may not have been specifically designed for the development of muscle power output, the reported increase in strength (MVC) would have been expected to induce an increase in power output. Indeed, following their own pre-training regression equation of PPO on MVC (which had a regression coefficient (slope) of 1.2), the mean training-induced increase in MVC in the male subjects (75 N) should have been associated with a mean increase in PPO of approximately 90 W (1.2 x 75); an increase of just 2 W was observed. This tends to suggest that their observed changes in MVC, whilst significant, may not have represented functional changes (an increase in the total contractile material) within the muscle.

In the present study, the training-induced increase in average peak power output was 11% greater than would have been predicted from the increase in MVC (R.Eq 14, results section). Further, if the increases in MVC did not represent a true change in the strength of the muscle (as intimated from the control group response),
the adaptation in power output is underestimated. Clearly then, the benefits of the isokinetic training were transferable to an activity requiring involvement of the same muscle group, employed in a completely different pattern of muscular coordination. This tends to suggest that true qualitative changes may have occurred within the muscle (rather than just neurogenic changes, relevant only to the training manoeuvre) possibly in the direction of accelerated contractile kinetics. A previous training study, utilising both isometric and dynamic training of the adductor pollicis muscle, demonstrated less of an improvement in maximum tension following dynamic than isometric training, but a significantly greater increase in the calculated maximal velocity of shortening and the rate of tension development and relaxation (Duchateau and Hainaut, 1984b). Such training-induced changes can be expected to necessitate an increase in myosin ATPase activity and/or calcium ion movements (Duchateau and Hainaut, 1984b) although this can only remain speculative at present.

The average work done over the resistive load range was significantly increased (8-9%) post-training. Part of this increase undoubtedly arises as a result of the increase in PPO, although a significant proportion was also accounted for by an increase in work done over the remainder of the test. Since the high intensity dynamic exercise test was time independent (terminated when pedal revs declined to < 60 rpm), an increase in total work done (MPO x test duration) could be achieved with either a) an increase in MPO for the same test duration or b) the same MPO maintained for a longer period of time or c) an increase in MPO and an increase in test duration. MPO increased by approximately 1.5% and the test duration by 7.3%, suggesting that the majority of the increase in work done was explained by mechanism (b). Training therefore resulted in a decrease in the fatiguability of the muscle i.e. an improved ability to maintain a given power output. Few studies have examined the effects of training on the capacity to maintain high intensity exercise, but those that have, report improvements consistent with the present study (e.g. Duchateau and Hainaut, 1984a; Troup et al, 1986). Hainaut and Duchateau (1989) suggest that the better resistance to fatigue of the trained muscles could be related to an increase in phosphagen stores and/or an enhanced rate of ATP turnover via increased activity of the specific enzymes which control phosphagen breakdown and glycolysis. This would be consistent with the proposed metabolic model of fatigue (Chapter 1; Fig. 1.6) but cannot, as yet, be substantiated from the results of the present study. It was also suggested that part of the training effect may be due to an increased muscle buffer value, limiting the extent of the pH decrement during exercise (Hainaut and Duchateau, 1989). This appears unlikely, in view of the non-
significant training-induced changes in the muscle buffer value (Bvit) (and the muscle carnosine concentration) reported in the present study.

In Chapter 3, it was concluded that the in vitro buffer value (Bvit) was likely to give the most accurate indication of the true muscle buffer value, and that values of Bvit must be interpreted with extreme caution. The results of the training study further emphasise these conclusions. In the 60 °/s training group, although mean Bvit determined from the regression of individual values of pH on [La] gave comparable values to Bvit, the % increase in Bvit calculated from individual values of $\Delta [\text{La}] / \Delta \text{pH}$, was twice that observed for Bvit. In previous studies, similar findings have tempted investigators to suggest that the additional improvement in Bvit arises from the increased efflux of H+ ions (in excess of lactate ions) out of the muscle (Bevan et al, 1985; Nevill et al, 1989). However, in the present study, Bvit was measured following isometric exercise to fatigue, in order to create a "closed system" for its determination. This therefore negates the involvement of any incalculable "buffering processes" such as the transmembrane flux of hydrogen ions. There are no other processes available which could have fully accounted for the apparently greater increase in Bvit than Bvit (see Chapter 3), and instead, it is suggested that the difference arises from the inappropriate use of individual in vivo buffer values $\Delta [\text{La}] / \Delta \text{pH}$. It cannot be confirmed with certainty that this applies also to the results of previous studies (e.g. Bevan et al, 1985; Nevill et al, 1989), but in view of the potential errors associated with the measurement of Bvit, it is recommended that conclusions concerning the trainability of H+ efflux mechanisms are reserved, until such techniques are available to examine this phenomenon more accurately in human muscle.

