

2000

The Relationship Between Functional And Histological Changes In Muscle Following Eccentric Exercise In Mice

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**THE RELATIONSHIP BETWEEN FUNCTIONAL AND HISTOLOGICAL
CHANGES IN MUSCLE FOLLOWING ECCENTRIC EXERCISE IN MICE**

By

Michael John Newton

Bachelor of Applied Science (Sports Science) Honours

A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of

Master of Science (Sports Science)

Faculty of Communications, Health and Science

School of Biomedical and Sports Science

Edith Cowan University

Western Australia

Date of Submission: 31-03-2000



ABSTRACT

Exercise-induced muscle damage (EIMD) is known to be produced by novel or unaccustomed exercise, especially high force eccentric contractions. Histological myofibre disruption, force loss and muscle soreness are associated with EIMD and have implications for sporting performance. Traditional practices of assessing the extent of disruption to the myofibres is by performing needle biopsies and subsequently analysing the histology of the fibres. Recently there has been interest in investigating whether changes in force production and contractile properties of muscle following damaging exercise correlate strongly with the magnitude of disruption to the myofibres. The main aim of this study was to investigate whether changes in force production and contractile properties of muscle following damaging eccentric exercise correlated with myofibre disruption. In order to test the hypotheses set down in the study 56 mice (C57 BL/ 10 strain) were randomly assigned to two groups (active and passive). Each main group was then divided into 5 subgroups. Anaesthetised mice performed either 120 active (eccentric contractions) or passive (no muscle contraction) lengthening repetitions after which they were allowed to recover. The right foot was fixed to a foot plate housing a force transducer which was directly attached to the axle of a stepping motor. A stimulating electrode was surgically placed around the peroneal nerve and P_0 and 1/ 150 Hz ratios were determined. Animals in the active group then performed 5 bouts of 24 stimulated lengthening repetitions at 0.3 amps with a stimulation frequency of 100 Hz. The passive group's protocol was identical with the exception that no stimulation was provided. One repetition for both active and passive groups consisted

of a 300 millisecond plantar flexion movement of the foot plate and a 4.7 second dorsiflexion recovery movement to the starting position. Active and passive subgroups were terminated at 3, 6, 10, 15 and 20 days following exercise, prior to which P_o and 1/ 150 Hz ratio were determined. Tibialis anterior (TA) muscles were excised at this time from both exercised and contralateral limbs and prepared for later histological examination.

Significant differences were evident between the two groups for P_o following each bout of 24 lengthening repetitions, 10 minutes following lengthening and on days 3 and 20 of recovery. The only significant differences between the groups in 1/ 150 Hz ratio occurred 10 minutes following lengthening and at day six of recovery ($p < 0.025$). A weak significant correlation was recorded between normalized P_o in the days following active lengthening and the magnitude of histological disruption to the TA muscle ($r = -0.35$; $p < 0.05$).

The major findings of this study were that a) there was a weak significant correlation between normalized P_o in the days following damaging exercise, and the magnitude of histological disruption to the TA muscle, b) average normalized P_o 10 minutes following damaging exercise does not seem to be a good predictor of the magnitude of histological disruption in the days following exercise, and c) The 1/ 150 Hz ratio immediately after, or in the days following damaging exercise does not seem to be a useful predictor of the magnitude of histological myofibre damage. Further investigation into any relationship between changes in P_o following eccentric exercise and the magnitude of myofibre disruption at the same time points will help to establish whether the use of force measures are valid to predict the quantity of muscle damage.

DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

- (i) incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education.
- (ii) contain any material previously published or written by another person except where due reference is made in the text; or
- (iii) contain any defamatory material.

Signature:

Date: 31-03-2000

ACKNOWLEDGEMENTS

There are many individuals who provided support and offered advice and constructive criticism during the preparation of this manuscript. This thesis would not be complete without including their names along with some brief comments about them.

To my supervisor, colleague, mentor and friend Dr Paul Sacco I thank you very much. I have learned an enormous amount from you in many aspects of physiology over the years it has taken to complete this piece of work. I thank you for offering to take over this project when my previous supervisor left for England. I aspire to be able to write half as clearly and concisely as you do.

To my initial supervisor, Dr Colin James, I express thanks for directing me into this field of research and for suggesting Dr Sacco as the excellent replacement supervisor to carry on the bulk of the work required to bring this project to completion. I also have you to thank for my introduction to the study of muscle physiology and exercise biochemistry. I hope that the passion you sparked in me for these topics is infectious and carries over to my current and future students.

Many thanks to Elaine Pascoe and one of my mentors Professor Alan Morton for their guidance with the statistics section of this paper. Alan, your help and discussions in the final few months of preparation have been much appreciated.

To Mary Cornelius, our resident gun triathlete and laboratory technician, many thanks for all of the assistance you have provided during the course of my research. I knew that if I requested something be ordered or manufactured you would be onto it immediately. It was tough for us at times in the early days with the complexity of the equipment required for the experiments but you shone through.

To Jenny Tollet. I thank you for the help you provided with the preparation of the pictomicrographs of my histology work. You saved me countless hours of work that would have been required for learning a new software program for manipulating images.

Thanks to Andrea Vis for her discussions on histological analysis and to Dr Peter Roberts for the use of his graticule while we were waiting for ours to arrive.

I am very appreciative of Dr Gary Thickbroom's help in adapting computer software he wrote to read the data files I collected in Waveview. I am also indebted to him for allowing me to use the computers at ANRI (QEII hospital) to crunch most of my pilot work data.

Associate professor Barry Gibson and Mr. Jerry Linsten were kind enough to take my masters research into account when setting up teaching and research loads on department. Thanks to both of you I managed to juggle research and teaching commitments for those three years.

I would like to thank another colleague, mentor and friend Associate professor Kazunori Nosaka from Yokohama City University in Japan. Your knowledge in this area is immense and the many discussions we have had have helped me enormously not only in the preparation of this manuscript but in my understanding of exercise-induced muscle damage in general.

I have left until last those that I love dearly. My parents, grandmother, and more recently my partner Taki, have been there providing support both emotionally and financially throughout this project. That has meant more to me than can be expressed in words. My father, Vern, stepped in when it looked like my machinery would never see the light of day and basically helped build the isokinetic dynamometer, a masters project in itself I have since been told. Without your electronics background and ability to build just about anything from any material just lying around, the data collection would have taken place much later or maybe not at all. Thanks Taki for drawing figures 1 and 2 from my rough sketches.

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LIST OF ABBREVIATIONS

A	ampere (the SI unit of electric current)
ANOVA	analysis of variance
AST	aspartate transaminase or aspartate aminotransferase
ATP	adenosine triphosphate
C	Celsius
Ca ²⁺ or Ca ⁺⁺	calcium ion
[Ca] _i	intracellular calcium concentration
CK or CPK	creatine kinase or creatine phosphokinase
CT	computerised tomography
DHP	dihydropyridine
E-C	excitation-contraction
EDL	extensor digitorum longus
H ⁺	hydrogen ion
Hz	hertz
kHz	kilohertz
LDH or LD	lactate dehydrogenase
LFF	low frequency fatigue
MRI	magnetic resonance imaging
ms	millisecond
n.s	non-significant
OCT	optimum cutting temperature

PCr	phosphocreatine
P _o	maximum isometric force production
r	correlation coefficient
S.E.M	standard error of the mean
TA	tibialis anterior
T-tubule	transverse tubule
V	volt
μs	microsecond
γ	gamma

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Chapter One

Introduction

1.1 Background

Novel or unaccustomed exercise has been shown to produce temporary and repairable damage to muscle (Armstrong, 1986). This phenomenon has been termed exercise-induced muscle damage (EIMD). Exercise involving a predominance of high force eccentric contractions has been found to produce the greatest magnitude of muscle damage in both human (Newham, Jones, & Clarkson, 1987) and other animal (Clarkson & Tremblay, 1988) models. A temporary decline in voluntary muscular force and power production (Davies & White, 1981; Sargeant & Dolan, 1987), impairment of fine motor control (Pearce, Sacco, Byrnes, Thickbroom, & Mastaglia, 1998), swelling (Ebbeling & Clarkson, 1989; Smith, 1991), reduction in range of motion (Clarkson, Nosaka, & Braun, 1992), and muscle soreness (Smith et al., 1994a) are all associated with damage resulting from eccentric exercise and have obvious implications for athletic performance.

A number of measures have been employed by researchers as indicators of EIMD. These include abnormally high plasma levels of intramuscular proteins such as creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate transaminase (AST), muscle strength loss, and altered muscle contractile properties (Ebbeling & Clarkson, 1989). In order to obtain quantitative measures of damage to myofibres it is customary to perform either needle or open biopsies and extract small samples of muscle tissue. Although this process is useful for experimentation purposes it does have its drawbacks. The

procedure is invasive, usually requires the services of a trained medical practitioner, and may not be indicative of damage to the whole muscle. Non-invasive approaches to this problem may be to use strength loss and/ or changes in contractile properties following eccentric exercise as a predictor of muscle fibre “damage”. Following exercise leading to myofibre damage it has been shown that force loss at low frequencies of stimulation is greater than that at higher frequencies, which has given rise to the term “low frequency fatigue” (LFF) (Davies & White, 1981; Jones, Newham, & Torgan, 1989). LFF is most pronounced immediately following exercise of an eccentric nature and full recovery can take up to two weeks (Newham et al., 1987). Sacco, Jones, Dick, & Vrbova (1992), using a murine model, reported that both loss of isometric force and reductions in the 40 Hz/ 100 Hz force ratio corresponded with the period of greatest fibre necrosis following eccentric exercise. Force losses at various stimulation frequencies have also been reported by others following eccentric exercise although many of these studies did not conduct histological analysis of myofibre damage (Davies & White, 1981; Sargeant & Dolan, 1987; Warren, Ingalls, Shah, & Armstrong, 1999a). Further investigation into the time course of changes in force following eccentric exercise, and subsequent histological examination of the exercised muscle would prove useful in establishing whether a relationship exists between force output and disruption to myofibres. In order to test the research hypotheses a study was designed utilizing two groups of 28 mice, of which one group underwent an active lengthening protocol of 120 stimulated maximum eccentric contractions of the dorsiflexor muscles (active group), the other, acting as a control (passive group), performed 120 non-stimulated passive lengthening movements. Force measurements were collected at various

frequencies of stimulation at specific periods during, and following, the two protocols, and histological examination of the tibialis anterior (TA) muscles were conducted.

1.2 Research Questions

1. Would there be a significant difference in maximum isometric force production (P_o) between the actively and passively lengthened dorsiflexor muscles in the course of, and during recovery from, 5 sets of 24 lengthening repetitions?
2. Would metabolic fatigue over the course of the active lengthening repetitions be a major cause of any decrease in P_o immediately following the last repetition?
3. Would the 1/150 Hz ratio be significantly different between active and passively lengthened dorsiflexor muscles at the common time points associated with decreased P_o , being immediately following and at 3 days following lengthening?
4. Would histological damage be greater in the exercised as compared to the non-exercised contralateral TA muscles during recovery following 120 lengthening repetitions?
5. Would normalized P_o over the course of, or during recovery from, 120 active lengthening repetitions be a predictor of myofibre damage?
6. Would the 1/150 Hz ratio following 120 active lengthening repetitions be a predictor of myofibre damage?

1.3 Specific Research Hypotheses

1. The normalized P_o of the actively lengthened dorsiflexor muscles would be significantly lower than those of the passively lengthened muscles during and 10 minutes following 5 sets of 24 lengthening repetitions ($p < 0.008$).
2. There would be no significant difference between normalized P_o of the foot dorsiflexors immediately following, and 10 minutes following, 120 active lengthening repetitions ($p < 0.05$).
3. There would be a significant difference in normalized P_o between actively and passively lengthened dorsiflexor muscles following 120 lengthening repetitions ($p < 0.01$).
4. There would be a significant difference in the 1/150 Hz ratio of foot dorsiflexors between active and passively lengthened muscle 10 minutes following, and at 3 days following 120 lengthening repetitions ($p < 0.025$).
5. There would be significantly greater histological damage in the actively lengthened compared to the contralateral TA muscles following 120 lengthening repetitions as measured by the average number of mononuclear cells plus the average number of central nuclei in the muscle, referred to subsequently as the damage index ($p < 0.01$).
6. There would be a significant negative correlation between normalized P_o 10 minutes following 120 active lengthening repetitions and myofibre damage at time of termination as measured by the damage index ($p < 0.05$).

7. There would be a significant negative correlation between the average normalized P_o over 120 active lengthening repetitions and myofibre damage at time of termination as measured by the damage index ($p < 0.05$).
8. There would be a significant negative correlation between normalized P_o following 120 active lengthening repetitions and myofibre damage at time of termination as measured by the damage index ($p < 0.05$).
9. There would not be a significant positive or negative correlation between the 1/ 150 Hz ratio 10 minutes following 120 active lengthening repetitions and myofibre damage at time of termination as measured by the damage index ($p < 0.05$).
10. There would not be a significant positive or negative correlation between the 1/ 150 Hz ratio at 3, 6, 10, 15, & 20 days following 120 active lengthening repetitions and myofibre damage at time of termination as measured by the damage index ($p < 0.05$).

1.4 Significance of the Study

In order to obtain an estimate of the magnitude of histological myofibre damage costly open or needle biopsies are currently employed in human and equine models, or whole muscles are assessed in many other animal models resulting in termination of the animal. Other methods such as non-invasive imaging techniques (e.g., magnetic resonance imaging (MRI), computerised tomography scans (CT), γ -camera imaging or ultrasound), are utilized to assess the extent of muscle damage, although most of these are expensive and have been questioned regarding their ability to correlate temporally

with muscle function (Warren, Lowe, & Armstrong, 1999b).

The current study examined the relationships between measures of muscle function, specifically P_0 and 1/150 Hz ratio, and the magnitude of histological damage imposed on the myofibres at varying times following active lengthening exercise. If the measures of muscle function proved valid predictors of histological myofibre damage then a cheaper and non-invasive means of damage determination would be available to the researcher. This would allow both functional (i.e., force measures) and histological effects of damaging exercise to be obtained using only force data which would prove economical in terms of time, and in human studies would preclude the use of medical practitioners required for muscle biopsy procedures.

Lieber & Friden (1999) commented that exercise in the form of rehabilitation is on the increase to expedite recovery after injury or hospitalization. This is the case in both sporting as well as non-athletic environments. From a physical rehabilitation viewpoint muscle function data combined with estimates of histological myofibre damage would provide useful information about the status of a patient's recovery and allow the therapist to fine tune the rehabilitation program.

Chapter Two

Review of Literature

2.1 Exercise-Induced Damage

It has been well established that novel or unaccustomed exercise can result in delayed damage to muscle fibres (Armstrong, Warren, & Warren, 1991; Clarkson et al., 1992; Kuipers, Drukker, Frederik, Geurten, & Kranenburg, 1983). The form of exercise shown to produce the greatest magnitude of delayed injury involves a predominance of eccentric actions (Newham et al., 1987; Sacco & Jones, 1992b; Triffletti, Litchfield, Clarkson, & Byrnes, 1988). This exercise-induced myofibre damage is temporary in nature and repairable (Armstrong, 1984; Clarkson & Tremblay, 1988; Davies & Barnes, 1972; Sacco & Jones, 1992b).

Although researchers have presented a number of possible explanations, the exact mechanism(s) by which high-force eccentric contractions produce delayed injury to muscle fibres remains unclear. Armstrong (1990) presented a four stage model of exercise-induced muscle injury derived from results obtained in his and other laboratories. In this model he suggests that exercise-induced muscular injury progresses sequentially through four separate stages, these being the “initial”, “autogenetic”, “phagocytic”, and “regenerative” stages.

The first, or initial, stage "includes the event that triggers the succeeding degenerative and regenerative phases in the injury process." (Armstrong 1990, p. 430). It has been postulated that an initiating event causes some disruption to the integrity of the muscle resulting in damage of the myofilament apparatus and/ or injury to the sarcolemma (Armstrong et al., 1991; Duncan & Jackson, 1987; Morgan, 1990). Several possible initiating events have been proposed and can be categorised as either mechanical or metabolic in nature (Armstrong, 1990). Mechanical events may manifest as "high specific tension" which could affect the sarcolemma, the sarcoplasmic reticulum (SR) and/ or the myofibrillar structures (Armstrong, 1990, p. 430). Potential damaging events of a metabolic nature include insufficient mitochondrial respiration (i.e., low ATP availability), lowered pH, free oxygen radical production and/ or high local muscle temperatures (Armstrong, 1990). Since eccentric work is associated with relatively low energy utilisation (compared with concentric or isometric work), Armstrong (1990) suggests that it is unlikely that the myofibre damage is caused by lowered pH or insufficient mitochondrial respiration. He does caution, though, that because damage to myofibres may be focal in nature it is possible that areas of low pH or energy depletion may not be resolved in whole muscle assays (Armstrong et al., 1991).

