THE RELATIONSHIP BETWEEN EXTRACELLULAR POTASSIUM CONCENTRATIONS AND MUSCLE MEMBRANE EXCITABILITY FOLLOWING A SUSTAINED SUBMAXIMAL ISOMETRIC QUADRICEPS CONTRACTION

THE RELATIONSHIP BETWEEN EXTRACELLULAR POTASSIUM CONCENTRATIONS AND MUSCLE MEMBRANE EXCITABILITY FOLLOWING A SUSTAINED SUBMAXIMAL ISOMETRIC QUADRICEPS CONTRACTION

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

October, 1994

MASTER OF SCIENCE **McMASTER UNIVERSITY**
(Human Biodynamics) Hamilton, Ontario

TITLE: The Relationship Between Extracellular Potassium Concentrations and Muscle Membrane Excitability Following a Sustained Submaximal Isometric Quadriceps Contraction

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NUMBER OF PAGES: XV-139

I am proud to dedicate this work to my family who have provided me with support, guidance, and a kick in the butt when I needed it!

ABSTRACT

The purpose of this study was to relate femoral venous plasma potassium concentrations ($[K^+]$) following a fatiguing submaximal isometric quadriceps contraction, to the excitability of the muscle cell membrane as assessed by the compound muscle action potential (M-Wave). Ten healthy male volunteers (22.0 ± .5 yrs) performed a unilateral 3 minute (min) sustained isometric quadriceps contraction at 30% of their maximum voluntary contraction (MVC). M-Waves, peak evoked twitch torque, plasma lactate concentration ($[La^-]$), and plasma potassium concentration ($[K^+]$) were measured before, and at predetermined times over the course of a 15 min recovery period following the fatigue paradigm. Immediately post-exercise, twitch torque decreased to 58% of baseline, femoral venous $[La^-]$ had risen to 10 ± 0.8 mmol/l, and $[K^+]$ was significantly increased from 4.0 ± 0.1 mmol/l to 5.9 ± 0.2 mmol/l. M-Wave amplitude illustrated a trend for potentiation increasing 9.5% from 13.9 ± 2.4 mV pre-exercise, to 15.3 ± 2.8 mV at 1 min 20 seconds post-exercise. M-Wave area exhibited a similar trend from baseline, but values showed no statistical significance during this time. These results suggest that in spite of increased extracellular $[K^+]$ following this type of

fatiguing exercise, muscle membrane excitability is maintained, which is probably due to the electrogenic nature of the highly active Na^+/K^+ pump.

This study was supported by the Natural Sciences and Engineering Research Council of Canada.

PREFACE

The following thesis is presented in two chapters. Chapter I is a literature review about the sites of muscle fatigue, the muscle cell membrane and its potential, the role of potassium in fatigue, Na^+/K^+ ATPase, and finally, the compound muscle action potential (M-Wave). Chapter II is the thesis research which is presented in a form suitable for publication.

ACKNOWLEDGEMENTS

In my past two years many people have influenced me and my work.

I must begin by thanking my supervisor Dr. Audrey Hicks. Dr. Hicks has had an unwavering commitment to me in finishing this project. She has tried to instill the scientific method in my brain somewhere with her guidance and simple explanations, and I greatly appreciate her tolerance spiked with effective criticism. Thanks Audrey!

I would also like to thank Dr. Bob Mckelvie for not only his time and skills at finding the right line to stick, but also for his inspiration which made me look at these two years from a different perspective.

It is difficult to describe the bond that has been made between each one of us in the two classes that made up our programme this year. Who can forget the sprinkler, the spinning globe, or the swinging chipmunk; concerts and the cottage; a conference that I don't really remember; baseball and DAVE!; A Phys Ed pub at the Rat; the M Club; a World Champion; Maria; The Phoenix; but most of all the support and companionship. We have all become much closer because of this past year, and we are better people for it. I am proud to say that I am your friend: Mo, Rob F, Katie, Kerry, Kevin, Marty, Lisa, Rob S, Shawn, George, Joan, Nikki, Wendy, Jennifer, and the Karens.

I would also like to thank my committee members for their time taken to evaluate this work and provide a stimulating defense--Dr. Vickie Galea, Dr. Digby Sale, and Dr. Jim Dowling!

There are a few people I would like to acknowledge for making my time here run much smoother: John Moroz for bailing me out numerous times in the lab with a flip of a switch or the turn of a wrist, and lots of knowledge; Joan Martin who kept this study on time, and the subjects conscious with her ability to chat; and Mary Cleland, for her stories of "back Home" to keep a smile on my face.

The rest of the faculty have been extremely supportive, especially this year. They are a very charismatic group and anyone who knows them is fortunate. Stats live Dig, thanks for your advice!

A special thanks is extended to Dr. McCartney who has the great ability to celebrate, and a wit matched by no one. Your help in my future has been invaluable!

Okay, thanks to Tim for his humility and Scott for his ability to talk! As well, thanks to the girls of 516 Bates for stimulating late night conversation--Michelle, Kelly, Shannon, and Tigger.

Finally, I'd like to thank my family who are persistent as hell and always keep their sense of humour.

CHEERS!

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List of Abbreviations

ACh	Acetylcholine
AP	Action potential
ATPase	Adenosine triphosphatase
$[Ca^{++}]$	Calcium ion concentration
CNS	Central nervous system
EC	Excitation-contraction
E_{K+}	Equilibrium potential for potassium ion
E_m	Resting muscle fibre membrane potential
EMG	Electromyogram
E_{Na+}	Equilibrium potential for sodium ion
ETTmax	Maximum evoked twitch torque
HFS	High frequency stimulation
Hz	Hertz
$[K^+]_{i/e}$	Potassium ion concentration (intra/extracellular)
kg	Kilogram
LFS	Low frequency stimulation
$[La^-]$	Lactate ion concentration
$[Na^+]$	Sodium ion concentration
µV•s	Microvolt seconds
µm	Micrometre
µs	Microsecond
min	Minutes
ml	Millilitre
mm	Millimetre
mmol/l	Millimole per litre
MN	Motoneuron
ms	Millisecond
MUA	Motor unit activation
mV	Millivolt
MVC	Maximum voluntary contraction
M-Wave	Compound muscle action potential
n	Number
NMJ	Neuromuscular junction
pK^+	Equilibrium potential for potassium ion
pNa^+	Equilibrium potential for sodium ion
SR	Sarcoplasmic reticulum
s	Seconds

CHAPTER I

Introduction

The cause of muscle fatigue is a complex topic. It is dependent on the duration, intensity, and nature of the exercise, fibre type composition of the muscles used, individual levels of fitness, and numerous environmental factors (Fitts and Metzger, 1988). As well, the fatigue experienced by a construction worker working all day in the sun is different from that experienced by a weight lifter, or an endurance athlete, however some of the physiological mechanisms are similar.

There have been numerous definitions of skeletal muscle fatigue (Asmussen, 1979; Edwards, 1981; Bigland-Ritchie and Woods, 1984) which have attempted to address aspects of physiology--especially force output--and time. The definition used in this study is one suggested by Edwards (1983), stating that fatigue is the "failure to maintain the required or expected power output."

The chain of events illustrated in Figure 1 is typical of a voluntary muscle contraction. Consequently, impairment at any one, or a combination of these sites could lead to

Figure 1

Chain of command for muscle contraction.
Adapted from Edwards, 1983

Central Drive from Motor Cortex

⇓

Excitation of Spinal Motor Neurons

⇓

Transmission of Impulse Across
Neuromuscular Junction

⇓

Excitation of Sarcolemma

⇓

Excitation-Contraction Coupling

⇓

Force Output

neuromuscular fatigue. In the brief review of muscle fatigue which follows, these sites will be considered in two distinct groups: 1) Central Sites of Fatigue--which includes central drive to the motor cortex, and motoneuron (MN) excitation; and 2) Peripheral Sites of Fatigue--which consists of transmission across the neuromuscular junction (NMJ), excitation of the sarcolemma, and excitation-contraction (EC) coupling.

1.1 MUSCLE FATIGUE

1.1.1 CENTRAL SITES OF FATIGUE

Central Nervous System Drive

The question of whether or not the central nervous system (CNS) is voluntarily capable of maximally activating skeletal muscle is an old one, and endures. Numerous methods have been developed and utilized to assess CNS drive including the following: 1) comparing the force in a maximum voluntary contraction (MVC) with a supramaximal tetanic stimulation (Merton, 1954; Bigland-Ritchie et al., 1978, 1986a, 1986c; Grimby et al., 1981a), 2) cortical stimulation during fatiguing MVC's (Merton et al., 1981) and 3) a less invasive assessment of interpolating a supramaximal stimulus over the course of an MVC (Merton, 1954; Belanger and McComas, 1981).

Results using these methods have generally led to the conclusion that in highly motivated and practiced individuals, it is possible to maintain adequate CNS drive--or attain it with brief extra efforts (Bigland-Ritchie et al., 1978)--to the appropriate MN's (Grimby et al., 1981a; Enoka and Stuart, 1992). There may however be exceptions. For example, it is still unclear why some muscles, such as the plantar flexors, are more prone to CNS drive failure than others (Belanger and McComas, 1981; Bigland-Ritchie et al., 1986c). Furthermore, there is evidence that subjects may be able to activate all the necessary motor units during repeated maximal concentric contractions, but not similar eccentric contractions (Eloranta and Komi, 1980; Tesch et al., 1990; Westing et al., 1991). In addition, these exceptions may be exacerbated by high altitudes (Garner et al., 1990).

The question of CNS drive as a factor in fatigue remains controversial. It would seem that motivation, feedback, practice of the subject, the muscle group tested, the type of contraction, altitude, and possibly pain in the limb being tested (Rutherford et al., 1986; Gandevia and McKenzie, 1988), all play an important role in central activation of the motor cortex. Consequently, changes from optimal conditions of any one or a combination of these factors may significantly contribute to the development of muscle fatigue in individuals.

Motoneuron Excitability

The reduction in electromyogram (EMG) activity reported over the course of fatigue and with declines in force generating capacity (Stephens and Taylor, 1972; Bigland-Ritchie et al., 1979; Garland, 1988), may be attributed to a decline in CNS drive, but may also be the result of impaired MN excitability.

Bigland-Ritchie and colleagues (1983) used tungsten microelectrodes to measure single motor unit potentials during MVC's. They found that the percent decline in mean MN firing rates paralleled, and probably accounted for, the similar decline in surface EMG recorded simultaneously. Bigland-Ritchie and Woods (1984) concluded that the results provide direct evidence for a reduction of MN firing rates in fatigue. Similarly, Marsden et al. (1971) and Grimby et al. (1981b) have shown decreases in the MN firing frequencies during sustained maximal efforts of adductor pollicis and extensor hallucis brevis muscles respectively.

There have been numerous explanations as to how the MN firing frequencies become depressed in fatigue. Many of them support the sensory feedback hypothesis of Bigland-Ritchie et al. (1986b) which states, "during fatigue, MN firing rates may be regulated by a peripheral reflex originating in response to fatigue-induced changes within the muscle."

Garland (1991) and Garland and McComas (1990) have provided evidence of MN inhibition resulting from activation of small diameter afferents (group III/IV metaboreceptors). Furthermore, a study on muscle spindle feedback by Hagbarth and colleagues (1986) showed MN discharge rates which were lower than expected during MVC's when the nerve innervating the muscle was partially blocked with a local anesthetic. Moreover, Macefield et al. (1991) directly measured muscle spindle afferents during a sustained contraction and showed a definite fall in their firing rates, especially in the first 5-10 seconds of this type of contraction. However, muscle vibration (which preferentially excites muscle spindle receptors) increased the MN discharge rates illustrating the importance of spindle support in muscle contractions.

MN excitability may also be influenced through modulation of descending signals. Maton (1991) had monkeys perform repetitive isometric elbow-flexion torques while recording from cortical cells. This study illustrated changes in cortical cell discharge with parallel changes in the EMG power spectrum, and supported the view that MN discharge may also be affected by signals from the motor cortex during fatiguing contractions.

Although the decline in MN excitation may contribute to the loss of force output in fatigue, the decreased firing rates actually preserve muscle function and delay the onset of

fatigue. Jones et al. (1979), and more recently Binder-Macleod (1990, 1991), have reported greater losses of force when adductor pollicis and quadriceps femoris muscles were stimulated continuously at the same frequency typically associated with the initiation of a voluntary effort. Therefore, a reduction of MN firing rates, combined with increased relaxation rates, and an increase in the time course of the muscle twitch in fatigue, probably ensure economical activation of fatiguing muscles by producing a fusion or synchronization of the active motor units (Bigland-Ritchie et al., 1983; Enoka and Stuart, 1992; Gandevia, 1992).

The result of the above discussed events is that the required force, or at least a minimum force, may be maintained for a longer period of time without affecting motor coordination, and may in fact be considered a built-in protective mechanism commonly referred to as 'muscle wisdom'.

As well, during a sustained submaximal isometric contraction to fatigue, any loss of muscle force generating capacity is compensated for by a progressive recruitment of additional motor units (Edwards and Lippold, 1956; Lippold et al., 1960; Enoka and Stuart, 1992; Fallentin et al., 1993). However, the recruitment pattern appears to be dependent upon the intensity of the effort. Using fine wire electrodes in the biceps brachii muscle, Fallentin et al. (1993) demonstrated that at 10% MVC there was a de novo recruitment of motor units

throughout the contraction as larger units replaced smaller ones. Furthermore, there is a rotation of motor units illustrated by fluctuating episodes of electrical activity during the effort which has been demonstrated previously (Sjogaard et al., 1986). Comparatively, at 40% MVC, the recruitment of motor units occurred early in the contraction and no new unfatigued units were seen at exhaustion.

1.1.2 PERIPHERAL SITES OF FATIGUE

The Neuromuscular Junction

The compound muscle action potential (M-Wave) has been used extensively to evaluate NMJ failure. The difficulty in interpreting M-Wave changes, however, relates to the inability to determine if it is the NMJ or the impulse propagation along the muscle membrane which is responsible.

Merton (1954) reported no NMJ impairment during a 3 minute MVC of the human adductor pollicis muscle because there were no changes in the M-Wave characteristics. Bigland-Ritchie et al. (1982) reported similar results with the first dorsal interosseous muscle. Contrary to this, Stephens and Taylor (1972) and Bellemare and Garzaniti (1988) found a decrease in the amplitude of M-Waves after fatiguing MVC's of the first dorsal interosseus muscle and the adductor pollicis,

respectively, suggesting NMJ failure. The dissimilar conclusions may be due to differences in measurement techniques in analyzing the M-Waves.

More recently, Fuglevand et al. (1993) found decreases in the amplitude of M-Waves elicited after submaximal fatiguing voluntary contractions of the first dorsal interosseus muscle. They proposed that NMJ and excitation failure of the membrane become more evident as the length of the fatiguing contraction increases. Contrasting this argument are the findings of Thomas et al. (1989) which showed no significant M-Wave changes after a 5 minute fatigue protocol.

Stimulation studies have been commonly used to provide information about NMJ failure during fatigue. Numerous investigations have compared the effects of high (30+ Hz) and low (10-30 Hz) frequency stimulation (HFS/LFS) on M-Wave properties. In general, HFS causes a rapid decline in force output compared to lower frequencies, and is associated with impairment of transmission at the NMJ. Decreased amplitudes and increased durations of the evoked action potentials are typical of HFS, and these changes parallel the decline and return of tension (Edwards et al., 1977; Bigland-Ritchie et al., 1979; Jones et al., 1979; Sandercock et al., 1985) However, at lower frequencies, the M-Wave behaviour does not follow the same pattern as HFS, and tension recovers in a much slower time course than membrane excitability (Edwards et al.,

1977; Sandercock et al., 1985). Because of this sequence, low frequency fatigue has been attributed to failure in EC coupling (Edwards et al., 1977; Bianchi and Narayan, 1982; Sandercock et al., 1985).

Although M-Wave changes are normally interpreted as NMJ failure, they may also reflect alterations in the muscle fibre action potential (AP). Hence, the most convincing evidence for NMJ integrity comes from reports of direct muscle fibre stimulation in which the NMJ is bypassed. Merton et al. (1981) directly stimulated the adductor pollicis fibres and found that the loss of force during a sustained MVC could not be restored. Furthermore, Bezanilla et al. (1972), Jones et al. (1979), and Jones (1981) stimulated isolated animal muscles and found similar characteristic changes in force as in high frequency nerve stimulation. Jones (1981) also found changes in the AP waveform which could be mimicked by reductions in the sodium concentration ($[Na^+]$), or increases in the potassium concentration ($[K^+]$) of the bathing medium, as occurs in fatigue. These results point to a reduced excitability of the membrane as a cause for high frequency fatigue. The question of excitability failure being causally related to voluntary fatigue, however, has yet to be answered.

The Sarcolemma

Many scientists have suggested that alterations in membrane function prevent cell activation and induce muscle fatigue (Lindinger and Sjogaard, 1991; Sjogaard, 1991; Fitts, 1994). The main theory of cell inactivation--which is discussed in section 1.3--is that an increase in the extracellular potassium concentration ($[K^+]_e$), and an inability of the Na^+/K^+ ATPase (Na^+/K^+ pump) to maintain ionic concentration gradients, result in cell depolarization and a reduced AP amplitude, or, complete inactivation of the muscle membrane (Fitts, 1994). Lindinger and Sjogaard (1991) suggest that depolarization permits contractions to continue at reduced rates and strengths while preventing catastrophic changes in cellular homeostasis. Furthermore, Edwards (1981) speculates that cell depolarization would serve as a safety mechanism to prevent further depletion of ATP stores or minimize their use. This mechanism would prevent disruption of the sarcolemma or cell organelles by destructive calcium-sensitive (Ca^{++}-sensitive) proteases and phospholipases activated by increased intracellular Ca^{++}.

Although muscle fatigue is frequently associated with changes in the sarcolemma AP, such as a reduced amplitude, a prolonged duration, and an increased amplitude of the early negative after potential (Bezanilla et al., 1972; Hanson,

1974; Jones, 1981; Metzger and Fitts, 1986; Lannergren and Westerblad, 1987; Fitts, 1994), some scientists question whether or not changes such as these compromise the muscle's ability to produce force. Bezanilla et al. (1972) found that even when the spike overshoot did not go over 0 mV, in isolated frog muscle, there was no effect on peak twitch tension. Furthermore, Sandow (1952) observed no relationship between the size of the AP and force output. Sandow's conclusion was that a safety margin may exist regarding the depolarization necessary for full activation.

In contrast, Ashley and Ridgway (1970) found that a reduction in the AP decreases force output--an argument supported by Sandercock et al. (1985) on high frequency stimulation of single motor units.

The sarcolemma could very well be a primary site for the development of fatigue. The results from several studies in this area support the hypothesis put forth by Jones (1981) that accumulation of K^+ in the extracellular space and the T-tubules could slow the conduction velocity, lower the spike amplitude, and eventually fail to propagate an AP in the cell.

Excitation-Contraction Coupling

When the performance of an exercise task is not impaired by subject motivation, changes in the neural strategy,

reductions in the M-Wave, or lack of metabolic substrates (Bigland-Ritchie et al., 1986a; Burke et al., 1973), the decreases in force must be considered a result of a disruption in the link between activation of the muscle fibre membrane and the force output (Enoka and Stuart, 1992).

Many factors may contribute to this EC coupling failure. Transmission of the AP between the T-tubules and the SR is accomplished by an allosteric change of the T-tubular charge sensor (T-tubule charge movement), initiated by Ca^{++} binding, which triggers Ca^{++} release from adjacent SR-Ca^{++} release channels (ryanodine receptors) (Rios and Pizarro, 1988, 1991; Rios et al., 1991). Prolonged depolarization of the membrane, which may be due to increased $[K^+]_e$ has been shown to cause inactivation of T-tubule charge movement (Chandler et al., 1976). The T-tubule voltage sensor has a key role in depolarization by means of its Ca^{++} binding site on the T-tubule lumen side (Rios and Pizarro, 1988, 1991). The AP conduction in the tubules is blocked if $[Ca^{++}]$ approaches 15 mmol/l (Howell and Snowdowne, 1981; Howell et al., 1987), which may occur during activity. On the other hand, low T-tubule $[Ca^{++}]$ seen in fatigue (Beam and Knudson, 1991) could directly reduce intramembranous T-tubule charge movement, reducing subsequent release of Ca^{++} by the SR, resulting in a decline of force output (Brum et al., 1988; Pizarro et al., 1989).

Release of Ca^{++} by the SR is also a potential candidate for EC coupling failure. Ashley and Ridgway (1970) and Vergara et al., (1978) used voltage clamping techniques to show that reduced Ca^{++} release (Ca^{++} transient) diminishes tension development. Recently, Gyorke (1993) attributed the reduced Ca^{++} transient to inhibition of the ryanodine receptor. Furthermore, myoplasmic Inositol Trisphosphate depletion may reduce the frequency of allosteric changes of the T-tubule/SR junction thereby reducing Ca^{++} release (Fitts, 1994).

Although the release of Ca^{++} may be hindered in fatigue, it is unlikely that the amount of stored Ca^{++} is a cause for EC failure, since caffeine studies have shown that the loss of tension can be significantly attenuated through direct stimulation of Ca^{++} release from the SR (Grabowski et al., 1972; Lannergren and Westerblad, 1989; Garcia et al., 1991; Westerblad and Allen, 1991). It may also be that a redistribution of Ca^{++} from the SR release site to parvalbumin and the SR pump occurs (Fitts, 1994). This redistribution would decrease the driving force for Ca^{++} release which would subsequently increase twitch and relaxation times, and put a greater burden on the ATP-dependent reuptake process which may already be compromised by H^{+} inhibition of Ca^{++} ATPase.

Finally, H^{+} is a particularly interesting fatigue agent in EC coupling failure as it may augment fatigue via inhibition at numerous sites. The increase in H^{+} during

exercise (Nakamura and Schwartz, 1972; Byrd et al., 1989) increases the Ca^{++} binding capacity of isolated SR membranes (Nakamura and Schwartz, 1972) thereby sequestering Ca^{++} in the SR. In addition to this, H^{+} attaches to the T-tubule voltage sensor and changes the affinity of Ca^{++}-activated binding sites. T-tubular charge is reduced and the rate and frequency of channel openings subsequently diminishes (Ma et al., 1988; Rousseau and Pinkos, 1990). H^{+} also competitively inhibit actomyosin ATPase and the binding of Ca^{++} to troponin C directly reducing cross-bridge action (Fuchs et al., 1970; Blanchard et al., 1984).

1.1.3 SUMMARY

The major sites of fatigue in a muscle contraction have been briefly reviewed in this section. Whether fatigue is brought on by central or peripheral sites is debatable and highly task dependent. However, fatigue is probably the result of a combination of physiological processes and not solely attributable to one impaired link in the chain of events of contraction. The rest of this review will focus mainly on the muscle membrane and the role that potassium plays in fatigue.

1.2 IONIC PROPERTIES OF THE MUSCLE CELL MEMBRANE

Transient electrical signals convey information from nerves to muscle resulting in movement. It is important to understand the components of this information pathway which include the structure of the muscle cell membrane, its electrophysiological properties, and AP propagation, before we can understand fatigue. These topics will be reviewed in this section.

Structure

The sarcolemma is an excitable, highly selective, permeability barrier which contains many channels and pumps. Channels are protein structures which span the membrane and form a pore. These channels are gated meaning they have an open and closed state which is dependent upon the availability of Ca^{++} ions, ATP, and changes in the membrane potential (Stryer, 1988). In the open state, ions are allowed to pass down their electrochemical gradients through the membrane. Of particular interest are the five following channels which control the movement of Na^{+} and K^{+} across the sarcolemma: 1) voltage-gated Na^{+} channel, 2) inward rectifier K^{+} channel, 3) delayed rectifier K^{+} channel, 4) Ca^{++}-sensitive K^{+} channel, and 5) ATP-sensitive K^{+} channel (Kolb, 1990).

Activation of the Na^+ channel permits the movement of Na^+ into the cell (Catterall, 1988; Koester, 1991), the opening of channels 3, 4, and 5 result in the movement of K^+ out of the cell (Lindinger and Sjogaard, 1991), and the inward rectifier K^+ channel which is responsible for the resting K^+ permeability (Sjogaard, 1991).

Pumps are also proteins in the membrane which expend energy to move substances opposite to that which they tend to move naturally (Woodbury, 1965). Important to this study is the Na^+/K^+ ATPase pump which is found most abundantly in the sarcolemma (Clausen, 1986). This pump moves Na^+ ions out of the cell, and K^+ ions back into the cell after an AP, thus attempting to restore ionic concentration gradients.

Another important part of the sarcolemma is the motor end-plate. This is a specialized region found on each muscle fibre which acts as a receptor for the Ca^{++} initiated release of the neurotransmitter Acetylcholine (ACh) from the end of the innervating nerve axon. ACh causes the opening of a common permembranous cation channel through which Na^+ rushes into the cell, and K^+ rushes out. This exchange creates an end-plate potential which then triggers a wave of depolarization along the fibre in both directions (Stryer, 1988; Kimura, 1989).