Conclusions

The major conclusions from the studies described in the present chapter are as follows:

1) The needle biopsy technique was reaffirmed as an effective research tool for the investigation of the histochemical and biochemical properties of human skeletal muscle. In the determination of group mean values for muscle fibre type distribution, fibre area, carnosine concentration and buffer value, no significant differences were observed between duplicate samples obtained over a period of 2 months.

2) In young, healthy, non-specifically trained individuals, a low correlation between the muscle carnosine concentration ([CAR]) and the in vitro muscle buffer value (Bvit) was observed ($r=0.30$, $p=0.016$). The buffering due to CAR (group mean concentration, 20.4 mmol.kg dm$^{-1}$) accounted for approximately 7% of the
total muscle buffering (group mean, 157 mmol H+ kg dm^-1 pH^-1), indicating that carnosine is of only limited importance as a physicochemical buffer in human skeletal muscle.

3) Both [CAR] and Bvit, demonstrated low correlations with the percentage area of the muscle occupied by type II fibres (r=0.32, p=0.034 and r=0.36, p=0.03 respectively). Therefore in human muscle, unlike that of various other vertebrate species, the glycolytic capacity and the muscle buffer value do not appear to be tightly coupled. Further correlative analyses of the activities of key glycolytic enzymes and the muscle buffer value, ideally through single fibre studies, are required to substantiate this conclusion.

4) Bvit was shown to moderate the decline in pH during high intensity (HI) dynamic and isometric exercise, but in contrast to previous suggestions, did not per se, determine the maximal permissible lactic acid accumulation, or the performance outcome during high intensity exercise. The muscle buffer value therefore appears to have only a secondary role as a limiting factor to high intensity exercise.

5) The extent of the muscle lactate accumulation during dynamic and isometric HI exercise, and the work done during dynamic HI exercise, were linearly dependent on the percentage area of the muscle occupied by type II fibres. It is proposed that this occurs as a result of the increased ability of the type II fibres to rapidly reporphosphorylate ADP during high intensity exercise, thereby delaying the point at which the rates of ATP utilisation and resynthesis are transiently uncoupled (suggested as the final stage in metabolic fatigue; Sahlin, 1986a). The ability to maintain an isometric contraction to fatigue (60% MVC) was not dependent on the % type II fibre area, and this can most likely be attributed to the lower economy of tension maintenance displayed by type II than type I fibres (i.e. increased energy cost for the maintenance of a given force).

6) The pH at fatigue following high intensity exercise was highly variable, both between individuals and between exercise modes (dynamic and isometric). The concept of a "critical muscle pH", inhibitory to further muscle contraction, therefore appears to be either fallacious, or subject to marked individual variability. It is unlikely that a decreased muscle pH exerts a direct inhibitory effect on the process of muscle contraction. Rather, it is suggested that the variable degree of acidosis, reflects the indirect effects of pH on the glycogenolytic and glycolytic flux rates. A lower intramuscular pH at fatigue was observed in muscle containing a high % type II fibre area. It is proposed that a greater accumulation of hydrogen ions is required to inhibit the inherently higher glycolytic activity of muscle containing a predominance of type II fibres (see (8)).

7) Significant improvements (p<0.05) in the performance of dynamic HI exercise (increased PPO and WORK during modified Wingate Anaerobic Test) were observed following 16 weeks of intensive, isokinetic training of the
quadriiceps (repeated maximal leg extensions). No concomitant improvements in the \textit{in vitro} buffer value, or the [CAR] were observed following training. Further research is required to elucidate the aetiology of the observed improvements in performance (see (8)).

8) It is suggested that future studies concerning the role of intramuscular pH as a limiting factor to HI exercise, concentrate not on the capacity of the muscle to sequester hydrogen ions and moderate the decline in pH, but rather, on the ability of the muscle to 'tolerate' a decreased muscle pH. This will most likely proceed through examination of the \textit{in vitro} pH sensitivities of key glycolytic enzymes, determined under conditions which closely reflect those observed during HI exercise \textit{in vivo}, in terms of known effector levels.
References


Appendix 1

Histological and histochemical staining procedures for skeletal muscle.*

1) Toluidine Blue

Stains the tissue components blue and is used to check the orientation and preservation of the block during sectioning.

