

**A COMPARISON OF MUSCLE DAMAGE,
SORENESS, MORPHOLOGY, T2 CHANGES AND
RUNNING PERFORMANCE FOLLOWING AN
ULTRAMARATHON RACE**

A DISSERTATION PREPARED BY WANDA VAN NIEKERK
(BYSWAN001) IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE MASTER OF PHILOSOPHY DEGREE IN
EXERCISE AND SPORTS PHYSIOTHERAPY (MPHIL IN EXERCISE
AND SPORTS PHYSIOTHERAPY) FROM THE UNIVERSITY OF
CAPE TOWN

FEBRUARY 2016

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ACKNOWLEDGEMENTS

This study would not have been possible without the support and assistance of certain people. I would like to thank them for their time and expertise on both a personal and professional level.

To Dr Theresa Burgess, my supervisor for always putting in so much time and dedication into guiding my research the last couple of years and for never giving up on me and always believing in my abilities.

To Dr Frances Robertson, for your guidance and expertise in helping with the analysis of the MRI scans.

To Willie van Niekerk, my husband for your support, calmness and faith in me as well as the many hours spent keeping the kids busy.

To my family for their faith in me and for all their support, interest and encouragement through this process.

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LIST OF ABBREVIATIONS

ACSA	Anatomical cross sectional area
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate-activated protein kinase
ATI	Acquired training intolerance
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BF	Biceps femoris
Ca²⁺	Calcium
CK	Creatine kinase
CSA	Cross sectional area
cm	Centimetre
DOMS	Delayed onset muscle soreness
ECM	Extracellular matrix
EIMD	Exercise induced muscle damage
EMG	Electromyography
GLUT-4	Glucose transporter type 4
h	Hours
HR	Heart rate
kg	Kilogram
km	Kilometre
LDH	Lactate dehydrogenase
MAPK	Mitogen activated protein kinase
MMP's	Matrix metalloproteinases
MRI	Magnetic resonance imaging
ms	Millisecond
MV	Muscle volume
MVC	Maximal voluntary contraction
Na⁺ - K⁺	Sodium Potassium pump
PCr	Phosphocreatine kinase
PCSA	Physiological cross sectional area
PTRS	Peak treadmill running speed
VO_{2max}	Maximal oxygen consumption

RER	Respiratory exchange ratio
RF	Rectus femoris
RPE	Rate of perceived exertion
TIMPs	Tissue inhibitor of metalloproteinases
TR	Repetition time
TE	Echo time
T2	Transverse relaxation time
VI	Vastus intermedius
VL	Vastus lateralis
VM	Vastus medialis
SM	Semimembranosus
ST	Semitendinosus

ABSTRACT

Background

Exercise induced muscle damage collectively describes the response to strenuous or unaccustomed exercise. It is well-established that endurance running causes muscle damage. Indirect indicators of muscle damage include the loss of muscle strength, increased levels of muscle proteins, such as creatine kinase, in the blood and delayed onset of muscle soreness. Magnetic resonance imaging has been used to gain insight into the underlying mechanisms associated with exercise induced muscle damage. The most common approach has focused on changes in transverse (T2) relaxation times after exercise. Given that inflammation and oedema are proposed as reasons for the changes in T2 times, there may be changes in morphological measurements such as muscle volume and peak cross sectional area. Few studies have utilised MRI morphological measurements to assess the effects of exercise induced muscle damage, and there is a lack of evidence regarding changes in muscle morphology after endurance running.

Aim

The aim of this study was to investigate changes in transverse (T2) relaxation times and muscle morphology in endurance runners after a 90 km ultramarathon race.

Specific objectives

(a) To determine the time course of recovery of muscle pain and plasma creatine kinase activity after a 90 km ultramarathon race; (b) to determine changes in 5 km time trial performance in an experimental group of endurance runners that took part in a 90 km ultramarathon race compared to a control group of endurance runners that did not take part in a 90 km ultramarathon race; (c) to compare changes in muscle morphology (volume and average cross sectional area) and T2 relaxation times of the quadriceps and hamstrings in an experimental group of endurance runners that took part in a 90 km ultramarathon race and a control group of endurance runners that did not take part in a 90 km ultramarathon race; and (d) to evaluate potential relationships between indicators of muscle damage (plasma creatine kinase levels and muscle pain measurements), morphological muscle changes, and T2 relaxation times in an experimental group of endurance runners that took part in a 90 km ultramarathon race and a control group of endurance runners that did not take part in a 90 km ultramarathon race.