The Resting Potential

The bulk of the cytoplasm and the extracellular fluid is electrically neutral, because of the equal number of positive and negative charges. However, charge separation occurs in a very narrow region less than 1 μm wide on either side of the cell membrane with the inside being more negative than the outside (Koester, 1991). The distribution of Na^+ and K^+ ions across the sarcolemma is vital to its excitability. In steady state, there must be an equilibrium so that if one ion moves into or out of the cell, another ion must move to maintain the charge.

The separation of charge is known as the membrane potential (E_m). Many factors contribute to E_m including ion concentrations, permeabilities, and electrochemical gradients (Woodbury, 1965; Katz, 1966; Kimura, 1989; Koester, 1991). The concentrations of Na^+ and K^+ in a typical mammalian muscle cell are found in Table 1 below (Woodbury, 1965; Kimura, 1989):

Table 1. Extracellular and intracellular Na^+ and K^+ ion concentrations

	Extracellular (mmol/l)	Intracellular (mmol/l)
Na^+	145	12
K^+	4	155

K^+ has the tendency to leak out of the cell, and Na^+ the tendency to leak into the cell down their concentration gradients. Opposing the efflux of K^+ is the negative potential inside the cell which pulls the positively charged K^+ back. Both the electrical and chemical driving forces for the Na^+ ion work to attract Na^+ inward, however the movement of this ion is limited by the very small permeability of the membrane to Na^+.

The membrane potentials at which ions are in equilibrium can be calculated from the Nernst equation which is derived from thermodynamic principles (Nernst, 1888):

$$E_{ion} = \frac{RT}{ZF} \ln \frac{[ion]_e}{[ion]_i}$$

where E_{ion} is the equilibrium potential of the ion, R is the universal gas constant, T is the absolute temperature, Z is the valency of the ion, and F is the Faraday constant (Koester, 1991). The difference in equilibrium potential

between K^+ (-97 mV) and Na^+ (+66 mV) results from the differences in the intra and extracellular concentrations of these two ions (Kimura, 1989).

E_m can be estimated from the Goldman-Hodgkin-Katz equation (Hodgkin and Katz, 1949):

$$E_m = \frac{RT}{F} \log_e \frac{pK[K^+]_e + pNa[Na^+]_e}{pK[K^+]_i + pNa[Na^+]_i}$$

which incorporates both the membrane permeabilities (p) of the ions as well as their concentrations on either side of the membrane. Because permeability of the ions determines E_m when total concentrations inside and outside the cell are roughly equal (Koester, 1991), E_m is -90 mV, which is closest to E_{K+}, because K^+ is the most permeable ion. E_m does not exactly equal E_{K+} due to the small but steady influx of Na^+, making E_m slightly more positive. Furthermore, this more positive value creates an imbalance in the forces acting on K^+ resulting in an efflux of K^+ at steady state (Kimura, 1989). These two passive fluxes of Na^+ and K^+ are carefully balanced by the Na^+/K^+ pump, the activity of which also contributes to the potential with its electrogenic effect (Hicks and McComas, 1989)--please see section 1.4.

The Action Potential

When Na^+ enters the cell at the end-plate, it causes the local E_m to become more positive (depolarization). This in turn opens the nearby voltage-sensitive Na^+ channels, consequently increasing the Na^+ conductance through the sarcolemma. Na^+ rushes into the cell, down its concentration and electrochemical gradients, until the membrane depolarizes by ~15 mV, which is termed threshold (Rush and Fulton, 1973). At this point, permeability of Na^+ increases approximately 500 fold (Kimura, 1989) giving rise to the characteristic steep slope of the muscle fibre membrane AP. At the peak of the AP, E_m is closer to E_{Na+}, reaching values between +20 and +30 mV (Kimura, 1989). After a delay of about 1 msec, two important events occur. First, the delayed rectifier K^+ channels start to open up and K^+ exits the cell. Secondly, Na^+ permeability begins to fall with the inactivation of the Na^+ channels as the K^+ conductance increases (Koester, 1991). These two events combine to bring the E_m back towards resting value (repolarization) which is actually surpassed (hyperpolarization) transiently due to the increased K^+ conductance.

The wave of depolarization influences Na^+ channels in adjacent non-active areas of the membrane to increase Na^+ conductance, and the process regenerates itself. In this way,

the impulse maintains its amplitude and propagates in both directions along the fibre from the site of initial depolarization. Repolarization follows the moving AP creating a dynamic regenerative cycle across the membrane (Clark and Plonskey, 1968).

1.3 THE ROLE OF POTASSIUM IN FATIGUE

Extracellular Potassium Accumulation

It is well documented that venous plasma [K^+] rises during muscular efforts (Fenn and Cobb, 1936; Medbo and Sejersted, 1990; Sjogaard, 1990; Lindinger and Sjogaard, 1991; Sjogaard, 1991), and that the source of the K^+ is principally from the exercising muscles (McKenna, 1992). Several mechanisms have been suggested for the K^+ efflux. The first is the electrically activated opening of the delayed rectifier K^+ channel which occurs during the repolarization of the membrane--please see section 1.2.

A second possible mechanism involves the ATP-sensitive K^+ channels which are very dense in skeletal muscle (Spruce et al., 1985). These channels open when ATP concentrations fall below 2 mmol/l (Spruce et al., 1985; Burton et al., 1988), however, it is unlikely that such low ATP concentrations would be seen in humans even at exhaustion (Sjogaard, 1991). In an

environment of adequate ATP, ADP may modulate the channel and make it accessible for K^+ efflux (Kakei et al., 1986). It is possible, however, that local reductions in ATP may be found in the cell if ATP is compartmentalized in a membrane pool as suggested by Spruce et al. (1985). In this case, ATP inhibition of the channel would be reduced, thereby increasing the possibility of open channels and K^+ efflux (Davies, 1990). Consequently, it has been reported that this channel probably accounts for most of the large K^+ conductance in metabolically exhausted mammalian muscle fibres (Fink and Luttgau, 1976; Castle and Haylett, 1987; Stewart and Bretag, 1991).

A third mechanism of K^+ efflux may be the opening of Ca^{++}-sensitive K^+ channels (Lindinger and Sjogaard, 1991; Sjogaard, 1991; McKenna, 1992). Juel (1988a) has shown that $[Ca^{++}]_i$ lower than those attained during activity are sufficient to allosterically alter these channels and open them.

Although the amount of K^+ lost per AP is very small, 0.23 mmol/l in frog sartorius muscle (Adrian and Peachy, 1973), during prolonged muscle activity when thousands of potentials are fired (Cupido, 1991) and the effects of both the ATP- and Ca^{++}- sensitive channels are included, there is opportunity for a significant efflux of K^+ (Fenn and Cobb, 1936; Hirche et al, 1980; Juel et al, 1990; Medbo and Sejersted, 1990).

A doubling of plasma $[K^+]$ has been reported after dynamic and isometric exercise, rising from a resting concentration of

4 mmol/l to a post exercise value of 8 mmol/l and above (Hirche et al., 1980; Hnik et al., 1986; Sjogaard, 1986; Sejersted and Hallen, 1987; Medbo and Sejersted, 1990). These plasma values may however be an underestimate of the true K^+ loss. McKelvie et al. (1991) reported that during maximal isokinetic cycling exercise with humans, the erythrocytes carried 57% of the total increase in whole blood $[K^+]$. Therefore plasma $[K^+]$ values are probably considerably less than what is present in the interstitial space surrounding the individual muscle fibres. For example, Vyskocil et al. (1983) measured interstitial $[K^+]$ with ion selective electrodes after brief maximal exercise of the human forearm and found values up to 15 mmol/l.

The Membrane Hypothesis

The increase in $[K^+]_e$ reduces the K^+ concentration gradient and depolarizes the membrane (Hodgkin and Horowicz, 1959)--causing E_m to move closer to E_{Na+}. In fact, an increase in $[K^+]_e$ to 8-9 mmol/l would depolarize the sarcolemma by 10-20 mV (Kwiecinski et al., 1984; Juel, 1986; Westerblad and Lannergren, 1986; Thompson et al., 1992). A depolarization sustained at this magnitude reduces the inward Na^+ current due to inactivation of up to 50% of the Na^+ channels (Hodgkin and Huxley, 1952). Consequently, the amplitude and conduction

velocity of the AP are reduced (Bigland-Ritchie and Woods, 1984; Sandercock et al., 1985; Juel, 1986; Kossler et al., 1989).

Most studies point to the T-tubule as the most probable location for transmission failure as there seems to be no direct temporal relationship between sarcolemma AP amplitude-duration changes, and muscle fatigue development or recovery (Bezanilla et al., 1972 ; Grabowski et al., 1972; Metzger and Fitts, 1986; Sjogaard, 1991). As well, since AP's recorded from the surface reflect the activity of the surface membrane, there could be major changes occurring in the T-tubules that go undetected by the surface recordings (Jones and Bigland-Ritchie, 1986). Furthermore, $[K^+]_e$ would be markedly increased in the T-tubules since there is a much greater surface to volume ratio, and a reduction in diffusion (Jones and Bigland-Ritchie, 1986). Therefore the sequence of fatigue may be the following: impairment of AP propagation in the T-tubule inhibits EC coupling in such a way that SR Ca^{++} channel opening, and/or Ca^{++} release are inhibited; the number of actin-myosin interactions decreases; and force output is attenuated (Ashley and Ridgway, 1970; Rios and Pizarro, 1988; Westerblad et al., 1990; Rios et al., 1991; Sjogaard, 1991).

1.4 Na^+/K^+ ATPase

The Na^+/K^+ ATPase is a membrane bound pump found in high concentrations in the sarcolemma but much more sporadically in the T-tubules (Venosa and Horowicz, 1981; Clausen, 1986). Affecting the Na^+/K^+ ATPase concentration are many factors such as: age (Dorup et al., 1988; Klitgaard and Clausen, 1989), thyroid status, caloric intake, K^+ availability, and muscle activity (Kjeldsen et al., 1986; Clausen, 1986; Clausen and Everts, 1989; Green et al., 1993).

When the pump is functioning normally, it utilizes one ATP (Glynn and Karlish, 1975) to move 3 Na^+'s out of the cell and 2 K^+'s into the cell (Thomas, 1972) in order to balance the ion fluxes which are continually occurring across the membrane. As discussed in section 1.3, the accumulation of K^+ in the extracellular space can be disastrous to the maintenance of E_m. Therefore the function of Na^+/K^+ ATPase is very important.

The actual capacity of the pump has been determined with the use of [^{3}H]-ouabain binding techniques (Clausen et al., 1987), as ouabain binds specifically to the Na^+/K^+ ATPase. In human skeletal muscle, the concentration of [^{3}H]-ouabain sites is about 280 nmol/kg wet weight (Norgaard et al., 1984), and with each binding site able to transport about 16 000 K^+ per minute, the maximal capacity of K^+ transport is 4.5

mmol/min/kg wet weight (Kjeldsen, 1987).

There are however many factors which will affect the activity of the pump and its capacity to move the ions. One is the availability of ATP as this is the metabolic energy source of the pump. Activity may be increased by augmenting the amount of insulin, aldosterone, epinephrine/norepinephrine, and intracellular [Na^+] (Flatman and Clausen, 1979; Bia and DiFronzo, 1981; Clausen, 1986; Clausen and Everts, 1989). In contrast, activity is attenuated by decreases in the same, cardiac glycosides such as ouabain (Matchett and Johnson, 1954; Johnson, 1956), a decrease in temperature (Clausen et al., 1987), and a low $[K^+]_e$ (Steinbach, 1940). Most important in acute control of the Na^+/K^+ ATPase during exercise are the adrenergic system, and $[Na^+]_i$--which is a potent stimulator of pump activity (Clausen, 1986; Lindinger and Sjogaard, 1991).

In particular, Na^+/K^+ ATPase is modulated by α- and β-adrenergic receptors (Clausen, 1986; Clausen and Everts, 1989). Increased activity of the pump results from β_2-adrenoreceptor stimulation (Brown et al., 1983; Juel, 1988b), whereas β_2-adrenoreceptor blockade (Clausen and Flatman, 1977; Katz et al., 1985), or stimulation of the α-adrenoreceptor cause a reduction in Na^+/K^+ ATPase activity (Williams et al., 1985; Lindinger and Sjogaard, 1991).

In spite of the many factors that stimulate the pump during exercise, there is evidence that it is unable to

maintain ionic homeostasis and, thus, K^+ accumulates in the extracellular space (Sjogaard, 1991; Lindinger and Sjogaard, 1991). It may be that the pump density is not high enough to fully compensate for the ionic fluxes and increased conductance, a condition which would be exacerbated in the T-tubules where the density of the pump is lower than in the sarcolemma (Sejersted, 1992), or the ATP availability may be insufficient (Sjogaard, 1991). The result is that the Na^+/K^+ ATPase is unable to restore ionic gradients quickly enough and $[K^+]_e$ increases.

By recognizing the electrogenic contribution of the Na^+/K^+ pump, Hicks and McComas (1989) have introduced another consideration for the hypothesis that cell depolarization mediates fatigue. They found that after stimulating rat soleus muscle (20 Hz for 4 seconds, every 5 seconds, for 5 minutes) there was a hyperpolarization of the membrane, and an increase in the amplitude of single AP's and M-Waves. The addition of ouabain to the bathing medium removed the hyperpolarization and supported their conclusion that the activity of the Na^+/K^+ ATPase during activity may add an electrogenic effect of up to -30 mV to the E_m thereby maintaining E_m and excitability of the cell, or at least delaying the onset of fatigue.

1.5 THE COMPOUND MUSCLE ACTION POTENTIAL

The M-wave is "the summation of nearly synchronous muscle fibre AP's recorded from a muscle commonly produced by stimulation of the nerve supplying the muscle" (AAEE Glossary of Terms, 1987), and is therefore an excellent indicator of changes in the propagation of the impulse between the nerve branches and the recording electrodes on the muscle (Bigland-Ritchie et al., 1979; Duchateau and Hainaut, 1985; Enoka and Stuart, 1992). Consequently, the M-wave offers useful information about the state of activity of the neuromuscular apparatus during fatigue (Cupido, 1991).

The M-wave amplitude is dependent upon the resting membrane potential of individual muscle fibres, the amplitudes of their AP's, and by the number of active muscle fibres responding to the stimulus. It therefore reflects the excitability of the muscle membrane (Hicks, 1988; Cupido, 1991).

The duration of the M-wave represents synaptic delay across the neuromuscular junction, synchronization of the muscle fibre AP's, and the conductance of the inward Na^+ channels and the outward K^+ channels (Cupido, 1991). Bigland-Ritchie et al. (1979) have suggested that a loss of M-wave amplitude, accompanied by a broadening of the waveform and no subsequent increase in area, indicates neuromuscular

transmission failure with a dispersion of muscle fibre action potential propagation. In contrast, broadening the waveform with little or no loss of amplitude results in an increase in total area which could be explained by a slowing of conduction velocity along the muscle fibre membrane illustrating a reduction of membrane excitability (Bigland-Ritchie et al., 1979).

Most studies utilizing the M-wave to confirm membrane excitability have found that it is unaffected by voluntary fatigue (Merton, 1954; Bigland-Ritchie, 1979; Bigland-Ritchie, 1982; Cupido, 1991). However others have reported changes in the M-Wave which point to membrane excitability or NMJ failure as causes for fatigue (Stephens and Taylor, 1972; Bellemare and Garzaniti, 1988; Fuglevand et al., 1993). Hicks et al. (1989) and Hicks and McComas (1989) have shown that M-wave amplitude and area may potentiate during both voluntary and stimulated fatigue protocols in human and animal models. Their findings suggest that the increased pump activity in response to muscle activity, produced an augmented electrogenic effect which hyperpolarized the individual muscle fibre membranes, resulting in potentiation of the M-Wave.

The M-Wave is an effective measurement of sarcolemma excitability and neuromuscular transmission integrity. It may also be possible to use the M-wave as a non-invasive method for assessing Na^+/K^+ pump activity fatigue of skeletal muscle.

1.6 SUMMARY AND STATEMENT OF PURPOSE

There are many places in the chain of events of muscle contraction associated with the onset of fatigue. It is tempting to select one as the primary cause, but it is more probable that impairments in numerous sites result in fatigue.

Of particular interest to this study is the maintenance of the muscle cell membrane potential and the dynamics of Na^+ and K^+ exchange which are instrumental in preserving sarcolemma excitability. One theory proposed here is that an increase in $[K^+]_e$ causes membrane depolarization, which decreases Ca^{++} release from the sarcoplasmic reticulum, and attenuates force output.

Many investigations have used the M-Wave to examine the role of the membrane and neuromuscular junction in fatigue. Furthermore, from M-Wave evaluation, the electrogenic activity of the Na^+/K^+ ATPase has been considered important in maintaining membrane potential during exercise, which may delay or even prevent fatigue.

To this researcher's knowledge, M-Wave characteristics and femoral plasma $[K^+]$ have not been investigated concomitantly during fatigue. Therefore, it is the purpose of this study to observe plasma $[K^+]$ changes with changes in M-Wave characteristics, and examine the role of membrane phenomena in fatigue.

CHAPTER II

THE RELATIONSHIP BETWEEN EXTRACELLULAR POTASSIUM CONCENTRATIONS AND MUSCLE MEMBRANE EXCITABILITY FOLLOWING A SUSTAINED SUBMAXIMAL ISOMETRIC QUADRICEPS CONTRACTION

2.1 INTRODUCTION

Muscle fatigue has been defined by Edwards (1983) as the failure to maintain the required or expected power output during an activity. The cause of exercise-induced muscle fatigue has been the focus of many investigations over the years (Merton, 1954; Stephens and Taylor, 1972; Bigland-Ritchie et al., 1979; Edwards, 1983; Sjogaard, 1990). The potential sites of fatigue may be grouped into three general categories (Bigland-Ritchie, 1984) 1) within the central nervous system (CNS) which includes excitatory input to higher motor centres, excitatory drive to lower motoneurons (MN), and MN excitability; 2) neuromuscular transmission which includes impulse transmission from nerve to muscle; and 3) sites within the individual muscle fibres including sarcolemma excitability, excitation-contraction (EC) coupling, contractile mechanisms and metabolic energy supply, and

metabolite accumulation. The site of voluntary fatigue in well motivated subjects does not appear to be at the level of the CNS nor the neuromuscular junction (NMJ) (Merton, 1954; Jones et al., 1971; Merton et al., 1981). This would suggest that the main site of fatigue may be located within the individual muscle fibres. Studies have examined muscle metabolism during exercise, and although there are decreases in energy supplies at the point of fatigue, the changes are small--ATP levels are depleted only by approximately 20%--and probably are not primarily responsible for the development of fatigue (Karlson and Saltin, 1970; Edwards et al., 1972; Katz et al., 1986; Miller et al., 1988). This has led to the speculation that fatigue may be occurring at another site within the muscle fibre which prevents the complete depletion of energy supplies within the cell, acting as a built-in safety mechanism preventing cell destruction (Sjogaard, 1991).

Impairment of the mechanical process of cross-bridging, or failure to maintain optimal muscle cell membrane excitability are two mechanisms that would produce muscle fatigue while not significantly depleting cellular energy supplies (Sjogaard, 1991). Of these two mechanisms, it has been postulated that failure to maintain an optimal resting membrane potential (E_m) is more likely responsible for the development of fatigue (Sjogaard, 1990, 1991).

The muscle cell membrane potential is mainly dependent

upon the ratio of the resting extracellular potassium concentration ($[K^+]_e$) to intracellular potassium concentration ($[K^+]_i$) (Hodgkin and Katz, 1949). Exercise has been shown to produce increases in $[K^+]_e$ that could cause a decline in the resting E_m of the sarcolemma (Hirche et al., 1980; Hnik et al., 1986; Sjogaard, 1986; Sejersted and Hallen, 1987; Medbo and Sejersted, 1990). An increase in $[K^+]_e$ can depolarize the membrane (Hodgkin and Horowicz, 1959) causing an impairment of AP propagation resulting in a decrease of the calcium (Ca^{++}) transient, and ultimately a decline in force output.

The evoked compound muscle action potential (M-Wave) is representative of muscle excitability as its size is dependent on the resting E_m and action potential (AP) amplitude of each single fibre responding to the stimulus (Bigland-Ritchie, 1982; Hicks, 1988; Cupido, 1991). As the membrane E_{K+} is the main determinate of E_m, changes in the $[K^+]_e$ or $[K^+]_i$ would potentially alter the size of the M-Wave. The resting E_m of the sarcolemma is maintained by the activity of the Na^+/K^+ ATPase which attempts to maintain the appropriate concentration gradients of K^+ and Na^+ on either side of the membrane (Clausen and Everts, 1991). Hicks and McComas (1989) have shown that the electrogenic nature of active Na^+/K^+ ATPase may delay or prevent the onset of fatigue, by offsetting the depolarizing effects of increased $[K^+]_e$ during muscular activity.

Although separate studies have investigated changes in $[K^+]_e$ (Vyskocil et al., 1983; Sjogaard et al., 1985; Sjogaard, 1986; Hnik et al., 1986; Juel, 1986; Lindinger and Heigenheuser, 1988; Sjogaard, 1988) and M-Wave responses (Stephens and Taylor, 1972; Bigland-Ritchie et al., 1979; Bigland-Ritchie et al., 1982; Bellemare and Garzaniti, 1988; Thomas et al., 1989; Fuglevand et al., 1993) during various fatigue protocols, there have been no studies performed which have examined these two variables during the same procedure. The purpose of this study was to relate femoral venous plasma $[K^+]$ to changes in membrane excitability as measured by evoked M-Waves during recovery after a fatiguing submaximal isometric quadriceps contraction. We hypothesize that in fatigue, membrane excitability will be maintained, due to the electrogenic nature of the highly active Na^+/K^+ pump.

2.2 METHODS

2.2.1 SUBJECTS

Ten healthy male university students (age: 22.0 ± .5 yrs, height: 179.9 ± 1.8 cm, weight: 77.3 ± 1.6 kg) volunteered to participate in this study. All ten subjects were recreational athletes so they were asked not to do any physical activity for three days prior to their test date. After the

experimental protocol and potential risks were outlined, informed consent was obtained from each subject (see appendix D). Procedures for the study were approved by the University Ethics Committee.

2.2.2 GENERAL PROTOCOL

Catheterization

After the skin was shaved at the electrode and catheter sites, the subject rested on a laboratory bed while the attending physician introduced the femoral and arterial catheters.

Following infiltration of the skin with 10 ml of 2% xylocaine without epinephrine (Astra Pharmaceuticals Canada LTD., Mississauga, Ontario) in the inguinal area, the Seldinger technique was used to insert a catheter (VC FN 7.5-38-J, Cook Canada Inc., Stouffville, Ontario) approximately 13 cm into the femoral vein. Once the femoral catheter was positioned, a rubber cathode (57mm x 103mm) was taped into place just inferior to where the catheter pierced the skin and over the course of the femoral nerve. The right brachial artery was catheterized percutaneously with a teflon catheter (20 gauge, 3.2 cm; Becton/Dickinson and Co., Sandy, Utah) after infiltration of the skin with 1 ml of 2% xylocaine

without epinephrine.

Patency of both catheters was maintained using nonheparinized isotonic saline (0.9% NaCl, Baxter Healthcare Corp., Deerfield, Illinois).

Stimulating and Recording

After the catheters were inserted and the rubber cathode positioned, the subjects walked approximately 10 metres to the neuromuscular laboratory where the experiment proceeded.

Once in the laboratory, the subjects sat with their right knee flexed at an angle of 90° and their hips and backs secured by a strap against an upright support. The hip angle was maintained at 100°. The lower right leg of each subject was affixed in a metal brace with two Velcro straps: one fastened around the proximal end of the lower right leg below the knee, and the second, immediately above the right ankle. Two different Velcro straps were fastened around the proximal and middle portions of the right thigh to stabilize it throughout the test (see Figure 2a). Finally, a blood pressure cuff (Baumanometer Calibrated V-Lok Cuff; W.A. Baum Co. Ltd., Copiague, New York) was wrapped loosely around the right leg just below the knee.