Method
1. Stain unfixed sections for about one minute.
2. Wash briefly in tap water then differentiate in 50% alcohol (ethanol) until excess dye is removed.
3. View specimen.
4. If required, dehydrate (i.e. dip in ascending alcohols), clear (in xylene) and mount in Practamount.

Stain
1% toluidine blue in 1% borax (disodium tetraborate) in water. Filter before use. Keeps well.

2) Haematoxylin and Eosin (H&E)

Stains sarcolemmal nuclei and basophilic material blue/black, muscle fibres pink and connective tissue a lighter pink. Used for the determination of fibre area.

Method
1. Air dry unfixed sections for approximately 30 minutes.
2. Stain unfixed sections in Harris's haematoxylin for 1-3 minutes depending on freshness of stain.
3. Blue by washing in tap water for 5 minutes.
4. Counterstain in eosin for 1 minute.
5. Wash briefly in cold tap water.
6. Dehydrate in ascending alcohols, clear and mount.

* from manual of routine staining procedures - Department of Cell and Structural Biology, Manchester University Medical School (adapted from Dubowitz and Brooke, 1973).
Harris's haematoxylin 2.5 litres. Filter before use. Keeps well.

Harris's alum haematoxylin 10 g
Absolute alcohol 200 ml
Ammonium or potassium alum 200 g
Mercuric oxide 6 g
Glacial acetic acid 80 ml
Distilled water make up to 2.5 litres

Dissolve haematoxylin in alcohol and add to alum solution. Bring almost to boil, remove from heat and add mercuric oxide. When cool add glacial acetic acid.

Eosin
0.5-1.0% eosin (alcohol soluble) in 70% alcohol.

3. Nicotinamide adenine dinucleotide (NADH) tetrazolium reductase

Intermyofibrillar material (mitochondria, sarcoplasmic reticulum, t-tubules) stains dark blue. Used as a supplement to the ATPase stain (see 4) for fibre typing (type I fibres stain darkly, IIA intermediate and IIB pale).

Method
1. Air-dry unfixed sections for approximately 30 minutes.
2. Incubate unfixed sections for 30 minutes at 37 °C.
3. Rinse gently in distilled water.
4. Mount in glycerine jelly.

Incubation medium
Tris buffer 0.2M pH 7.4 30 ml
NADH 24 mg
Nitro blue tetrazolium 30 mg
Make fresh.

Tris buffer (0.2M pH 7.4 - 2 litres)
tris (hydroxymethyl) methylamine 12.1 g
HCl (1M) 82.8 ml
Distilled water make up to 2 litres
Adjust pH to 7.4 with NaOH/HCl
Glycerine jelly
Gelatine 10 g
Distilled water 60 ml
Glycerine 70 ml
Phenol 0.25 g
Melt in a vessel of hot water.

4) Myofibrillar adenosine triphosphatase (ATPase)

Used to differentiate muscle fibre types. Serial sections are preincubated at pH 4.35, 4.6 or 10.2 prior to incubation and development.

<table>
<thead>
<tr>
<th>Pre-incubation pH</th>
<th>Type I</th>
<th>Type IIa</th>
<th>Type IIb</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.35</td>
<td>Dark</td>
<td>Pale</td>
<td>Pale</td>
</tr>
<tr>
<td>4.6</td>
<td>Dark</td>
<td>Pale</td>
<td>Intermediate</td>
</tr>
<tr>
<td>10.2</td>
<td>Pale</td>
<td>Dark</td>
<td>Dark</td>
</tr>
</tbody>
</table>

Method
1. Air dry unfixed frozen sections for 2 hours minimum.
2. Pre-incubate (acid pH 15 min, alkali 25 min) at room temperature.
3. Wash 3 times in tap water; rinse in distilled water.
4. Incubate (acids 45 min, alkali 30 min) at 37 °C.
5. Wash in 2 changes of 1% calcium chloride.
6. Place in 2% cobalt nitrate for 3 minutes.
7. Quickly wash in 3 changes of tap water and 3 changes of distilled water.
8. Develop in dilute (1%) ammonium polysulphide for about 5 minutes.
9. Wash well in tap water and then distilled water.
10. Mount in aqueous mountant (e.g. Aquamount).
Pre-incubation solutions