Regardless of the nature of the initiating event, there follows an influx of extracellular calcium which proceeds to disrupt the intracellular calcium homeostasis (Armstrong, 1990; Jackson, Jones, & Edwards, 1984). From a mechanical standpoint, it has been suggested that high eccentric forces acting on the muscle may cause an increase in sarcolemmal permeability (Armstrong et al., 1991) or a disruption of sarcoplasmic reticulum function, allowing for the influx of calcium (Amelink, Van der Kallen,

Wokke, & Bar, 1990b; Byrd, 1992). Numerous studies have shown that an accumulation of intracellular calcium causes subcellular damage to the affected myofibres, both in vivo and in vitro (Duncan, 1987; Duncan & Jackson, 1987; Jackson et al., 1984). Duncan et al. (1987) reported that ultrastructural damage can also occur to the myofilament apparatus which is not obligatorily dependent on calcium. Signs of focal disturbances to the banding patterns of some fibres have been reported immediately following exercise involving eccentric contractions with these disturbances taking the form of widened A- and I-bands (Armstrong, Ogilvie, & Schwane, 1983; Newham, McPhail, Mills, & Edwards, 1983b). In addition, Friden, Sjostrom, & Ekblom (1981, 1983) suggested that the high myofibrillar tension developed during eccentric exercise may result in some disturbance to the Z-bands, although it is not clear whether high intramuscular tension was primarily responsible for the Z-band disruption. Friden & Lieber (1992) state that "Z-disk streaming appears to be a primary myofibrillar response to altered physical and metabolic situations" (p. 523). They also mention that a number of investigators have demonstrated "a clear connection between Z-disk streaming and high-tension physical exercise" (p. 523). Newham et al. (1983b) noted Z-line disruption of the eccentrically worked muscles immediately after a group of subjects had completed a twenty minute step test. They implied that this was mechanically induced via the high tensions developed during the exercise.

The increased intracellular concentration of calcium ($[Ca]_i$) produced by the initiating event has been implicated in further destructive processes within myofibres (Jones & Round, 1990). This on-going destructive phase in the damage/ repair process of muscle is referred to by some researchers as the "autogenetic stage" (Armstrong, 1990;

Clarkson, 1992). It is characterised by activation of calcium-activated enzymes and other substances such as free radicals which have been implicated in the lysis of cell and intracellular organelle membranes, and cellular ultrastructure (Armstrong, 1984; Duncan, 1987). Once lysis of the cell membrane has passed a critical stage, intracellular proteins previously contained within the cell leak out into the extracellular spaces and eventually appear in the blood (Evans & Cannon, 1991; Nosaka & Clarkson, 1992).

2.2 Mononuclear Cell Infiltration

One to four hours after exercise designed to produce injury researchers have observed mononuclear cells invading the site(s) of damage (Armstrong, 1990; Smith et al., 1994b). Substantial numbers have been reported at twenty four hours post exercise and are maintained through 48 hours (Smith, 1991; Stauber, Fritz, Vogelbach, & Dahlmann, 1988). This phase of the damage/ repair process in muscle has been termed the "phagocytic stage" due to the phagocytic nature of many of the invading mononuclear cells. It has been suggested that the infiltrating mononuclear cells, which mainly comprise monocytes/ macrophages, both T helper and T suppressor cells, and neutrophils, are all intricately involved in the disposal of necrotic tissue (Round, Jones, & Cambridge, 1987; Smith, 1991). What exactly attracts these mononuclear cells to the site of injury has been the focus of much research over the years. Although far from fully resolved it has been suggested that many products from damaged myofibres could leach out through a compromised sarcolemma and become directly or indirectly

attractants to the previously quiescent mononuclear cells (Evans & Cannon, 1991). At some point early into the injury process complement is activated, components of which serve as chemoattractants for both neutrophils (Evans & Cannon, 1991) and macrophages (Grounds, 1991).

Macrophages are believed to play an essential role in the repair process (Evans & Cannon, 1991; Grounds, 1991). Carlson and Faulkner (1983) state that "unless the damaged muscle fibre becomes invaded by macrophages, it remains arrested in the stage of intrinsic degeneration, and the activation of satellite cells and regeneration proceed no further." (p. 190).

2.3 Muscle Fibre Regeneration

Once removal of the original damaged segment, or whole fibre, is complete, myogenic regeneration proceeds (Carlson & Faulkner, 1983; Hurme & Kalimo, 1992). Macrophages are believed to play a direct role in the repair of damaged connective tissue, and "influence the ingrowth of new capillaries into the injured area" (Almekinders & Almekinders, 1992). It has also been suggested that macrophages "synthesise and secrete" some factor(s) which cause satellite cells to proliferate (Hurme & Kalimo, 1992, p. 202).

Darr and Schultz (1987) state that "the primary role of satellite cells in nongrowing, mature muscle is to repair or regenerate damaged myofibres" (p. 1820). Upon activation, satellite cells proliferate and then differentiate into myoblasts (Bischoff, 1989; Schultz, 1989; White & Esser, 1989). These myoblasts fuse in the space

previously filled with necrotic tissue and form a hollow cylindrical structure known as a myotube. The newly formed multinucleated myotubes begin to produce muscle specific proteins and are readily distinguished by their centrally located nuclei (Carlson & Faulkner, 1983; Hurme & Kalimo, 1992). In time the centrally nucleated myotubes develop into myofibres, characterised by their peripherally located nuclei (Campion, 1984; White & Esser, 1989).

2.4 Indicators and Quantification of Exercise-Induced Muscle Damage

2.4.1 Intracellular protein release.

Following high intensity or long duration exercise involving eccentric, concentric, and/or isometric contractions, elevated levels of muscle proteins may be detected in the blood (Clarkson, Byrnes, McCormick, Turcotte, & White, 1986; Newham, Jones, & Edwards, 1986; Noakes, 1987; Nosaka, Clarkson, & Apple, 1992). The more commonly studied of these proteins include creatine kinase (CPK or CK), lactate dehydrogenase (LDH or LD), aspartate aminotransferase (AST), and myoglobin (Nosaka & Clarkson, 1992; Roxin, Hedin, & Venge, 1986; van der Meulen, Kuipers, & Drukker, 1991). The blood concentrations of these proteins have been used as markers of muscle damage, though it is CK which has received the most attention (Jones, Newham, Round, & Tolfree, 1986; Nosaka & Clarkson, 1992; van der Meulen et al., 1991). CK remains the most commonly used marker of muscle damage (Nosaka & Clarkson, 1992).

Factors such as the type of exercise employed, the exercise intensity, and the degree of specific training of the subjects have been shown to influence both the magnitude and time course of protein efflux from muscle and influx to blood (Byrnes, Clarkson, & Katch, 1985a; Byrnes et al., 1985b; Clarkson et al., 1986; Clarkson, Litchfield, Graves, Kirwan, & Byrnes, 1985; Newham, Jones, & Edwards, 1983a; Schwane & Armstrong, 1983; Triffletti et al., 1988). Although isometric and isotonic contractions have been linked to elevated levels of blood borne muscle proteins, it is those of an eccentric nature which produce the greatest response and also exhibit a delayed peak (Manfredi et al., 1991; Newham et al., 1986; Schwane, Johnson, Vandenakker, & Armstrong, 1983). The relationship between enzyme efflux and myofibre damage has attracted much attention but remains to be elucidated. It has been suggested that the initial peak in CK shortly after exercise may be due to increases in the permeability of the sarcolemma from contractile activity, and observable damage may not yet be evident (Armstrong et al., 1983). In contrast, the delayed rise in CK does seem to coincide with observable myofibre damage. Clarkson and Tremblay (1988) have proposed that the influx of calcium may cause, among other things, impairment of sarcoplasmic reticulum and mitochondrial functions, and activation of sarcoplasmic protease's which aid in lysing of the sarcolemma causing the delayed CK response. Although CK is widely used as an indicator of muscle damage, questions have arisen as to the sensitivity of the marker (van der Meulen et al., 1991). The magnitude of CK efflux into the blood has been used to determine the extent of damage to the muscle on the basis that the larger the CK efflux the more substantial is the damage (Nosaka & Clarkson, 1992; van der Meulen et al., 1991). Recent research has cast doubt on the accuracy of CK as an estimate of the

damage in muscle. van Der Meulen et al. (1991) state that "the actual volume of histological muscle damage was significantly less than the calculated muscle damage based on enzyme release." (p. 999). Janssen et al. (1989) work with runners revealed no significant relationship between histological changes and the increase in CK activity following damaging exercise.

In addition to the poor correlation reported between the level of CK response and magnitude of damage, research has also shown wide variability in CK response among subjects (Clarkson & Ebbeling, 1988; Newham et al., 1983a; Nosaka & Clarkson, 1996) and a difference in magnitude of response between sexes (Amelink, Koot, Erich, Van Gijn, & Bar, 1990a; van der Meulen et al., 1991).

2.4.2 Histological analysis.

Light or electron microscopy combined with specialised staining techniques are commonly used to assess the histological and histochemical changes in myofibres following injurious exercise. In fact, as noted by Brooks, Zebra, & Faulkner (1995), "demonstration of morphological damage to fibres is vital to confirm directly that an injury has occurred" (p. 459). Myofibres are sectioned either transversely or longitudinally in order to ascertain and/ or quantify the extent of damage. At the light microscope level there are certain indicators that individual or groups of myofibres are abnormal following active lengthening exercise. These include fibres that appear more rounded, stain more lightly with hematoxylin and eosin and are swollen, sometimes up to four times their normal size (Lieber, Woodburn, & Friden, 1991). Alterations to A

and I bands (Newham et al., 1983b), clotted fibre lesions (Ogilvie, Armstrong, Baird, & Bottoms, 1988), depleted intracellular glycogen stores (Lieber & Friden, 1999), Z-line alterations (Friden et al., 1981) and mononuclear cell infiltration (McCully & Faulkner, 1985) are evident at both the light and electron microscope level. The greater power of electron microscopy reveals clearer damage to Z-lines as evidenced by streaming, smearing or even total disruption of these structures (Lieber & Friden, 1988).

Various methods have been employed to quantify the magnitude of damage to muscle following exercise-induced injury. These range from manual counting of the number of damaged fibres per field of view (Sacco & Jones, 1992b) to combinations of planimetry and automated counting using image analysers (Faulkner, Jones, & Round, 1989).

Although variations of both of the above methods are frequently used to quantify the extent of myofibre damage, Warren et al. (1999b) argue that “it is technically difficult to perform quantitative histological analyses on an entire muscle at either the light or electron microscopic level” (p. 53). They suggest that establishing clear cut criteria for the presence or extent of a histological abnormality is problematic and that histological measurements are subject to observer and sampling bias.

2.4.3 Imaging techniques.

Other techniques have also been employed to assess the site and extent of damage to exercise-injured muscle and have been used in a number of human studies. These techniques are often utilised due to the difficulty in obtaining large samples of muscle tissue for examination purposes in the human. As the present study involved mice

where whole muscles were available for histological purposes only brief mention of the imaging techniques will be made.

Most imaging techniques such as MRI, CT scans and ultrasound are non-invasive and provide indirect measures of muscle injury. A form of imaging that is invasive is that employing injection of a radioactive substance with a short half-life into the body and using a γ -camera to provide a measure of the spatial distribution of the γ -emitting radionucleotide. Jones et al. (1986) reported that γ -camera images stored as a matrix can be manipulated to quantify the area of damage. McCully, Shellock, Bank, & Posner, (1992) wrote that MRI provides excellent determination of the anatomical location of the sites of injury and allows two-dimensional and even three dimensional images to be constructed. Warren et al. (1999b) noted that few studies have been conducted using the above methods to examine exercise-damaged muscle. Continued research in this area will provide needed information regarding the usefulness of these tools to assess muscle injury.

2.5 Fibre Types Affected During Exercise-Induced Damage

Work with human and other animal models has produced interesting observations in regard to the fibre types damaged during eccentric exercise. Friden, Sjostrom, & Ekblom (1981) noted damage in the predominantly slow-twitch soleus muscle of humans following eccentric down stairs running. In a subsequent human study (1983), the same researchers reported damage to predominantly fast twitch muscle fibres following eccentric exercise cycling for 30 minutes. Type II (fast twitch) fibres were

also found to be preferentially damaged in humans following eccentric exercise involving the calves and forearm flexors (Jones et al., 1986). Following 90 minutes of downhill running in rats, Armstrong et al. (1983) noted that "the deeply located, predominantly slow-twitch muscles were most affected." (p. 80). Schwane et al. (1983) also noted damage to rat slow twitch muscles after 90 minutes of intermittent downhill running. During the above study no predominantly fast-twitch muscles were analysed, therefore, it cannot be ruled out that these were also affected. McCully and Faulkner (1985), using an electrical stimulation protocol, recorded damage to the predominantly fast twitch extensor digitorum longus (EDL) muscle of mice following eccentric contractions. Likewise, Lieber and Friden (1988) reported damage to only the fast twitch fibres following electrical stimulation of the rabbit tibialis anterior (TA) muscle. Sacco et al. (1992a), though, reported damage to both slow and fast twitch TA fibres following eccentric exercise using an electrical stimulation protocol that allowed greater recovery between contractions.

Results from the above studies present differences which have proved difficult to clearly explain. Lieber et al. (1988) suggested that the fast twitch fibres could fatigue early in the exercise period and reach a state of rigor depending upon their ability to regenerate ATP. If muscle fibres in a state of rigor were subjected to subsequent stretch, then it is possible that damage could result. Alternatively, motor unit recruitment patterns may be responsible for the selective damage seen in specific fibre types although glycogen depletion studies of eccentrically exercised muscles do not support this (O'Reilly et al., 1987).

2.6 Effect of Exercise-Induced Damage on Force Production

The effect of damaging exercise has been studied on force production immediately following the damaging bout, and during the ensuing recovery period using both human (Child, Saxton, & Donnelly, 1998; Davies & White, 1981; Friden et al., 1983) and other animal models (Lieber & Friden, 1988; McCully & Faulkner, 1985; Warren et al., 1999a). Few studies have reported changes in dynamic strength, instead, most have chosen to display their data in the form of isometric force changes.

In all of the studies reviewed, P_0 was significantly reduced immediately following the damaging exercise. Decrements in P_0 of up to 78% have been reported following active lengthening exercise (McCully & Faulkner, 1985). The nadir in P_0 has been shown to occur from immediately following the damaging exercise in some studies (Faulkner et al., 1989; Jones et al., 1989; Lowe, Warren, Ingalls, Boorstein, & Armstrong, 1995) to around three days (Brooks & Faulkner, 1990; Brown, Child, & Donnelly, 1997b; McCully & Faulkner, 1985; Sacco et al., 1992) in others. The recovery time course for P_0 has varied between the studies with some showing near full recovery in as little as 24 hours (Newham, Mills, Quigley, & Edwards, 1983c), with others taking between two weeks to over a month (Faulkner et al., 1989; McCully & Faulkner, 1985; Newham et al., 1987). The discrepancy in P_0 recovery may be due to the type of damaging exercise undertaken as those involving stepping usually recovered much more rapidly than those employing maximal eccentric contractions performed on isokinetic dynamometers or resistance training type apparatus (Brown et al., 1997b; Clarkson & Tremblay, 1988;

Davies & White, 1981; Faulkner et al., 1989; Newham et al., 1983c; Nosaka, Clarkson, McGuiggin, & Byrne, 1991).