Methods

This was a descriptive, correlational study that involved secondary analysis of previously collected data. No new participants were recruited for the study. Participants were allocated to groups, based on whether they took part in a 90 km ultramarathon. The experimental group (n = 11) completed a 90 km ultramarathon. The control group (n = 11) consisted of endurance runners, who ran a minimum of 60 km.wk⁻¹, but did not take part in the ultramarathon. Magnetic resonance images were taken seven days before and 10 -15 days after an ultramarathon as part of an earlier study. The magnetic resonance images analysis included the digital segmentation and reconstruction of the rectus femoris, combined quadriceps and combined hamstrings muscle groups. Muscle volume and peak cross sectional area was calculated as well as T2 relaxation times. These measurements were correlated with muscle pain and plasma creatine kinase activity measurements obtained during the initial study.

Results

There was a significant difference in hamstrings muscle volume between the experimental and control groups. The experimental group had a significantly lower muscle volume compared to the control group (p =0.03). There was also a significant positive relationship between the T2 relaxation time and plasma CK activity. (r = 0.74; p = 0.04)

Conclusion

Changes in muscle morphology in endurance runners are evident after a 90 km ultramarathon. The significant relationship between T2 relaxation times and plasma creatine kinase activity confirms that T2 relaxation time may be used as a non-invasive direct indicator of exercise induced muscle damage.

CHAPTER 1: INTRODUCTION AND SCOPE OF THE DISSERTATION

1.1 INTRODUCTION

Exercise induced muscle damage (EIMD) is associated with strenuous or unaccustomed exercise. It is particularly associated with activities involving lengthening muscle actions⁽¹⁾. This scenario is common during downhill running, during the activation of an antagonist muscle group or when muscles are exposed to high levels of repeated or unaccustomed activity⁽²⁾. Distance running imposes severe stress on the body and it is well documented that muscle damage occurs during distance running^(3,4).

The mechanisms responsible for these types of muscle injuries are physical strain induced by lengthening muscle actions and post-exercise inflammatory processes⁽⁵⁾. Cytoskeletal, sarcolemmal and microtubular lesions have been documented after exercise such as downhill running^(5,6). Together with these lesions there are also signs of sarcoplasmic reticulum fragmentation and mitochondrial swelling^(5,6). A few hours after the end of exercise there is an accumulation of local inflammatory mediators. There is also a significant increase in blood enzyme activities two to five days after exercise which is implemented as a sign of systemic inflammatory response. At four to six days after exercise muscle regeneration starts and may last for several weeks^(3,4).

Indirect indicators of muscle damage include prolonged losses in muscle strength⁽⁷⁻¹¹⁾, alterations in range of motion^(10,12), increased levels of muscle proteins in the blood, such as plasma creatine kinase levels^(10,13,14) and delayed onset muscle soreness⁽¹⁵⁾. However, it is recognised that these indirect indicators of exercise induced muscle damage provide limited insight regarding the extent of muscle damage⁽¹⁶⁾. In addition, changes in indirect indicators of muscle damage are most evident in the initial, acute period after the exercise bout that induced damage⁽¹⁷⁾. However, it is known that signs of muscle damage are still evident well beyond the presence of any indirect markers of damage^(3,4).

Muscle biopsies are used to investigate exercise induced muscle damage directly by analysing the histological and biochemical components of muscle, but the invasive nature of muscle biopsies limits repeated measurements⁽¹⁷⁾.