**Acid (per coplin jar)**

**pH 4.35**
- Sodium acetate 0.2M 9 ml
- Acetic acid 0.2M 21 ml

**pH 4.6**
- Sodium acetate 0.2 M 15 ml
- Acetic acid 0.2 M 15 ml
Adjust pH to 4.35 or 4.6 with 1M KOH

**Alkaline (per coplin jar)**
- 0.18 M CaCl₂ 6 ml
- 0.1 M sodium barbitone 6 ml
- distilled water 18 ml
Carefully adjust pH to 10.2 with 0.1M KOH or HCl

Incubation solution

ATP (disodium salt) 45 mg

Tris buffer 0.1M plus 0.018M calcium chloride 30 ml
Adjust to pH 9.5 with 0.1 M KOH or HCl

Tris buffer 0.1M plus 0.018 M calcium chloride
- tris (hydroxymethyl) methylamine 6.05 g
- calcium chloride 1.32 g
- distilled water make up to 500 ml

Store at 4 °C

1% calcium chloride 0.2 M acetic acid
2% cobalt nitrate 0.2 M sodium acetate
0.1 M tris calcium buffer 0.18 M CaCl₂
Appendix 2

Muscle metabolite assays.

Phosphocreatine (PCr) and adenosine triphosphate (ATP)

\[
\begin{align*}
\text{CK} & : \text{P-Creatine} + \text{ATP} \rightarrow \text{Creatine} + \text{ATP} \\
\text{HK} & : \text{ATP} + \text{glucose} \rightarrow \text{ADP} + \text{Glucose-6-P} \\
\text{G-6-PDH} & : \text{Glucose-6-P} + \text{NADP}^+ \rightarrow \text{6-P-gluconolactone} + \text{NADPH} + \text{H}^+
\end{align*}
\]

Stock reagents

<table>
<thead>
<tr>
<th>Stock reagents</th>
<th>Final concentration</th>
<th>Cocktail per 100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIS-HCl, 50 mM (25 mM TRIZMA base + 25 mM TRIZMA-HCl) + 0.02% BSA; pH 8.1</td>
<td>50 mM (refrigerated)</td>
<td>100 ml 100 ml 100 ml</td>
</tr>
<tr>
<td>Magnesium chloride, 1M (in water, <em>care! - exothermic</em>)</td>
<td>1 mM (fresh)</td>
<td>100 ul 100 ul 100 ul</td>
</tr>
<tr>
<td>NADP, 50 mM (in distilled water)</td>
<td>50 µM (-20 °C)</td>
<td>100 ul 100 ul 100 ul</td>
</tr>
<tr>
<td>ADP, 100 mM (in water, pH 7)</td>
<td>100 µM (-20 °C)</td>
<td>100 ul 100 ul 100 ul</td>
</tr>
<tr>
<td>Glucose, 100 mM (in distilled water, fresh)</td>
<td>100 µM (fresh)</td>
<td>100 ul 100 ul 100 ul</td>
</tr>
<tr>
<td>Dithiothreitol, 0.5M (in distilled water)</td>
<td>500 µM (refrigerated)</td>
<td>100 ul 100 ul 100 ul</td>
</tr>
<tr>
<td>G-6-P dehydrogenase (yeast)</td>
<td>100 U/ml (refrigerated)</td>
<td>50 ul 50 ul 50 ul</td>
</tr>
<tr>
<td>Hexokinase (yeast)</td>
<td>200 U/ml (refrigerated)</td>
<td>75 ul 75 ul</td>
</tr>
<tr>
<td>Creatine kinase (rabbit muscle)</td>
<td>100 U/ml (refrigerated)</td>
<td>900 ul</td>
</tr>
</tbody>
</table>
Calibration standards: Glucose-6-P, 0 - 200 µM in distilled water, stored at -20 °C.
Additional reagent: Carbonate buffer, 20 mM, pH 10 (10mM sodium carbonate, 10mM sodium hydrogen carbonate, fresh).