Few studies have evaluated the effect of active lengthening exercise on subsequent measures of dynamic force. Those that have focused on this parameter have recorded significant decrements in maximum torque. Thus, Friden et al. (1983) had subjects perform eccentric bicycle exercise for 30 min at between 80 to 100 percent of maximal oxygen uptake. Prior to, shortly after, three days, and six days following exercise maximal voluntary knee extension torque was determined via an isokinetic dynamometer with pre-set angular velocities of 90, 180, and 300 degrees per second. Strength at all of the angular velocities decreased soon after exercise, gradually returning over the subsequent days. Crenshaw, Thornell, & Friden (1994) recorded an average decline in maximum concentric torque of approximately 20% in their subjects following one legged active lengthening exercise to exhaustion using a Biodex seated isokinetic dynamometer. Eston, Finney, Baker, & Baltzopoulos (1996) had subjects perform a downhill run consisting of five bouts of eight minutes at a gradient of -10% and a speed that corresponded to 80% of their maximal heart rate. Prior to, immediately after and at two, four and seven days following the downhill run maximal concentric and eccentric torque were measured at slow and fast angular velocities. Immediately following the damaging run decrements of between 10 and 23% were measured in maximal torque for both eccentric and concentric modes and at both angular velocities. With the exception of the slow velocity eccentric torque there were similar decrements

at two days following the run with all dynamic forces slowly returning to baseline by day seven.

2.7 Effect of Exercise-Induced Damage on Force-Frequency Responses

One way of assessing the contractile properties of muscle following exercise is to study the relationship between force and the frequency of electrical stimulation applied either directly to the motor nerve or percutaneously over the surface of the muscle(s) in question. By electrically stimulating a muscle at selected incremental frequencies and measuring the resulting isometric force production a chart can be constructed illustrating individual or group average relationships between the variables. When the force-frequency chart is presented in the form of a line chart it usually takes on a sigmoidal appearance and is referred to as a force-frequency curve.

Following active lengthening exercise numerous studies have reported that the force-frequency curve shifts in relation to one plotted prior to exercise (Davies & White, 1981; Faulkner et al., 1989; Jones et al., 1989; McCully & Faulkner, 1985; Sacco et al., 1992). Both high and low frequency forces are usually lower than those obtained for the same muscle prior to damaging exercise with the decrement in stimulated force being more pronounced at the lower stimulation frequencies (Davies & White, 1981; Ebbeling & Clarkson, 1989; Newham et al., 1987). The force-frequency curve is usually shifted to the right of the original curve for at least the first few days following active lengthening exercise (Faulkner et al., 1989; McCully & Faulkner, 1985; Sacco et al., 1992). The exact cause of this phenomenon is not known although there have been

suggestions that it may in part be due to a failure site in the excitation-contraction (E-C) coupling pathway located between the T-tubular voltage sensor and the sarcoplasmic reticulum calcium release channel (Warren et al., 1999a).

In a study by Faulkner et al. (1989) the force-frequency curve was shown to shift upward and to the left of the original curve at five days following damaging exercise giving increased relative forces at low frequencies of stimulation. However, this response at five days following active lengthening exercise was not observed in a study by Warren et al. (1999a) who reported that the curve was still shifted downward and to the right at this time. In fact in their study the curve was still below and to the right of the baseline at 14 days following active lengthening.

As mentioned above, following damaging exercise the relative decrement in stimulated force is usually greater at lower frequencies of stimulation. This phenomenon is referred to as low frequency fatigue. A ratio of force production, determined by dividing the force produced at a low frequency into that produced at a higher frequency, is normally used to report the decrement associated with LFF. Both the low and high stimulation frequencies have varied between studies with lower frequencies of one, 10 or 20 Hz, being used in both human and other animal studies and higher frequencies of 50 to 100 Hz being employed in human studies and up to 400 Hz in other animal models (Newham et al., 1987; Newham et al., 1983c; Sacco et al., 1992; Sargeant & Dolan, 1987). The resulting ratios commonly reported are 1/ 150, 20/ 50, 20/ 100, 40/ 100 (Brown, Child, Day, & Donnelly, 1997a; Sacco et al., 1992; Sargeant & Dolan, 1987).

Although the exact mechanism causing low frequency fatigue remains unclear, it is thought to be the result of damage caused by high forces in the active muscle fibres (Jones & Round, 1990; McCully & Faulkner, 1985). These high forces may affect some of the dihydropyridine (DHP) channels in the T-tubules and ryanodine receptors in the sarcoplasmic reticulum resulting in a lower calcium release with each action potential as has been suggested to occur in exercise of a fatiguing nature (Fitts, Balog, & Thompson, 1991; Jones, 1981).

The time course of recovery from low frequency fatigue seems to be dependant upon the type of exercise undertaken. Thus, recovery from running and uphill walking has been found to occur within two hours (Davies & White, 1981), whereas, with active lengthening type exercise it has taken from 24 hours to two weeks or longer (Jones et al., 1989; Newham et al., 1987; Newham et al., 1983c). In addition, local muscular eccentric exercise (i.e., dynamometry or weight training) appears to require longer periods for complete recovery as compared to box stepping (Brown et al., 1997a; Jones et al., 1989; Newham et al., 1987; Newham et al., 1983c). This may be due, in part, to the intensity of the exercise as most eccentric dynamometry work has employed maximal voluntary contractions (Brown et al., 1997b).

2.8 Correlation Between Force Loss and Magnitude of Histological Muscle Damage

Limited research has been conducted in correlating measures of force following active lengthening exercise with the magnitude of injury to the muscle. Warren et al. (1999b) reviewed seven studies that quantified histological abnormalities in humans and 18 that

used other animal models. The findings of a few of these studies led them to suggest that histological damage correlated poorly with functional measurements (i.e., P_o) both temporally and in terms of magnitude for both human and other animal models. They argued that the maximum decrement in P_o occurred immediately following active lengthening exercise whereas the greatest number of abnormal fibres were evident two to four days following injury at a point when large numbers of inflammatory cells have invaded the tissue. Some studies, however, have shown decrements in P_o to correspond with the magnitude of myofibre damage. McCully & Faulkner (1985) and Sacco et al. (1992) found P_o at 3 days following active lengthening exercise in mice to correspond with the magnitude of myofibre damage at the same time point. Following damaging exercise in rabbits, Lieber, Schmitz, Mishra, & Friden (1994) determined that a significant negative correlation existed between P_o and myofibre damage as measured by the percent of desmin negative fibres. Further studies looking at the relationship between P_o at varying time periods following active lengthening exercise and the magnitude of myofibre damage will aid in establishing if predicting the magnitude of damage from collected P_o values is possible.

To date no studies have attempted to correlate changes in the 1/ 150 Hz ratio following active lengthening exercise with the magnitude of histological myofibre damage. As with P_o , if significant correlations between the 1/ 150 Hz ratio and muscle damage were shown to exist this could be used to predict the magnitude of histological damage to myofibres.

Chapter Three

Method

3.1 Experimental Animals

Fifty six female mice (C57 BL/ 10 strain) aged 18-22 weeks were obtained from the Animal Resource Centre at Murdoch University and were housed in accordance with the Australian Code for the Care and Use of Animals for Scientific Purposes. Food and water were available ad libitum.

3.2 Design

The study population consisted of two randomly assigned groups of 28 mice. A 2x7 factorial design was used to investigate the effect of the manipulation of two independent variables on three dependent variables. The independent variables were a) type of treatment (active or passive lengthening), and b) recovery times following active or passive lengthening. The dependent variables were a) normalized P_o , b) 1/ 150 Hz ratio, and c) the damage index.

Type of treatment	Recovery Interval						
	0	10 min	3 days	6 days	10 days	15 days	20 days
Active lengthening	X	X	XY	XY	XY	XY	XY
Passive lengthening	X	X	X	X	X	X	X

X = P_o and 1/ 150 Hz ratio.

Y = damage index.

3.3 Procedure

3.3.1 Preparation of animals.

Animals were anaesthetised with 25% pentobarbitone sodium (0.03 ml per gram body weight) injected into the peritoneal cavity and the right knee was clamped to restrict movement of the lower limb. An incision was made laterally and slightly below the level of the patella, the peroneal nerve exposed and a hook electrode positioned around the nerve. The animal's foot was taped to a foot plate containing a force transducer which was directly attached to the axle of an Oriental Motor Co T.T.D. Vexta stepping motor (Fig 1). The foot was positioned so that rotation of the axle caused it to move through approximately 100° from a dorsiflexed to plantarflexed position in 300 milliseconds (ms) [Fig 2]. The contraction velocity and range of motion of the stepping motor were controlled via an Oregon Micro Systems Inc MH10 Microstep Drive (10 μ steps/ step) which received its instructions from a batch program containing stepper motor commands run through a digital to analogue board housed in an IBM 286 personal computer (Fig 1).

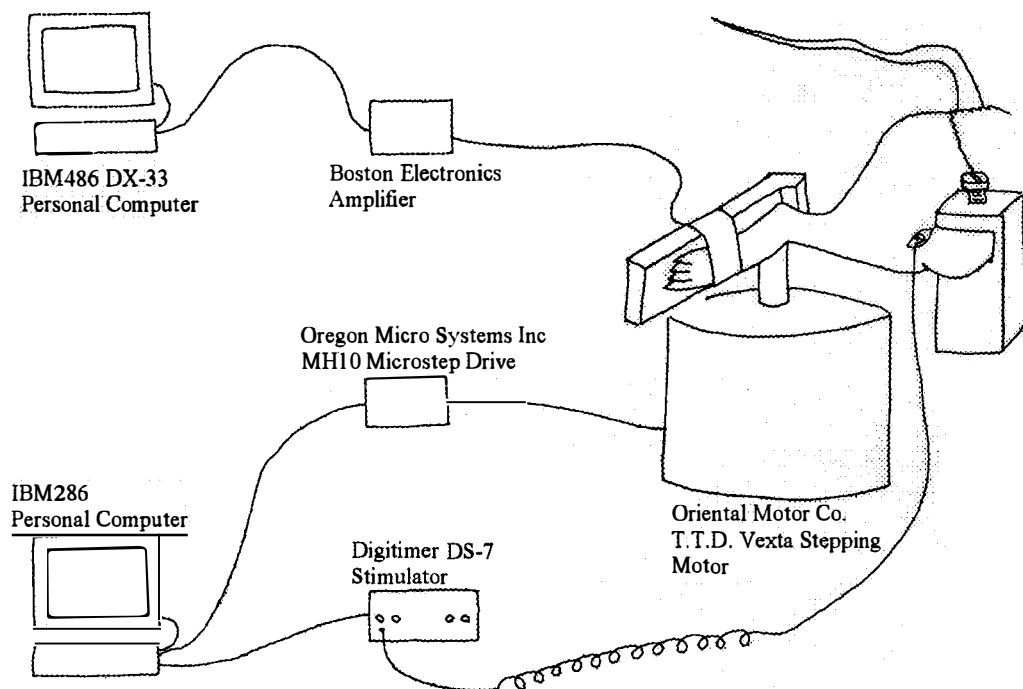


Figure 1. Apparatus used for stimulating, lengthening, and recording force production from dorsiflexor muscles.

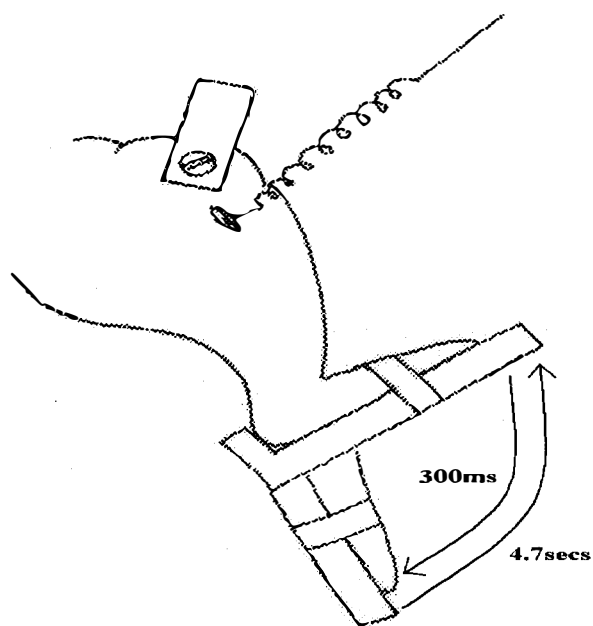


Figure 2. Time taken to complete plantar and dorsiflexion components of cycle.

During active lengthening the peroneal nerve was stimulated using a Digitimer DS-7 electrical stimulator for 310 ms at 100 Hz using 100 μ s square wave pulses. Voltage was fixed at 200 V and the optimal amperage of 0.3 A was determined, in previous pilot work, by increasing from zero until maximum force production of the dorsiflexor muscles was recorded at the foot plate in the absence of any noticeable co-contraction of the plantarflexor muscles or drop in P_o with increasing amperage. The stimulation commenced 10 ms prior to and throughout muscle lengthening, the return phase being entirely passive. The duration of the entire cycle, from a fully flexed starting position to a fully flexed final position was approximately five seconds (Fig 2). The electrical stimulator was operated by separate commands programmed into the batch program that controlled the stepping motor.

Depending upon the treatment group the lengthening phase was either active or passive (i.e., stimulated or non-stimulated) and was repeated every 5 seconds for five sets of twenty four repetitions (total of 120 repetitions). Following the lengthening protocol (active or passive) the electrode was removed, the incision area was sutured and cleaned, and the animals were allowed to recover.

3.3.2 Division of animals into groups.

Prior to active or passive lengthening the animals were randomly assigned to two groups. Each group was then further divided into five subgroups of four to eight mice each. Animals in group 1 performed 5 sets of 24 maximal eccentric contractions (active lengthening), each set separated by a one minute interval to allow for P_o to be recorded.

P_0 was determined as the peak force generated during a short (~ one second) stimulation of the dorsiflexor muscles (via the peroneal nerve) at 150 Hz.

Group 2 consisted of animals that underwent operations followed by passive lengthening. The operations were performed and the electrode was positioned around the peroneal nerve. Each animal then performed 120 plantar and dorsi flexion movements in an identical fashion to those of Group 1 with the exception that no stimulation was applied to the animals during these movements. This essentially constituted passive movements which resulted in no eccentric work being performed by this group.

3.3.3 Muscle function.

Force output was determined via a force transducer mounted into a foot plate which was attached to the axle of a stepping motor. Analogue signals from the force transducer were amplified and sent for processing to an analogue to digital converter, located in an IBM 486DX-33 personal computer (Fig 1). Converted digital signals were captured by a software program (Waveview – 1993, Eagle Appliances Pty LTD) at 1000 data points per second (1000 Hz) and displayed visually in a format which allowed measurement of the captured forces. The foot was positioned at a fixed angle of 90 degrees to the foreleg and the dorsiflexor muscles were stimulated via the peroneal nerve. Previous pilot work examining force-angle relationships at 10 degree intervals from 70 to 100 degrees revealed that a fixed foot angle of 90 degrees to the foreleg produced a force of the greatest magnitude.

3.3.4 Maximum isometric force (P_o).

Based on data from preliminary studies, 150 Hz was chosen as the frequency of stimulation for determination of P_o . No further increases in P_o were obtained when stimulation frequencies above 150 Hz were employed.

The P_o at 150 Hz was collected immediately prior to exercise, following each bout of 24 lengthening movements, immediately following the 120th lengthening movement, ten minutes following the final lengthening movement, and on the day the animal was sacrificed (i.e., day 3, 6, 10, 15, or 20). The muscle was stimulated for approximately one second at 150 Hz allowing time for maximum force production to occur.