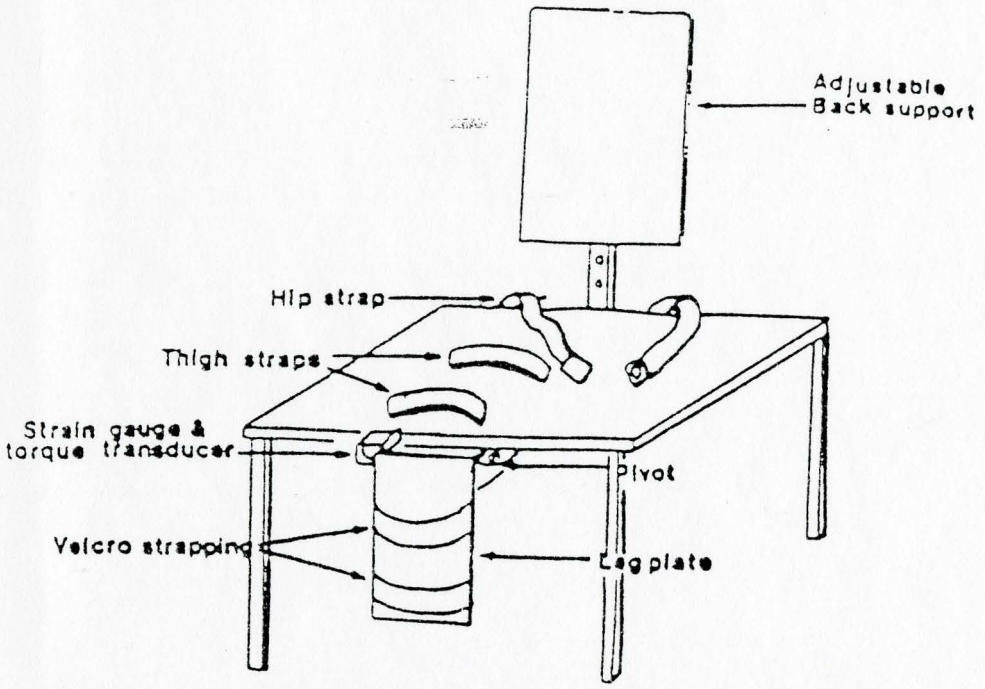
Prior to all the electrode placements, the skin was prepared by rubbing it with an abrasive, and cleaning it with

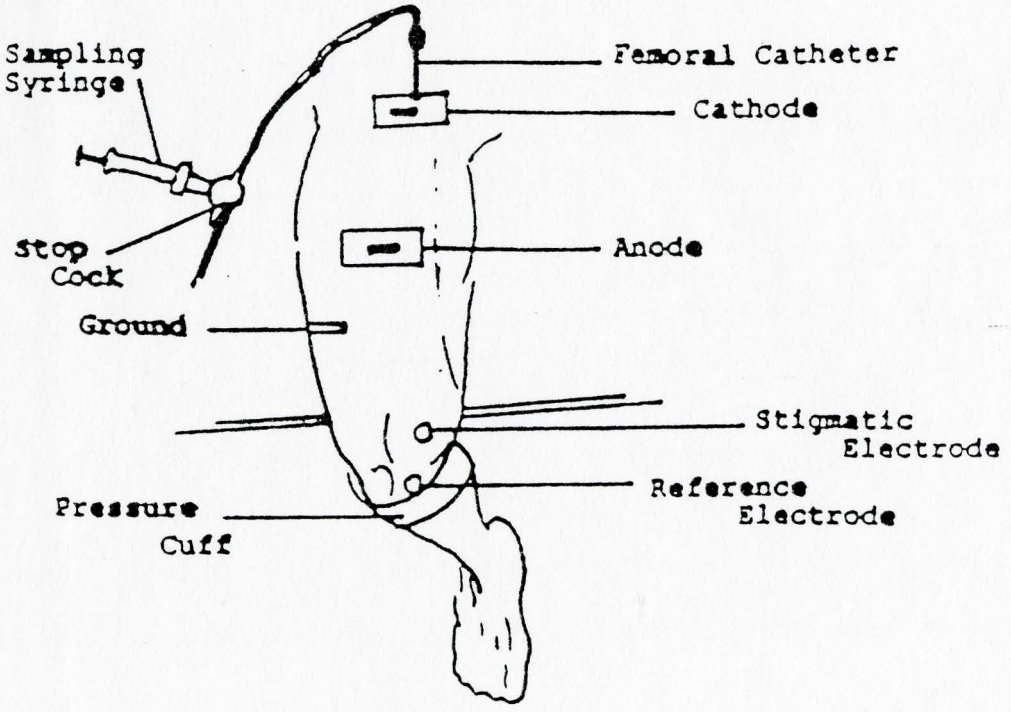
Figure 2a

Leg apparatus with restraining straps used in the protocol

Figure 2b

Electrode placements, pressure cuff position, and femoral catheter site


Adjustable
Back support
Hip strap
Thigh straps
Strain gauge &
torque transducer
Pivot
Velcro strapping
Leg plate


Sampling
Syringe
Femoral Catheter
Cathode
Stop
Cock
Anode
Ground
Stigmatic
Electrode
Reference
Electrode
Pressure
Cuff

alcohol. The rubber anode (57mm x 103mm) was taped onto the anterior aspect of the middle of the right thigh.

Voluntary electromyogram (EMG) activity and M-wave characteristics were recorded with a monopolar arrangement using two disposable silver/silver chloride monitoring electrodes (3M No.2248). The stigmatic electrode was placed over the belly of the right vastus medialis muscle, and the reference electrode was secured approximately 2 cm distal to this and medial to the patella. A silver strip (6mm X 50mm) ground electrode was placed on the antero-lateral aspect of the right thigh, between the anode and the stigmatic electrode (see Figure 2b for all electrode placements and position of the pressure cuff).

A high-voltage stimulator (Devices Stimulator 3072, Medical Systems Corp.) was used to deliver single rectangular pulses (pulse width: 200-500 μs) to the femoral nerve. The EMG signals from the recording electrodes were fed into a Honeywell Accudata EMG Amplifier (model #135A) at a sampling rate of 2.7 KHz, filtered (.004-2.5 KHz), and were displayed in real time on a VGA monitor. The data were continuously streamed to disc through a Dataq waveform scrolling board (AT CODAS Interface Card; Dataq Instruments Inc., Akron, Ohio) in an IBM-compatible computer.

Baseline Assessments

Baseline (BL) arterial and venous blood samples were drawn immediately prior to determining the maximum twitch torque. The maximum twitch torque was determined by progressively increasing the stimulator voltage until a maximum muscle twitch was evoked. This voltage was then used for the remainder of the experiment to produce the maximum evoked twitch torque (ETTmax). Subjects were then encouraged to perform a maximum voluntary contraction (MVC), without using their hands or arms for leverage. The best of three MVC's (approximately 1 min rest between each 4s attempt) was used to calculate the torque required for the subsequent fatigue test.

Motor unit activation (MUA) was assessed by interpolating a twitch during the MVC (Belanger and McComas, 1981). The theoretical MUA was calculated as follows:

$$MUA(\%) = \frac{\text{Twitch Torque - Interpolated Twitch Torque}}{\text{Twitch Torque}} \times 100$$

Approximately 3 min after these baseline assessments were made, another blood sample was taken from the arterial and venous catheters, and a twitch was evoked. This time point in the experiment is referred to as immediately before contraction (IBC).

Fatigue Paradigm

Subjects were required to sustain an isometric contraction of the right quadriceps at an intensity of 25-30% MVC for 3 min. Visual feedback on the computer monitor allowed the subject to monitor his torque output, and the experimenter provided constant verbal feedback to ensure that the appropriate torque range was adhered to. The during contraction (DC) blood sample was drawn at the 2 min point of exercise. During the final 2s of this 3 min exercise protocol, subjects were required to perform another MVC (MVC2).

Over the 3 min fatigue paradigm, seven windows of EMG were saved to disc. The first six were 5s in duration starting at 0s and saved every 30s, however the final window started at 2 min 53s and lasted 7s, to incorporate both the end of the sustained contraction and MVC2.

At the end of the 3 min fatigue paradigm, a blood pressure cuff was inflated to a pressure of approximately 100 mmHg which allowed arterial blood flow into the limb, but prevented venous admixture from the lower leg diluting the electrolyte concentrations obtained from the femoral vein.

Recovery

For the ensuing recovery period, subjects remained as

relaxed as possible while fastened in the apparatus (Figure 2a). M-waves were evoked, and blood samples were taken concurrently, with the first at 5s, the second at 20s, then the following 8 samples at 20s intervals to the 3 min mark, then every 30s for the next 3 min, then every min for the following 4 min, and the final sample at 15 min. Each stimulation was preceded by a tone to ensure the blood sampling was timed simultaneously with the twitch. The timing and delivery of the tones and twitches were controlled by a Stoelting Laboratory Controller (Stoelting Laboratory Corp.) interfaced with the computer. Following completion of the testing, the subjects returned to the laboratory bed where the physician removed the catheters. A schematic diagram of the prefatigue, fatigue, and recovery protocols are outlined in Figure 3.

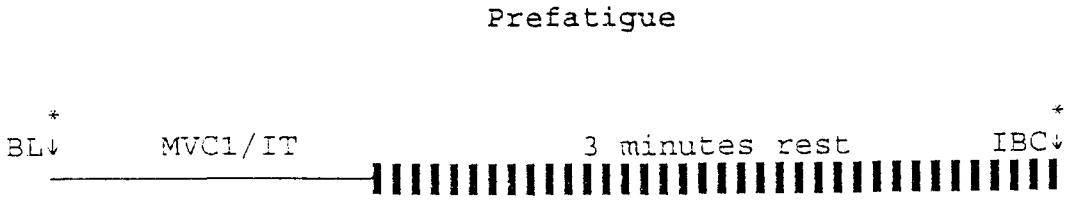
2.2.3 ANALYSES

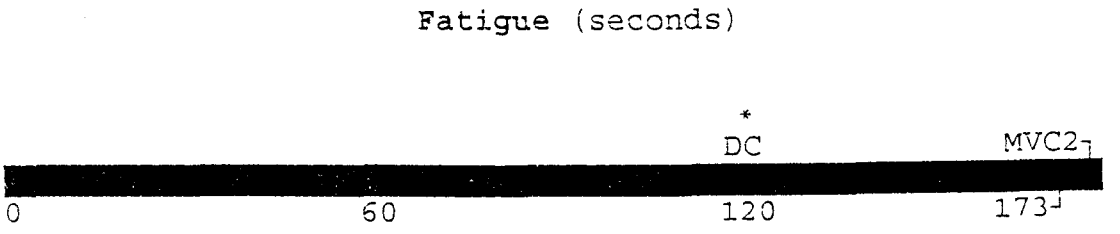
Blood Analysis

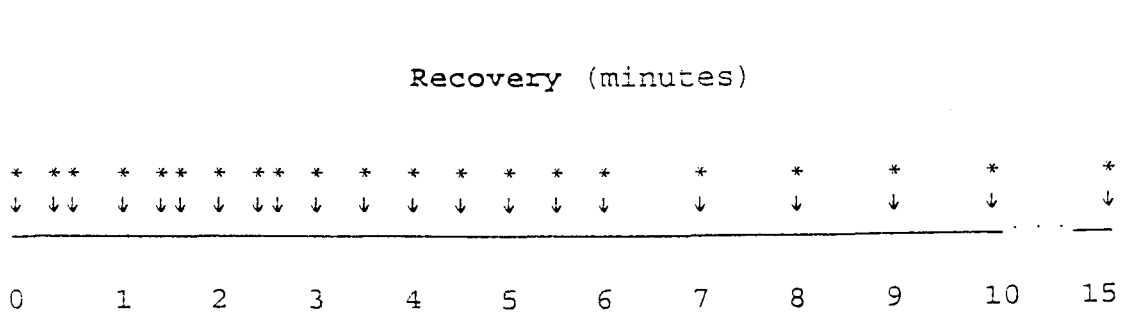
Each blood sample (approximately 4.0 ml) was collected into a 4.5 ml safety Monovette Li-Heparin plastic syringe (Sarstedt Inc., St. Laurent, Quebec). Arterial and venous blood samples were handled identically throughout the study. Each sample was immediately shaken to mix the blood and

Figure 3

A schematic diagram of the pre-fatigue, fatigue, and recovery protocols







BL	Baseline
MVC	Maximum Voluntary Contraction (1 and 2)
IT	Interpolated Twitch
DC	During Contraction
IBC	Immediately Before Contraction
*	Blood Sample Drawn
↓	Twitch Evoked

heparin. Blood was transferred into prelabelled microcentrifuge tubes (Fisher Scientific, 1.8 ml, cat. no. 05-664-10, Ottawa, Ontario; Sarstedt Test Tubes, 1.5 ml, no. 72.690.075), and then centrifuged at 12,400 rpm (Fisher Scientific Micro Centrifuge model #235C). Plasma was drawn off, and stored at -20 °C for later analysis of ion concentrations.

Prior to analysis, plasma in the microcentrifuge tubes was thawed in water. The [K^+] was determined with an automated NA^+/K^+ analyzer (Radiometer KNA2, Copenhagen, Denmark) and values were reported in mmol/l. An automated lactate analyzer (Yellow Springs Instruments model 23L, Yellow Springs, Ohio) was used to determine plasma lactate concentration ([La^-]) and values were similarly reported in mmol/l.

EMG Analysis

One second epochs of EMG recordings were cut from the 5s windows of data saved during the MVC's and sustained muscle contractions. These epochs were selected based on both the value of the corresponding torque, and on how closely they represented the 5s of saved data. All 1s epochs were then integrated by the CODAS programme, and normalized to the EMG associated with MVC1.

M-Wave Analysis

M-Waves were analyzed for peak to peak amplitude, duration, and area. Amplitude was calculated by adding the absolute magnitude of the two peaks of the wave form. Duration was determined as the time from the beginning of the M-Wave, when the trace departed from baseline, until the point where the wave form was at 5% of its peak amplitude after the positive and negative phases. Area was calculated by integrating the entire M-Wave (Figure 4).

Torque Measurements

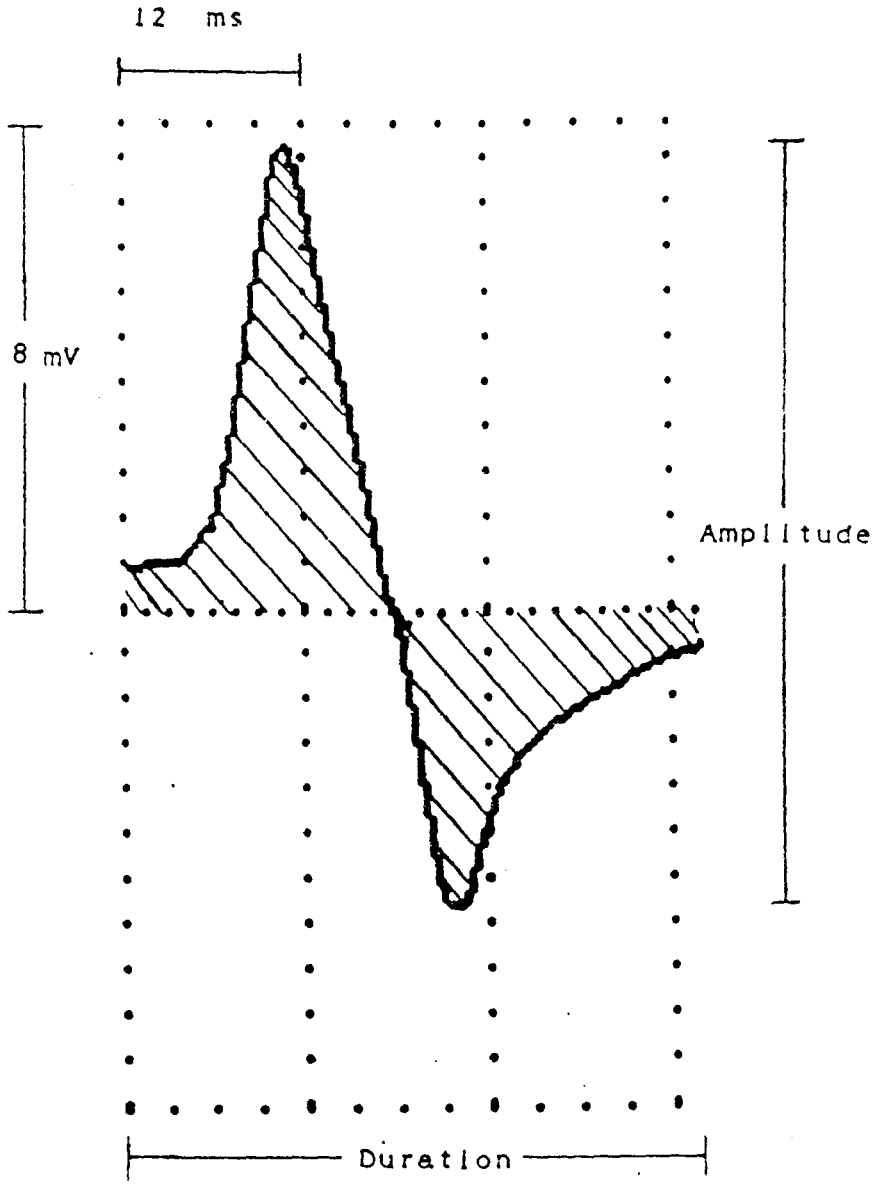
All of the torques recorded during the fatiguing contraction were normalized to MVC1. All elicited twitches were analyzed for peak twitch torque and half relaxation time by a custom designed software programme.

Statistical Analyses

Data were analyzed using a one way analysis of variance (ANOVA) with repeated measures. Any comparisons of means to a related baseline measure were examined using a Dunnett test. Significant differences between any other means were examined using a Tukey A post hoc test. Correlations were performed

Figure 4

An enlarged sample M-Wave and its components recorded from the right vastus medialis muscle of a subject, prior to his fatiguing isometric contraction


12 ms
8 mV
Amplitude
Duration
Area

using stepwise regression analysis in the Regress II software package. Significance was set at $p < 0.05$.

All values in the text are presented as means ± standard error of the mean unless stated otherwise.

2.3 RESULTS

2.3.1 SUSTAINED CONTRACTION ANALYSES

Voluntary Torque

The mean MVC at baseline (MVC1) was 239.5 ± 22.9 Nm. Theoretical motor unit activation was derived using the equation described in section 2.2.2. As can been seen from Table 2, the mean motor unit activation was 94.7 ± 3.4%. Torques during the 3 min fatigue protocol were normalized to MVC1 and maintained between 29.0 ± .6% and 23.0 ± 1.9% with a mean torque of 26.5 ± 1.2%. The mean torque output during the fatigue protocol is illustrated in Figure 5.

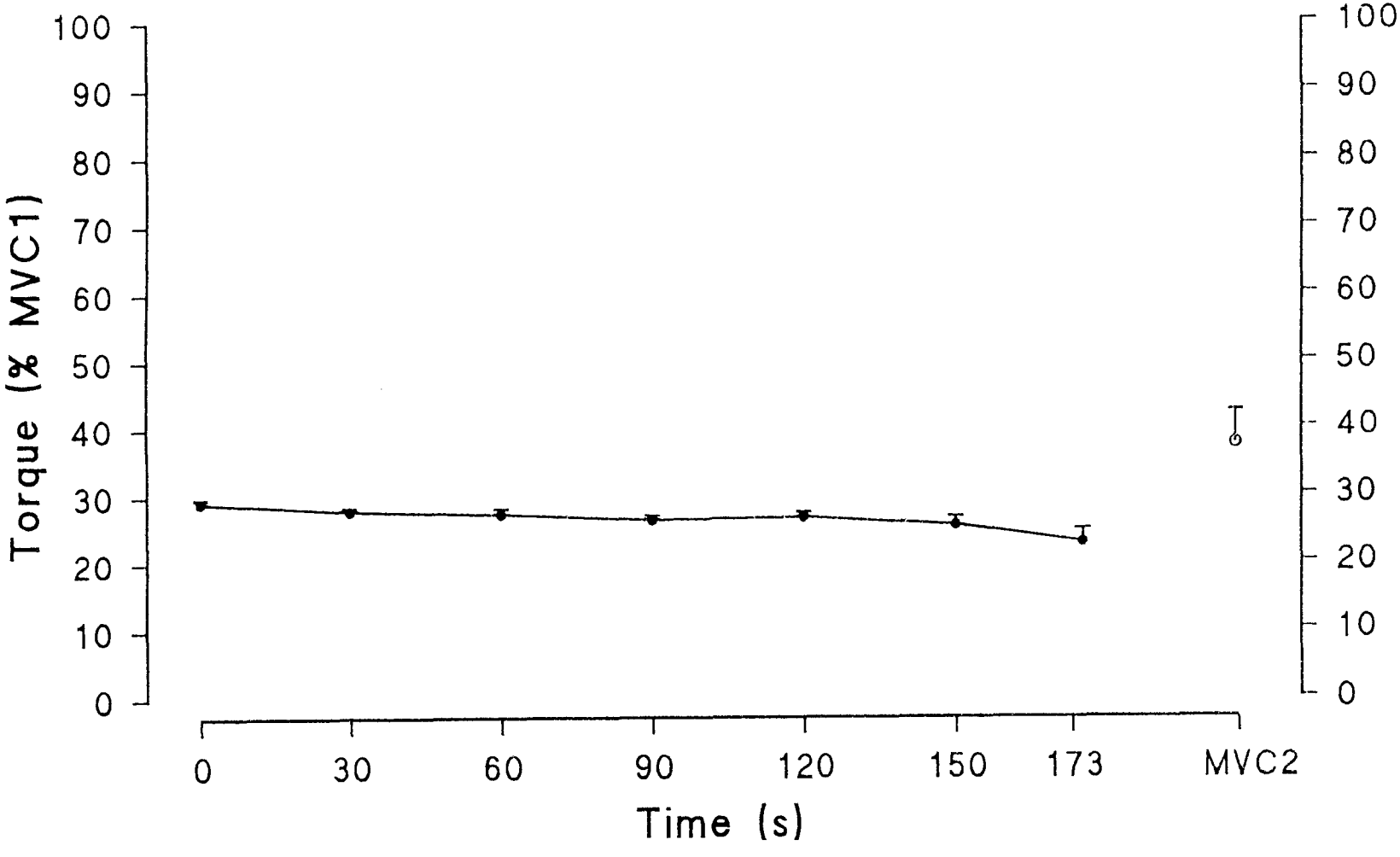
For the final 2s of the sustained contraction, subjects were asked to give another maximum effort (MVC2). As can be seen in Figure 5, the torque output for MVC2 rose to only 37.4 ± 5.0%, confirming the very significant fatigue that had occurred over the 3 min sustained contraction.

Table 2. Individual subject values and group means (± SEM) for maximum evoked twitch torque (ETTmax), interpolated twitch torque (ITT), and theoretical percent motor unit activation (% MUA). Units for both twitch torques are Nm.

Subject	ETTmax	IT	% MUA
JK	19.7	1.9	90.3
RC	45.5	1.7	96.2
SI	58.4	5.9	89.9
BR	49.6	3.7	92.5
BS	38.3	1.6	95.9
SC	34.0	1.6	95.3
SE	61.1	0.3	99.5
EF	39.2	2.7	93.1
MB	45.5	11.4	75.0
MG	15.3	0.0	100
$\bar{X}$	40.7	3.1	94.7
± SEM	± 14.1	± 3.2	± 3.4

Figure 5

Torques recorded at 30s intervals during the sustained isometric quadriceps contraction, and for MVC2. All values are means ± SEM, and normalized to MVC1. n=10


Torque (% MVC1)
100
90
80
70
60
50
40
30
20
10
0
0
30
60
90
120
150
173
MVC2
Time (s)

Integrated EMG

All IEMG values were normalized to that obtained during MVC1. At the start of the sustained contraction, the integrated EMG (IEMG), had a value of 33.7 ± 4.8% and increased slightly to 36.1 ± 4.2% at the 90s mark (Figure 6). From here, the IEMG increased more rapidly between sampling times until the final value of 66.9 ± 10.3% at 173s. The IEMG for MVC2 increased by 22.3% from the 173s time interval, to 89.3 ± 9.6%.