A non-invasive alternative to muscle biopsies is magnetic resonance imaging (MRI). Magnetic resonance imaging allows for both detailed anatomical analysis and assessment of physiological changes in muscles following exercise⁽¹⁸⁾. Magnetic resonance imaging may be used to investigate the underlying mechanisms associated with exercise induced muscle damage. Specifically, there may be changes in transverse (T2) relaxation times^(19, 20) and morphological measurements, such as muscle volume and peak cross sectional area, following exercise⁽²¹⁾. There is some evidence to suggest that T2 relaxation times are increased following eccentric training⁽²²⁾, and that there are associations between changes in plasma CK activity and T2 relaxation times^(23, 24). However, few studies have utilised MRI morphological measurements to assess the effects of exercise induced muscle damage, and there is a lack of evidence regarding changes in both T2 relaxation times and muscle morphology after endurance running. Accordingly, the aim of this study was to investigate changes in T2 relaxation times and muscle morphology following an endurance race.

1.2 AIMS AND OBJECTIVES

1.2.1 AIM

The aim of this study was to investigate changes in transverse (T2) relaxation times and muscle morphology in endurance runners after a 90 km ultramarathon race.

1.2.2 SPECIFIC OBJECTIVES

The specific objectives were:

- (a) To determine the time course of recovery of muscle pain and plasma creatine kinase activity after a 90 km ultramarathon race;
- (b) To determine changes in 5 km time trial performance in an experimental group of endurance runners that took part in a 90 km ultramarathon race compared to a control group of endurance runners that did not take part in a 90 km ultramarathon race;
- (c) To compare changes in muscle morphology (volume and average cross sectional area) and T2 relaxation times of the quadriceps and hamstrings in an experimental group of endurance runners that took part in a 90 km ultramarathon race and a control group of endurance runners that did not take part in a 90 km ultramarathon race; and
- (a) To evaluate potential relationships between indicators of muscle damage (plasma creatine kinase levels and muscle pain measurements), morphological muscle

changes, and T2 relaxation times in an experimental group of endurance runners that took part in a 90 km ultramarathon race and a control group of endurance runners that did not take part in a 90 km ultramarathon race.

1.3 SIGNIFICANCE OF THIS DISSERTATION

There is a lack of evidence for the extent of the recovery period after an ultramarathon. The findings of this study will provide information regarding the extent of exercise induced muscle damage and non-invasive measurements to quantify this muscle damage. It will also provide information on and insight into morphological changes that occurs in skeletal muscle after an ultramarathon.

1.4 PLAN OF DEVELOPMENT

In preparation for the secondary data analysis study of this dissertation, a comprehensive review of the literature examining exercise induced muscle damage as well as the indicators thereof will be presented (Chapter 2). This will be followed by a description of the study designed to investigate and compare changes in muscle damage, soreness, morphology and T2 relaxation times after an ultramarathon race (Chapter 3). A summary and conclusion section, with future research recommendations (Chapter 4) will complete this dissertation.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

Exercise induced muscle damage (EIMD) is a well-documented phenomenon that occurs after the performance of novel or unaccustomed exercise, or after exercise with increased intensity and/or prolonged duration^(25, 26). Muscle damage can be classified as a state where one or more of the indicators of muscle damage are present. These indicators of muscle damage can be direct or indirect. However, as increasingly sophisticated studies seek a more complete understanding of the mechanisms underlying the muscle damage response, it is becoming evident that the biology of exercise induced muscle damage (EIMD) is much more complex than first thought⁽²⁷⁾.

Symptoms of muscle damage include the disruption of the intracellular muscle structure, sarcolemma and the extracellular matrix⁽²⁸⁻³¹⁾, prolonged impairment of muscle function and strength⁽⁸⁾; and delayed onset of muscle soreness (DOMS)^(17, 32) stiffness and swelling^(10, 33, 34). It is also characterised by an increase in the activity of intramuscular enzymes in the blood plasma or serum, such as creatine kinase^(12, 35-38). An increase in circulating concentrations of troponin I, myoglobin and myosin heavy chains are also documented^(37, 39-42). It is well-documented that eccentric or lengthening muscle actions result in more severe muscle damage and is the main factor responsible for exercise induced muscle damage⁽⁴³⁾. During movement eccentric contractions rarely occur in isolation. Muscle function occurs in a sequence where an active eccentric (lengthening) contraction is followed by an active concentric (shortening) contraction. This phenomenon is known as the stretch shortening cycle (SSC)⁽⁴⁴⁾. Various studies documented exercise induced muscle damage after marathon running^(3, 4, 36). During an exhaustive exercise such as marathon running a large amount of stretch shortening cycles occur. Lengthening or eccentric muscle actions are a component of the SSC and contribute to muscle damage occurring during endurance exercise such as distance running^(3, 4, 36, 45).