3.3.5 Force frequency responses.

In order to collect force frequency responses the peroneal nerve was stimulated for approximately one second at frequencies of 1, 10, 20, 50, 80, 100 and 150 Hz utilising identical voltages and amperages employed during the stimulated (active) lengthening protocol (Fig 3). A ten-second interval was provided between successive frequencies to allow for muscle recovery. Force frequency responses were collected immediately prior to exercise, immediately following the 120th lengthening repetition, ten minutes post exercise, and on the day the animal was sacrificed. From the force frequency data

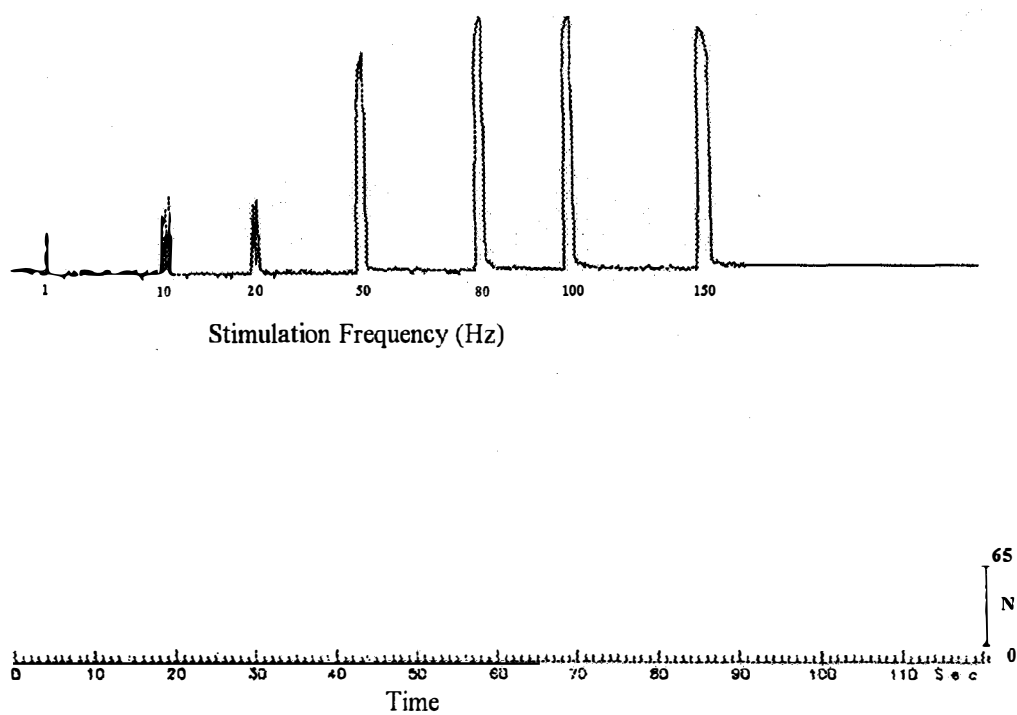


Figure 3. Force responses in Newtons (N) from mouse dorsiflexor muscles stimulated at increasing frequencies (Hz).

collected the force at one Hz was divided into the force associated with 150 Hz to obtain the 1/ 150 Hz ratio.

3.3.6 Histological preparation and analysis.

Mice were terminated with a lethal intraperitoneal dose of pentobarbital sodium. Treatment and contralateral TA muscles were carefully dissected out and a portion was separated from the belly of the muscles, mounted adjacent to and in contact with each other on a corkboard with the base of the muscles surrounded by Optimum Cutting Temperature (OCT) fluid, and frozen in isopentane cooled in liquid nitrogen. The muscle blocks were oriented in such a way that the long axis of the muscle fibres were lying perpendicular to the base of the cork board allowing transverse sections to be separated in a cryostat.

Eight μm transverse sections were separated from all muscle blocks in a Tissue Tek cryostat at minus 20°C, fixed in 10% formalin and stained with haematoxylin and eosin. The stained tissue samples were viewed via microscopy under x40 magnification, and histological data were gathered on every second field of each muscle section. Ten fields were counted for all treatment and contralateral muscle sections using a Leitz Wetzlar 42 point graticule. The total number of myofibres and central nuclei per field of view were recorded as were the number of points falling on myoplasm, mononuclear cells or “other structures” (“other structures” being defined as those intracellular components other than myoplasm or mononuclear cells).

3.4 Calibration of force transducer

To determine the relationship between load applied to the foot plate (in grams) and the resulting force signal (in millivolts), known weights were hung from a marked point on the foot plate. Five weights of increasing mass were weighed on a microbalance (Mettler TM 4600) and then hung one at a time, from smallest to largest, from the foot plate. A short interval of approximately five seconds was allowed between removing one known mass and applying the next to allow the foot plate to return to a non-moving state. The process was then repeated in the reverse order with weights being hung from largest to smallest. The resulting analogue signals were amplified, relayed to an analogue to digital board housed in an IBM 486 DX-33 personal computer and subsequently captured in a software viewing program (Waveview) at a sampling rate of one kHz. The load values, in millivolts, were manually read from the Waveview program via a cross-hair cursor and plotted against the corresponding loads in grams (Fig 4). Two-tailed Pearson product moment correlations were used to test for any relationship between the variables. Correlation coefficients of 0.99 revealed almost perfect linear relationships between the load in millivolts and grams for small to large and reverse order protocols ($p < 0.05$).

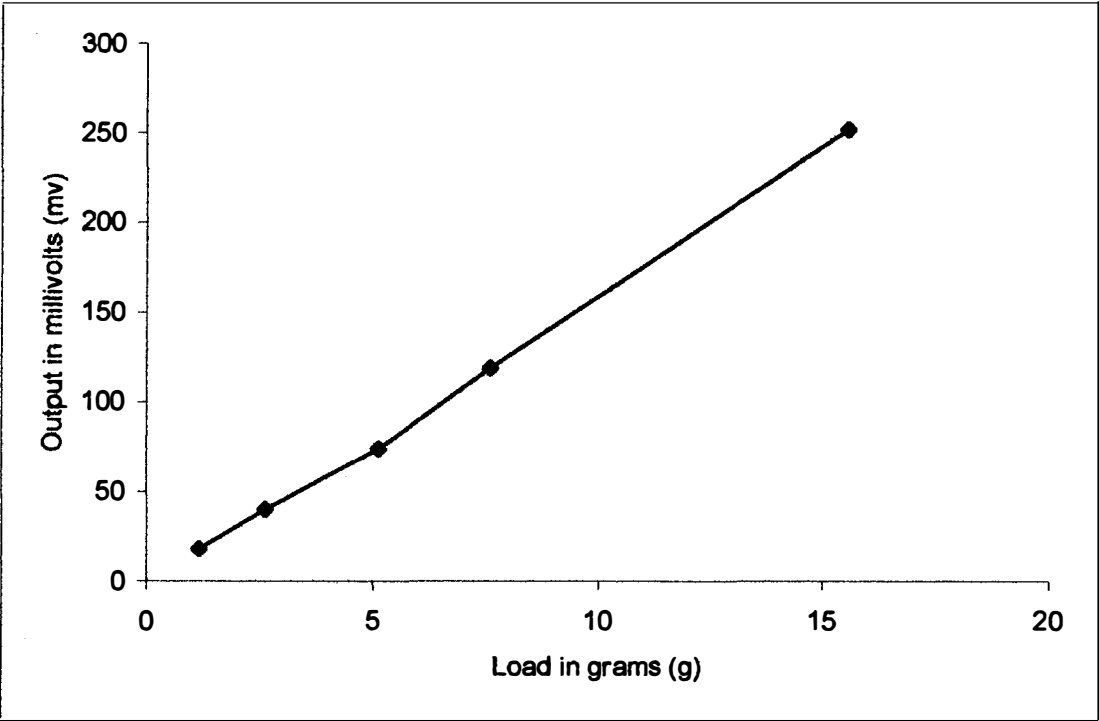


Figure 4. Calibration figures for the strain gauge showing load in grams versus output in millivolts. Two-tailed $r = 0.99$ ($p < 0.05$); $n = 5$.

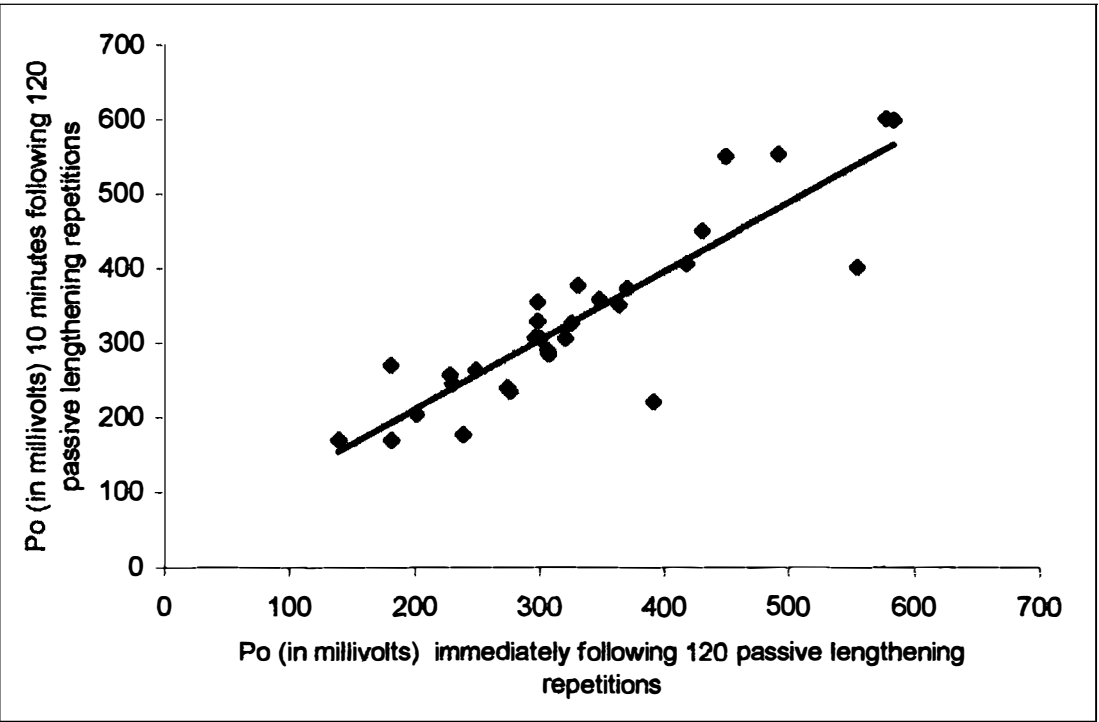


Figure 5. Scatterplot of reproducibility showing P_o immediately following 120 passive lengthening repetitions versus 10 minutes later. Two-tailed $r = 0.90$ ($p < 0.05$); $n = 30$.

3.5 Reproducibility of force measurements

In order to determine the reproducibility of the force transducer housed in the foot plate the known weights were applied to the foot plate in an identical manner on two separate occasions (trial one and two). A two-tailed Pearson product moment correlation revealed an 'r' of 0.99 between the two trials ($p < 0.05$).

3.6 Reproducibility of P_o following lengthening

Reproducibility of P_o in passively lengthened dorsiflexor muscles was determined in order to assess the reliability of force measures and to determine whether the operative procedure itself caused a decrement in P_o .

Reproducibility was determined using two measures of P_o for all passively lengthened mice. The first P_o measure was obtained immediately following passive lengthening and the second measure was obtained ten minutes later. The hook electrode was removed from the peroneal nerve following the first measure and re-positioned immediately prior to the second. Figure 5 shows that there was a significant correlation of 0.90 ($p < 0.05$) between the two groups of measures demonstrating a strong positive linear relationship (Ellery & Strickland, 1997). A coefficient of variation for the repeated measurements of 11.3% was calculated. Taken together with the significant positive correlation coefficient these data suggest good stability of P_o in non actively lengthened dorsiflexor muscles following the operative procedure.

3.7 Analysis of Results

All statistical analyses were carried out using the software program SPSS for windows release 7.5. Scatterplots, column graphs and linear regression lines were prepared using Microsoft Excel 97. Independent samples t-Tests were used to test hypothesis one, three, four and five. The chosen alpha level of 0.05 was adjusted for each hypothesis tested to account for the number of t-Tests performed. This was accomplished by dividing 0.05 by the number of t-Tests required for the specific hypothesis being assessed. One-way ANOVAs with Bonferroni post-hoc comparisons were used to test for significant differences within the passive and active groups 1) during the course of lengthening (i.e., pre-lengthening to 10 minutes post lengthening) and 2) over the recovery days. The alpha level was set at 0.05 for all one way ANOVAs. Hypothesis two was tested using appropriate post-hoc results from the one way ANOVA of the active group during the course of lengthening. Hypotheses six, seven and eight were tested using one-tailed Pearson product moment correlations with two-tailed Pearson product moment correlations being employed to test hypotheses nine and ten. The choice of two tailed tests for hypotheses nine and ten was due to the uncertainty of a) whether there would be significant correlations between the variables and b) whether any significant correlations, if evident, would be positive or negative. Significance was set at an alpha level of 0.05 for all correlation analysis. All significant t-Test, ANOVA and correlation results are displayed in appendix A, B and C, respectively.

Chapter Four

Results

4.1 Muscle Force and Contractile Properties

4.1.1 Short-term changes in P_o

Figure 6 shows the mean P_o of the foot dorsiflexion muscles, normalized to the pre-exercise value, during and 10 minutes following active and passive lengthening. During the active lengthening protocol normalized P_o declined significantly, being 55.3% (± 2.8) of the baseline value immediately following the 120th repetition ($p < 0.05$). The normalized P_o decline was greatest over the first 48 active lengthening repetitions (70% of baseline) with only a 15% decline occurring thereafter (Fig 6). Following 10 minutes of passive recovery there were no significant changes in normalized P_o . Mean P_o of the passively lengthened group was elevated above the pre-exercise value at the testing points immediately following 24, 48, 72, and 96 repetitions (Fig 6; $p < 0.05$).

It can be seen from figure 6 that following each bout of 24 active lengthening repetitions normalized P_o was significantly lower than that of the passive group ($p < 0.008$). The difference in maximum isometric force between the active and

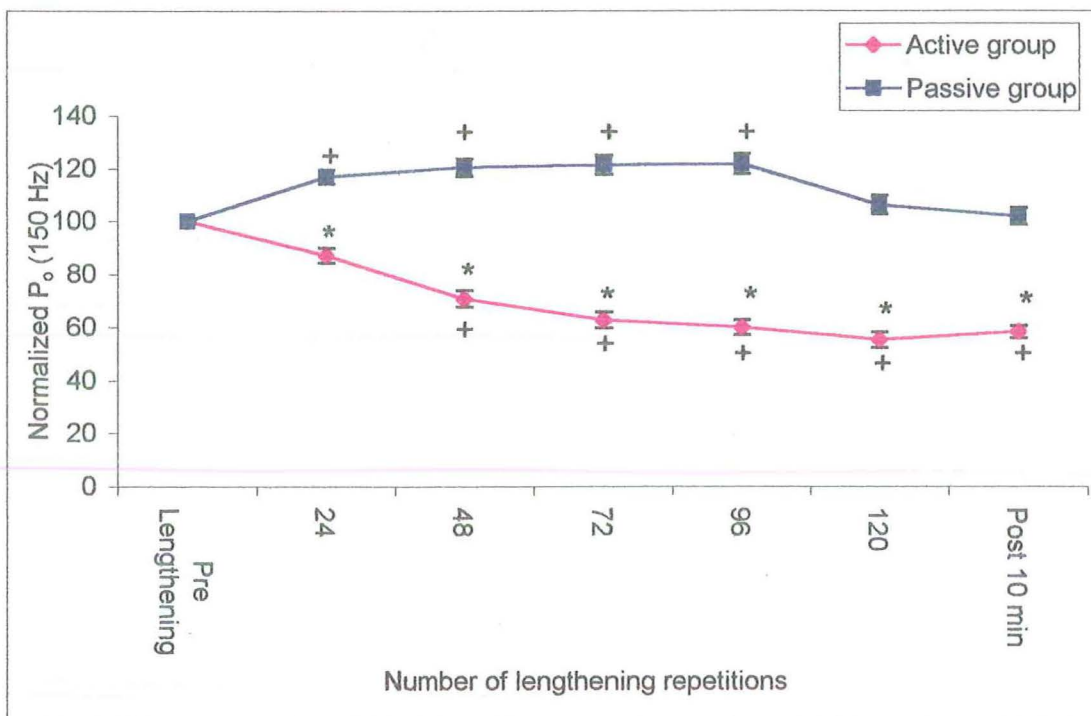


Figure 6. Normalised P_0 (% of pre) for active and passively lengthened dorsiflexor muscles of mice over 120 repetitions and after 10 minutes of recovery (\pm S.E.M). * Mean values are significantly different from passive ($p < 0.008$). + Mean values significantly different from pre lengthening value ($p < 0.05$); for each mean $n = 4-8$.