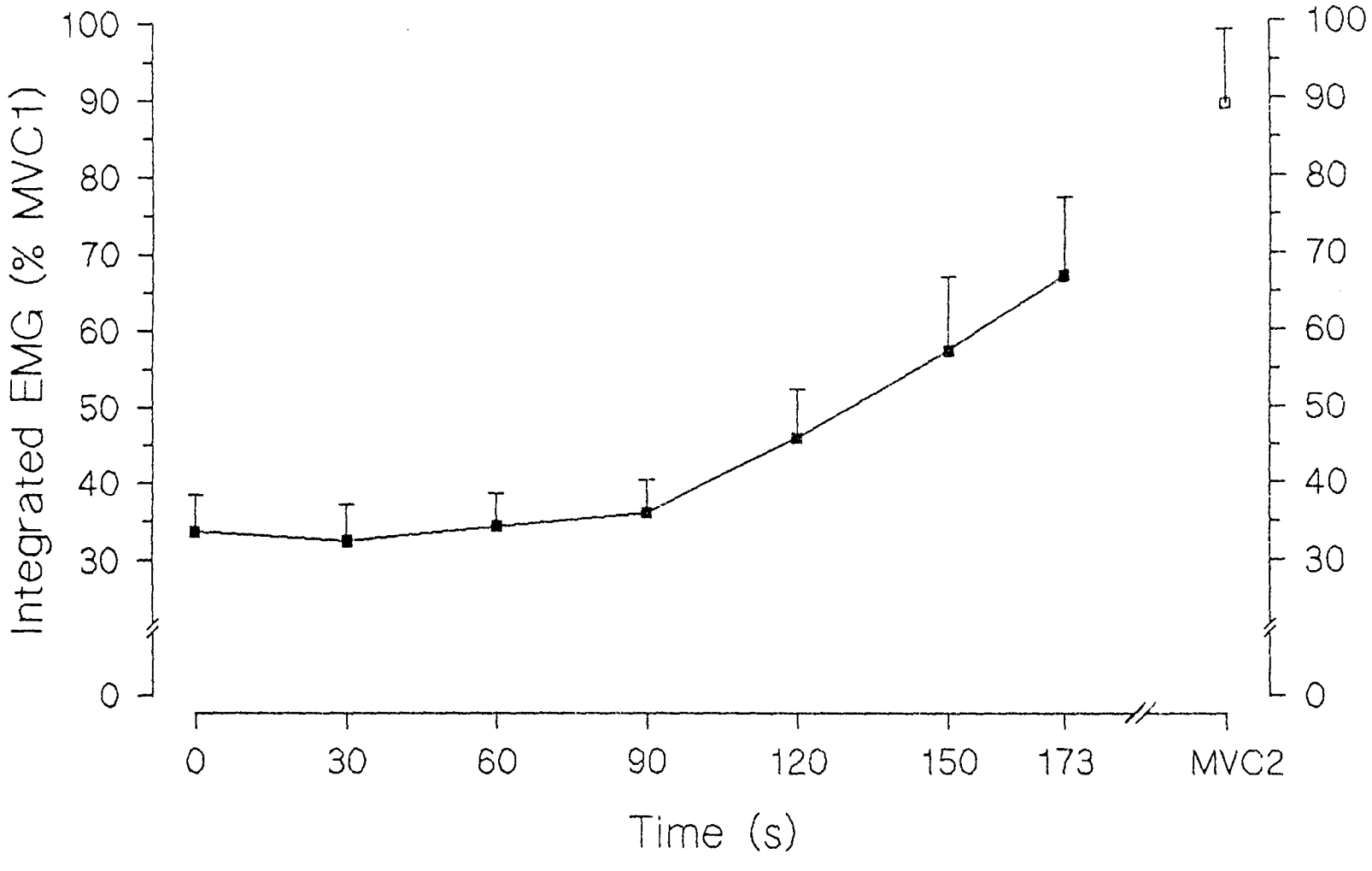
2.3.2 TWITCH ANALYSES

Twitch Torque

The mean twitch torque at baseline was 40.7 ± 4.7 Nm. Twitch torque decreased significantly following the 3 min sustained contraction; at 5s post-exercise the twitch torque was 23.6 ± 5.6 Nm, representing a 41.9% decrease from baseline (Figure 7a). Twitch torque recovered almost completely by 3 min post-exercise, but then tended to decrease gradually over the remainder of the recovery period.

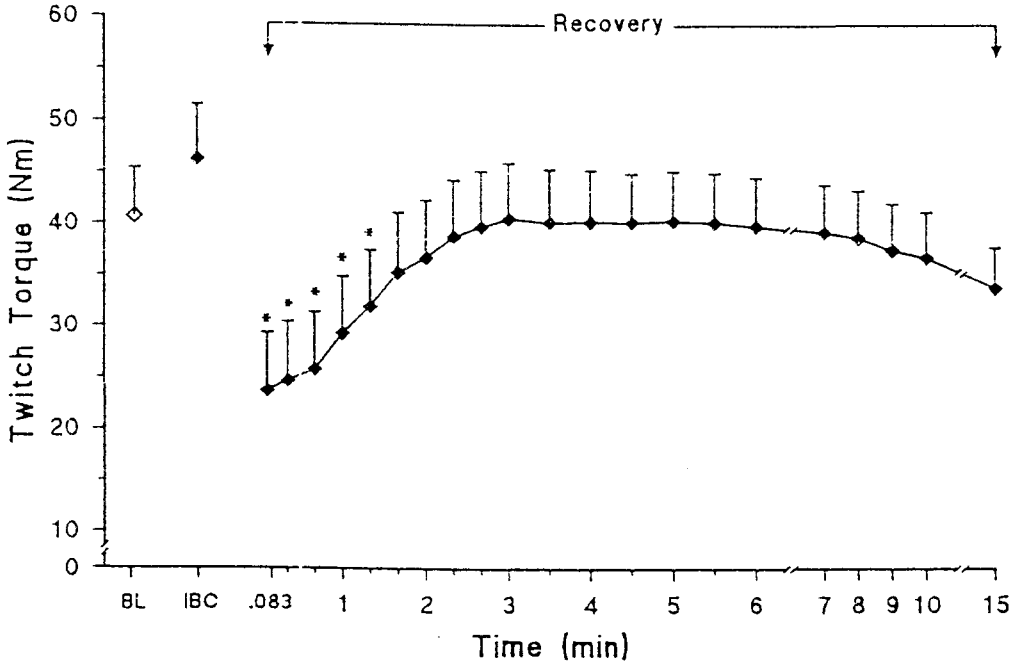
Figure 6

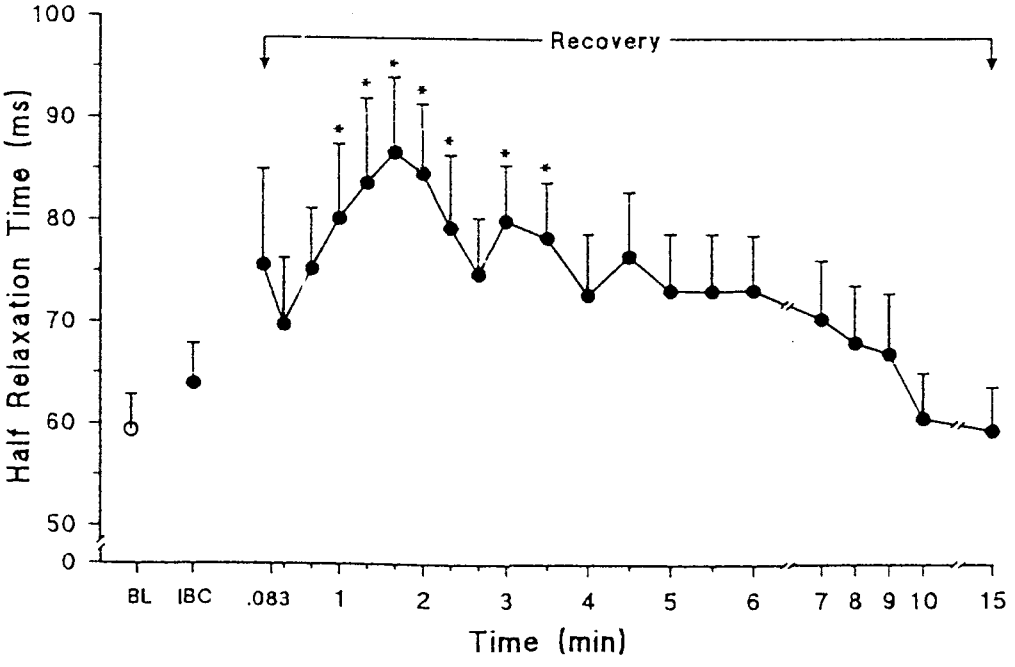
Changes in integrated EMG at 30s intervals during the sustained isometric quadriceps contraction and MVC2. All values are means ± SEM, and are normalized to the integrated EMG of MVC1. n=10


Integrated EMG (% MVC1)
100
90
80
70
60
50
40
30
0
0
30
60
90
120
150
173
MVC2
Time (s)

Figures 7a, 7b

Torque and half relaxation time of twitches evoked for the baseline (BL), immediately before contraction (IBC), and during recovery. All values are means ± SEM. * indicates different from baseline at $p<0.05$ n=10


Recovery
Twitch Torque (Nm)
60
50
40
30
20
10
0
BL
IBC
.083
1
2
3
4
5
6
7
8
9
10
15
Time (min)


Recovery
Half Relaxation Time (ms)
100
90
80
70
60
50
0
BL
IBC
.083
1
2
3
4
5
6
7
8
9
10
15
Time (min)

Half Relaxation Time

Twitch half relaxation time increased from baseline (59.4 ± 3.4 ms) to a peak of 86.7 ± 7.4 ms at 1 min 40s post-exercise (Figure 7b), representing a 45.9% increase. Throughout the remainder of the recovery period, half relaxation time gradually decreased, and was very similar to baseline by the 15 min mark.

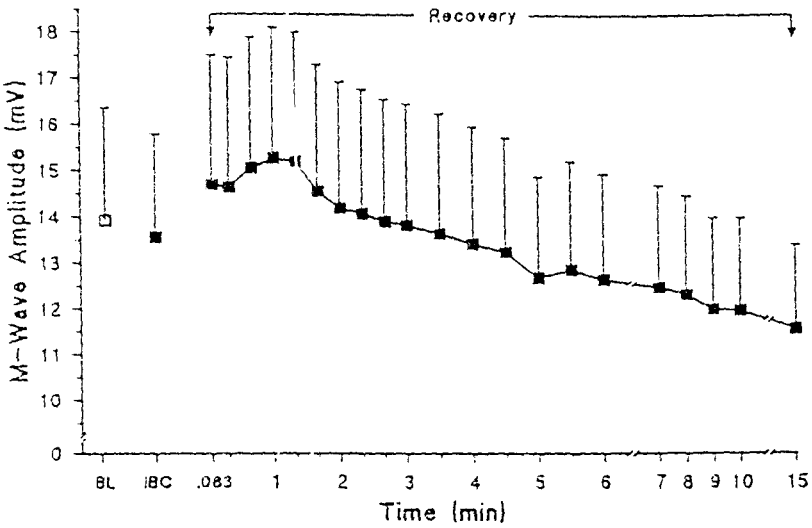
2.3.3 M-WAVE ANALYSES

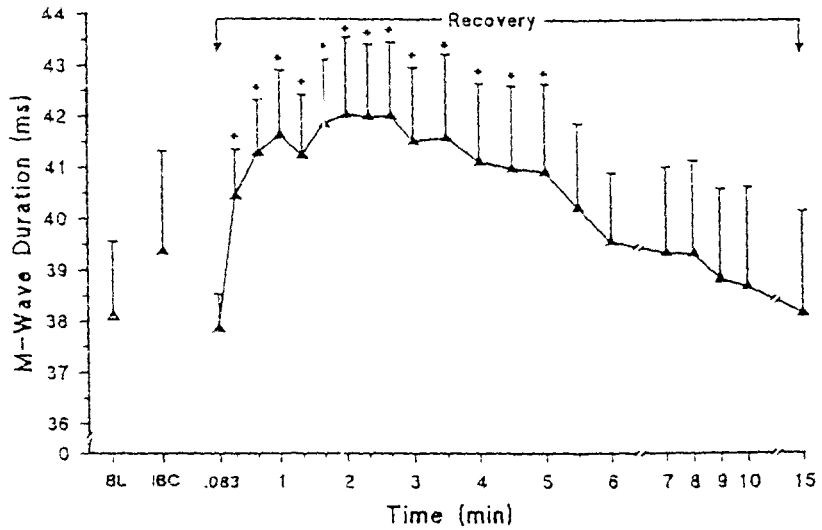
M-Wave Amplitude

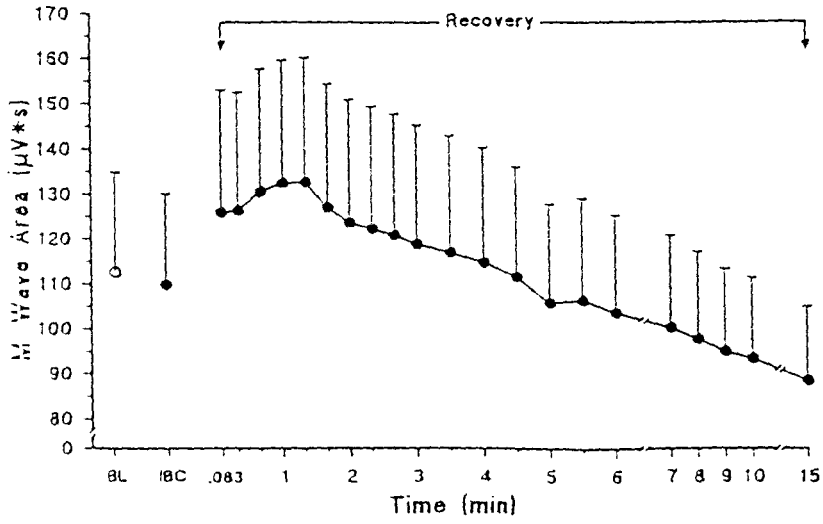
There were no statistically significant changes in amplitude from the mean baseline value of 13.9 ± 2.4 mV throughout the experiment (Figure 8a). However there was a trend of potentiation between 5s and 1 min 40s of recovery with a peak of 15.3 ± 2.8 mV (9.5% increase) reached at 1 min 20s of recovery. From 5 min to the end of the recovery period there was a progressive decrease in the M-Wave amplitude, however, the changes were not statistically significant. Amplitude demonstrated a main effect for time.

Figures 8a, 8b, 8c

Amplitude, duration, and area of M-Waves evoked for the baseline (BL), immediately before contraction (IBC), and during recovery. All values are means ±SEM. * indicates different from baseline at $p<0.05$. n=10


Recovery
M-Wave Amplitude (mV)
18
17
16
15
14
13
12
11
10
0
BL
IBC
.083
1
2
3
4
5
6
7
8
9
10
15
Time (min)


Recovery
M-Wave Duration (ms)
44
43
42
41
40
39
38
37
36
0
BL
IBC
.083
1
2
3
4
5
6
7
8
9
10
15
Time (min)


Recovery
170
160
150
140
130
120
110
100
90
80
0
BL
IBC
.083
1
2
3
4
5
6
7
8
9
10
15
Time (min)

M-Wave Duration

M-Wave duration became significantly longer during the first 5 min of the recovery period, reaching a peak of 42.0 ± 1.5 ms at 2 min post-exercise (Figure 8b). For the remainder of recovery, duration gradually decreased back to baseline. A main effect for time was also exhibited for duration.

M-Wave Area

The M-Wave area did not increase significantly from the baseline measure of 112 ± 22.2 μV•s (Figure 8c). Area did, however, exhibit a trend of potentiation from 5s to 2 min post-exercise before declining for the remainder of recovery. Similarly, area had a main effect for time.

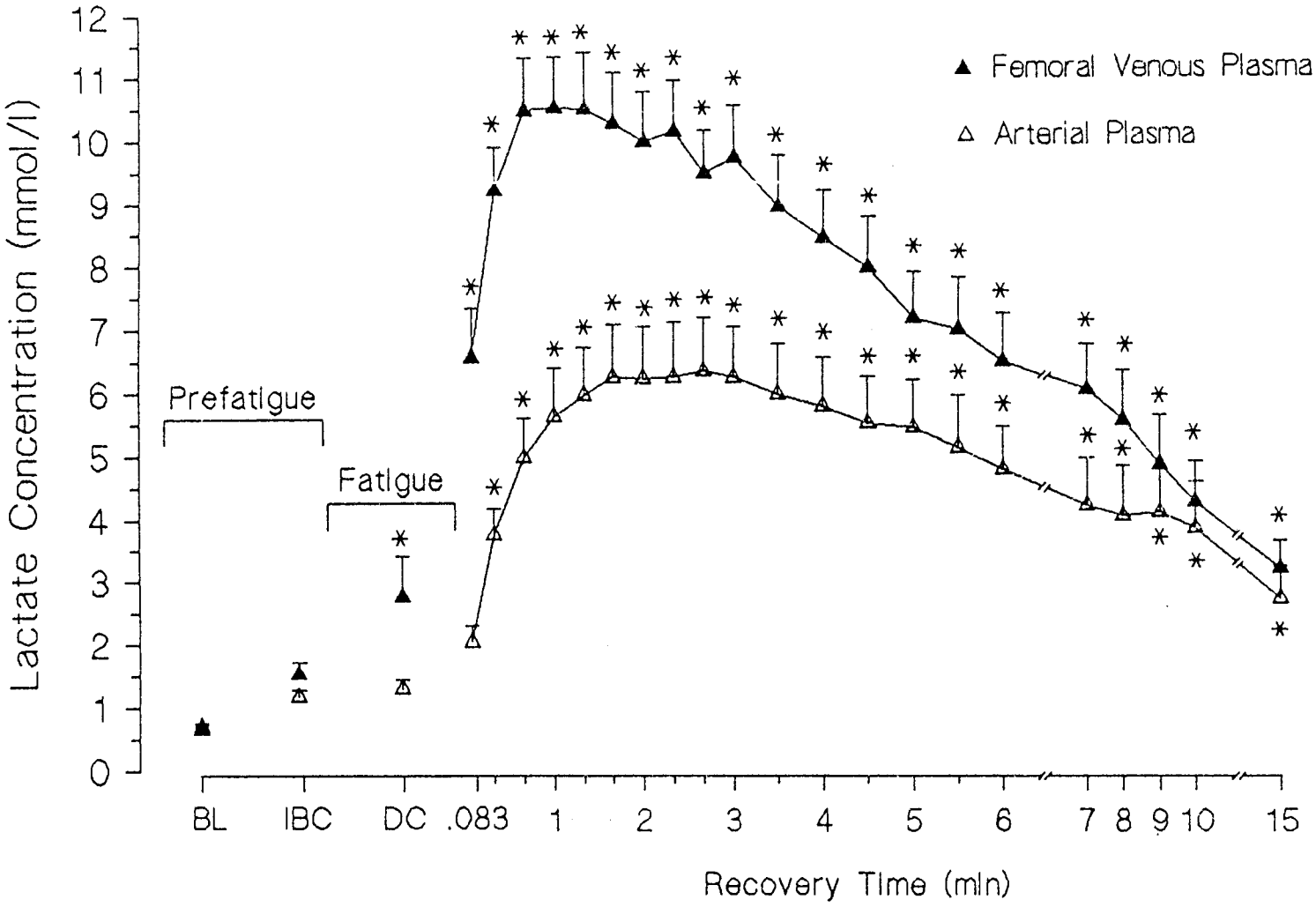
2.3.4 PLASMA ION ANALYSES

Femoral Venous Plasma Lactate Concentration

The [La^-] (Figure 9) increased from a baseline of 0.7 ± 0.1 mmol/l, to a peak value of 10 ± 0.8 mmol/l at 1 min post-exercise. From that point on, [La^-] gradually declined but was still significantly higher than baseline at the 15 min mark of recovery.

Figure 9

Levels of arterial and femoral venous plasma lactate concentration recorded before (prefatigue), during (fatigue), and after (recovery) the sustained isometric quadriceps contraction. All values are means ± SEM. * indicates different from baseline (BL) concentration at $p<0.05$. n=10. immediately before contraction (IBC); during contraction (DC)


Lactate Concentration (mmol/l)
0
1
2
3
4
5
6
7
8
9
10
11
12
Prefatigue
Fatigue
▲ Femoral Venous Plasma
△ Arterial Plasma
BL
IBC
DC
.083
1
2
3
4
5
6
7
8
9
10
15
Recovery Time (min)

Arterial Plasma Lactate Concentration

The arterial [La^-] changes (Figure 9) followed a similar pattern as femoral venous [La^-] changes except the arterial values were lower. Starting at a mean baseline value of 0.7 ± 0.1 mmol/l, concentrations only reached a peak of 6.4 ± 0.8 mmol/l at 2 min 40s. All measurements were significantly greater than baseline from 5s post-exercise to the end, and indicated the [La^-] in the arterial circulation.

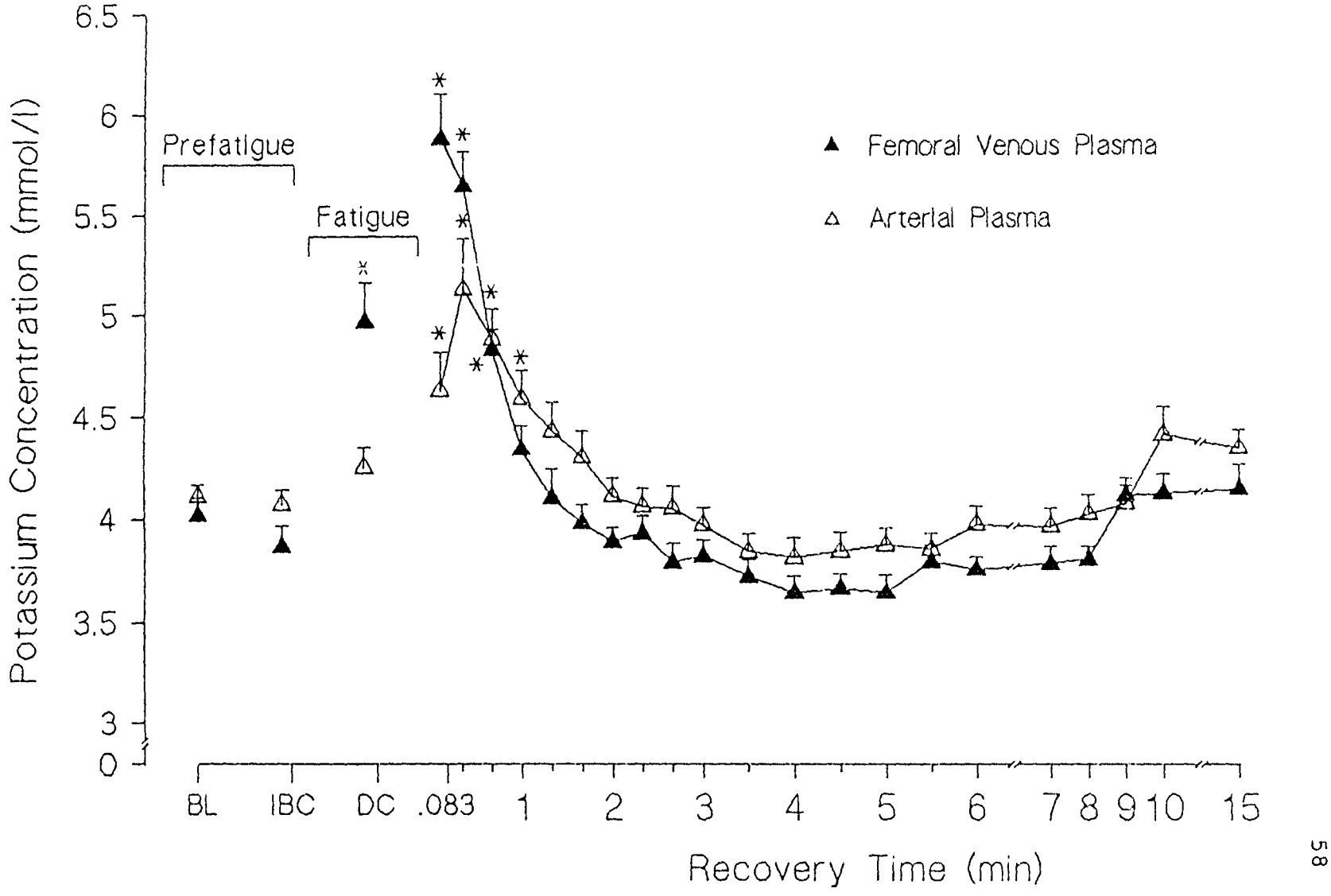
Femoral Venous Plasma Potassium Concentration

As illustrated in Figure 10, the baseline femoral venous plasma [K^+] was 4.0 ± 0.1 mmol/l. At DC, the [K^+] increased significantly to a concentration of 5.0 ± 0.2 mmol/l. The peak value of 5.9 ± 0.2 mmol/l was obtained 5s post-exercise, representing an increase in concentration of 46.3%. Femoral venous plasma [K^+] then decreased rapidly from 5s to 40s of recovery but remained significantly greater than baseline.

There was a trend for [K^+] to drop below baseline between 1 min 40s and 8 min into the recovery period, however, these differences were not statistically significant.

Figure 10

Levels of arterial and femoral venous plasma potassium concentration observed before (prefatigue), during (fatigue), and after (recovery) the sustained isometric quadriceps contraction. All values are means ± SEM. * indicates different from baseline (BL) levels at $p<0.05$. n=10. immediately before contraction (IBC); during contraction (DC)


Potassium Concentration (mmol/l)
6.5
6
5.5
5
4.5
4
3.5
3
0
Prefatigue
Fatigue
Femoral Venous Plasma
Arterial Plasma
BL
IBC
DC
.083
1
2
3
4
5
6
7
8
9
10
15
Recovery Time (min)

Arterial Plasma Potassium Concentration

The arterial plasma [K^+] increased from a baseline of 4.1 ± 0.1 mmol/l (Figure 10), to a peak of 5.1 ± 0.3 mmol/l at 20s post-exercise. Arterial plasma [K^+] steadily decreased during early recovery but remained significantly greater than baseline until the 1 min sample. From 2 to 9 min during recovery, the plasma [K^+] tended to be lower than the baseline value, but was not significantly different. These samples indicated the arterial [K^+] entering the quadriceps from the arterial circulation.