Procedure
Prepare reaction mixtures as detailed above.
Take 10 µl of neutralised perchloric acid extract of muscle and add 50 µl of distilled water. Vortex mix.
Repeat a - f as follows:
a. Pipette aliquots (10 µl) of diluted samples/standards into 6 reaction vials (1.5 ml capped conical tubes, Sarstedt, U.K.)
b. Add 200 µl of reaction mixture A to 2 of the 6 reaction vials. Gently mix.
c. Add 200 µl of reaction mixture B to 2 of the 6 reaction vials. Gently mix.
d. Add 200 µl of reaction mixture C to the remaining 2 reaction vials. Gently mix.
e. Incubate for 30 minutes at room temperature.
f. Add 800 µl of carbonate buffer, vortex mix and read relative fluorescence (excitation 340 nm, emission 460 nm).
Subtract relative fluorescence of reaction B from reaction C to determine phosphocreatine, and A from reaction B to determine ATP. No attempt is made to determine G-6-P. Interpolate sample concentration from calibration graph of G-6-P standards.
Lactic acid

LDH
Lactate + NAD+ → Pyruvate + NADH + H+

Stock reagents | Final concentration | Cocktail per 100 ml (40 assays)
--- | --- | ---
Hydrazine buffer, 1.1M, pH 9.0 (containing disodium EDTA, 1mM). | 1.1 mM (refrigerated) | 10 ml
NAD, 50 mM (in distilled water) | 50 µM (-20 °C) | 10 µl
Lactate dehydrogenase (beef heart) | 1000 U/ml (refrigerated) | 100 µl

Calibration standards: lactic acid, 0 - 1000 µM in distilled water, stored at -20 °C.
Additional reagent: Carbonate buffer, 20 mM, pH 10 (10mM sodium carbonate, 10mM sodium hydrogen carbonate, fresh).

Procedure
Prepare reaction mixtures as detailed above.
Repeat a - d.
a. Pipette aliquots (10 µl) of diluted samples/standards into 2 reaction vials (1.5 ml capped conical tubes, Sarstedt, U.K.).
b. Add 200 µl of reaction mixture to the reaction vials. Gently mix.
c. Incubate for 30 minutes at room temperature.
d. Add 800 µl of carbonate buffer, vortex mix and read relative fluorescence (excitation 340 nm, emission 460 nm).
Interpolate sample concentration from calibration graph of lactate standards.
**Creatine**

CK
Creatine + ATP $\rightarrow$ P-creatine + ADP

PK
ADP + PEP $\rightarrow$ ATP + pyruvate

LDH
Pyruvate + NADH + H+ $\rightarrow$ lactate + NAD+

<table>
<thead>
<tr>
<th>Stock reagents</th>
<th>Final Concentration</th>
<th>Cocktail A (per 10 ml) (50 assays)</th>
<th>Cocktail B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazole-HCl buffer, 50 mM, pH 7.5 (40 mM imidazole, 10 mM imidazole-HCl).</td>
<td>50 mM (refrigerated)</td>
<td>10 ml</td>
<td>10 ml</td>
</tr>
<tr>
<td>Magnesium chloride, 1M (in water, care-exothermic)</td>
<td>5 mM (fresh)</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Potassium chloride, 1M (in water)</td>
<td>30 mM (room temp)</td>
<td>300 µl</td>
<td>300 µl</td>
</tr>
<tr>
<td>Phospho-enol-pyruvate, 10 mM (in water)</td>
<td>25 µM (-20 °C)</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>NADH, 10 mM (in carbonate buffer)</td>
<td>25 µM (-20 °C)</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>ATP, 200 mM (in water, pH 7)</td>
<td>200 µM (-20 °C)</td>
<td>10 µl</td>
<td>10 µl</td>
</tr>
<tr>
<td>Lactate dehydrogenase (beef heart)</td>
<td>500 U/ml (refrigerated)</td>
<td>4 µl</td>
<td>4 µl</td>
</tr>
<tr>
<td>Pyruvate kinase (rabbit muscle)</td>
<td>200 U/ml (refrigerated)</td>
<td>37.5 µl</td>
<td>37.5 µl</td>
</tr>
<tr>
<td>Creatine kinase (rabbit muscle)</td>
<td>100 U/ml</td>
<td>-----</td>
<td>360 µl</td>
</tr>
</tbody>
</table>

*Calibration standards:* Creatine, 0 - 1000 µM in distilled water, stored at -20 °C.

*Additional reagent:* Carbonate buffer, 20 mM, pH 10 (10 mM sodium carbonate, 10 mM sodium hydrogen carbonate, fresh).
**Procedure**

Dilute creatine standards and samples (10 µl) by the addition of distilled water (90 µl). Vortex mix.