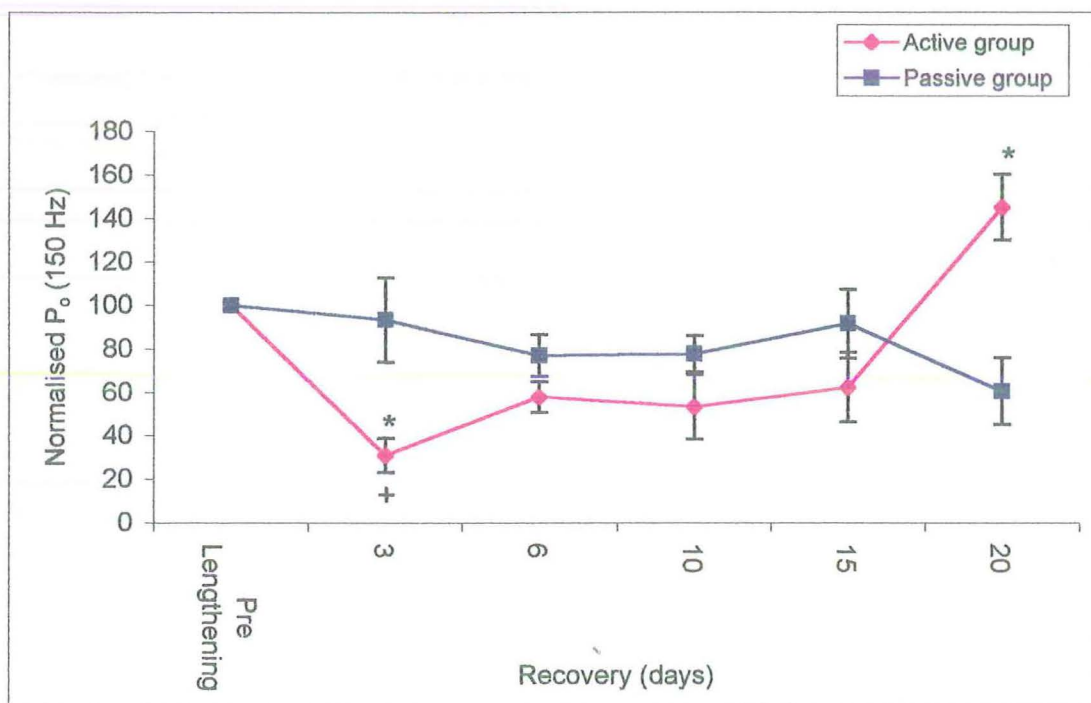


Figure 7. Normalised P_0 (% of subgroup pre lengthening) for active and passively lengthened dorsiflexor muscles of mice at varying times pre and post lengthening (\pm S.E.M). * Mean values are significantly different from passive ($p < 0.01$). + Mean values significantly different from subgroup's pre lengthening value ($p < 0.05$); for each mean $n = 4-8$.

passively lengthened groups progressively increased following each of the first four bouts of 24 lengthening repetitions up to the 96th repetition.

4.1.2 Normalized P_o on the days following muscle lengthening.

Figure 7 illustrates the time course of normalized P_o during 20 days of recovery from active or passive lengthening. The P_o of each sub-group was normalized to the mean P_o of all animals in its main group (i.e., active or passive) at the pre-exercise point. Normalized P_o declined following active lengthening and remained below baseline levels for 15 days. This difference proved statistically significant only for day 3, where normalized P_o was on average $31 \pm 8\%$ of the pre-exercise level ($p < 0.05$). Subsequent to day 3 there was a trend for normalized P_o to return toward pre-exercise levels (Fig 7). Twenty days after active lengthening, P_o was $145 \pm 15\%$ greater than the pre-exercise level although this difference was not statistically significant. Normalized P_o of the actively lengthened sub-groups were significantly different from the passive sub-groups at three and 20 days following lengthening (Fig 7; $p < 0.01$).

4.1.3 Twitch/ tetanus force ratio (1/ 150 Hz).

The 1/ 150 Hz force ratios for the active and passively lengthened groups are shown in Figure 8. The ratio decreased in both groups 10 minutes following 120 lengthening repetitions with the active group decreasing significantly compared to the passive ($p<0.025$). By day 3 of recovery 1/ 150 Hz ratios recovered substantially in both groups, approaching pre-lengthening ratios. Both groups ratios decreased again at day 6, although to a lesser extent than at 10 minutes following lengthening, with the difference between groups being significant ($p<0.025$). The 1/ 150 Hz ratio of the actively lengthened group recovered to baseline by day 10 and varied little over the rest of recovery. The passively lengthened group's 1/ 150 Hz ratio also approximated the pre lengthening value by day 10, and remained so for the rest of recovery. On days 10 through 20 following lengthening there were no significant differences between the two treatment groups.

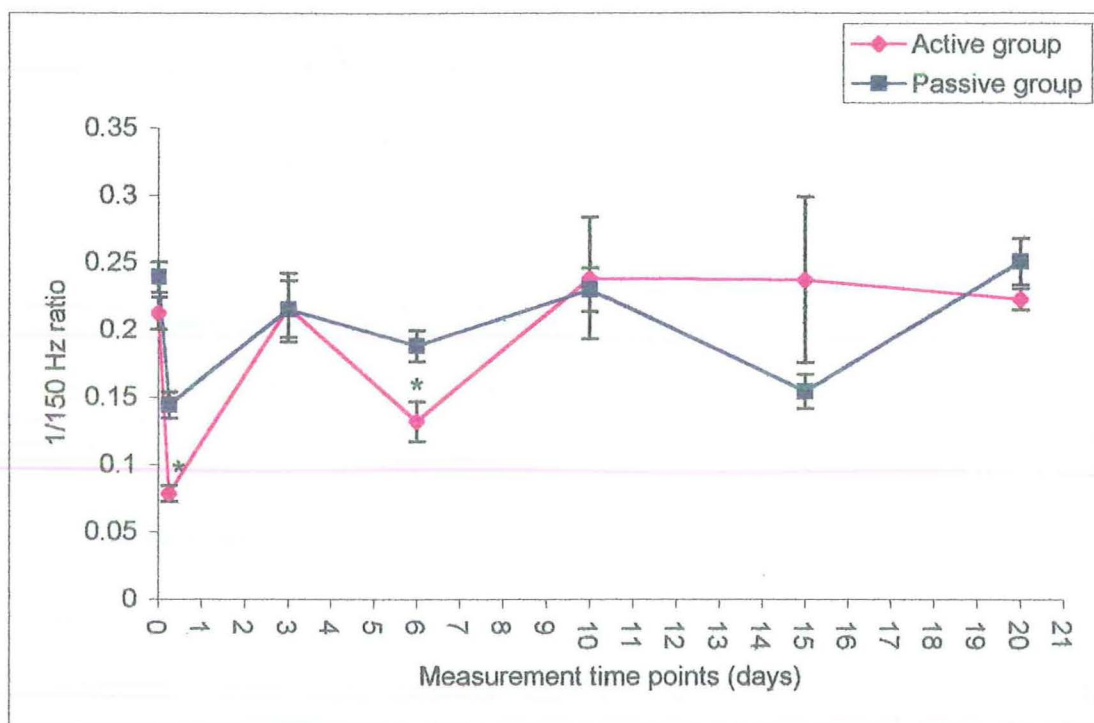


Figure 8. 1/ 150 Hz ratio for active and passively lengthened dorsiflexor muscles of mice pre, and at varying times post lengthening (\pm S.E.M). * Mean values are significantly different from passive ($p < 0.025$); for each mean $n = 4-8$.

4.2 Histology

Histological examination of the TA muscles at 3 days post-exercise showed marked mononuclear cell infiltration for those actively lengthened (Fig 9a) as compared to contralateral muscles (Fig 9b). In order to compare the extent of histological disruption to different muscles across time the average number of mononuclear cells + the average number of central nuclei (damage index) for both TA muscles were compared. Pilot work revealed that the damage index of the non-lengthened contralateral muscles did not significantly differ from the passively lengthened muscles, therefore the contralateral muscles were used in subsequent histological comparisons. This allowed mice to act as their own age matched controls.

Employing the quantitative damage index measure revealed that the actively lengthened TA muscles had significantly higher values at days 3, 6, 10 and 15 following lengthening (Fig 10; $p < 0.01$). Figure 10 also shows the damage index for the contralateral TA muscles. The histology of these muscle sections were unremarkable in respect to evidence of myofibre injury with the damage index remaining low and stable over all recovery days. In contrast, the damage index of the actively lengthened TA muscles steadily increased from days 3 through 10, decreasing again at day 15 and further at day 20, to the extent that there was no significant difference between actively lengthened and contralateral muscles by day 20 (Fig 10).

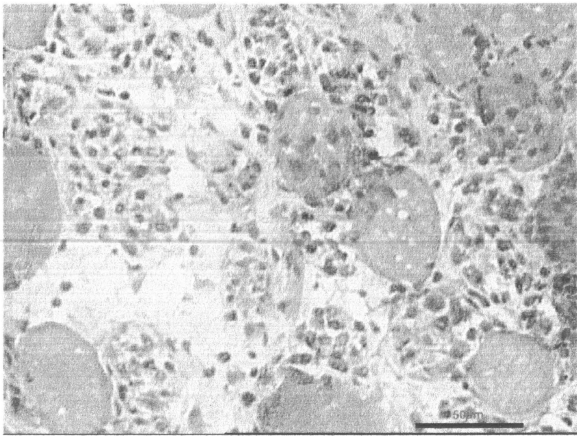
Figure 9c and d show pictomicrographs of actively lengthened and contralateral TA muscle at 6 days following lengthening. It can be seen from these micrographs that there were large numbers of infiltrating mononuclear cells and several myofibres

containing what appear to be centrally located nuclei in the actively lengthened TA muscle. In contrast the contralateral muscle lacks any evidence of damage (Fig 9d). By day 15 histological examination revealed minimal infiltrating mononuclear cells in actively lengthened TA muscles (Fig 9e) plus a greater presence of myofibres with centrally located nuclei. In comparison, contralateral muscles were unremarkable in respect to evidence of damage (Fig 9f).

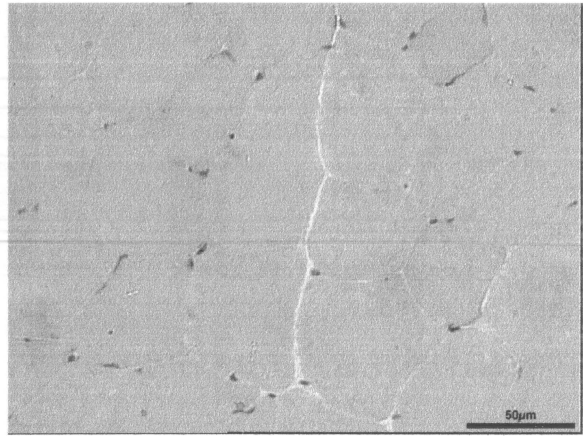
Figure 11 shows that when the average number of mononuclear cells and the average number of central nuclei were considered separately, there were more of the former in the actively lengthened muscles compared to the contralateral muscles at days 3, 6 and 10 following lengthening ($p < 0.01$). The average number of mononuclear cells peaked at day 6, following which there was a trend to decrease through to day 20. The average number of central nuclei per field of view were significantly greater for the active lengthened muscles for days 6, 10 and 15 following lengthening. The trend for the central nuclei was different to that of the mononuclear cells, increasing with each day to peak at day 10, and decreasing through to day 20.

Actively Lengthened TA

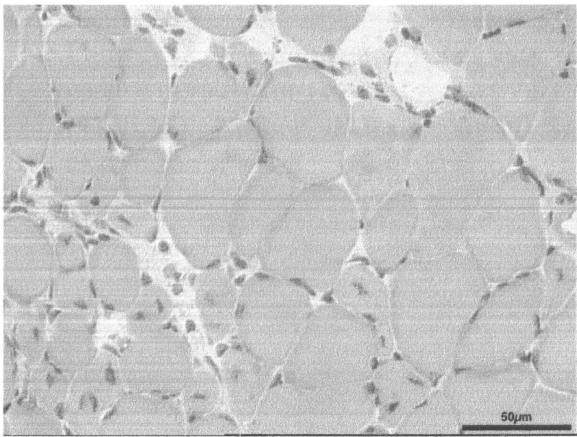
Contralateral TA



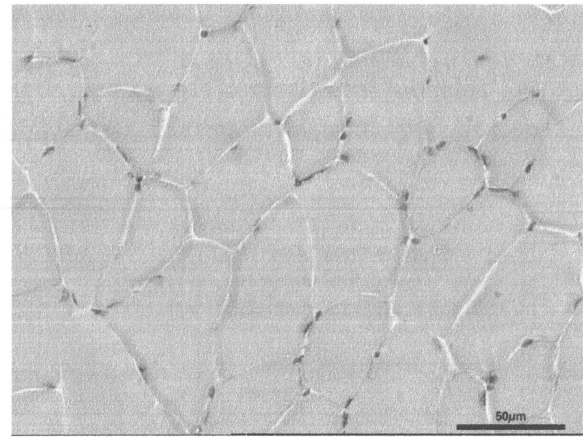
a



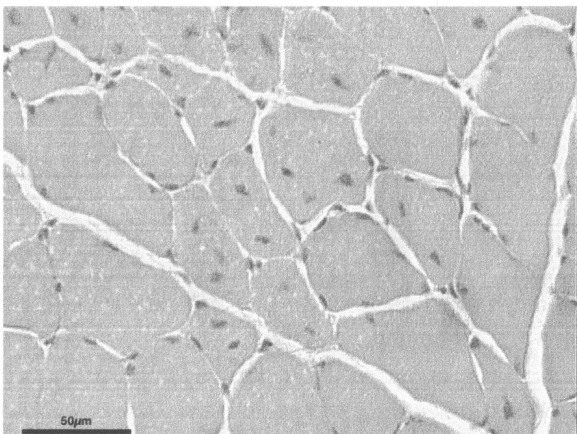
b



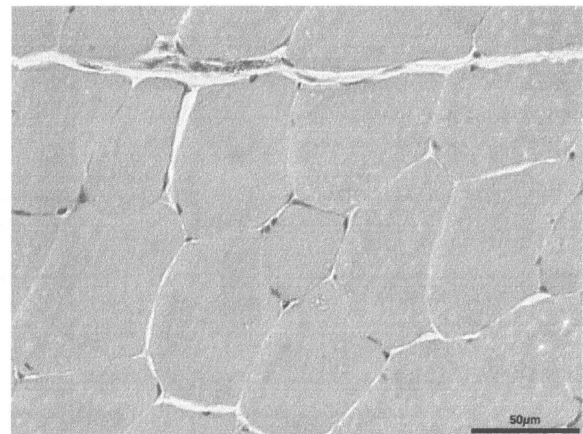
c



d



e



f

Fig. 9. Histological appearance of actively lengthened mouse TA muscle 3 (a), 6 (c) and 15 (e) days following 120 lengthening repetitions. Contralateral TA muscle for the same time periods are shown in b, d and f.

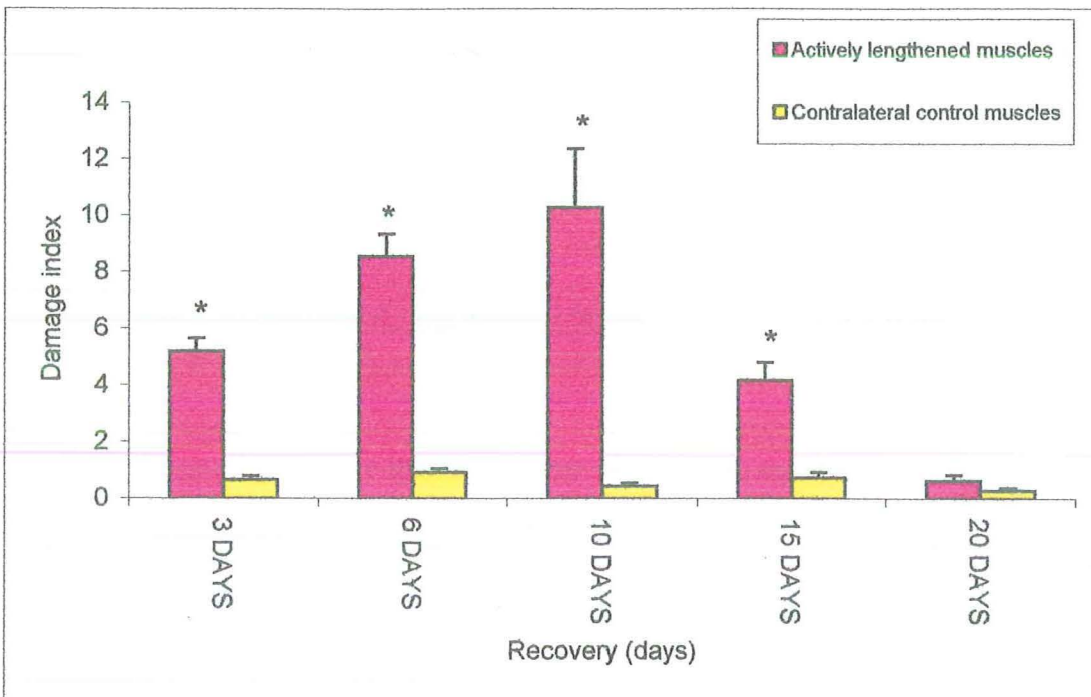


Figure 10. The damage index at varying days following 120 lengthening repetitions of mouse dorsiflexor muscles for actively lengthened versus contralateral muscles (\pm S.E.M). * Mean values are significantly different from contralateral ($p < 0.01$); for each mean $n = 4-8$.