Relationships Between $[K^+]_e$ and Other Variables

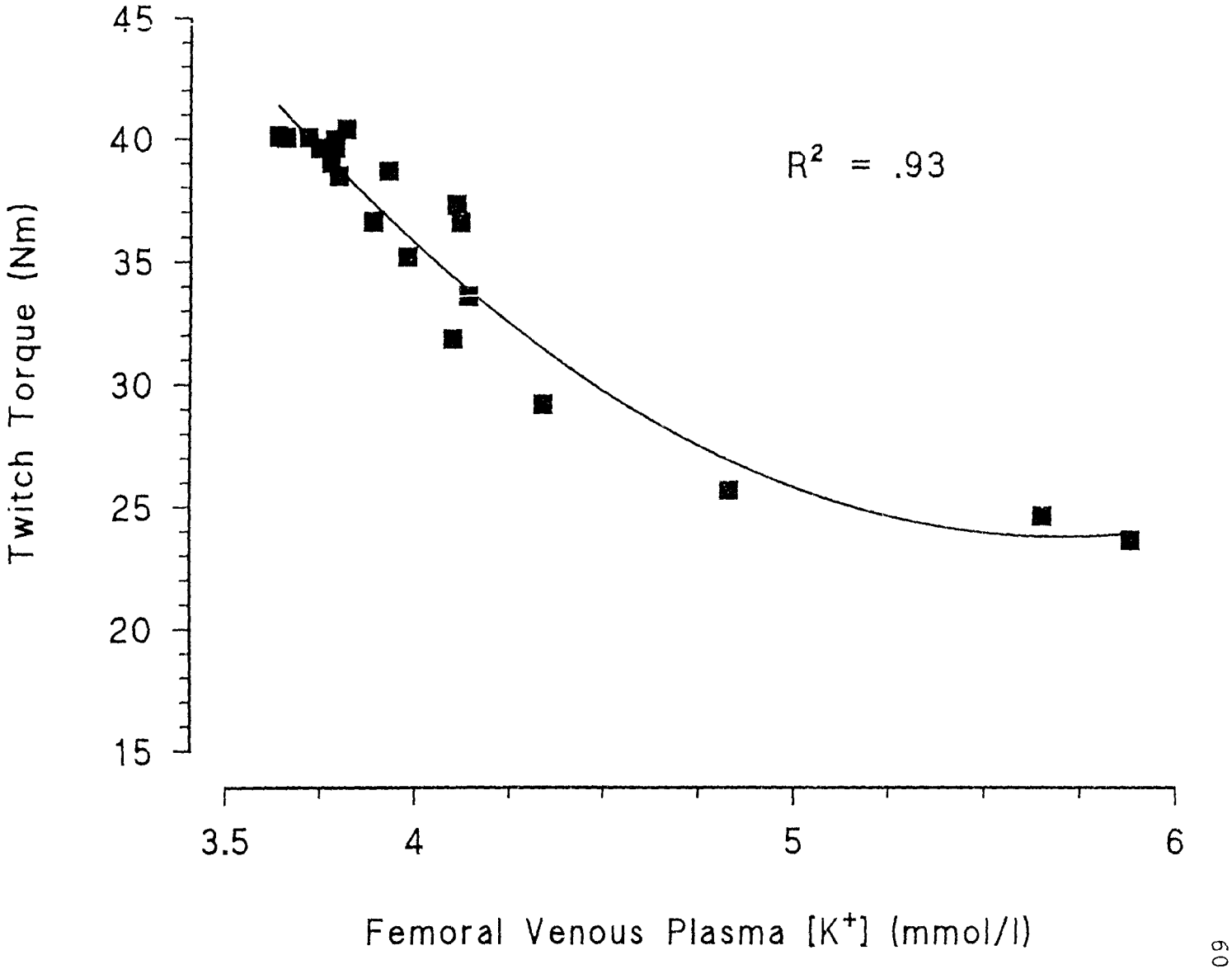
The relationship between $[K^+]_e$ and twitch torque in recovery (Figure 11) was quadratic (R^2=.93), and is described by the equation:

$$\text{Twitch Torque} = 158.37 - 47.20\ ([K^+]) + 4.14\ ([K^+])^2$$

No significant relationship was found in any analyses relating [K^+] and M-Wave characteristics.

Figure 11

The relationship between venous plasma potassium concentration and evoked twitch torque in recovery


45
40
35
30
25
20
15
Twitch Torque (Nm)
R^2 = .93
3.5
4
5
6
Femoral Venous Plasma [K^+] (mmol/l)

2.4 DISCUSSION

There are numerous sites in the chain of events for muscle contraction which could be named as possible mechanisms for the etiology of fatigue. These potential sites may be grouped into three general categories: 1) within the CNS which includes MN excitability; 2) NMJ transmission; and 3) sites within the individual muscle fibres which includes sarcolemma excitability, EC coupling, contractile mechanisms and metabolic energy supply, and metabolite accumulation.

The methods used in this study have concentrated on evaluating sites within the muscle cell, with the primary focus being on membrane excitability, estimated by femoral venous plasma [K^+] and corresponding M-Wave characteristics.

2.4.1 IEMG

The 3 min contraction was maintained at an intensity of 25-30% of each subjects MVC. Throughout the 3 min, IEMG increased significantly from 34% to 67% of MVC1. Increased IEMG activity can be attributed to augmented motor unit recruitment and firing frequency to maintain a constant force as previously discharging units fatigue (Person and Kudina, 1972; Bigland-Ritchie et al., 1986b; Garland et al., 1989).

The observation that IEMG only reached 67% of MVC1 is

similar to findings in other studies using various muscles where sustained submaximal isometric contractions were part of the protocol (Lind and Petrofsky, 1979; Petrofsky and Phillips, 1986; Fuglevand et al., 1993). Neuromuscular propagation failure (Fuglevand et al., 1993), alterations in descending neural drive (Maton, 1991), sensory feedback inhibition of the MN pool (Hannertz and Grimby, 1979; Bigland-Ritchie et al., 1986b), and decreased firing rate of MN's are potential reasons for the less than 100% increase in IEMG activity.

If there was a failure in propagation of the impulse across the NMJ, one would expect to find corresponding changes in the M-Wave. M-Wave amplitude is dependent upon the resting membrane potential of individual muscle fibres, the amplitudes of their AP's, and the number of active muscle fibres responding to the stimulus (Hicks, 1988; Cupido, 1991). A decrease in amplitude is commonly used as an indication of NMJ transmission failure (Merton, 1954; Stephens and Taylor 1972; Bigland-Ritchie et al., 1982; Bellemare and Garzaniti 1988; Thomas et al., 1989; Fuglevand et al., 1993). In this study, there was no evidence of NMJ failure as the amplitude was not depressed, in fact, there was a trend of potentiation early in recovery.

The results from this study, however, do support the idea of afferent inhibition of the MN pool as contributing to the

depressed IEMG activity. The very significant increases in both [La^-] and [K^+] at the end of the contraction would be expected to cause some reflex inhibition of the spinal MN's through activation of small diameter afferents (group III/IV metaboreceptors) (Garland and McComas, 1990; Garland, 1991). As well, there is evidence in the literature for the loss of spindle support to MN's causing a decline in their firing rates (Hagbarth et al., 1986; Macefield et al., 1991), and pain associated with the contraction could decrease the descending drive to the MN pool thereby attenuating motor unit discharge (Gandevia, 1992). All of these mechanisms could account for the decreased IEMG activity.

It is not known whether the failure of IEMG to reach its predicted maximum contributes to the force failure, or is simply an adaptive response to various processes associated with the contraction. The design of this experiment does not allow for determination of the mechanism, but feedback inhibition of the MN pool would be most likely in view of the maintenance of the M-Wave amplitude, and accumulation of metabolites.

2.4.2 THE EFFECT OF POTASSIUM CONCENTRATIONS ON M-WAVE CHARACTERISTICS

As mentioned previously, our techniques allowed us to

assess $[K^+]_e$ and membrane excitability in recovery after a fatiguing sustained submaximal isometric quadriceps contraction. The protocol used here was based upon the pilot study preceding this work (Appendix C). Consistent with the results of the pilot study, all the subjects in this investigation were significantly fatigued as illustrated by not only the small increase in voluntary torque for MVC2 at the end of the sustained contraction (up to only 37% of MVC1), but also by the significant increases in venous plasma $[La^-]$ which surpassed levels described in previous fatigue studies with similar contractions (Sjogaard, 1986; Sjogaard, 1988).

At the intensity of contraction used in this study, blood flow through the muscle is reduced due to the elevated intramuscular pressure causing compression of the blood vessels (Sjogaard et al., 1988; Gaffney et al., 1990). This results in accumulation of K^+ in the extracellular space (Sjogaard, 1990). Compounding the increase in $[K^+]$, caused by the partial occlusion of blood flow, is the inability of the Na^+/K^+ pump to manage the K^+ efflux, resulting in significant release of K^+ once the circulation is restored.

The mean femoral venous plasma $[K^+]$'s reported over the first min post exercise were in the range of 4.3 to 5.9 mmol/l. It has been demonstrated that these concentrations may actually be doubled at the muscle membrane as illustrated by Vyskocil et al. (1983). In the present study, $[K^+]$ could

therefore be higher than 10 mmol/l interstitially. At this $[K^+]_e$, the sarcolemma would be depolarized by close to 20 mV. Depolarization of the membrane to this extent has been shown to cause inactivation of most of the voltage-sensitive Na^+ channels (Hodgkin and Huxley, 1952). Inactivation of these channels subsequently decreases the Na^+ conductance through the sarcolemma and severely impairs or even inhibits the initiation and propagation of the AP, eventually leading to a loss of Ca^{++} transient, and a decline in force output.

The expected decline of membrane excitability should be illustrated by changes in the M-Wave characteristics. However, in this study, there was no evidence of M-Wave failure, nor was there any statistical relationship between $[K^+]_e$ and any of M-Wave amplitude, duration, or area changes.

Most importantly, M-Wave amplitude was not compromised by the increased $[K^+]_e$, but was actually potentiated when $[K^+]_e$ was highest. This characteristic of the M-Wave has been reported previously. Hicks and McComas have argued that the electrogenic nature of a highly stimulated Na^+/K^+ pump may add up to -30mV to the membrane potential during activity, which would explain an increased M-Wave amplitude. Pump activity is acutely controlled by numerous factors and is stimulated most commonly by increased $[Na^+]_i$ and elevated circulating epinephrine levels (Clausen and Flatman, 1977; Williams et al., 1985; Clausen, 1986; Clausen and Everts, 1991). Kjaer

(1989) has shown, that epinephrine levels remain elevated for up to 5 min post-exercise, which would keep the pump stimulated in the early phases of recovery. Our results support this argument and suggest that the preservation and possible potentiation of the M-Wave, is due to the Na^+/K^+ pump-induced hyperpolarization of individual muscle fibres.

If the Na^+/K^+ ATPase was not contributing to the excitability of the membrane during the exercise-induced hyperkalemia, one would have expected to see a relationship between plasma $[K^+]$ and M-Wave characteristics. This was not found. However, the very strong quadratic relationship (R^2=.93) between femoral venous plasma $[K^+]$ and evoked twitch torque in recovery deserves some discussion. It is obvious that muscle contractile function is highly influenced by $[K^+]_e$, but the inhibiting effects of K^+ must be occurring at a site distal to surface membrane action potential propagation. The most likely candidate is the T-tubules, where an elevated $[K^+]$ would be exacerbated due to the increased surface to volume ratio, poor diffusion, and the decreased density of Na^+/K^+ ATPase compared to the sarcolemma (Venosa and Horowicz, 1981; Jones and Bigland-Ritchie, 1986). Recently, Renaud and Light (1992) have suggested that the T-tubules reach a critical $[K^+]$ higher than those detected at the sarcolemma which abruptly causes depression of the Ca^{++} transient leading to force failure. It has also been reported that in fatigue $[Ca^{++}]_i$ is

greater near the outside of the muscle where the sarcolemma conducts the impulse, and much lower in the interior fibres of the muscle where the T-tubules are responsible for the conduction of the AP (Westerblad et al., 1990). These two studies lend support to the hypothesis that conduction block in the T-tubules decreases the Ca^{++} transient in the SR which eventually compromises cross-bridge function and attenuates force output (Ashley and Ridgway, 1970).

2.5 SUMMARY

This study has illustrated that after a fatiguing isometric quadriceps contraction, $[K^+]_e$ increases to a level expected to cause sufficient depolarization of the sarcolemma to impair AP propagation.

The M-Wave, however, showed no evidence of a decline in membrane excitability, perhaps owing to the electrogenic nature of the Na^+/K^+ ATPase hyperpolarizing the individual muscle cell membranes.

The increased $[K^+]_e$ is still a very probable candidate for the ultimate failure in force generating capacity, but its effect is likely being manifested in the T-tubules, affecting EC coupling through a reduction in the Ca^{++} transient. The very strong relationship seen between $[K^+]$ and torque output in recovery supports this hypothesis.

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APPENDIX A

Spreadsheet of individual subject data

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				BL	IBC	DC	:05
SI	ARTERIAL	WB	K+	58.70	56.10	58.10	50.00
			La-	1.20	2.10	1.80	1.80
			HEM	52.50	58.50	52.70	52.90
		PL	K+	4.40	4.00	4.10	4.30
			La-	0.90	1.90	1.70	2.00
	VENOUS	WB	K+	56.70	56.70	58.30	56.90
			La-	1.20	1.50	4.10	4.20
			HEM	53.30	51.40	50.00	53.60
		PL	K+	4.50	4.50	5.20	5.10
			La-	1.00	1.40	5.00	4.90
M-WAVE AMPLITUDE (mV)				11.41	11.42		12.55
M-WAVE DURATION (S)				0.03	0.04		0.03
M-WAVE AREA (mV*S)				0.08	0.09		0.09
PEAK TWITCH TORQUE (Nm)				58.35	67.55		33.72
HALF RELAXATION TIME (ms)				42.94	46.44		68.91
BR	ARTERIAL	WB	K+	55.30	54.10	57.40	59.60
			La-	1.00	0.90	1.20	1.00
			HEM	43.60	44.40	46.80	42.90
		PL	K+	4.10	3.90	4.00	4.40
			La-	0.70	1.20	1.10	1.60
	VENOUS	WB	K+	52.70	51.30	53.70	55.60
			La-	1.20	1.50	1.50	3.40
			HEM	42.70	41.20	43.30	46.10
		PL	K+	3.90	3.50	5.10	5.40
			La-	0.70	1.60	1.60	4.00
M-WAVE AMPLITUDE (mV)				7.18	7.18		6.87
M-WAVE DURATION (S)				0.04	0.05		0.04
M-WAVE AREA (mV*S)				0.06	0.06		0.05
PEAK TWITCH TORQUE (Nm)				49.60	54.89		40.16
HALF RELAXATION TIME (ms)				57.26	56.55		55.06
RC	ARTERIAL	WB	K+	50.10	50.20	53.90	54.00
			La-	1.30	1.10	1.60	2.20
			HEM	44.10	46.30	47.60	46.30
		PL	K+	3.90	3.80	4.10	4.20
			La-	0.80	1.20	1.20	1.50
	VENOUS	WB	K+	50.60	51.20	55.40	62.60
			La-	1.40	1.80	2.10	5.60
			HEM	45.00	47.10	45.20	48.60
		PL	K+	3.90	3.90	5.10	6.20
			La-	0.80	1.20	1.60	7.80
M-WAVE AMPLITUDE (mV)				8.84	8.78		7.98
M-WAVE DURATION (S)				0.03	0.04		0.04
M-WAVE AREA (mV*S)				0.06	0.06		0.06
PEAK TWITCH TORQUE (Nm)				45.46	52.13		5.41
HALF RELAXATION TIME (ms)				56.59	62.17		144.94

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				BL	IBC	DC	:05
EF	ARTERIAL	WB	K+	51.60	53.80	53.40	46.80
			La-	0.80	1.60	1.50	1.50
			HEM	43.90	44.70	42.70	44.40
		PL	K+	4.30	4.00	4.40	4.10
			La-	0.50	1.30	1.40	1.50
	VENOUS	WB	K+	49.30	50.00	54.30	58.00
			La-	0.80	2.30	3.30	4.30
			HEM	46.20	46.70	49.20	47.10
		PL	K+	4.10	3.90	5.70	6.10
			La-	0.60	2.70	4.20	5.80
M-WAVE AMPLITUDE (mV)				14.87	15.26		14.94
M-WAVE DURATION (S)				0.05	0.04		0.03
M-WAVE AREA (mV*S)				0.14	0.13		0.10
PEAK TWITCH TORQUE (Nm)				39.24	47.07		53.05
HALF RELAXATION TIME (ms)				50.93	47.57		82.77
SE	ARTERIAL	WB	K+	54.70	55.90	57.60	59.20
			La-	1.00	1.00	1.10	2.30
			HEM	48.80	47.40	51.20	51.20
		PL	K+	4.10	4.40	4.50	5.60
			La-	0.70	0.80	1.00	3.00
	VENOUS	WB	K+	54.40	54.80	57.00	63.40
			La-	0.90	1.30	1.10	5.20
			HEM	49.30	48.50	50.00	53.80
		PL	K+	4.10	4.10	4.30	6.30
			La-	0.60	1.40	0.90	8.10
M-WAVE AMPLITUDE (mV)				24.34	22.85		29.27
M-WAVE DURATION (S)				0.03	0.03		0.04
M-WAVE AREA (mV*S)				0.19	0.17		0.24
PEAK TWITCH TORQUE (Nm)				61.11	59.73		9.78
HALF RELAXATION TIME (ms)				71.90	76.78		62.55
BS	ARTERIAL	WB	K+	55.70	56.10	56.30	59.80
			La-	0.90	0.90	0.90	1.20
			HEM	47.20	48.80	48.80	50.00
		PL	K+	4.20	4.10	4.30	5.20
			La-	0.50	0.70	0.50	1.60
	VENOUS	WB	K+	52.00	52.50	55.90	58.40
			La-	0.70	1.00	0.90	5.00
			HEM	47.40	46.60	47.90	51.30
		PL	K+	4.00	3.90	4.70	6.20
			La-	0.50	0.80	0.70	7.20
M-WAVE AMPLITUDE (mV)				19.14	17.36		17.53
M-WAVE DURATION (S)				0.04	0.04		0.04
M-WAVE AREA (mV*S)				0.15	0.14		0.19
PEAK TWITCH TORQUE (Nm)				38.32	39.70		16.92
HALF RELAXATION TIME (ms)				53.26	68.54		89.14

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				BL	IBC	DC	:05
MB	ARTERIAL	WB	K+	52.00	55.30	56.90	55.00
			La-	1.70	1.50	1.30	1.50
			HEM	45.80	46.40	45.90	45.90
		PL	K+	3.80	3.80	4.00	4.10
			La-	1.10	1.20	1.20	1.60
	VENOUS	WB	K+	47.50	50.40	52.60	56.60
			La-	1.20	1.70	1.60	2.20
			HEM	42.30	44.60	45.10	46.20
		PL	K+	3.50	3.50	4.20	4.50
			La-	1.00	1.60	2.30	2.80
M-WAVE AMPLITUDE (mV)				23.34	23.30		24.26
M-WAVE DURATION (S)				0.04	0.04		0.03
M-WAVE AREA (mV*S)				0.16	0.16		0.15
PEAK TWITCH TORQUE (Nm)				45.69	60.88		44.54
HALF RELAXATION TIME (ms)				51.93	54.68		86.89
SC	ARTERIAL	WB	K+	52.90	51.60	53.20	57.50
			La-	0.90	1.50	1.60	2.00
			HEM	45.00	46.30	45.90	46.90
		PL	K+	4.20	4.20	4.20	4.50
			La-	0.50	1.20	1.60	2.00
	VENOUS	WB	K+	49.00	48.70	53.20	55.20
			La-	0.90	1.40	2.40	4.30
			HEM	43.20	42.90	46.20	46.20
		PL	K+	4.00	3.50	5.10	5.70
			La-	0.50	1.10	3.10	6.00
M-WAVE AMPLITUDE (mV)				19.72	19.72		20.01
M-WAVE DURATION (S)				0.04	0.04		0.04
M-WAVE AREA (mV*S)				0.20	0.20		0.20
PEAK TWITCH TORQUE (Nm)				33.95	40.16		18.53
HALF RELAXATION TIME (ms)				78.56	83.52		78.28
JK	ARTERIAL	WB	K+	50.80	51.00	56.10	54.70
			La-	0.90	1.40	2.00	3.10
			HEM	44.30	45.10	45.60	46.10
		PL	K+	4.10	4.50	5.00	5.60
			La-	0.50	1.30	2.20	4.00
	VENOUS	WB	K+	48.30	48.40	55.70	54.20
			La-	0.80	1.70	4.40	5.10
			HEM	43.10	43.10	47.50	46.20
		PL	K+	4.30	4.00	6.00	6.40
			La-	0.90	2.20	7.00	8.00
M-WAVE AMPLITUDE (mV)				4.85	5.38		5.93
M-WAVE DURATION (S)				0.04	0.05		0.04
M-WAVE AREA (mV*S)				0.04	0.04		0.04
PEAK TWITCH TORQUE (Nm)				19.68	22.90		10.24
HALF RELAXATION TIME (ms)				62.25	68.65		47.57

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				BL	IBC	DC	:05
MG	ARTERIAL	WB	K+	46.20	49.70	50.50	50.90
			La-	0.90	1.50	1.50	1.90
			HEM	42.90	44.20	44.00	44.00
		PL	K+	4.00	4.00	3.90	4.30
			La-	0.50	1.00	1.10	1.70
	VENOUS	WB	K+	47.60	48.50	50.30	59.90
			La-	0.90	1.50	1.40	7.00
			HEM	43.10	43.20	46.20	44.00
		PL	K+	3.90	3.90	4.30	6.90
			La-	0.50	1.40	1.40	11.20
M-WAVE AMPLITUDE (mV)				15.87	15.71		17.30
M-WAVE DURATION (S)				0.04	0.03		0.04
M-WAVE AREA (mV*S)				0.11	0.11		0.14
PEAK TWITCH TORQUE (Nm)				15.31	16.46		3.80
HALF RELAXATION TIME (ms)				68.24	74.12		39.70

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				:20	:40	1:00	1:20
SI	ARTERIAL	WB	K+	59.20	59.90	59.30	59.40
			La-	2.90	3.90	4.30	4.60
			HEM	52.50	52.10	51.40	52.90
		PL	K+	4.60	4.90	4.60	4.10
			La-	3.50	5.20	5.30	6.00
	VENOUS	WB	K+	65.80	64.10	61.70	61.30
			La-	9.50	9.80	9.10	9.20
			HEM	55.20	56.70	56.30	55.20
		PL	K+	6.10	4.70	4.40	4.20
			La-	12.60	14.60	14.10	14.70
M-WAVE AMPLITUDE (mV)				12.39	12.44	12.38	12.22
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.10	0.10	0.10	0.10
PEAK TWITCH TORQUE (Nm)				34.87	37.40	42.00	47.07
HALF RELAXATION TIME (ms)				67.79	60.67	71.91	51.31
BR	ARTERIAL	WB	K+	54.40	59.00	56.40	59.80
			La-	2.80	3.40	4.00	4.30
			HEM	45.90	46.30	46.80	45.00
		PL	K+	5.00	4.70	4.50	4.40
			La-	2.90	3.90	4.60	5.00
	VENOUS	WB	K+	56.80	58.20	57.10	56.60
			La-	5.40	6.30	6.10	6.30
			HEM	45.90	46.90	46.60	48.10
		PL	K+	5.50	5.10	4.50	4.60
			La-	7.40	8.90	8.70	9.50
M-WAVE AMPLITUDE (mV)				6.83	7.00	7.04	7.07
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				44.08	42.93	48.91	50.75
HALF RELAXATION TIME (ms)				66.29	63.30	66.67	74.53
RC	ARTERIAL	WB	K+	48.00	57.90	60.90	56.70
			La-	2.90	4.50	5.00	4.80
			HEM	46.70	47.60	47.70	50.00
		PL	K+	5.50	5.20	4.40	4.80
			La-	5.20	6.10	6.60	7.70
	VENOUS	WB	K+	63.80	62.80	60.80	55.60
			La-	8.90	8.40	8.90	8.50
			HEM	50.00	48.80	48.90	48.80
		PL	K+	6.10	5.20	4.70	4.50
			La-	11.30	13.40	13.50	15.00
M-WAVE AMPLITUDE (mV)				7.69	8.05	8.44	8.53
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				8.40	11.62	18.76	23.59
HALF RELAXATION TIME (ms)				76.78	68.91	66.67	60.67