Prepare reaction mixtures as detailed above.

Repeat a - e.

a. Pipette aliquots (20 µl) of diluted samples/standards into 4 reaction vials (1.5 ml capped conical tubes, Sarstedt, U.K.).

b. Add 200 µl of reaction mixture A to 2 of the 4 reaction vials. Gently mix.

c. Add 200 µl of reaction mixture B to 2 of the 4 reaction vials. Gently mix.

d. Incubate for 30 minutes at room temperature.

e. Add 800 µl of carbonate buffer, vortex mix and read relative fluorescence (excitation 340 nm, emission 460 nm).

Subtract relative fluorescence of reaction B from reaction A. Interpolate sample concentration from calibration graph of creatine standards.
Appendix 3

Comparison of human muscle homogenate pH using either an iodoacetic acid cocktail or 0.01M NaF as the homogenising solution.

The pH of portions of 26 resting, 11 post-dynamic and 17 post-isometric, freeze-dried human muscle samples* was determined following homogenisation:
(i) at 15 mg dm\textsuperscript{-1}, in an iodoacetic acid (IAA) cocktail solution (145mM KCl, 10 mM NaCl, 5mM IAA, adjusted to pH 7 with 0.1M bicarbonate-free NaOH), and
(ii) at 30 mg dm\textsuperscript{-1}, in a 0.01M sodium fluoride (NaF) solution.

Comparison of the pH values obtained using method (i) and (ii) was made by means of a paired "t" test. Resting muscle homogenate pH was significantly higher when measured by method (ii) (NaF solution) than method (i) (IAA cocktail) (p<0.00001) (Table A3.1). There was no significant difference between the two methods, for the measurement of post-exercise muscle homogenate pH (post-isometric: method (i) vs (ii), p=0.153; post-dynamic: method (i) vs (ii), p=0.283). When all resting and post-exercise samples were considered together, a significant difference between methods was observed (p=0.0001). The higher the pH value, the greater the disparity between the two methods (See Table A3.1).

* For details of methods for biopsy sampling, sample preparation and muscle homogenate pH measurement, see Chapter 2. For details of the exercise tasks employed, see Chapter 3 (Section 3.3).
Table A3.1  Comparison of muscle homogenate pH with IAA cocktail or NaF as the homogenising solution.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Resting</td>
<td>7.09</td>
<td>0.06</td>
</tr>
<tr>
<td>Post isometric exercise</td>
<td>6.94</td>
<td>0.11</td>
</tr>
<tr>
<td>Post dynamic exercise</td>
<td>6.74</td>
<td>0.16</td>
</tr>
<tr>
<td>All samples (rest + exercise)</td>
<td>6.97</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*a significantly different from pH measured in IAA homogenising cocktail (p<0.05)*
Appendix 4

Relationship between resistive load and variables derived from performance of the modified Wingate Anaerobic Test.

The mean values for each mWAnt performance parameter over the resistive load range 9 - 14% MVC, are shown in Table A4.1 and Figs. A4.1 (a) to (j) (standard deviations are omitted from Fig. A4.1 for clarity, but are presented in Table A4.1). Uncorrected values of power (U.PPO and U.MPO) were determined according to method A, Chapter 2.1.1, and corrected values (C.PPO and C.MPO) according to method B, Chapter 2.1.1.

![Graph](image)

Fig. A4.1 (a) Relationship between resistive load and test duration during performance of the modified Wingate Anaerobic test.
Fig. A4.1 (b) Relationship between resistive load and work done during performance of the modified Wingate Anaerobic test.

Fig. A4.1 (c) Relationship between resistive load and peak velocity during performance of the modified Wingate Anaerobic test.
Fig. A4.1 (d) Relationship between resistive load and uncorrected mean power output (U.MPO) during performance of the modified Wingate Anaerobic test.

Fig. A4.1 (e) Relationship between resistive load and corrected mean power output (C.MPO) during performance of the modified Wingate Anaerobic test.
Fig. A4.1 (f) Relationship between resistive load and uncorrected peak power output (U.PPO) during performance of the modified Wingate Anaerobic test.

Fig. A4.1 (g) Relationship between resistive load and corrected peak power output (C.PPO) during performance of the modified Wingate Anaerobic test.
Fig. A4.1 (h) Relationship between resistive load and time to uncorrected peak power output (U.Tpp) during performance of the modified Wingate Anaerobic test.