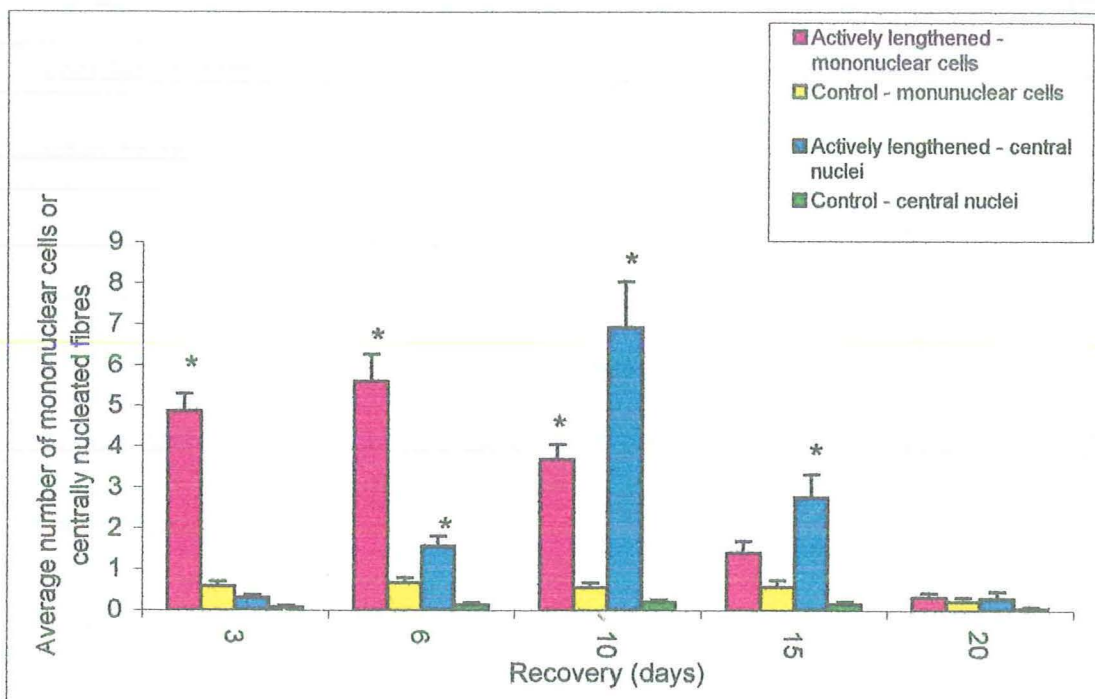


Figure 11. The average number of mononuclear cells and central nuclei per field of view at varying days following 120 lengthening repetitions of mouse dorsiflexor muscles for actively lengthened versus contralateral muscles (\pm S.E.M). * Mean values are significantly different from contralateral ($p < 0.01$); for each mean $n = 4-8$.

4.3 Force and Contractile Properties Versus Damage Index

4.3.1 Normalized P_o and damage index.

Figure 12 displays the relationship between normalized P_o 10 minutes following active lengthening and the damage index of actively lengthened TA muscles. A one-tailed correlation coefficient of -0.20 ($n = 32$) indicated that no significant relationship exists between the variables.

In order to gauge whether the average normalized P_o over the entire 120 active lengthening repetitions would be a predictor of the magnitude of histological myofibre disruption this parameter was plotted against the damage index (Fig 13). A one-tailed correlation coefficient of -0.19 ($n = 32$) suggested no significant relationship between these variables.

When the normalized P_o at varying days following active lengthening was plotted against the damage index (Fig 14) a significant negative relationship between the variables was evident in the direction predicted (one tailed $r = -0.35$; $n = 27$; $p < 0.05$). As the damage index increased there was a trend for normalized P_o at varying days to decrease.

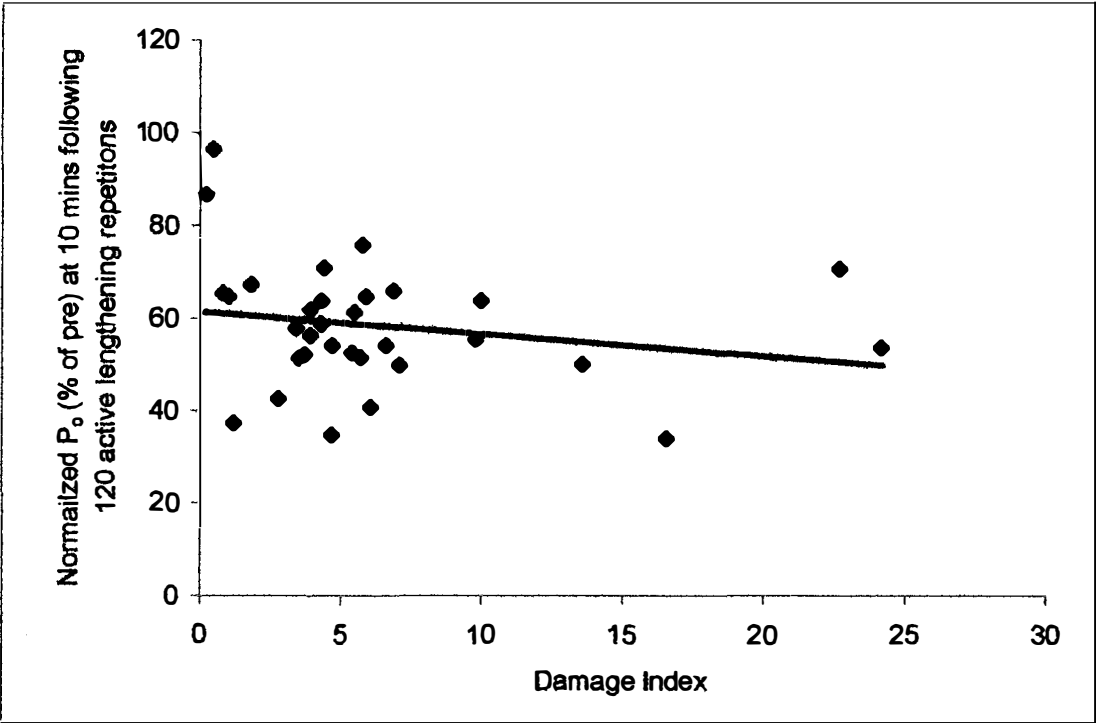


Figure 12. Scatterplot showing normalized P_o (150 Hz) at 10 minutes following 120 active lengthening repetitions versus the damage index. $r = -0.20$ (n.s).

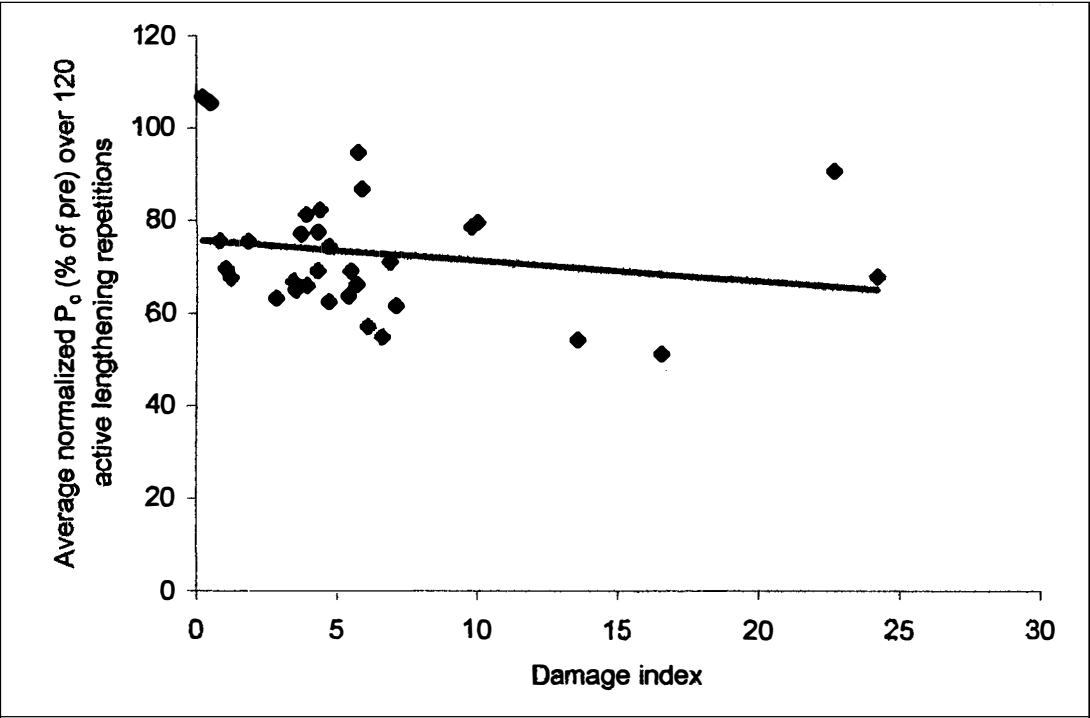


Figure 13. Scatterplot showing average normalized P_o (150 Hz) over 120 active lengthening repetitions versus the damage index. $r = -0.19$ (n.s).

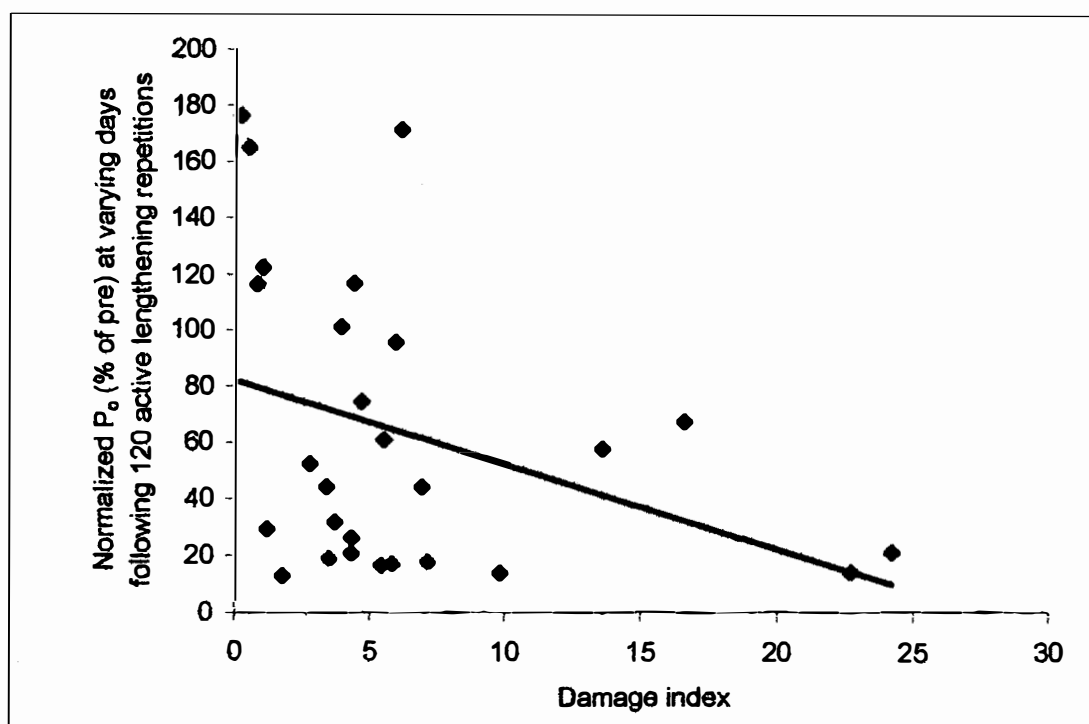


Figure 14. Scatterplot showing normalized P₀ (150 Hz) at varying days post 120 active lengthening repetitions of mice dorsiflexor muscles versus the damage index. One tailed $r = -0.35$ ($p < 0.05$).

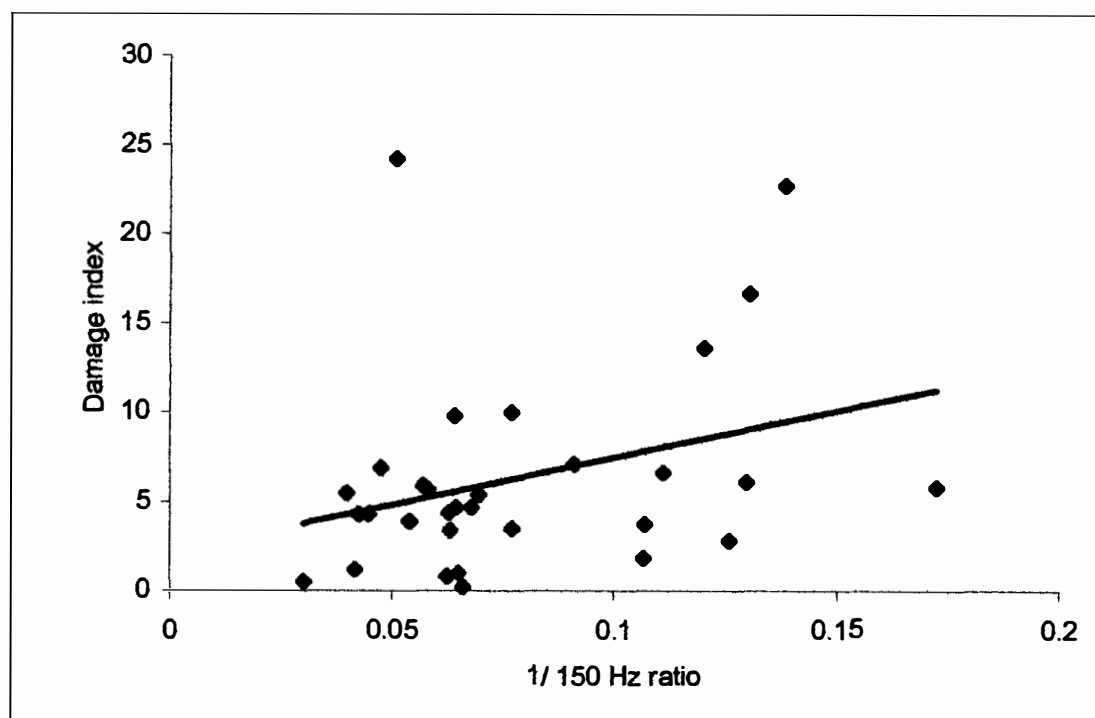


Figure 15. Scatterplot showing the damage index versus the 1/ 150 Hz ratio 10 minutes following 120 active lengthening repetitions of mice dorsiflexor muscles. $r = 0.32$ (n.s).

4.3.2 1/ 150 Hz ratio and damage index.

Two tailed correlations were employed to determine if there were any significant relationships between damage indices and 1/ 150 Hz ratios at 10 minutes and various periods following 120 active lengthening repetitions of mouse dorsiflexor muscles. The 1/ 150 Hz ratio 10 minutes following 120 active lengthening repetitions showed no significant relationship with histological damage (Fig 15). Similarly, there was a lack of a significant relationship between the damage index and the 1/ 150 Hz ratio at 3, 6, 10, 15 and 20 days following active lengthening (Fig 16).