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				:20	:40	1:00	1:20
EF	ARTERIAL	WB	K+	55.10	54.40	49.80	54.20
			La-	2.70	3.10	3.50	3.90
			HEM	46.30	46.40	45.50	46.40
		PL	K+	4.60	4.20	4.00	3.90
			La-	2.70	3.40	4.10	4.30
	VENOUS	WB	K+	58.40	56.00	56.10	55.80
			La-	6.20	6.10	7.20	6.80
			HEM	50.60	49.30	50.00	48.30
		PL	K+	5.60	4.40	4.10	4.00
			La-	8.80	8.40	9.60	8.70
M-WAVE AMPLITUDE (mV)				6.87	6.93	7.04	6.90
M-WAVE DURATION (S)				0.04	0.04	0.03	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.05	0.06
PEAK TWITCH TORQUE (Nm)				51.44	50.52	52.13	52.36
HALF RELAXATION TIME (ms)				86.89	79.40	78.65	89.51
SE	ARTERIAL	WB	K+	60.80	60.60	60.90	59.50
			La-	4.20	4.30	7.70	7.80
			HEM	51.90	52.20	52.50	53.80
		PL	K+	5.70	5.20	5.00	4.50
			La-	6.20	9.80	12.10	12.00
	VENOUS	WB	K+	61.70	60.70	41.90	48.80
			La-	5.70	7.60	6.20	7.40
			HEM	55.00	52.60	44.10	44.90
		PL	K+	5.40	4.80	3.50	3.00
			La-	8.60	13.20	12.20	11.20
M-WAVE AMPLITUDE (mV)				28.37	28.26	28.23	27.98
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.23	0.24	0.24	0.24
PEAK TWITCH TORQUE (Nm)				9.78	11.85	16.92	22.90
HALF RELAXATION TIME (ms)				50.56	95.51	108.99	114.23
BS	ARTERIAL	WB	K+	59.00	61.60	60.70	60.60
			La-	2.80	3.40	4.10	4.40
			HEM	50.00	50.80	50.00	50.00
		PL	K+	5.40	5.40	5.10	4.80
			La-	3.40	4.60	6.00	6.10
	VENOUS	WB	K+	63.10	62.10	61.10	59.80
			La-	6.50	7.40	8.60	7.60
			HEM	52.30	52.20	52.50	51.50
		PL	K+	6.10	5.40	4.90	4.60
			La-	9.80	11.70	12.30	11.90
M-WAVE AMPLITUDE (mV)				18.61	19.28	19.62	19.53
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.17	0.17	0.17	0.17
PEAK TWITCH TORQUE (Nm)				16.46	15.77	16.92	18.53
HALF RELAXATION TIME (ms)				84.64	86.89	88.76	90.64

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				:20	:40	1:00	1:20
MB	ARTERIAL	WB	K+	51.70	54.20	55.80	61.70
			La-	1.90	2.80	3.10	3.30
			HEM	46.10	45.00	45.80	45.20
		PL	K+	4.50	4.40	4.20	4.10
			La-	2.30	3.30	3.60	3.30
	VENOUS	WB	K+	54.30	56.20	57.10	55.50
			La-	3.50	4.60	4.80	4.50
			HEM	47.30	48.20	47.00	47.30
		PL	K+	4.70	4.60	4.30	4.10
			La-	5.00	6.80	5.90	6.00
M-WAVE AMPLITUDE (mV)				13.82	10.42	10.47	10.32
M-WAVE DURATION (S)				0.03	0.04	0.03	0.03
M-WAVE AREA (mV*S)				0.09	0.07	0.07	0.07
PEAK TWITCH TORQUE (Nm)				47.76	50.52	52.82	54.89
HALF RELAXATION TIME (ms)				104.49	62.55	59.55	67.42
SC	ARTERIAL	WB	K+	55.90	56.70	55.70	53.20
			La-	2.70	3.10	3.60	3.80
			HEM	47.20	48.10	45.70	45.90
		PL	K+	4.30	4.50	4.30	4.20
			La-	3.20	3.90	4.50	5.00
	VENOUS	WB	K+	57.20	56.60	54.50	55.30
			La-	6.30	6.80	6.70	6.90
			HEM	47.60	46.40	46.70	46.30
		PL	K+	5.50	4.80	4.20	3.90
			La-	10.50	10.90	10.80	10.80
M-WAVE AMPLITUDE (mV)				19.96	21.44	21.73	21.93
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.21	0.23	0.23	0.24
PEAK TWITCH TORQUE (Nm)				16.69	18.07	21.52	24.28
HALF RELAXATION TIME (ms)				77.45	80.90	87.64	94.76
JK	ARTERIAL	WB	K+	52.50	52.70	51.00	51.40
			La-	3.70	4.10	4.40	4.70
			HEM	47.40	47.10	48.80	47.50
		PL	K+	7.00	5.70	5.40	5.40
			La-	5.10	5.80	5.40	5.80
	VENOUS	WB	K+	52.70	47.80	46.40	44.00
			La-	6.30	6.00	6.30	6.70
			HEM	47.40	44.30	42.70	41.20
		PL	K+	6.40	4.70	4.40	4.00
			La-	10.00	8.90	9.40	9.40
M-WAVE AMPLITUDE (mV)				5.49	5.69	5.88	5.91
M-WAVE DURATION (S)				0.05	0.05	0.05	0.05
M-WAVE AREA (mV*S)				0.04	0.04	0.05	0.05
PEAK TWITCH TORQUE (Nm)				12.31	12.54	14.16	15.08
HALF RELAXATION TIME (ms)				40.07	45.69	49.06	59.93

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				:20	:40	1:00	1:20
MG	ARTERIAL	WB	K+	53.20	53.30	53.30	53.10
			La-	2.70	3.20	3.30	3.40
			HEM	46.50	45.00	45.80	46.30
		PL	K+	4.70	4.60	4.40	4.10
			La-	3.10	3.80	4.10	4.50
	VENOUS	WB	K+	57.60	55.60	54.70	54.50
			La-	5.70	5.60	5.60	5.70
			HEM	47.50	47.60	47.20	47.30
		PL	K+	5.10	4.60	4.40	4.10
			La-	8.30	8.10	8.80	8.00
M-WAVE AMPLITUDE (mV)				17.68	18.20	18.71	18.26
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.14	0.14	0.15	0.15
PEAK TWITCH TORQUE (Nm)				4.26	5.64	7.94	9.09
HALF RELAXATION TIME (ms)				42.32	108.61	123.97	133.71

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				1:40	2:00	2:20	2:40
SI	ARTERIAL	WB	K+	59.30	59.20	57.30	57.80
			La-	4.70	4.50	4.80	4.90
			HEM	53.10	51.50	47.20	55.10
		PL	K+	4.10	4.00	3.90	3.90
			La-	6.30	6.10	7.10	6.80
	VENOUS	WB	K+	51.40	59.30	59.30	54.30
			La-	8.80	8.40	8.20	7.70
			HEM	54.50	53.30	52.70	50.00
		PL	K+	3.50	3.80	3.90	3.30
			La-	12.70	12.60	13.00	10.50
M-WAVE AMPLITUDE (mV)				12.08	11.84	11.65	11.49
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.10	0.09	0.09	0.09
PEAK TWITCH TORQUE (Nm)				50.06	52.59	55.81	58.12
HALF RELAXATION TIME (ms)				54.31	50.19	49.44	53.56
BR	ARTERIAL	WB	K+	61.40	65.00	54.60	58.80
			La-	4.30	4.40	4.00	4.00
			HEM	45.80	44.70	46.20	44.00
		PL	K+	4.50	4.30	4.10	4.00
			La-	5.20	5.30	5.00	4.80
	VENOUS	WB	K+	59.20	56.30	54.00	57.00
			La-	6.20	6.10	5.70	5.80
			HEM	45.50	45.80	45.00	46.20
		PL	K+	4.30	4.20	4.10	4.10
			La-	8.90	8.30	8.30	8.60
M-WAVE AMPLITUDE (mV)				7.09	7.00	7.00	6.97
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				59.96	59.73	60.88	61.57
HALF RELAXATION TIME (ms)				78.28	77.15	73.78	70.04
RC	ARTERIAL	WB	K+	57.50	46.00	55.70	55.50
			La-	5.30	4.60	5.70	6.40
			HEM	46.30	47.60	46.40	48.20
		PL	K+	4.40	4.00	4.40	4.30
			La-	7.40	7.70	8.30	8.10
	VENOUS	WB	K+	59.90	63.30	56.50	59.90
			La-	8.90	8.60	9.20	9.20
			HEM	48.80	47.40	48.80	48.30
		PL	K+	4.10	4.00	3.90	3.70
			La-	14.00	13.00	13.00	11.90
M-WAVE AMPLITUDE (mV)				8.69	8.70	8.70	8.66
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				27.73	30.04	34.18	35.79
HALF RELAXATION TIME (ms)				77.53	79.03	79.40	76.78

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				1:40	2:00	2:20	2:40
EF	ARTERIAL	WB	K+	54.90	44.70	54.00	53.50
			La-	4.10	3.10	3.80	3.90
			HEM	46.50	46.50	45.20	45.50
		PL	K+	3.60	3.80	3.80	3.70
			La-	4.10	4.20	4.10	4.10
	VENOUS	WB	K+	55.00	55.20	56.90	51.40
			La-	6.50	6.30	6.10	5.90
			HEM	50.00	48.60	48.20	48.30
		PL	K+	3.90	3.80	3.70	3.70
			La-	8.60	7.80	7.70	7.30
M-WAVE AMPLITUDE (mV)				6.86	6.81	6.84	6.76
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				53.97	54.66	54.20	53.74
HALF RELAXATION TIME (ms)				92.13	98.13	97.38	96.25
SE	ARTERIAL	WB	K+	61.00	60.50	60.90	60.10
			La-	8.20	8.20	8.30	8.30
			HEM	53.80	53.80	52.20	52.60
		PL	K+	4.30	4.20	3.90	3.80
			La-	13.00	12.70	12.70	12.80
	VENOUS	WB	K+	60.30	58.00	61.50	60.30
			La-	8.60	8.70	8.90	8.80
			HEM	55.10	52.50	54.20	54.30
		PL	K+	3.80	3.50	3.80	3.70
			La-	13.60	13.60	14.10	13.40
M-WAVE AMPLITUDE (mV)				28.11	27.58	27.35	26.82
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.24	0.23	0.23	0.23
PEAK TWITCH TORQUE (Nm)				27.27	31.19	35.33	39.01
HALF RELAXATION TIME (ms)				111.61	107.49	105.99	98.13
BS	ARTERIAL	WB	K+	60.10	59.50	61.00	59.30
			La-	4.50	4.60	4.60	4.70
			HEM	48.60	50.00	48.80	50.00
		PL	K+	4.60	4.60	4.40	4.40
			La-	5.90	6.50	6.60	6.90
	VENOUS	WB	K+	59.40	59.90	57.80	57.90
			La-	7.30	7.10	7.10	6.90
			HEM	50.60	51.30	51.20	50.00
		PL	K+	4.40	4.20	4.10	4.00
			La-	11.20	10.70	10.00	9.60
M-WAVE AMPLITUDE (mV)				19.05	19.10	18.71	18.51
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.17	0.17	0.16	0.16
PEAK TWITCH TORQUE (Nm)				21.06	23.36	27.27	29.12
HALF RELAXATION TIME (ms)				94.01	93.26	55.06	53.18

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				1:40	2:00	2:20	2:40
MB	ARTERIAL	WB	K+	59.60	53.90	52.50	56.30
			La-	3.30	3.50	3.70	3.10
			HEM	47.10	46.50	46.40	45.70
		PL	K+	4.10	3.90	3.90	3.90
			La-	4.20	4.10	4.30	4.20
	VENOUS	WB	K+	54.10	54.30	55.20	54.70
			La-	5.20	5.10	4.80	4.60
			HEM	43.40	46.50	46.90	46.80
		PL	K+	4.00	3.90	3.90	3.90
			La-	7.00	6.70	6.70	6.30
M-WAVE AMPLITUDE (mV)				10.19	9.93	9.74	9.62
M-WAVE DURATION (S)				0.04	0.03	0.04	0.04
M-WAVE AREA (mV*S)				0.07	0.07	0.07	0.07
PEAK TWITCH TORQUE (Nm)				57.89	56.74	57.66	56.28
HALF RELAXATION TIME (ms)				73.41	68.16	68.54	62.17
SC	ARTERIAL	WB	K+	50.20	58.60	58.10	53.00
			La-	3.70	4.00	4.00	4.30
			HEM	46.70	47.50	47.60	46.40
		PL	K+	4.40	4.00	4.00	4.20
			La-	5.50	5.00	5.10	5.60
	VENOUS	WB	K+	52.30	55.70	55.50	53.50
			La-	6.80	7.00	7.20	7.00
			HEM	45.20	47.70	47.70	45.00
		PL	K+	3.70	4.00	3.80	3.60
			La-	10.80	10.50	11.10	10.50
M-WAVE AMPLITUDE (mV)				21.64	21.75	21.42	21.31
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.23	0.23	0.23	0.23
PEAK TWITCH TORQUE (Nm)				26.58	28.20	30.50	30.96
HALF RELAXATION TIME (ms)				95.51	92.88	90.64	82.77
JK	ARTERIAL	WB	K+	50.60	50.20	49.60	49.60
			La-	4.60	4.50	4.40	4.50
			HEM	46.20	47.10	46.80	45.50
		PL	K+	5.10	4.50	4.50	4.70
			La-	6.60	6.50	6.00	6.20
	VENOUS	WB	K+	45.20	44.70	49.80	49.70
			La-	5.90	5.90	6.30	6.40
			HEM	42.90	46.30	47.30	46.30
		PL	K+	4.20	3.80	4.50	4.30
			La-	8.00	9.10	9.70	9.10
M-WAVE AMPLITUDE (mV)				5.93	5.91	5.83	5.76
M-WAVE DURATION (S)				0.05	0.05	0.05	0.05
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				16.92	17.84	18.53	18.76
HALF RELAXATION TIME (ms)				59.18	60.67	57.30	61.42

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				1:40	2:00	2:20	2:40
MG	ARTERIAL	WB	K+	52.60	52.10	51.60	51.60
			La-	3.40	3.50	3.90	3.40
			HEM	45.80	45.70	45.20	44.40
		PL	K+	3.90	3.80	3.70	3.60
			La-	4.40	4.30	3.40	4.10
	VENOUS	WB	K+	53.30	52.50	53.10	52.00
			La-	5.60	5.50	5.10	5.00
			HEM	42.50	46.50	46.20	42.50
		PL	K+	3.90	3.70	3.60	3.60
			La-	8.00	7.70	7.90	7.70
M-WAVE AMPLITUDE (mV)				13.71	11.51	11.79	11.59
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.11	0.09	0.09	0.09
PEAK TWITCH TORQUE (Nm)				10.47	11.85	12.31	13.00
HALF RELAXATION TIME (ms)				130.71	119.10	115.36	93.63

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				3:00	3:30	4:00	4:30
SI	ARTERIAL	WB	K+	57.20	56.30	56.80	56.00
			La-	4.90	4.90	4.90	4.60
			HEM	51.60	51.50	54.50	53.70
		PL	K+	3.70	3.70	3.70	3.60
			La-	6.80	6.10	5.80	5.70
	VENOUS	WB	K+	57.50	48.90	57.30	56.50
			La-	7.70	6.50	7.50	6.90
			HEM	53.30	53.10	50.00	52.50
		PL	K+	3.70	3.60	3.60	3.60
			La-	12.30	11.00	10.20	9.80
M-WAVE AMPLITUDE (mV)				11.36	11.15	10.97	10.86
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.09	0.09	0.08	0.08
PEAK TWITCH TORQUE (Nm)				60.65	60.19	60.88	59.73
HALF RELAXATION TIME (ms)				50.56	49.06	48.31	44.57
BR	ARTERIAL	WB	K+	56.80	57.70	58.00	55.60
			La-	3.90	3.80	3.60	3.20
			HEM	45.20	45.50	45.10	45.10
		PL	K+	4.10	4.10	4.00	4.00
			La-	4.70	4.30	4.70	4.10
	VENOUS	WB	K+	55.80	53.00	52.60	53.80
			La-	5.40	5.30	5.10	4.70
			HEM	45.70	52.50	46.10	46.40
		PL	K+	4.20	3.90	3.90	4.00
			La-	8.00	7.60	7.10	6.60
M-WAVE AMPLITUDE (mV)				6.93	6.87	6.87	6.86
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				61.80	57.20	54.89	52.59
HALF RELAXATION TIME (ms)				75.66	74.16	79.40	82.77
RC	ARTERIAL	WB	K+	50.60	58.90	55.50	49.00
			La-	4.90	5.70	6.10	4.70
			HEM	47.70	46.60	47.00	44.40
		PL	K+	4.20	4.20	4.10	4.10
			La-	8.40	8.30	8.00	7.40
	VENOUS	WB	K+	57.20	56.90	54.60	66.10
			La-	9.00	8.60	8.70	8.10
			HEM	46.40	47.00	46.30	46.60
		PL	K+	3.80	3.90	3.70	3.80
			La-	14.10	13.30	12.70	12.20
M-WAVE AMPLITUDE (mV)				8.70	8.69	8.64	8.69
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				35.79	36.48	36.25	40.16
HALF RELAXATION TIME (ms)				76.78	82.77	82.40	76.78

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				3:00	3:30	4:00	4:30
EF	ARTERIAL	WB	K+	52.70	52.00	51.80	47.50
			La-	3.30	3.30	3.40	2.90
			HEM	45.20	45.50	44.20	45.50
		PL	K+	3.70	3.60	3.50	3.60
			La-	3.80	3.60	3.40	3.30
	VENOUS	WB	K+	53.10	52.70	52.00	51.80
			La-	5.40	5.00	5.20	4.40
			HEM	48.20	46.50	48.80	47.60
		PL	K+	3.60	3.50	3.50	3.60
			La-	6.90	6.20	5.90	4.70
M-WAVE AMPLITUDE (mV)				6.73	6.62	6.67	6.67
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				52.13	52.36	51.44	48.45
HALF RELAXATION TIME (ms)				95.13	95.88	97.38	95.88
SE	ARTERIAL	WB	K+	59.40	60.00	58.60	58.20
			La-	8.40	8.30	7.40	7.80
			HEM	52.50	52.60	51.20	52.60
		PL	K+	3.90	3.50	3.40	3.50
			La-	12.20	11.90	11.50	11.10
	VENOUS	WB	K+	59.70	60.10	58.20	56.50
			La-	8.50	8.50	7.90	7.90
			HEM	52.50	53.80	51.20	49.40
		PL	K+	3.50	3.60	3.40	3.30
			La-	12.90	11.90	11.30	10.90
M-WAVE AMPLITUDE (mV)				26.68	26.22	25.63	25.13
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.23	0.22	0.22	0.21
PEAK TWITCH TORQUE (Nm)				42.24	42.93	44.31	44.08
HALF RELAXATION TIME (ms)				100.37	91.39	94.01	95.88
BS	ARTERIAL	WB	K+	58.60	58.60	58.60	57.10
			La-	4.80	4.60	4.60	4.60
			HEM	50.00	50.00	50.60	49.40
		PL	K+	4.20	4.20	4.20	4.20
			La-	6.90	6.70	6.60	6.00
	VENOUS	WB	K+	57.70	57.50	57.50	57.30
			La-	6.60	6.40	6.20	6.00
			HEM	49.40	50.00	50.60	50.00
		PL	K+	4.00	4.00	3.90	3.80
			La-	9.50	9.30	8.90	8.50
M-WAVE AMPLITUDE (mV)				18.61	18.32	18.15	17.76
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.16	0.16	0.15	0.15
PEAK TWITCH TORQUE (Nm)				28.89	31.42	33.72	36.25
HALF RELAXATION TIME (ms)				94.01	94.38	50.19	95.88

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				3:00	3:30	4:00	4:30
MB	ARTERIAL	WB	K+	53.50	59.30	52.70	52.10
			La-	3.20	3.20	2.80	2.20
			HEM	46.30	46.30	45.20	45.90
		PL	K+	3.80	3.80	3.80	3.80
			La-	4.20	3.70	3.50	3.50
	VENOUS	WB	K+	51.80	56.30	50.40	48.00
			La-	4.70	4.20	3.30	3.70
			HEM	46.80	45.50	47.30	38.60
		PL	K+	3.90	3.70	3.70	3.70
			La-	6.50	5.60	5.00	4.90
M-WAVE AMPLITUDE (mV)				9.65	9.60	9.52	9.59
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.07	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				56.97	56.28	55.12	54.43
HALF RELAXATION TIME (ms)				70.41	70.04	64.42	63.67
SC	ARTERIAL	WB	K+	54.20	55.50	53.40	54.10
			La-	4.30	4.10	3.90	3.90
			HEM	45.10	45.50	45.20	45.70
		PL	K+	4.10	3.80	3.70	3.80
			La-	5.50	5.00	4.80	4.90
	VENOUS	WB	K+	54.20	52.90	52.50	52.10
			La-	6.80	6.40	6.20	6.10
			HEM	47.80	45.30	43.80	46.20
		PL	K+	3.90	3.70	3.50	3.50
			La-	10.50	9.90	9.20	9.00
M-WAVE AMPLITUDE (mV)				20.97	20.82	20.29	20.04
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.22	0.23	0.22	0.21
PEAK TWITCH TORQUE (Nm)				31.88	30.27	30.73	31.19
HALF RELAXATION TIME (ms)				87.27	67.04	61.80	61.80
JK	ARTERIAL	WB	K+	49.60	49.30	48.80	48.30
			La-	4.30	4.20	4.10	4.20
			HEM	44.40	46.30	46.20	46.10
		PL	K+	4.40	4.00	4.20	4.30
			La-	5.60	5.80	5.60	5.40
	VENOUS	WB	K+	48.90	48.60	47.10	46.00
			La-	6.40	5.80	6.40	5.30
			HEM	47.60	46.30	46.60	45.10
		PL	K+	4.10	4.10	4.00	3.90
			La-	9.00	8.50	7.90	7.50
M-WAVE AMPLITUDE (mV)				5.76	5.66	5.55	5.49
M-WAVE DURATION (S)				0.05	0.05	0.05	0.05
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				19.68	19.68	19.68	19.45
HALF RELAXATION TIME (ms)				55.81	59.18	56.18	52.43

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				3:00	3:30	4:00	4:30
MG	ARTERIAL	WB	K+	51.60	51.70	51.00	50.40
			La-	3.50	3.40	3.40	3.10
			HEM	46.10	44.20	45.20	44.00
		PL	K+	3.60	3.50	3.50	3.50
			La-	4.50	4.50	4.00	3.80
	VENOUS	WB	K+	51.10	48.90	51.00	51.50
			La-	5.00	4.70	4.70	4.40
			HEM	46.20	46.30	46.40	44.30
		PL	K+	3.50	3.20	3.20	3.40
			La-	7.70	6.30	6.30	5.80
M-WAVE AMPLITUDE (mV)				11.46	11.29	11.20	11.03
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.09	0.09	0.08	0.08
PEAK TWITCH TORQUE (Nm)				13.92	13.69	13.92	14.16
HALF RELAXATION TIME (ms)				93.63	99.63	94.01	96.25

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				5:00	5:30	6:00	7:00
SI	ARTERIAL	WB	K+	56.70	55.70	55.20	59.60
			La-	4.60	4.50	4.40	0.80
			HEM	52.50	51.60	51.30	54.50
		PL	K+	3.90	3.60	4.30	4.00
			La-	5.40	5.40	5.00	1.00
	VENOUS	WB	K+	57.30	57.00	56.30	58.80
			La-	7.10	6.10	5.90	5.50
			HEM	52.80	52.60	52.70	51.70
		PL	K+	3.60	3.80	3.70	3.90
			La-	8.90	7.80	7.20	6.50
M-WAVE AMPLITUDE (mV)				10.69	10.64	10.50	10.41
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.08	0.08	0.08	0.08
PEAK TWITCH TORQUE (Nm)				59.04	58.58	58.58	57.66
HALF RELAXATION TIME (ms)				44.94	48.31	48.31	45.32
BR	ARTERIAL	WB	K+	54.00	54.10	53.30	58.10
			La-	3.30	2.90	2.70	2.50
			HEM	46.70	46.20	46.30	43.90
		PL	K+	4.00	4.00	4.10	3.90
			La-	3.70	3.50	3.30	3.00
	VENOUS	WB	K+	54.80	55.00	54.20	52.80
			La-	4.80	4.10	3.60	3.40
			HEM	46.80	44.70	43.80	43.40
		PL	K+	4.00	4.20	4.10	4.20
			La-	6.20	6.10	5.20	5.40
M-WAVE AMPLITUDE (mV)				6.81	6.84	6.75	6.90
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				54.89	55.58	53.05	52.59
HALF RELAXATION TIME (ms)				74.91	71.16	67.04	63.30
RC	ARTERIAL	WB	K+	55.80	53.50	55.80	56.70
			La-	4.90	5.40	5.30	4.40
			HEM	48.80	47.20	45.20	45.30
		PL	K+	4.00	4.10	4.40	4.20
			La-	7.80	7.70	5.30	6.60
	VENOUS	WB	K+	57.80	53.70	55.80	56.30
			La-	7.80	7.70	7.60	7.00
			HEM	46.50	47.90	45.50	45.60
		PL	K+	3.70	4.10	3.80	4.10
			La-	10.80	11.60	9.40	9.50
M-WAVE AMPLITUDE (mV)				8.64	8.56	8.44	8.49
M-WAVE DURATION (S)				0.04	0.03	0.03	0.03
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				42.47	44.54	45.23	45.23
HALF RELAXATION TIME (ms)				73.41	76.03	75.66	72.28