Fig. A4.1 (j) Relationship between resistive load and time to corrected peak power output (C.Tpp) during performance of the modified Wingate Anaerobic test.
Table A4.1  Mean and standard deviation of each mWAnt performance parameter * over the resistive load range 9 - 14% MVC.

<table>
<thead>
<tr>
<th>Resistive load (% MVC)</th>
<th>Test duration (s)</th>
<th>Work done (J.kg(^{-1}))</th>
<th>Peak velocity (rpm)</th>
<th>Uncorrected mean power (W.kg(^{-1}))</th>
<th>Corrected mean power (W.kg(^{-1}))</th>
<th>Uncorrected peak power (W.kg(^{-1}))</th>
<th>Corrected peak power (W.kg(^{-1}))</th>
<th>Time to U.PPO (s)</th>
<th>Time to C.PPO (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>40.4 SD 9.5</td>
<td>279.5 SD 96.0</td>
<td>145.1 SD 12.2</td>
<td>7.0 SD 1.4</td>
<td>6.9 SD 1.4</td>
<td>10.4 SD 2.3</td>
<td>13.7 SD 2.9</td>
<td>3.2 SD 0.7</td>
<td>0.4 SD 0.3</td>
</tr>
<tr>
<td>10</td>
<td>36.7 SD 9.1</td>
<td>269.5 SD 71.0</td>
<td>139.3 SD 10.4</td>
<td>7.6 SD 1.4</td>
<td>6.9 SD 1.4</td>
<td>11.1 SD 2.2</td>
<td>13.8 SD 2.5</td>
<td>3.2 SD 0.7</td>
<td>0.5 SD 0.5</td>
</tr>
<tr>
<td>11</td>
<td>32.5 SD 7.6</td>
<td>255.0 SD 69.0</td>
<td>132.4 SD 12.1</td>
<td>8.0 SD 1.6</td>
<td>7.4 SD 1.4</td>
<td>11.6 SD 2.4</td>
<td>13.7 SD 2.7</td>
<td>3.1 SD 0.7</td>
<td>0.7 SD 0.6</td>
</tr>
<tr>
<td>12</td>
<td>29.6 SD 7.7</td>
<td>240.5 SD 67.0</td>
<td>124.7 SD 11.4</td>
<td>8.3 SD 1.6</td>
<td>7.9 SD 1.6</td>
<td>11.8 SD 2.4</td>
<td>13.8 SD 2.7</td>
<td>2.9 SD 0.7</td>
<td>0.6 SD 0.5</td>
</tr>
<tr>
<td>13</td>
<td>26.8 SD 7.1</td>
<td>229.5 SD 73.0</td>
<td>116.7 SD 11.7</td>
<td>8.7 SD 1.7</td>
<td>8.2 SD 1.6</td>
<td>12.0 SD 2.4</td>
<td>13.6 SD 2.6</td>
<td>3.0 SD 0.7</td>
<td>0.7 SD 0.7</td>
</tr>
<tr>
<td>14</td>
<td>23.5 SD 6.9</td>
<td>210.0 SD 73.0</td>
<td>107.8 SD 12.8</td>
<td>9.1 SD 1.8</td>
<td>8.9 SD 1.7</td>
<td>11.9 SD 2.5</td>
<td>13.2 SD 2.6</td>
<td>2.9 SD 0.9</td>
<td>0.9 SD 0.9</td>
</tr>
</tbody>
</table>

* all mass-dependent variables (MPO, PPO and WORK) are expressed per kg body mass.
Appendix 5

Table A5.1 contains the results of the resting and post-exercise muscle lactate concentration and muscle homogenate pH values, pre and post-training (from which Table 4.19 was constructed), including the anomalous results obtained from 7 subjects. These 7 subjects demonstrated post-exercise [La] and pH values atypical of muscle priorly engaged in high intensity exercise. The performance times (60% MVC to fatigue) post-habituation and on the day of the biopsy were 48.7 ± 16.0 and 46.1 ± 10.8 s respectively (n=7, p=0.170; paired "t" test). The anomalous results were therefore not considered to be the result of a non-maximal effort on the behalf of the subjects. Similarly, they were unlikely to be the result of an error in the measurement of either [La] or homogenate pH, because when the measured post-exercise lactate was low, the pH was always correspondingly high (measurements made on different portions of the same muscle sample). Rather, it is suggested that this may have been the result of sampling from an area of the muscle, damaged by the insertion of the biopsy needle during the acquisition of the first two resting muscle samples, and rendered "inactive" during the exercise trial. Katz et al (1986b) reported similar findings following multiple biopsy sampling. When considering the choice of sites for repeated sampling, these data highlight a major disadvantage of sampling from too confined an area of muscle, and through the same incision.