In recent years studies have implemented magnetic resonance imaging (MRI) to investigate exercise induced muscle damage. Magnetic resonance imaging is a non-invasive method to indicate muscle damage and the extent thereof⁽¹⁷⁾.

The aims of this review are to summarise the effects of exercise induced muscle damage on skeletal muscle as well as the various indicators used to assess muscle damage.

The methods used as indicators of muscle damage such as muscle protein levels, muscle soreness and the use of imaging indicators (specifically MRI) will be reviewed. In addition the effect exercise induced muscle damage has on muscle morphology and the effect it has on T2 relaxation times will be investigated.

Data were sourced from sports medicine and science literature, incorporating medical literature sourced through online databases including Academic search premier, Pubmed, Web of Science, CINAHL and Google Scholar. Keywords included in the search were “exercise induced muscle damage”, “endurance running”, “marathon”, “ultramarathon”, “MRI”, “muscle volume”, “cross sectional area”, “muscle morphology”, “quadriceps”, “hamstrings”, “T2 relaxation times”, “plasms creatine kinase activity” and “running performance.”

2.2 MUSCLE DAMAGE AND ENDURANCE RUNNING

Various studies have investigated the physiological effects of marathon and ultramarathon races. Specifically, studies on runners of the 90 km Comrades marathon provided information regarding serum enzyme activities⁽⁴⁶⁾, C – reactive protein levels⁽⁴⁷⁾ and the decrease of muscle power associated with muscle damage⁽⁴⁵⁾. Furthermore, various studies investigated and observed ultrastructural skeletal muscle damage in relation to marathon and ultramarathon races^(3, 4, 48).

2.2.1 ULTRASTRUCTURAL DAMAGE AFTER ENDURANCE RUNNING

Hikida et al⁽³⁾ documented the effects of a 42 km marathon on the muscle morphology of ten male distance runners. Muscle biopsy samples were taken before the marathon, at fifteen minutes after the marathon and on days one, three, five, and seven after the marathon. The pre-race and post-race samples showed signs of repetitive trauma and muscle damage. This included muscle fibre necrosis and inflammation, leukocytic and phagocytic activity, and the presence of erythrocytes and mitochondria in the interstitial space.

Muscle fibre changes included disruptions of the sarcolemma, degeneration and streaming of the z-lines, “contractile knots”, as well as, empty basal lamina tubes in which the contents of the muscle fibre and the sarcolemma had broken down.

An accumulation of phagocytes, erythrocytes and mitochondria within the muscle extracellular spaces were also found. In the muscle samples collected after the marathon, the mitochondria were observed to be actively engulfed by phagocytes in the basal lamina. The abnormalities were most evident on days one and three after the marathon and lasted for the seven day testing period after the marathon⁽³⁾.

In addition, degenerative peripheral myofibrils were observed, with an associated decrease of z-lines as well as mal-aligned sarcomeres and the existence of bodies containing only microfilaments in the muscle biopsy. In the later biopsy samples, atrophic fibres and satellite cells were observed⁽³⁾. The authors suggested that as structural abnormalities were observed in the pre-race muscle biopsy samples and prevailed for the seven day period of the marathon, that the intense training for the marathon as well as the marathon itself were sufficient to induce inflammation and fibre necrosis. The authors also proposed that the inflammatory response after the marathon may contribute to the sensation of delayed onset of muscle soreness⁽³⁾.