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				5:00	5:30	6:00	7:00
EF	ARTERIAL	WB	K+	52.10	50.70	52.20	51.80
			La-	3.10	2.80	3.00	2.70
			HEM	44.00	44.30	45.50	43.40
		PL	K+	3.60	3.70	3.80	3.70
			La-	3.10	2.80	2.70	2.50
	VENOUS	WB	K+	49.30	51.80	52.20	50.60
			La-	3.80	3.90	3.70	3.30
			HEM	43.60	46.30	45.20	46.50
		PL	K+	3.30	3.60	3.80	3.60
			La-	4.10	4.20	3.90	3.50
M-WAVE AMPLITUDE (mV)				6.59	6.62	6.44	6.55
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				48.22	47.07	45.46	43.85
HALF RELAXATION TIME (ms)				93.63	90.64	90.26	89.51
SE	ARTERIAL	WB	K+	58.40	58.40	58.20	57.10
			La-	7.90	7.60	7.70	7.30
			HEM	51.20	51.90	52.40	51.90
		PL	K+	3.50	3.60	3.60	3.70
			La-	10.90	10.80	10.30	9.50
	VENOUS	WB	K+	54.60	56.80	56.90	56.80
			La-	7.90	7.80	7.90	7.50
			HEM	49.70	52.80	53.50	51.30
		PL	K+	3.20	3.60	3.60	3.70
			La-	9.90	10.90	11.00	9.90
M-WAVE AMPLITUDE (mV)				21.92	24.08	23.69	23.14
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.18	0.20	0.19	0.18
PEAK TWITCH TORQUE (Nm)				44.31	43.85	43.16	41.77
HALF RELAXATION TIME (ms)				89.14	90.64	88.76	88.76
BS	ARTERIAL	WB	K+	56.80	57.60	58.30	55.50
			La-	4.50	4.30	3.50	3.10
			HEM	48.80	50.00	50.70	50.70
		PL	K+	4.20	4.10	4.00	4.00
			La-	5.80	5.50	4.70	3.80
	VENOUS	WB	K+	56.90	55.90	55.30	55.00
			La-	5.80	5.30	4.50	3.90
			HEM	48.80	50.00	49.40	50.00
		PL	K+	3.80	3.70	3.60	3.70
			La-	6.60	5.40	5.30	5.30
M-WAVE AMPLITUDE (mV)				17.70	17.42	17.08	16.85
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.15	0.15	0.14	0.14
PEAK TWITCH TORQUE (Nm)				35.56	35.33	35.79	36.02
HALF RELAXATION TIME (ms)				90.64	95.88	93.26	97.00

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				5:00	5:30	6:00	7:00
MB	ARTERIAL	WB	K+	54.50	56.80	51.80	52.10
			La-	2.00	1.60	2.60	2.60
			HEM	46.50	45.10	46.50	45.20
		PL	K+	3.80	3.80	3.90	3.90
			La-	3.60	1.80	3.10	3.00
	VENOUS	WB	K+	50.20	51.20	45.30	47.60
			La-	3.60	2.70	3.20	3.00
			HEM	45.20	45.00	45.90	44.70
		PL	K+	3.70	3.70	3.80	3.80
			La-	4.50	4.20	4.00	3.60
M-WAVE AMPLITUDE (mV)				9.54	9.42	9.43	9.23
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				53.97	52.59	53.05	52.13
HALF RELAXATION TIME (ms)				62.92	63.30	59.18	51.31
SC	ARTERIAL	WB	K+	54.40	55.00	55.80	54.30
			La-	3.80	3.90	3.90	3.70
			HEM	45.80	46.70	44.90	46.10
		PL	K+	4.00	4.00	4.00	4.00
			La-	5.50	5.20	5.10	4.80
	VENOUS	WB	K+	52.60	52.60	52.80	52.60
			La-	5.90	5.90	5.90	5.10
			HEM	45.00	45.00	45.10	44.30
		PL	K+	3.70	3.70	3.60	3.60
			La-	8.70	8.40	8.00	7.00
M-WAVE AMPLITUDE (mV)				19.51	19.08	18.65	18.01
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.21	0.20	0.19	0.18
PEAK TWITCH TORQUE (Nm)				29.58	29.58	29.81	30.04
HALF RELAXATION TIME (ms)				56.55	52.43	55.06	52.06
JK	ARTERIAL	WB	K+	46.10	45.20	45.90	49.50
			La-	3.90	3.60	3.50	3.50
			HEM	45.80	45.10	46.30	46.20
		PL	K+	4.20	4.10	4.10	4.60
			La-	5.30	5.20	5.00	4.50
	VENOUS	WB	K+	46.10	50.50	49.60	48.30
			La-	5.30	5.10	4.80	4.30
			HEM	45.70	44.70	45.80	44.90
		PL	K+	4.00	4.10	4.00	3.90
			La-	6.80	6.40	6.20	6.00
M-WAVE AMPLITUDE (mV)				5.43	5.41	5.43	5.27
M WAVE DURATION (S)				0.05	0.05	0.05	0.05
M-WAVE AREA (mV*S)				0.04	0.04	0.04	0.04
PEAK TWITCH TORQUE (Nm)				19.22	18.76	18.30	17.84
HALF RELAXATION TIME (ms)				55.06	55.43	62.55	62.92

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				5:00	5:30	6:00	7:00
MG	ARTERIAL	WB	K+	50.40	50.70	50.10	50.10
			La-	2.80	3.00	2.80	2.70
			HEM	46.30	45.20	44.90	45.00
		PL	K+	3.50	3.50	3.50	3.60
			La-	3.40	3.40	3.30	3.40
	VENOUS	WB	K+	49.30	48.10	49.80	47.20
			La-	3.80	3.80	3.50	3.10
			HEM	44.90	44.40	43.90	44.00
		PL	K+	3.40	3.40	3.50	3.30
			La-	5.30	5.00	4.80	3.80
M-WAVE AMPLITUDE (mV)				10.72	10.77	10.55	10.59
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.08	0.08	0.07	0.07
PEAK TWITCH TORQUE (Nm)				14.16	13.69	13.69	12.77
HALF RELAXATION TIME (ms)				90.64	87.64	92.13	81.27

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				8:00	9:00	10:00	15:00
SI	ARTERIAL	WB	K+	58.10	58.10	58.10	57.40
			La-	1.80	3.40	3.20	2.40
			HEM	55.20	52.80	51.40	52.90
		PL	K+	4.00	4.00	4.10	4.10
			La-	2.10	3.70	3.50	2.40
	VENOUS	WB	K+	55.00	59.00	58.30	56.00
			La-	4.60	1.30	0.60	1.80
			HEM	52.60	51.60	52.80	50.00
		PL	K+	3.90	4.30	4.30	4.30
			La-	5.40	1.60	0.90	1.80
M-WAVE AMPLITUDE (mV)				10.30	10.21	10.15	10.07
M-WAVE DURATION (S)				0.04	0.04	0.04	0.03
M-WAVE AREA (mV*S)				0.07	0.07	0.07	0.07
PEAK TWITCH TORQUE (Nm)				56.97	54.20	53.97	49.37
HALF RELAXATION TIME (ms)				46.07	43.07	43.45	52.81
BR	ARTERIAL	WB	K+	49.40	53.40	53.30	52.40
			La-	2.20	2.60	2.40	1.80
			HEM	44.00	45.00	44.60	44.70
		PL	K+	3.90	4.10	4.20	4.00
			La-	2.70	2.50	2.40	1.70
	VENOUS	WB	K+	53.90	52.90	52.00	51.30
			La-	3.00	3.30	2.90	2.20
			HEM	43.90	44.40	43.40	42.10
		PL	K+	3.90	4.10	4.00	4.00
			La-	3.90	3.70	3.40	2.20
M-WAVE AMPLITUDE (mV)				6.87	6.81	6.84	6.95
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				52.36	50.06	49.37	44.08
HALF RELAXATION TIME (ms)				61.05	57.68	64.04	50.94
RC	ARTERIAL	WB	K+	53.30	57.30	55.30	52.50
			La-	4.70	4.50	4.50	3.40
			HEM	49.40	47.70	46.70	47.30
		PL	K+	4.60	4.10	5.50	4.90
			La-	6.20	5.20	5.00	3.70
	VENOUS	WB	K+	56.60	60.90	54.20	52.30
			La-	6.20	6.30	4.90	3.60
			HEM	44.40	46.40	45.10	46.60
		PL	K+	4.10	4.70	4.90	5.00
			La-	9.80	7.90	6.10	4.20
M-WAVE AMPLITUDE (mV)				8.42	8.39	8.41	8.41
M-WAVE DURATION (S)				0.03	0.03	0.03	0.03
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				45.23	44.77	43.85	39.24
HALF RELAXATION TIME (ms)				71.16	72.28	65.17	63.67

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				8:00	9:00	10:00	15:00
EF	ARTERIAL	WB	K+	50.80	51.60	53.00	51.50
			La-	2.40	2.20	2.10	1.50
			HEM	44.70	42.90	42.10	43.20
		PL	K+	3.80	3.80	4.30	4.40
			La-	2.10	2.00	2.20	1.30
	VENOUS	WB	K+	51.30	50.60	53.80	50.20
			La-	2.90	2.70	2.50	1.70
			HEM	47.10	45.00	44.40	44.20
		PL	K+	3.60	3.90	3.90	3.70
			La-	2.80	2.70	2.70	2.00
M-WAVE AMPLITUDE (mV)				6.41	6.34	6.56	6.41
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.05	0.05	0.05	0.05
PEAK TWITCH TORQUE (Nm)				43.16	43.85	40.39	39.01
HALF RELAXATION TIME (ms)				89.14	86.52	79.78	79.40
SE	ARTERIAL	WB	K+	56.90	57.20	56.50	55.90
			La-	7.30	7.40	7.20	5.50
			HEM	51.80	51.30	48.80	51.40
		PL	K+	4.00	3.70	4.30	4.40
			La-	10.20	9.60	9.80	7.00
	VENOUS	WB	K+	56.90	56.70	56.80	54.10
			La-	7.30	7.00	7.00	5.10
			HEM	52.60	50.70	51.20	49.40
		PL	K+	3.80	4.00	3.90	4.00
			La-	10.00	9.60	7.90	6.50
M-WAVE AMPLITUDE (mV)				22.65	21.07	21.38	19.70
M-WAVE DURATION (S)				0.04	0.04	0.03	0.03
M-WAVE AREA (mV*S)				0.17	0.16	0.16	0.14
PEAK TWITCH TORQUE (Nm)				40.62	39.93	39.24	39.24
HALF RELAXATION TIME (ms)				86.89	78.65	55.06	55.06
BS	ARTERIAL	WB	K+	54.30	55.60	56.30	55.30
			La-	2.90	2.60	2.40	2.10
			HEM	49.40	47.40	47.10	48.80
		PL	K+	4.00	4.00	4.10	4.20
			La-	3.50	3.30	2.00	2.10
	VENOUS	WB	K+	54.20	55.10	54.40	53.30
			La-	3.60	3.60	3.40	2.90
			HEM	47.60	49.40	50.70	48.60
		PL	K+	3.70	4.00	4.00	3.90
			La-	4.90	4.70	3.90	3.70
M-WAVE AMPLITUDE (mV)				16.78	16.61	16.50	16.40
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.14	0.14	0.14	0.14
PEAK TWITCH TORQUE (Nm)				36.48	33.26	33.72	31.42
HALF RELAXATION TIME (ms)				93.26	89.14	87.64	78.65

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				8:00	9:00	10:00	15:00
MB	ARTERIAL	WB	K+	52.20	50.10	49.90	51.70
			La-	2.60	2.60	2.40	2.10
			HEM	46.30	45.60	45.40	44.70
		PL	K+	3.90	3.90	4.00	4.00
			La-	2.80	2.60	2.40	2.00
	VENOUS	WB	K+	50.80	48.80	47.90	50.60
			La-	2.90	2.70	2.50	2.10
			HEM	46.40	45.30	44.70	44.30
		PL	K+	3.90	3.90	3.90	3.90
			La-	3.30	3.00	2.80	2.10
M-WAVE AMPLITUDE (mV)				9.31	9.26	9.23	9.49
M-WAVE DURATION (S)				0.04	0.04	0.03	0.04
M-WAVE AREA (mV*S)				0.06	0.06	0.06	0.06
PEAK TWITCH TORQUE (Nm)				50.29	49.60	48.68	42.00
HALF RELAXATION TIME (ms)				50.19	43.82	47.57	40.45
SC	ARTERIAL	WB	K+	52.90	51.90	50.60	47.90
			La-	3.40	3.30	3.20	2.20
			HEM	45.80	45.00	44.90	44.40
		PL	K+	3.90	4.00	4.60	4.30
			La-	4.20	4.20	4.40	2.70
	VENOUS	WB	K+	52.70	49.40	49.90	48.40
			La-	4.80	4.60	4.60	2.90
			HEM	45.00	44.40	43.80	42.10
		PL	K+	3.60	3.80	4.00	3.90
			La-	6.40	6.20	6.40	3.60
M-WAVE AMPLITUDE (mV)				17.65	17.27	17.06	16.27
M-WAVE DURATION (S)				0.04	0.04	0.04	0.04
M-WAVE AREA (mV*S)				0.17	0.17	0.16	0.15
PEAK TWITCH TORQUE (Nm)				29.12	28.43	27.50	24.97
HALF RELAXATION TIME (ms)				49.44	51.31	49.81	49.06
JK	ARTERIAL	WB	K+	46.10	46.30	48.90	48.70
			La-	3.10	2.60	2.70	2.00
			HEM	47.10	45.80	45.10	45.10
		PL	K+	4.50	4.50	4.70	4.70
			La-	3.80	4.00	3.50	2.30
	VENOUS	WB	K+	48.00	48.20	48.90	48.50
			La-	3.90	3.70	3.40	2.20
			HEM	44.30	44.20	43.80	44.20
		PL	K+	4.00	4.10	4.10	4.20
			La-	5.50	4.70	4.20	2.40
M-WAVE AMPLITUDE (mV)				5.32	5.21	5.20	5.07
M-WAVE DURATION (S)				0.05	0.05	0.05	0.05
M-WAVE AREA (mV*S)				0.04	0.04	0.04	0.04
PEAK TWITCH TORQUE (Nm)				17.61	17.15	16.46	15.31
HALF RELAXATION TIME (ms)				56.18	55.85	52.81	51.69

NAME	ART/VEN	PART	ION/HEM	SAMPLE			
				8:00	9:00	10:00	15:00
MG	ARTERIAL	WB	K+	50.70	49.50	48.10	47.50
			La-	2.50	3.10	3.00	2.30
			HEM	45.00	44.90	45.50	45.50
		PL	K+	3.60	4.60	4.30	4.40
			La-	2.80	3.90	3.30	2.00
	VENOUS	WB	K+	49.00	47.10	47.30	45.80
			La-	3.00	3.90	3.50	2.80
			HEM	44.30	42.20	43.70	43.90
		PL	K+	3.50	4.30	4.20	4.50
			La-	3.60	4.50	4.30	3.50
M-WAVE AMPLITUDE (mV)				10.35	10.22	10.08	9.49
M-WAVE DURATION (S)				0.04	0.04	0.04	0.03
M-WAVE AREA (mV*S)				0.07	0.07	0.06	0.06
PEAK TWITCH TORQUE (Nm)				12.77	11.62	12.77	11.39
HALF RELAXATION TIME (ms)				77.15	91.01	60.30	71.91

APPENDIX B

Analysis of variance tables

Torque over 3 minute fatigue protocol

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	615.75	9	68.41666	1.860215
TIME	1248.008	7	178.2868	4.847531
Error	2317.07	63	36.77889	

F (7 , 63) = 4.847531 Probability = 0.00036

IEMG over 3 minute fatigue protocol

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	18960.78	9	2106.753	7.403753
TIME	29092.42	7	4156.06	14.60562
Error	17926.78	63	284.5521	

F (7 , 63) = 14.60562 Probability = 0.00000

Twitch torques in recovery

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	47583.5	9	5287.056	155.8482
TIME	7329.375	22	333.1534	9.820466
Error	6717.031	198	33.9244	

F (22 , 198) = 9.820466 Probability = 0.00000

Half relaxation times in recovery

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	39190.13	9	4354.458	22.71353
TIME	13174	22	598.8182	3.123528
Error	37959	198	191.7121	

F (22 , 198) = 3.123528 Probability = 0.00005

M-Wave amplitude post-exercise

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	7707.5	7	1101.071	595.7324
TIMEamp	209.7422	22	9.533736	5.158208
Error	284.6328	154	1.848265	

F (22 , 154) = 5.158208 Probability = 0.00000

M-Wave duration post-exercise

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	2.640486E-03	7	3.772122E-04	159.5743
TIMEdur	3.423095E-04	22	1.555952E-05	6.582234
Error	3.640354E-04	154	2.363866E-06	

F (22 , 154) = 6.582234 Probability = 0.00000

M-Wave area post-exercise

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	.7171628	7	.1024518	499.0534
TIMEarea	.0307157	22	1.396168E-03	6.800879
Error	3.161502E-02	154	2.052923E-04	

F (22 , 154) = 6.800879 Probability = 0.00000

Arterial lactate concentration

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	776.4326	9	86.27029	76.66927
TIMEapl	722.6816	23	31.42094	27.9241
Error	232.9219	207	1.125226	

F (23 , 207) = 27.9241 Probability = 0.00000

Venous lactate concentration

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	716.1738	9	79.57487	37.79683
TIMEvpl	2087.801	23	90.77395	43.11622
Error	435.8037	207	2.105332	

F (23 , 207) = 43.11622 Probability = 0.00000

Arterial potassium concentration

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	14.74414	9	1.638238	22.02689
TIMEapk	27.21582	23	1.183297	15.90999
Error	15.39551	207	7.437444E-02	

F (23 , 207) = 15.90999 Probability = 0.00000

Venous potassium concentration

Source	Sum Sqr.	df	Mean Sqr.	F
Ss/Blocks	7.279785	9	.808865	9.039807
TIMEvpk	83.85742	23	3.645975	40.74711
Error	18.52197	207	8.947813E-02	

F (23 , 207) = 40.74711 Probability = 0.00000

APPENDIX C

Pilot study

DETERMINING THE INTENSITY OF A FATIGUING SUSTAINED ISOMETRIC QUADRICEPS CONTRACTION AND ITS EFFECT ON THE AMPLITUDE, DURATION, AND AREA OF M-WAVES EVOKED DURING RECOVERY: A PILOT STUDY

INTRODUCTION

Skeletal muscle fatigue has been defined as "an inability to generate the required or expected force" (Edwards, 1981). Whether the site and mechanism of fatigue are central or peripheral in origin, however, is debatable.

Potassium ion (K^+) has become a focus of fatigue because of the importance of its distribution across the muscle membrane in maintaining cell excitability and propagating electrical impulses (Hodgkin and Horowicz, 1959). The rate of K^+ efflux into the extracellular space increases with increasing intensity of dynamic exercise--as a % of VO_2 max--and escalates in static contractions until approximately 25% of the related maximum voluntary contraction (MVC) is attained (Sjogaard, 1990). However, the total amount of K^+ released depends upon both the intensity and duration of the exercise (Sjogaard, 1990). Isometric forms of muscle contraction provide an excellent static environment for evaluating increases in extracellular K^+ concentration ($[K^+]_e$) during

exercise, due to the reduced blood flow evident when the contraction intensity is greater than 15% of the related MVC (Sjogaard, 1990).

An increase in plasma K^+ concentration ($[K^+]_p$) to 8 mmol/l causes: 1) a decrease in the K^+ conductance across the membrane (Hodgkin and Horowicz, 1959), 2) inactivation of up to 50% of the inward voltage-dependent sodium (Na^+) channels, and 3) a reduction in the size of the action potential (Hodgkin and Huxley, 1952). However, muscle cells are probably exposed to higher $[K^+]$'s in the extracellular space, than values attained in the venous blood (Hirche et al., 1980; Vyskocil et al., 1983; Hnik et al., 1986).

In order to maintain the ionic gradients necessary for excitability, the membrane is equipped with Na^+/K^+ ATPase--a pump that returns Na^+ and K^+ ions, which have exchanged during an action potential, back across the membrane in the ratio of 3:2 respectively (Thomas, 1972).

The rate of active Na^+/K^+ transport is determined by many factors including: 1) availability of ATP, 2) concentration of Na^+ and K^+ on either side of the plasma membrane, 3) the concentration of Na^+/K^+ ATPase, and 4) the short-term activation of the pump by insulin, epinephrine and norepinephrine (Clausen and Flatman, 1977; Clausen, 1986). The

activity of the pump has an electrogenic effect by transporting more Na^+ ions out of the cell than K^+ ions in. This unequal transport contributes to the membrane potential, which during exercise, helps to offset the depolarizing effects of increased $[K^+]_e$. The action of this pump has been shown to add 10 mV to the membrane potential at rest, and up to 30 mV to the potential during exercise, when the activity of the pump is heightened (Hicks and McComas, 1989). It may be possible to evaluate the activity of the Na^+/K^+ pump by analyzing the compound muscle action potential (M-wave).

The M-wave is "the summation of nearly synchronous muscle fibre action potentials recorded from a muscle commonly produced by stimulation of the nerve supplying the muscle" (AAEE Glossary of Terms, 1987). Consequently, the M-wave offers useful information about the state of activity of the neuromuscular apparatus during fatigue (Cupido, 1991).

The M-wave amplitude is dependent upon the resting membrane potential of individual muscle fibres and the size of their action potentials. As well, the M-wave amplitude is affected by the number of active muscle fibres thereby refecting the integrity of neuromuscular transmission (Cupido, 1991).

The duration of the M-wave represents the synaptic delay across the neuromuscular junction, synchronization of the muscle fibre action potentials, and the conductance of the

inward Na^+ channels and the outward K^+ channels (Cupido, 1991).

It has been proposed that a loss of M-wave amplitude, accompanied by a prolongation of the waveform, but no increase in area, indicates neuromuscular transmission failure with a dispersion of motor unit firing (Bigland-Ritchie et al., 1979). In contrast, maintenance of the amplitude in spite of increases in the duration and area, could be explained by a slowing of conduction velocity along the muscle fibre membrane illustrating a reduction of membrane excitability (Bigland-Ritchie et al., 1979).

Most studies utilizing the M-wave to confirm membrane excitability have found that it is unaffected by voluntary fatigue (Merton, 1954; Bigland-Ritchie, 1979; Bigland-Ritchie, 1982; Cupido, 1991). Hicks et al (1989) have shown that M-wave amplitude and area may even potentiate during an intermittent MVC fatigue protocol of the thenar and extensor digitorum brevis muscles. Furthermore, Hicks and McComas (1989) showed that the augmented electrogenic effect of the stimulated Na^+/K^+ pump, in response to muscle activity, temporarily hyperpolarized the single muscle fibre membranes--ultimately causing the M-wave potentiation in rat soleus muscle fibres. Therefore, it may be possible to use the M-wave as a non-invasive method for assessing Na^+/K^+ pump activity during fatigue in skeletal muscle.

Work by Cupido (1994) preceding this pilot study

evaluated the M-wave response to a fatiguing quadriceps paradigm during which intermittent isometric MVC's were performed with short periods of rest between. No significant changes to the M-wave were found post-exercise when compared to baseline values of amplitude, duration, and area. However, we propose that the fatigue protocol used may have allowed sufficient blood flow during the relaxation phase to wash out some of the muscle metabolites, especially extracellular K^+, thereby decreasing the depolarizing effect it may have had on the muscle cell membrane, and thus, producing no effect on the M-wave characteristics.

The purpose of this pilot study is to change the protocol used by Cupido (1994), and assess the time and contraction intensity necessary to cause fatigue using a sustained isometric quadriceps contraction, while at the same time producing the highest rate of K^+ efflux possible from the muscle (approximately 25% MVC). Furthermore, this study will evaluate the succeeding evoked M-waves during recovery for significant changes in amplitude, duration, and area from baseline measures which may arise due to the effects of the preceding fatiguing contraction.