Table A5.1 Lactate concentration ([La]) and homogenate pH at rest and post isometric exercise, pre and post training (all values including anomalous post-exercise [La] and pH values)

<table>
<thead>
<tr>
<th>TRAINING GROUP</th>
<th>CONTROL (n=10)</th>
<th>240 DEG.s⁻¹ (n=8)</th>
<th>60 DEG.s⁻¹ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LACTATE CONCENTRATION ([La]) (mmol.kg dm⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>Rest</td>
<td>4.7</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>68.0</td>
<td>14.6</td>
</tr>
<tr>
<td>POST</td>
<td>Rest</td>
<td>8.5</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>56.1</td>
<td>27.2</td>
</tr>
<tr>
<td>MUSCLE HOMOGENATE pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td>Rest</td>
<td>7.22</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>6.82</td>
<td>0.09</td>
</tr>
<tr>
<td>POST</td>
<td>Rest</td>
<td>7.20</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Post-exercise</td>
<td>6.86</td>
<td>0.20</td>
</tr>
</tbody>
</table>
Appendix 6

Estimation of range of muscle protein contents from mean and SD.

An estimate of the range of values for a given variable can be obtained by multiplying the SD (of a group mean value) by the *mean range value* for the appropriate sample size (assuming the data is normally or near normally distributed) (Sokal and Rohlf, 1981). The *mean range* is equivalent to the number of standard deviations included in the range and is dependent on the sample size (Downie and Heath, 1974.)

In the following studies from the literature, sample sizes of 6, 8 and 10 were employed, for which *mean range values* of 2.691, 2.909 and 3.078 respectively were used, in estimating the range of protein contents (range = SD x *mean range value*; Sokal and Rohlf, 1981). Where the protein content was measured per kg wet weight, values were converted to dry weight assuming a muscle water content of 77% (Hultman and Sahlin, 1980).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Mean g/kg dm</th>
<th>SD</th>
<th>n</th>
<th>estimated range (SD x <em>mean range value</em>) (g/kg dm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahlin and Henriksson, 1984</td>
<td>718*</td>
<td>90</td>
<td>8</td>
<td>262 (587-849)**</td>
</tr>
<tr>
<td>Bevan, 1985</td>
<td>709*</td>
<td>44</td>
<td>6</td>
<td>118 (650-768)</td>
</tr>
<tr>
<td>Kelso et al, 1987: Rat white gastocnemius</td>
<td>843</td>
<td>62</td>
<td>10</td>
<td>190 (748-938)</td>
</tr>
<tr>
<td>Rat plantaris</td>
<td>818</td>
<td>40</td>
<td>10</td>
<td>123 (757-880)</td>
</tr>
<tr>
<td>Rat soleus</td>
<td>726</td>
<td>102</td>
<td>10</td>
<td>314 (569-883)</td>
</tr>
<tr>
<td>Horse mid-gluteal</td>
<td>675</td>
<td>57</td>
<td>10</td>
<td>175 (588-763)</td>
</tr>
</tbody>
</table>

**average range for all muscles** = 197

* converted from wet weight to dry weight (77% water content).
** value in brackets = estimated lowest and highest value (= mean value plus or minus estimated range/2)
The average range in total protein (average of all studies cited above) = 197 g/kg dry muscle.

Protein histidine content = 2.7 g.100 g⁻¹ protein, and protein cysteine content = 3.1 g.100g⁻¹ protein (Furst et al, 1970).

Therefore, the estimated range in protein histidine = 5.3 g.kg dm⁻¹ (2.7 x 197/100), and in protein cysteine = 6.1 g.kg dm⁻¹ (3.1 x 197/100) (assuming histidine and cysteine represent a constant proportion of total protein). The molecular masses of histidine and cysteine are 156 and 121, respectively.

Therefore, the estimated concentration ranges of histidine and cysteine are =

34.0 mmol.kg dm⁻¹ (histidine)
50.4 mmol.kg dm⁻¹ (cysteine)