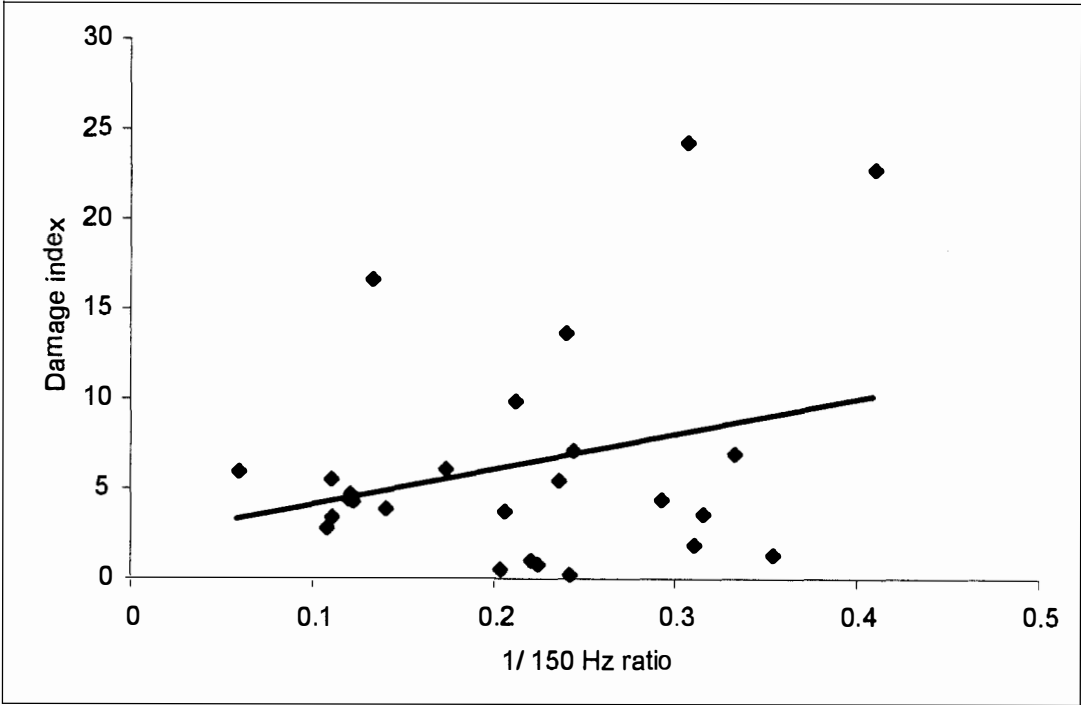


Figure 16. Scatterplot showing the damage index versus the 1/ 150 Hz ratio for varying days following 120 active lengthening repetitions of mice dorsiflexor muscles. $r = 0.28$ (n.s).

Chapter Five

Discussion

The present study demonstrated significant decreases in normalized P_o for actively exercised muscles following each of the 5 sets of 24 lengthening repetitions, and at 10 minutes post-exercise. Thus the first hypothesis of the study stating that the normalized P_o of the actively lengthened dorsiflexor muscles would be significantly lower than those of the passively lengthened muscles following each of the 5 sets of 24 lengthening repetitions, and at 10 minutes following the final bout of 24 repetitions was accepted. The magnitude of the normalized P_o decrement in the active group was within the range previously reported. McCully & Faulkner (1985) presented values as low as 22% of control muscles following lengthening contractions of the mouse EDL muscle. Using a similar technique of active lengthening to that of the present study Sacco et al. (1992) exercised mouse foot dorsiflexor muscles with 240 repetitions and recorded average P_o values of up to 56% of contralateral muscles 3 days later. It is interesting to note that Sacco et al. (1992) used twice the number of contractions than in the present study yet the decrement in normalized P_o was practically identical. This could be explained if the majority of the mechanical damage to the myofibre and/ or surrounding connective tissue occurs early in the series of active lengthening contractions. According to Armstrong et al. (1991), the damage could be manifest after a single high stress active lengthening (normal stress theory) or following multiple active lengthening repetitions (materials fatigue theory). The materials fatigue theory, when employing maximally

stimulated active lengthening repetitions, may require significantly fewer repetitions than that used in the present study (i.e., 120) or that of Sacco et al. (1992) (i.e., 240). Warren, Lowe, Hayes, Karwoski, Prior, & Armstrong (1993), using an in vitro rat soleus model, produced evidence to support the materials fatigue theory showing that marked decrements in force transmission can occur after as little as eight active lengthening repetitions. In earlier work Warren, Hayes, Lowe, Guo, & Armstrong (1991) reported a 14% reduction in P_o using a similar in vitro rat soleus set-up to that of their latter (1993) study. In this work they employed as few as five actively lengthened repetitions. McCully & Faulkner (1986) have also reported active lengthening to result in decrements in P_o with as little as 15 lengthening contractions. In the same study they reported decrements in P_o that grew in magnitude with increasing repetitions up to 150 active lengthening repetitions. Beyond 150 repetitions, however, no further decreases in P_o were noted.

The 20% increase in normalized P_o of the passive group over the first 96 lengthening repetitions was an interesting and unexpected observation. McCully & Faulkner (1985) noted an increase in force of passively lengthened muscles in their study although the magnitude was larger, approximately 50%. Unlike this study the increase was a peak dynamic measure and represented entirely passive force as no stimulation was performed to assess P_o . Unfortunately they did not speculate as to the cause of the increase in passive force. They also found that in muscles that underwent sham operations and were left untreated (i.e., did not undergo lengthening) for the time the active lengthened muscles took to complete their protocol there was a significant increase in P_o of 4% (McCully & Faulkner, 1985). This suggests that a small

proportion of the increase in the P_o of the passively lengthened muscles in our study may be due to some factor associated with the operation although the majority of the increase we noted (i.e., ~15%) cannot be explained by this mechanism.

There was no significant recovery of P_o in the actively lengthened muscles 10 minutes following the 120th repetition (Fig 6), which led to the second hypothesis being accepted. Combined with the relatively long period between active lengthening contractions (~ 5 seconds) this result supports the contention that the decrease in normalized P_o of the active group over the 120 lengthening repetitions was not due to metabolic fatigue. The period between contractions would be expected to allow for adequate phosphocreatine (PCr) resynthesis and negligible hydrogen ion (H^+) accumulation in the active myofibres. If PCr depletion played a major role then one could reasonably expect to see a significant recovery of normalized P_o following the 10-minute recovery period. As a single maximum isometric contraction would derive the majority of its energy from immediate sources (i.e., the phosphagen or ATP-PCr system) and with the half time of recovery of PCr being in the vicinity of 30 seconds (Hultman, Bergstrom, & McLennan-Anderson, 1967), PCr stores following 120 active lengthening repetitions should be fully restored within the 10 minutes of recovery. In a review of anaerobic metabolism in high-intensity exercise Spriet (1995) noted that “PCr and ATP are almost completely restored following four minutes of recovery” from work that depletes PCr levels by ~ 75% (p. 18). In addition, it is firmly established that negative work is much less costly metabolically than either concentric or isometric exercise (Bonde-Petersen, Knuttgen, & Henriksson, 1972; Davies & Barnes, 1972; Infante, Klaupiks, & Davies, 1964). Considered together, this evidence would suggest

that the decrement in P_o 10 minutes following active lengthening was not related to metabolite buildup and/ or significant phosphagen depletion due to high metabolic stress. Instead, as suggested by others (Balnave & Allen, 1995; Warren et al., 1993), the majority of the pronounced decrement in normalized P_o immediately following exercise is more likely a result of E-C failure. In an experiment designed to determine the contribution of E-C coupling failure to the P_o decrement in mouse EDL muscles following eccentric contractions Ingalls, Warren, Williams, Ward, & Armstrong (1998) injured the anterior crural muscles in vivo with 150 active lengthening contractions. P_o , caffeine and potassium-induced contracture forces, sarcoplasmic reticulum Ca^{2+} release and uptake rates, and intracellular Ca^{2+} concentration were then measured in vitro in the damaged and contralateral EDL muscles at varying times post active lengthening. Following analysis of the collected data they determined that E-C coupling impairment could account for at least 75% of the reduction in P_o immediately after the injury and at least 57% of the decrement in P_o following 5 days of recovery.

The hypothesis that there would be a significant difference in normalized P_o between actively and passively lengthened dorsiflexor muscles following the 120 lengthening repetitions was accepted for day's 3 and 20 of recovery. At 3 days post exercise the normalized P_o of the muscles of the active group were significantly lower than the passive group (Fig 7). This time point also coincided with the lowest normalized P_o recorded following lengthening. This is in agreement with the work of Sacco et al. (1992) who reported that the normalized P_o nadir occurred 3 days following 240 active lengthening repetitions of mouse foot dorsiflexor muscles. In the present study, normalized P_o began to increase after the third day following lengthening and was not

significantly different from the passive group on days 6, 10 and 15 of recovery. It is interesting to note that, although non-significant, the average normalized P_o value of the actively lengthened group was still approximately 30% lower than the corresponding value of the passive group at 15 days following active lengthening (Fig 7). These findings are in line with some studies that report protracted decrements in P_o , taking from 12 to 30 days to approximate control values (Brooks & Faulkner, 1990; Faulkner et al., 1989; Sacco et al., 1992; Warren et al., 1999a). In the present study sub-groups on days 6, 10 and 15 had a small number of mice that produced unusually high P_o s, creating significant outliers. This was unexpected and not encountered in the pilot work. Therefore an improvement for future studies would be to increase the number of animals in each sub group. If the number of mice in each sub group were larger then it may have been possible to reduce the standard deviation and demonstrate statistical significance between the two groups at more of the days following lengthening.

At 20 days following lengthening the active group's normalized P_o was 85% greater than that of the passive group. This normalized P_o , which also exceeds the active group's pre-lengthening value by approximately 45%, is not in line with previous findings of Faulkner et al. (1989) and McCully & Faulkner (1985). Komulainen, Kalliokoski, Koskinen, Takala, Kuipers & Hesselink. (1998), in a study involving eccentrically exercised rat TA muscle, produced evidence that a single session of forced lengthening contractions caused a long-lasting hypertrophic effect on the treated skeletal muscle. They noticed hypertrophy in the actively lengthened, that was not evident in the contralateral TA muscle, for the first time 50 days following the active lengthening session. This has also been reported by Sacco & Jones (1992a) who found

that TA muscles measured 42 and 84 days following damaging eccentric exercise were significantly larger and stronger than non-exercised contralateral muscles. If, by 20 days of recovery from injurious active lengthening the regenerating myofibres undergo a supercompensating hypertrophy, and the regenerated muscle tissue has similar relative force production capability to the pre-injured muscle, this would lead to an increase in P_o . Goldspink (1991) stated that “the maximum force production of a muscle is related to the myofibril cross-sectional area” (p. 217). He also noted that increased protein synthesis has been detected as early as six hours after stretch has been applied to muscle (p. 220). In a study examining the effects of combined eccentric/ concentric and concentric only weight training programs on subsequent muscle size, Pearson & Costill (1988) found that only the leg that included an eccentric component increased in size following training. Wong & Booth (1990) have also found that protein synthesis rates were greater in eccentrically exercised than concentrically exercised rat muscle. However, at this time there are a lack of studies linking increases in hypertrophy following damaging exercise leads to increases in P_o . Further studies investigating fibre cross sectional areas and concurrent P_o at various time points into recovery from damaging exercise would provide data required to reinforce that found by Sacco and Jones (1992a).

The fourth hypothesis, stating that there would be a significant difference in the 1/ 150 Hz ratio between active and passively lengthened dorsiflexor muscles 10 minutes following, and at 3 days following each lengthening protocol was not supported. Although the results revealed a significant difference between the groups 10 minutes following lengthening this did not extend to the results obtained at 3 days following

treatment (Fig 8). As the hypothesis was inclusive of both 10 minutes and 3 days following lengthening the hypothesis could not be accepted. The finding of a significantly lower ratio 10 minutes following active lengthening is in agreement with that of McCully & Faulkner (1985) who reported similar results. Faulkner et al. (1989) noted a shift to the right of the frequency-force relationship one-hour following lengthening contractions which was associated with larger decreases in force at low frequencies of stimulation. As no frequency-force measurements were recorded 10 minutes following active lengthening in the study of Faulkner et al. it cannot be unequivocally stated that they were significantly depressed at this time. Results from studies in humans agree with the findings of greater reductions in force production at low as compared to high frequency in actively lengthened muscle (LFF) (Davies & White, 1981; Newham et al., 1987). This LFF, that is suggested to be caused by damage to the sarcoplasmic reticulum leading to a smaller release of calcium following each action potential passed down the T tubule (Fitts et al., 1991; Jones, 1981), was recorded by Newham et al. (1983c) as early as 2 minutes following active lengthening exercise in humans.

In contrast, the findings of no significant difference for the 1/ 150 Hz ratios between the groups at 3 days following lengthening were unexpected and are not in agreement with the findings of mouse (Sacco et al., 1992) and human (Jones et al., 1989) studies. The 1/ 150 Hz ratio for the active group at 3 days following lengthening (Fig 8) is doubtful and not at all in line with what was expected based on previous studies. In a typical recovery pattern there is usually a large decrease in the low/ high force frequency ratio immediately following active lengthening followed by a gradual recovery over days

(Newham et al., 1987; Sacco et al., 1992; Sargeant & Dolan, 1987). Further support for doubting the 3-day value obtained in this study is that the ratio decreased once again at 6 days following active lengthening. This is in line with other studies of mouse (Warren et al., 1999a) and human (Brown et al., 1997a; Jones et al., 1989) recovery patterns. Aside from the possibility of human error an explanation for this doubtful ratio recording is not forthcoming.

The hypothesis that there would be greater histological damage in the actively lengthened as compared to the contralateral TA muscles following the 120 lengthening repetitions was accepted. With the exception of day 20, the damage index of the actively lengthened TA muscles was significantly higher during recovery than the control muscles (Fig 10). A common observation following active lengthening of rodent muscle is a large increase in mononuclear cells at and around the site of injury at 1-3 days post exercise, peaking between 2 and 5 days and then slowly returning to pre-injury levels (Armstrong et al., 1983; Faulkner et al., 1989; McCully & Faulkner, 1985; Sacco et al., 1992). In the present study, an increase in mononuclear cell numbers occurred up to six days following active lengthening which slowly returned to normal by 20 days (Fig 11). Also in line with previous reports, this study revealed greater numbers of centrally located myo-nuclei following active lengthening (Lowe et al., 1995; McCully & Faulkner, 1985). The numbers were significantly greater than control TA muscle from day 6, through 15 following active lengthening (Fig 11). I expected to see higher numbers of central nuclei 20 days following active lengthening but this was not the case. McCully & Faulkner (1985) reported immature muscle fibers with central nuclei at 7 and 14 days following active lengthening with some fibers still possessing

central nuclei 30 days later. Why significantly higher numbers of central nuclei at 20 days following lengthening were not seen is unclear. It is possible that many may have migrated back to the periphery of the fibre by this stage. It is known that as a regenerating myofibre fills with newly synthesized contractile proteins the centrally located nuclei are usually pushed to the periphery (Lieber, 1992). Brooks & Faulkner (1990) results showed that the centrally located nuclei in mice of similar age to those used in this study had migrated back to the periphery by 28 days following active lengthening. Jones & Round (1990, p. 173), however, pointed out that following active lengthening centrally located nuclei usually remain for protracted periods, although they do not provide a time frame for this process. Lieber (1992) related that although it takes about six months for the entire regeneration process to take place in most higher mammals it is much more rapid in rats taking approximately two months. If the smaller size of the mouse compared to the rat is associated with an increased metabolic rate then this process could be even speedier in this rodent.

Another possibility is evident upon scrutiny of the 20-day subgroup's normalized P_o immediately following active lengthening. Where the average P_o immediately following active lengthening for all of the groups combined was $55.3 \pm 2.8\%$, that of the 20-day subgroup's was 81.4 ± 12.0 . The smaller decrement in normalized P_o of this subgroup may be reflected in a reduced magnitude of damage, which would lead to lower levels of myofibre regeneration and central nuclei.

The hypotheses stating that 1) the normalized P_o 10 minutes following, and 2) the average normalized P_o over, 120 active lengthening repetitions would produce significant negative correlations with the quantity of myofibre damage were not

accepted. The low and non significant 'r' value returned for the first of these correlations ($r = -0.20$) suggests that the change in normalized P_o 10 minutes following active lengthening failed to predict the extent of histological muscle damage (Fig 12). Similarly, there seems to be no value in using the average normalized maximum isometric force over 120 active lengthening repetitions to predict the extent of subsequent muscle damage, as evidenced by a non significant and even lower 'r' value of -0.19 (Fig 13). These results were interesting and somewhat surprising as it was hypothesized that the cause of the large decrements in P_o over the course of, and at 10 minutes following, the 120 active lengthening repetitions would be manifest in some form of disruption to the myofibre leading either directly or indirectly to loss of integrity of the sarcolemma. This reasoning was based on the argument of Armstrong et al. (1991) that physical stresses associated with eccentric contractions are imposed on the sarcolemma and "cause disruption of the normal permeability barrier provided by the cell membrane and basal lamina" (p. 184). I speculated that the larger the P_o decrement the larger the damage to the sarcolemma and other cytoskeletal structures. It was also reasoned that the loss of sarcolemmal integrity and subsequent release of quantities of products from the 'injured' fibres would lead to the attraction of immune mononuclear cells via a chemotactic mechanism. Grounds (1991) noted that complement cleavage products and metabolites from membrane phospholipid breakdown are chemoattractant mediators and chemotactic in nature. More specifically, she related that certain components of complement activation serve as powerful chemotactic and stimulating agents to macrophages. In addition, she continued that "changes in levels of Ca^{++} and proteases are often involved in the production of

chemoattractants” (p. 2). As discussed in chapter two it is well established that there is an increase in intracellular calcium homeostasis following active lengthening exercise (Amelink et al., 1990b; Duarte, Soares, & Appell, 1992) as well as possible increases in activity levels of cytosolic bound proteases (Armstrong, 1984).