METHODS

Subjects. One female and four male university students (mean

age 23.8 ± .75 (SEM) yrs) volunteered for the study and agreed to the procedures.

Stimulating and Recording. The subjects sat with their right knee flexed at an angle of 90 degrees for maximum quadriceps EMG activity (Haffajee et al., 1972), and their backs against an upright support. The lower right leg of each subject was secured in a metal brace with two Velcro straps: one fastened around the proximal end of the lower leg just below the knee, and the second, just above the right ankle. Two different Velcro straps were fastened around the proximal and middle portions of the right thigh to secure it throughout the test.

The stimulating electrodes consisted of a rubber cathode (57mm x 103mm) which was placed in the inguinal crease over the femoral nerve, and a rubber anode (57mm x 103mm) which was placed on the anterior aspect of the middle of the thigh. Voluntary EMG activity and M-wave characteristics were recorded with a monopolar arrangement using two disposable silver/silver chloride monitoring electrodes (3M-No.2248). The stigmatic electrode was placed over the belly of the vastus medialis and the reference electrode was secured approximately 2 cm distal to this and medial to the patella. A silver strip (6mm X 50mm) ground electrode was placed on the postero-lateral aspect of the thigh, between the anode and the stigmatic electrode (Cupido, 1991). Prior to all the electrode

placements, the skin was prepared by rubbing it with an abrasive and cleaning it with alcohol. Where necessary, the leg was also shaved.

A high-voltage stimulator (Devices Stimulator 3072, Medical Systems Corp.) was used to deliver single rectangular pulses (pulse width: 200-500 μs) to the femoral nerve. The EMG signals from the recording electrodes were fed into a Honeywell Accudata EMG Amplifier (model #135A) at a sampling rate of 2.7 KHz, and were real time displayed on a VGA monitor. The data was streamed continuously to disc through a Dataq waveform scrolling board (AT CODAS Interface Card: Dataq Instruments Inc.; Akron, Ohio) in an IBM-compatible computer.

Experimental Protocol. After the subject's skin was prepared, the electrodes positioned, and the leg secured in the apparatus, maximum twitch torque was determined by progressively increasing the stimulator voltage until a peak twitch was evoked. This voltage was then used to indicate motor unit activation (MUA) by interpolating the supramaximal twitch during a briefly sustained MVC (Belanger and McComas, 1981). The theoretical MUA was calculated as follows:

$$\text{MUA}(\%) = \frac{\text{Twitch Torque} - \text{Interpolated Twitch Torque}}{\text{Twitch Torque}} \times 100$$

Three MVC's, lasting approximately 4 seconds each with one minute rest between each were performed and saved for

analysis. While performing these MVC's, the subjects were not allowed to use their hands or arms to gain leverage. The highest MVC was used to calculate the torque required during the sustained isometric contraction.

The subject rested for 3 minutes and immediately before the sustained contraction was initiated, the baseline twitch was evoked. Two black lines were scribed on the VGA monitor at the subject's 25% and 30% MVC levels to allow visual feedback for the subject, and the experimenter constantly monitored the voluntary torque to ensure this range was adhered to. The subjects were asked to increase their sustained torque, for the last 30 seconds, up to almost 30% MVC to ensure fatigue by three minutes, and with intensive motivation from the experimenter, it was possible for the subjects to barely reach 3 minutes. When the contraction was finished, the subjects remained strapped into the apparatus throughout the 15 minute recovery phase.

Recovery. During the 15 minute recovery phase, the subjects sat comfortably with their hands at their sides. M-waves were evoked at various times throughout the recovery; every 20s for the first three minutes, every 30s for the next three minutes, every minute for the following four minutes, and the final one at 15 minutes. No twitches were elicited between 10 and 15 minutes, and each stimulation was preceded by a tone. The

timing and delivery of these tones and twitches were controlled by a Stoelting Laboratory Controller (Stoelting Laboratory Corp.) interfaced with the computer.

M-wave Analysis. M-waves were analyzed using custom-designed CODAS analysis software. Amplitude was calculated by adding the absolute magnitude of the two peaks of the wave form. For consistency, duration was determined as the time from the beginning of the M-wave--when the trace departed from baseline--until the point where the waveform was within 5% of its peak amplitude after the positive and negative phases. Area was then calculated by integrating the entire wave form.

Statistical Analysis. Data were analyzed using repeated measures analysis of variance (ANOVA) in the Practical Statistics software programme (D. DeCatanzaro, McMaster University; version 3.5). Significant differences between means and their related baseline measures were examined using a Dunnett test. Effects were deemed significant at $p < 0.05$.

RESULTS

Motor Unit Activation. The mean theoretical motor unit activation was 90.1% ± 7.02 (SEM). This figure indicates each subject was exerting a true maximal voluntary contraction from

which 25% was used for the sustained isometric contraction during the fatigue protocol.

Sustained Contraction Intensity. As indicated in the methods section, the mean torque increased above the 25% goal from 2.5 to 3 minutes in order to ensure the subjects' fatigue. All the subjects acknowledged they found it difficult to continue from 1.5 minutes and, except for the short burst of activity from 2.5 to 3 minutes, could not continue at that workload past 3 minutes. Figure 1. illustrates the consistency of the contractions between 25 and 30% of the related MVC, and Table 1. contains the torque values observed every 30s during the contraction. The mean torque maintained over the three minute protocol was 24.73 ± 1.86 (SEM) Nm.

M-wave Analysis. Table 2. contains the values of M-wave amplitude, duration, and area at the various sampling times previously explained in the methods. We found no significant changes in the amplitudes, however, there was a trend of potentiation for the M-waves sampled from 5 secs to 2 min.

Duration of the M-waves decreased significantly from their baseline values at all times during recovery.

All M-wave areas were less than baseline during recovery, however, only at 20 secs, 40 secs, and 1 min was the difference not significant. Similar to amplitude values during

recovery, a trend emerged for area during the first two minutes where some potentiation is evident compared to the other recovery values.

Figures 2A, 2B, and 2C illustrate the three M-wave characteristics measured over time.

DISCUSSION

One of the purposes of this pilot study was to assess the maximum duration of a sustained isometric quadriceps contraction of 25% - 30% MVC, which would appear to be 3 minutes. Because of the variability in fatigue at 2.5 minutes between the five subjects, they were asked to increase the intensity so the contraction could not be held past 3 minutes. The differences in fatigue may be due to pain tolerance, individual training, muscle fibre types, and the consistency of the subject's contraction throughout the protocol.

It may be preferable, in the following thesis, for the subjects to maintain the contraction closer to 30% MVC from the beginning. With this simple modification, all the subjects will be contracting maximally by 3 minutes, and maintaining torques close to 25% MVC--thus ensuring fatigue.

The second purpose of this study was to evaluate M-wave changes during recovery for this type of sustained submaximal effort. We found no significant differences in mean amplitude

of M-waves between the values during the recovery period and baseline, although there seems to be some potentiation for the first two minutes. Maintenance of the amplitude suggests no depolarization of the membrane, nor a decrease in the number of muscle fibres or motor units firing. The potentiation illustrated during the first two minutes is probably due to the electrogenic effect produced by the increased activity of the Na^+/K^+ pump in trying to maintain the ion concentrations on either side of the muscle cell membrane.

M-wave duration was significantly less at all times during recovery compared to baseline. This would suggest some type of volume conduction change after the fatiguing protocol.

M-wave area was also significantly less for all times during recovery compared to baseline, except for three at the beginning of recovery: 20 secs, 40 secs, and 1 min. The overall decrease in area can be attributed to the shorter duration of the wave form throughout recovery, and the slight potentiation of the amplitude for the first two minutes would explain the augmented values in area over the same time.

Based on the relatively minor changes in M-wave characteristics following this type of fatiguing exercise three assumptions can be made at this point: 1) there is not enough potassium in the extracellular space to cause a depolarization of the muscle membrane, 2) the fatigue protocol is not stressing the muscle sufficiently to induce excitation

failure, and elicit the anticipated changes in the M-wave, or finally, 3) the Na^+/K^+ ATPase is producing a substantial electrogenic effect that is sufficient to offset the depolarization caused by the $[K^+]_e$ and prevent the anticipated changes in the M-wave.

There is good evidence this type of fatigue protocol causes significant increases in $[K^+]_e$. Sjogaard (1991) has evaluated this contraction type and intensity and discovered that together they produced the greatest rate of K^+ efflux from the quadriceps.

Furthermore, it is interesting to note that using a variety of analytical approaches, numerous researchers (Sreter, 1963; Bergstrom et al., 1971; Hnik et al., 1986; and Lindinger and Heigenheuser, 1988) have reported that substantial amounts of K^+ are lost from contracting muscle. In particular, Vyskocil et al. (1983) have measured interstitial K^+ concentrations up to 15 mmol/l with ion sensitive electrodes during maximal static contractions. This level of K^+ illustrates the substantial difference between interstitial and venous concentrations--peak venous $[K^+]$'s have been in the order of 6-8 mmol/l (Sjogaard, 1991). Doubling $[K^+]_e$ from a resting value of 4 mmol/l to 8 mmol/l, would result in a severe depolarization (18mV) of the muscle membrane as described by the Goldman-Hodgkin-Katz equation (Hodgkin and Katz, 1949). In the present study, it is reasonable to assume

the $[K^+]_e$ are high enough to cause a depolarization of the muscle membrane, but the M-wave appears unaffected.

We are also satisfied the fatigue protocol utilized was sufficient to cause fatigue; all our subjects were visibly spent at the end of 3 minutes, and they claimed they could not have continued any longer. Furthermore, the endurance time for this intensity of isometric contraction is consistent with previous work done in this muscle group (Sjogaard, 1991).

The most likely explanation for the membrane's resistance to fatigue is that the Na^+/K^+ pump is managing to offset the depolarizing effects of increased $[K^+]_e$ on the membrane potential. Indeed it has been shown previously that increased activity of the Na^+/K^+ pump can result in the maintenance of near-normal action potentials and M-waves even when the $[K^+]_e$ is four times its normal value (Hicks and McComas, 1989).

This pilot study was necessary to determine an appropriate fatigue protocol and analyze the M-wave changes during the ensuing recovery period. This information and the basic protocol will be incorporated into my thesis work in which blood samples will be drawn--both arterial and venous--to clarify the levels of potassium in the blood, and correlate these values with characteristics of the elicited M-waves. The work will test our hypothesis that the Na^+/K^+ pump is important in delaying, or even offsetting, the effects of increased $[K^+]_e$ on muscle excitability.

Table 1. Mean % MVC sampled at 30s intervals during a 3 minute sustained isometric quadriceps contraction

	Time (min)						
	0	.5	1	1.5	2	2.5	3
% MVC	25.05 ±.83	24.12 ±.58	23.95 ±.43	23.27 ±.77	24.01 ±.40	25.03 ±.26	27.69 ±.92

All values are expressed as mean percentages of MVC ± SE of the mean.

n=5 for all values and $p < 0.05$.

Mean % MVC torque = 24.73 ± 1.86 Nm.

Table 2. Absolute values for amplitude, duration, and area of M-waves elicited during the 15 minute recovery period

	Amplitude (mV)	Duration (mS)	Area (μV*S)
Time (min)			
BL	11.97 ±1.86	41.48 ±1.19	108.52 ±18.91
:05	12.67 ±1.86	34.96 ±1.15‡	99.16 ±16.89‡
:20	12.65 ±2.02	36.84 ±.98‡	103.48 ±18.96
:40	12.54 ±2.01	37.22 ±.94‡	101.70 ±18.20
1:00	12.52 ±2.02	37.16 ±1.07‡	101.34 ±18.51
1:20	12.34 ±2.02	37.38 ±.68‡	99.36 ±18.10‡
1:40	12.27 ±2.01	37.30 ±.83‡	98.36 ±17.88‡
2:00	12.14 ±2.00	37.50 ±.63‡	97.98 ±18.16‡
2:20	12.40 ±1.89	37.12 ±.61‡	96.08 ±17.46‡
2:40	12.16 ±2.02	37.42 ±.62‡	96.54 ±17.73‡
3:00	12.11 ±2.07	37.18 ±.63‡	96.40 ±18.16‡
3:30	12.11 ±2.08	36.98 ±.81‡	96.16 ±18.13‡
4:00	12.01 ±2.06	37.26 ±.54‡	95.66 ±18.09‡
4:30	11.98 ±2.08	37.18 ±.63‡	94.32 ±17.87‡
5:00	12.03 ±2.11	36.76 ±.91‡	95.24 ±18.43‡
5:30	11.86 ±2.08	37.04 ±.82‡	94.44 ±18.03‡
6:00	11.78 ±2.06	36.82 ±1.00‡	94.00 ±18.19‡
7:00	11.79 ±2.06	37.20 ±.79‡	93.30 ±17.67‡
8:00	11.58 ±2.01	37.44 ±.78‡	92.58 ±17.25‡
9:00	11.32 ±1.91	37.00 ±1.12‡	90.80 ±16.89‡
10:00	11.31 ±1.92	37.52 ±.73‡	89.86 ±16.23‡
15:00	11.45 ±1.96	37.08 ±1.19‡	93.88 ±17.30‡

All values are absolute means ± SEM ($p < 0.05$)

n=5 for all values.

‡ mean is significantly different from related baseline value.

FIGURE 1

Mean % MVC sampled at 30s intervals during a 3 minute sustained isometric quadriceps contraction. Values are means ± SEM for five subjects.

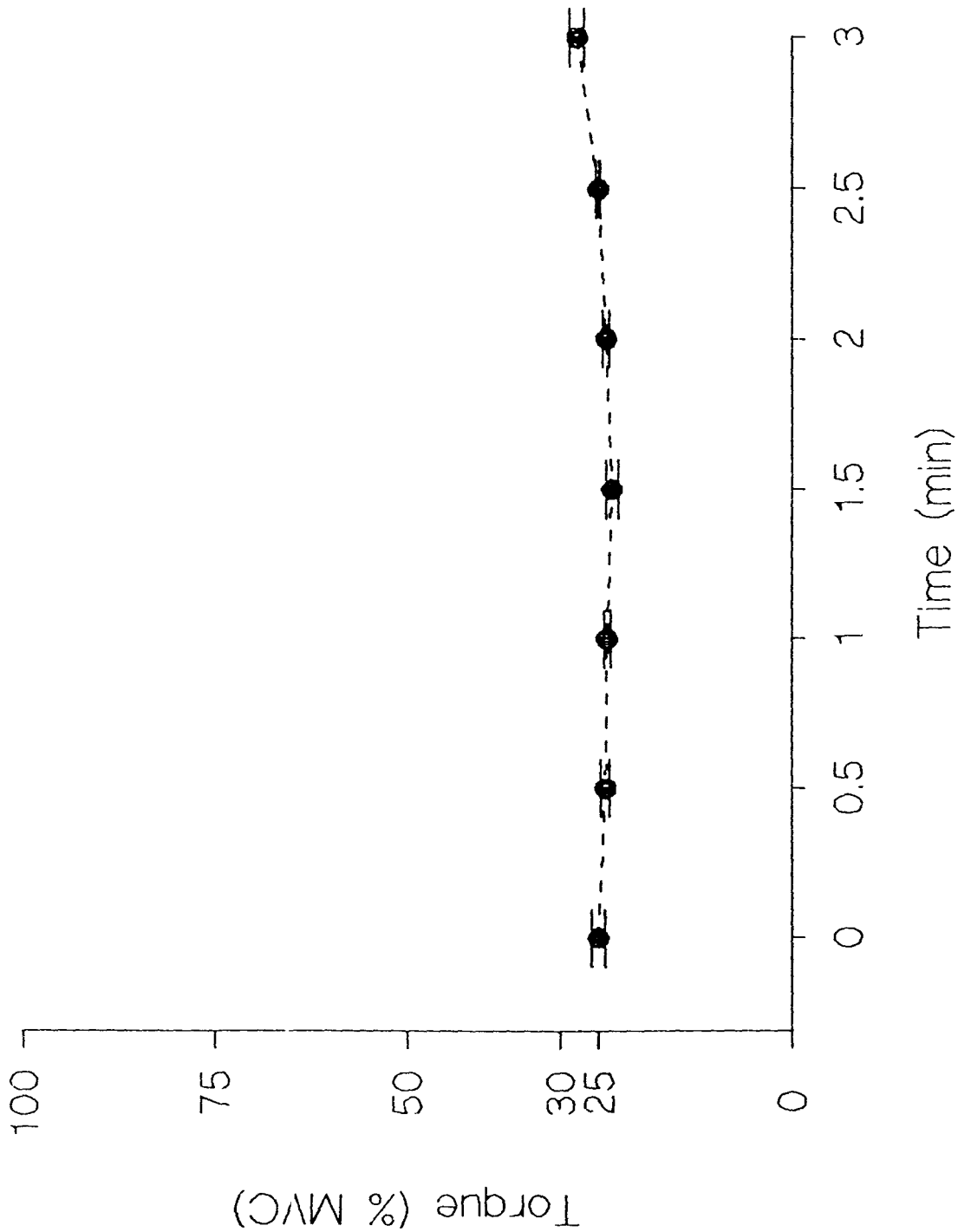

Torque (% MVC)
100
75
50
30
25
0
0
0.5
1
1.5
2
2.5
3
Time (min)

FIGURE 2A

Mean M-wave amplitude in millivolts. *

FIGURE 2B

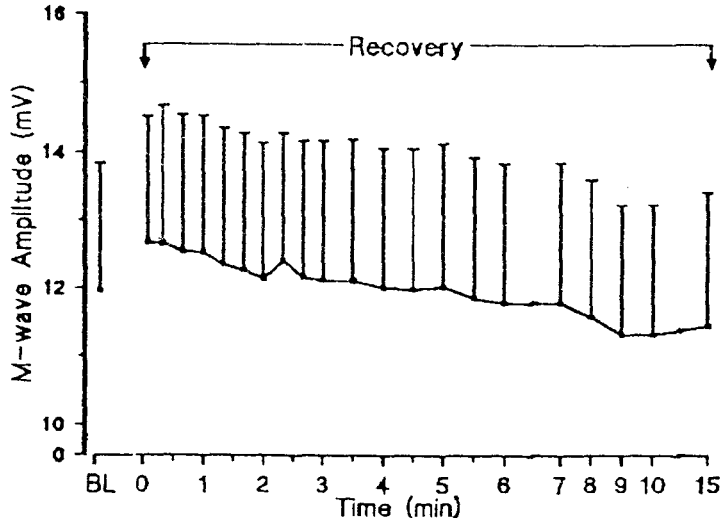
Mean M-wave duration in milliseconds. *

FIGURE 2C

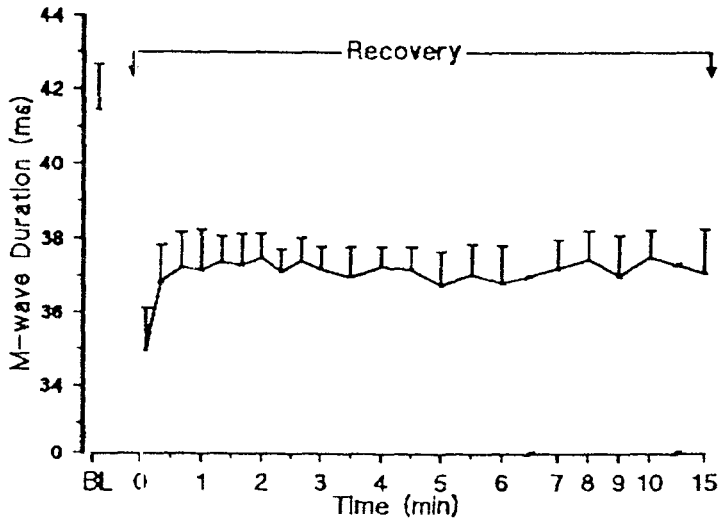
Mean M-wave area in microvolt*seconds. *

* Values are means ± SEM for 5 subjects.
BL represents prefatigue baseline measurement.

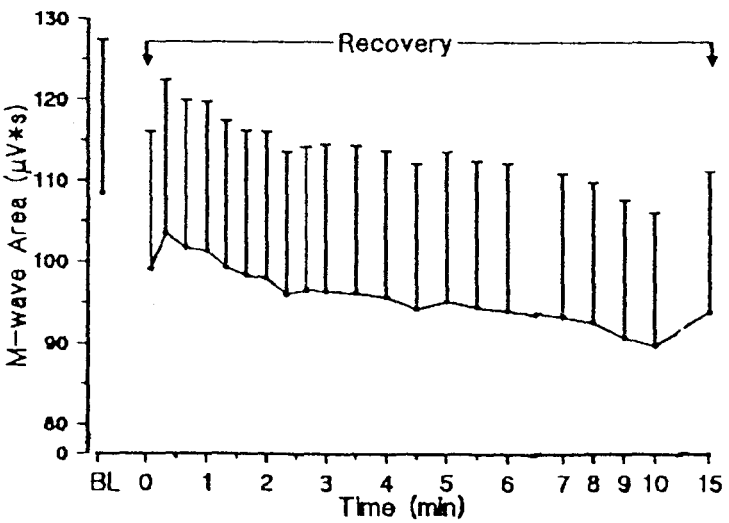
A


Recovery
M-wave Amplitude (mV)
16
14
12
10
0
BL 0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

B


Recovery
M-wave Duration (ms)
44
42
40
38
36
34
0
BL 0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

C


Recovery
M-wave Area (μV*s)
130
120
110
100
90
80
0
BL 0 1 2 3 4 5 6 7 8 9 10 15
Time (min)

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APPENDIX D

Subject consent form

McMASTER UNIVERSITY
Department of Kinesiology
1280 Main Street West, Hamilton, ON L8S 4K1
Telephone (416) 525-9140
FAX: (416) 523-6011
Area code will be (905) after Oct. 4, 1993

CONSENT FORM

THE RELATIONSHIP BETWEEN PLASMA POTASSIUM CONCENTRATIONS, SKELETAL MUSCLE EXCITABILITY AND MUSCLE FATIGUE DURING STATIC EXERCISE

I, _____, consent to participate in a study directed by Dr. Audrey Hicks and Dr. Robert McKelvie. The purpose of this study is to investigate some of the mechanisms that may contribute to skeletal muscle fatigue during activity. The results of this study will be made available to the scientific community but participation in this study will offer no direct benefit to me.

For the purposes of this study I will have two catheters inserted and I will sustain a submaximal isometric contraction of my right quadriceps muscles for 3 min. I will then have my muscle twitched by an electrical stimulation at varying time intervals during a 15 minute recovery period and blood samples will be taken.

I am aware that several measurements will be taken during the study. Surface electrodes will be placed over the muscles of the right thigh to measure electrical activity (recording electrodes). As well, surface electrodes will be secured to my right thigh which will deliver electrical stimulation to my right femoral nerve (stimulating electrodes). Catheters will be inserted into my right femoral vein and my left brachial artery before the exercise begins by a physician (Dr. B. McKelvie) qualified to perform these procedures. Approximately 5 cc (1 teaspoon) of blood will be taken from each catheter before the test begins, as a baseline measure, and 30 will be taken from each catheter site during the 15 minute recovery period. Throughout the study, a maximum of 350 cc of blood will be drawn which is less than half of a normal blood donation.

I am aware that the protocol is as follows: First, catheters will be inserted by Dr. McKelvie followed by a baseline drawing of blood from both catheters; I will then be strapped into the isometric leg extension chair in the lab and both sets of electrodes will be placed on my right quadriceps muscles and femoral nerve; following this, my maximum twitch torque will be determined by manipulating the intensity of the stimulator, then I will perform 3 maximal voluntary contractions (MVC's) with a twitch interpolated on top to indicate motor unit activation; I will then sustain 25% of my best MVC for 3 minutes at which time I will perform another MVC to indicate my muscle fatigue; and finally, at predetermined times during the ensuing recovery period, I will be twitched by the stimulator and the remaining 60 blood samples will be taken.

I understand that there is a slight risk (less than 1 in 1,000) of a blood clot forming at the catheter sites. Also, there is a very slight risk of developing a localized infection at the puncture site, but this has never occurred in similar studies of this kind. There may also be some slight bruising and/or redness around the puncture sites, but these are temporary and should recover within several days of testing. There may be temporary discomfort associated with the muscle stimulation, however this procedure has no apparent side effects. I have been assured that the physician (Dr. McKelvie) will be available to respond to any of the side effects related to this study.

Neither my name nor any reference to me will be used in compiling the results nor in publication in any form whatsoever.

I understand that I may withdraw from the study at any time without prejudice, even after signing this form.

Name (print)	Signature	Date

Witness (print)	Signature	Date

I have explained the nature of the study to the subject and believe that he has understood it.

Name (print)	Signature	Date