The number of mononuclear cells and central nuclei were chosen as the quantitative measures of histological damage for the following reasons. 1) the presence of mononuclear cells has been used by other researchers to measure histological damage (Sacco et al., 1992), and 2) that concurrent with the latter stages of mononuclear cell infiltration and subsequent to their migration away from the previously necrotic tissue, the second quantitative marker, centrally located nuclei, appear as new myotubes begin to form (Carlson & Faulkner, 1983). As mouse (Brooks & Faulkner, 1990; Ingalls et al., 1998; McCully & Faulkner, 1985) and human (Clarkson et al., 1992; Clarkson & Tremblay, 1988; Nosaka et al., 1991) studies clearly show, the largest P_o decrements can be measured in the first three days following active lengthening. Based on the reasoning presented above it seemed plausible that muscle demonstrating the lower average normalized P_o over the 120 actively lengthened repetitions, and/ or the lower P_o 10 minutes following, would be those that presented with the larger magnitude of histological damage upon subsequent evaluation.

Another possibility, however, that opposes my argument and may explain the lack of a significant correlation lies in the work of Balnave & Allen (1995) and Warren et al. (1993) who have provided data to support the idea that decrements in P_o following active lengthening are probably related to E-C failure. Both of these studies provided evidence to suggest that the sarcolemma was not compromised following the active

lengthening exercise. If in fact, as discussed in the review of literature, a compromised sarcolemma is required as a prerequisite for immune mononuclear cell invasion then these studies indirectly provide support for the argument that P_o following active lengthening is not significantly correlated with myofibre damage.

It was hypothesized that there would be a significant negative correlation between normalized P_o following active lengthening and the quantity of histological muscle damage as measured at the time of termination of the mice. The significant one-tailed correlation of -0.35 allowed the hypothesis to be accepted (Fig 14). McCully & Faulkner (1985) reported a correlation between the decline in P_o at 3 days post active lengthening and damage at the same time point, as measured by the decline in the number and total cross-sectional area of fibers. Unfortunately, no 'r' value was reported to provide supporting evidence that this correlation was significant. Sacco et al. (1992) also reported that the time course for loss in isometric force and histological damage were similar suggesting a strong relationship existed between these variables. They used the number of damaged foci/ per field as their quantitative measure, with 'damaged foci' being defined as "fibres which were infiltrated by mononuclear cells" (p. 229). As in the previous study of McCully & Faulkner (1985) no correlation figures were reported to relate the significance of any apparent relationship between these variables. Lieber et al. (1994), however, did perform correlation analyses in their active lengthening study involving rabbit TA and EDL muscle. In this study their assessment of myofibre damage was based on the magnitude of 'desmin-negative' fibers present in the actively lengthened muscles. They reported a significant negative correlation between maximum tetanic tension (P_o) and the percent of desmin-negative fibers.

It is important to note that the methods of assessing histological damage in this and the previously mentioned studies of McCully & Faulkner (1985) and Sacco et al. (1992) were different. This makes it somewhat difficult when trying to compare relationships between P_o and histological damage between the studies. Warren et al. (1999b), in their review of measurement tools used in the study of eccentric contraction-induced injury, noted that histological measurements of damage are difficult to quantitate, subject to observer and sampling bias, and are problematic to perform quantitatively on an entire muscle. They even suggest that it is difficult to perform quantitative histological analyses on as little as 10 mg of murine muscle (Warren et al., 1999b). In addition, they suggest that “it is difficult to establish clear-cut criteria for the presence or extent of a histological abnormality” (p. 54).

Warren et al. (1999b) reviewed 18 studies that made an attempt to quantify the observed abnormalities and reported that neither the time-course nor the magnitude of the histopathology in these studies corresponded with the changes in muscle function. It is interesting to note that the above studies of Sacco et al. (1992) and McCully & Faulkner (1985) were included in the 18 reviewed studies but they both reported that histopathology “corresponded” and were “correlated”, respectively, with changes in muscle function.

Warren et al. (1999b) argued that in many studies the nadir in P_o occurs immediately following active lengthening but that the greatest number of fibre abnormalities have been observed at 2 to 4 days following active lengthening. Maximum decrements in P_o immediately following active lengthening have been reported by their group (Lowe et al., 1995) in a mouse model. Although no corresponding abnormalities to the myofibres

were reported in this study it should be noted that electron microscopy was not performed in order to assess damage at an ultrastructural level. Many studies use transverse sections of muscle tissue and light microscopy to search for evidence of muscle injury. It may be that using longitudinal sections of tissue and/ or employing electron microscopy may reveal damage not evident by viewing transverse sections under light microscopy. Ogilvie et al. (1988) examined rat soleus muscle within 30 minutes of downhill running exercise and noted histological abnormalities when longitudinal sections of muscle were examined via light microscopy. Lieber & Friden (1988), using an active lengthening rabbit model, noted both light and electron microscopic abnormalities of TA muscle 1 hour following exercise. In that study P_o was measured concurrently and was shown to be 67% below the pre-exercise values. In addition Friden et al. (1983), employing a human model, noted the nadir in P_o within 20 minutes following active lengthening. Electron micrograph evaluation at this stage revealed Z-band streaming, broadening and at places total disruption. The studies of Ogilvie et al. (1988) and Lieber & Friden (1988) did not report the time of maximum fibre abnormality therefore it is possible that there may have been a greater magnitude of disruption at 2 to 4 days following active lengthening as mentioned by Warren et al. (1999b).

In contrast to Warren et al's. (1999b) contention that the maximum deficit in P_o usually occurs immediately following active lengthening exercise a number of studies (Brooks & Faulkner, 1990; Brown et al., 1997b; McCully & Faulkner, 1985; Sacco et al., 1992) have reported that using their damage protocols the nadir in P_o occurred after three days. This time frame coincides with the window (2-4 days) that Warren et al. (1999b)

notes has been associated with the greatest magnitude of fibre abnormalities and invasion of the tissue by inflammatory cells.

In support of Warren et al. (1999b) contention, E-C failure has been implicated in the reductions in P_o following active lengthening exercise. As stated above, this cause of P_o decrement may not be associated with damage to myofibrillar structures, or infiltration of immune mononuclear cells due to maintenance of sarcolemmal integrity. Why then, in the present study, was there only a weak correlation between normalized P_o at these days and the magnitude of myofibre damage (Fig 14)? I reasoned that the correlation would not only be significant but more convincing by way of a larger correlation coefficient, whereas Warren et al. (1999b) would suggest no significant relationship between these variables at all. The answer may well lie in the aetiology of the P_o decrement. In cases where the P_o decrement following active lengthening was caused more by E-C failure then it could be argued that there would be a weakening or complete loss of correlation between the two variables. In contrast, based on our earlier arguments, when the reduction in P_o is due largely to significant damage to force bearing structures, including the sarcolemma, then it could be reasoned that there would be a significant correlation between the variables in question.

The lack of any significant correlation between the 1/ 150 Hz ratio at 10 minutes (Fig 15) and 3, 6, 10, 15, and 20 days (Fig 16) following active lengthening and the degree of myofibre damage caused me to accept the final two hypotheses put forward in this study. As with P_o , reductions in the 1/ 150 Hz ratio have been attributed largely to E-C failure possibly due to damage to the sarcoplasmic reticulum or T-tubular system (Jones, 1981). If damage to the sarcoplasmic reticulum at the level of the ryanodine

receptors (channels) leads to LFF without compromising T-tubular integrity, release of intracellular components and the signalling of immune mononuclear cells may not occur, resulting in a reduced 1/ 150 Hz ratio without subsequent mononuclear cell invasion. Although a possibility, the present study and others have noted LFF immediately following active lengthening that persisted for a period thereafter and was associated with mononuclear cell invasion at varying times post lengthening (Faulkner et al., 1989; Sacco & Jones, 1992b). It could be that mechanical stress due to high active lengthening forces causes damage to both sarcolemmal and sarcoplasmic reticulum structures to differing degrees depending on the exercise protocol employed and/ or the susceptibility of myofibres to exercise-induced injury. Hypothetically, depending upon the degree of damage to each of the above structures LFF could result with or without subsequent mononuclear cell invasion of the myofibres. As shown, LFF and histological damage can occur alone or concurrently. Unfortunately there is paucity in the research literature of studies that have attempted to correlate these two variables. In fact this seems to be the first study to examine if a relationship exists between LFF and histological myofibre damage.

In summary, evaluation of the results of this study caused me to accept 7 of the 10 hypotheses put forward and report the major findings of the study to be:

- 1) Normalized P_o of actively lengthened mouse dorsiflexor muscles are reduced significantly below that of passively lengthened dorsiflexor muscles during the execution of 120 lengthening repetitions.

- 2) The reduction in normalized P_o following 120 active lengthening repetitions of mouse dorsiflexor muscles does not recover to any significant extent following 10 minutes of passive recovery, suggesting that the contribution of metabolic fatigue to these force losses is negligible.
- 3) The 1/ 150 Hz ratio of the actively lengthened group were significantly lower than the passively lengthened group at 10 minutes following 120 lengthening repetitions of mouse dorsiflexor muscles suggesting that there may be some E-C failure at this time following active lengthening resulting in the LFF experienced.
- 4) Histological damage to mouse TA muscle was significantly greater in actively lengthened than non lengthened contralateral muscle at 3, 6, 10 and 15 days following lengthening.
- 5) There is a weak but significant negative correlation between the normalized P_o at days 3, 6, 10, 15 and 20 days following 120 active lengthening repetitions and histological damage to mouse TA muscle. This suggests that there may be value in using normalized isometric force in the days following active lengthening to predict the magnitude of histological damage, as measured by the method used in this study.
- 6) Average normalized P_o at 10 minutes following active lengthening or over the 120 active lengthening repetitions does not seem to be a good predictor of subsequent histological myofibre damage at days 3, 6, 10, 15 and 20 following active lengthening.

- 7) The 1/ 150 Hz ratio immediately after, or in the days following, active lengthening does not seem to be a useful predictor of the magnitude of histological myofibre damage.

This study has provided the first correlation data between the 1/ 150 Hz ratio and histological myofibre damage and suggests that measurement of this ratio either before, during or subsequent to active lengthening is not useful in predicting the magnitude of histological damage following lengthening.

It has also provided supporting evidence that there may be value in using P_o in the days following active lengthening to predict the magnitude of histological myofibre damage. Future studies could focus on determining whether a specific day following active lengthening provides stronger correlation data than combining the days as was done in the present study. If stronger correlation data than obtained in this study can be gathered for P_o and histological myofibre damage then a linear function could be calculated from the data to provide a prediction of the magnitude of myofibre damage for any given P_o . Being able to determine the extent of myofibre damage without resorting to open or needle biopsy techniques would be of enormous benefit in terms of time, difficulty, and cost.

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APPENDIX A

STATISTICALLY SIGNIFICANT INDEPENDENT t-TEST RESULTS

Pre lengthening to Post 10 Minutes - Active Versus Passive

Measurement Variable	Independent t-Test Result
24 repetitions	$t(2) = 7.82, p < 0.008$
48 repetitions	$t(2) = 11.09, p < 0.008$
72 repetitions	$t(2) = 12.49, p < 0.008$
96 repetitions	$t(2) = 13.36, p < 0.008$
120 repetitions	$t(2) = 11.40, p < 0.008$
Post 10 minutes	$t(2) = 11.33, p < 0.008$

Recovery Days – Active Versus Passive

Measurement Variable	Independent t-Test Result
3 days	$t(2) = 5.63, p < 0.01$
20 days	$t(2) = 3.93, p < 0.01$

1/150 Hz Ratio – Active Versus Passive

Measurement Variable	Independent t-Test Result
Post 10 minutes	$t(2) = 5.83, p < 0.025$
6 days recovery	$t(2) = 3.0, p < 0.025$

Damage Index – Actively Lengthened TA Versus Contralateral TA Muscles

Measurement Variable	Independent t-Test Result
3 days recovery	$t(2) = 9.64, p < 0.01$
6 days recovery	$t(2) = 8.20, p < 0.01$
10 days recovery	$t(2) = 7.35, p < 0.01$
15 days recovery	$t(2) = 5.16, p < 0.01$

Mononuclear Cells – Actively Lengthened TA Versus Contralateral TA muscles

Measurement Variable	Independent t-Test Result
3 days recovery	$t(2) = 9.62, p < 0.01$
6 days recovery	$t(2) = 7.23, p < 0.01$
10 days recovery	$t(2) = 8.27, p < 0.01$

Centrally Nucleated Fibres – Actively Lengthened TA Versus Contralateral TA

Muscles

Measurement Variable	Independent t-Test Result
6 days recovery	$t(2) = 5.60, p < 0.01$
10 days recovery	$t(2) = 6.03, p < 0.01$
15 days recovery	$t(2) = 4.69, p < 0.01$

APPENDIX B

STATISTICALLY SIGNIFICANT ONE-WAY ANOVA RESULTS

Pre Lengthening to Post 10 Minutes for Active Group

Measurement Variable	F-ratio and Bonferroni Post-Hoc t-Test
Pre lengthening to post 10 minutes	$F(6,224) = 41.41, p < 0.05$
Pre lengthening versus 48 repetitions	$t(2) = 29.18, p < 0.05$
Pre lengthening versus 72 repetitions	$t(2) = 37.18, p < 0.05$
Pre lengthening versus 96 repetitions	$t(2) = 40.04, p < 0.05$
Pre lengthening versus 120 repetitions	$t(2) = 44.69, p < 0.05$
Pre lengthening versus post 10 minutes	$t(2) = 41.72, p < 0.05$
24 repetitions versus 48 repetitions	$t(2) = 16.43, p < 0.05$
24 repetitions versus 72 repetitions	$t(2) = 24.43, p < 0.05$
24 repetitions versus 96 repetitions	$t(2) = 27.30, p < 0.05$
24 repetitions versus 120 repetitions	$t(2) = 31.94, p < 0.05$
24 repetitions versus post 10 minutes	$t(2) = 28.98, p < 0.05$
48 repetitions versus 120 repetitions	$t(2) = 15.51, p < 0.05$

Pre Lengthening to Post 10 Minutes for Passive Group

Measurement Variable	F-ratio and Bonferroni Post-Hoc t-Test
Pre lengthening to post 10 minutes	$F(6,203) = 9.83, p < 0.05$
Pre lengthening versus 24 repetitions	$t(2) = -16.92, p < 0.05$
Pre lengthening versus 48 repetitions	$t(2) = -20.45, p < 0.05$
Pre lengthening versus 72 repetitions	$t(2) = -21.26, p < 0.05$
Pre lengthening versus 96 repetitions	$t(2) = -21.73, p < 0.05$
48 repetitions versus post 10 minutes	$t(2) = 18.53, p < 0.05$
72 repetitions versus post 10 minutes	$t(2) = 19.34, p < 0.05$
96 repetitions versus post 10 minutes	$t(2) = 19.81, p < 0.05$
96 repetitions versus 120 repetitions	$t(2) = 15.58, p < 0.05$

Recovery Days (Active Lengthening)

Measurement Variable	F-ratio and Bonferroni Post-Hoc t-Test
Pre lengthening to 20 days of recovery	$F(5,25) = 9.72, p < 0.05$
Pre lengthening versus 3 days	$t(2) = 68.95, p < 0.05$
3 days versus 20 days	$t(2) = -114.09, p < 0.05$
6 days versus 20 days	$t(2) = -87.13, p < 0.05$
10 days versus 20 days	$t(2) = -91.87, p < 0.05$
15 days versus 20 days	$t(2) = -82.72, p < 0.05$

APPENDIX C

STATISTICALLY SIGNIFICANT CORRELATION RESULTS

Measurement variable	Correlation result
Calibration figures: load in grams versus output in millivolts	$r = 0.99, p < 0.05$ (Two-tailed)
Foot plate reproducibility (trial 1 versus 2)	$r = 0.99, p < 0.05$ (Two-tailed)
Reproducibility of P_o in passively lengthened mice: immediately following 120 th repetition versus post 10 minutes.	$r = 0.90, p < 0.05$ (Two-tailed)
Normalized P_o at varying days following active lengthening versus the damage index.	$r = -0.35, p < 0.05$ (One-tailed)