Warhol et al⁽⁴⁾ studied 40 male marathon runners who completed a marathon race of 42.2 km. Muscle biopsies were taken from the lateral gastrocnemius muscle on the same day of the race and on days one, two, three, five, seven and ten after the race as well as at week two, three, four, six, eight, ten and twelve after the race. Biopsies taken 48 hours after exercise showed myofibrillar lysis and the disappearance of the sarcoplasmic reticulum. Mitochondrial changes were also documented. It was of interest that these mitochondrial changes occurred in the fibres with normal myofibrils, indicating that these changes are independent of the myofibrillar changes. Further damage was also noted in endothelial cells, with a loss of cytoplasmic matrix and intracellular constituents. Endothelial damage was only seen 24 and 48 hours after competition⁽⁴⁾.

The biopsies taken one week after competition showed a beginning resolution of the acute injury. Most fibres reconstituted their glycogen. Some fibres appeared as empty myotubes with scattered mitochondria. Within one month after the competition myofibrillar damage have been resolved. A prominent feature in these biopsies was the appearance of central nuclei, which correlates with regenerative activity. Eight to ten weeks after competition biopsies showed resolution of the fibre injury and changes of regeneration and repair. Prominent in these biopsies was the appearance of satellite cells⁽⁴⁾.

All the biopsies found injury to the sarcoplasmic units. The extent of the damage varied, which is understandable as the participants varied in age, conditioning and running speed. All participants showed some degree of myofibrillar lysis and the damage was focal and restricted to the sarcomeric units. Less than 10% of the sarcomeric units were damaged, even in the most extreme case. It is noticeable that the damage occurred in the fibres with glycogen and lipid depletion. Warhol et al⁽⁴⁾ findings suggest cell injury but not necrosis, as the absence of inflammatory cells indicates that acute necrosis did not happen. In the biopsies taken ten to twelve weeks after the race, central nuclei were found, an increase in the content of the endoplasmic reticulum as well as prominent Golgi areas, indicating an ongoing regenerative process⁽⁴⁾.

St Clair Gibson et al⁽⁴⁹⁾ described a case study of a 28 year old male who experienced a sudden decline in performance as well as being unable to cope with high training loads. On examination of a muscle biopsy of the vastus lateralis, predominantly type I muscle fibres were found. There were no signs of inflammation or necrosis or excessive regeneration. What stood out was that the mitochondria were highly irregular and abnormal. The biopsy taken from the athlete's triceps muscle showed no signs of any abnormality in the mitochondria⁽⁴⁹⁾.

There seems to be a relationship between long term, high volume endurance training and skeletal muscle damage⁽⁵⁰⁾. Derman et al⁽⁵¹⁾, described a group of endurance athletes with exercise associated fatigue, as well as a decline in performance. The clinical condition for this was called "fatigued athlete myopathic syndrome".

Common characteristics associated with this condition included: history of high volume of endurance training as well as racing, a decline in running performance not related to ordinary aging, and an inability to cope with previous accustomed training loads. These athletes showed clinical signs of skeletal muscle symptoms, such as excessive delayed onset of muscle soreness, muscle stiffness, tenderness and weakness. Structural and ultrastructural changes associated with exercise induced muscle degeneration and regeneration of the skeletal muscle were documented. The physical symptoms of the athletes did not correlate with the acute effects of overtraining as their training loads were significantly less than normal and long periods of rest did not reduce the symptoms⁽⁵¹⁾.

To further investigate this Grobler et al⁽⁵⁰⁾ compared the presence of chronic skeletal muscle ultrastructural damage between endurance athletes with acquired training

intolerance (ATI) and asymptomatic athletes. Athletes were matched for age and years of endurance training. Grobler et al⁽⁵⁰⁾ showed that significantly more athletes with ATI, had structural and ultrastructural skeletal muscle disruption than the control group of asymptomatic athletes. Damage to the internal nuclei, z-disc streaming and variations in muscle fibre size were documented. The other significant finding of this study was the athletes with ATI had a significantly higher volume of endurance training⁽⁵⁰⁾.

Overgaard et al⁽⁵²⁾ investigated membrane leakage and content of Na⁺-K⁺ pumps and Ca²⁺ in humans after a 100 km run. During endurance exercise changes in the ionic environment in and around the muscle can cause fatigue or lead to muscle damage. Overgaard et al⁽⁵²⁾ found that after the race there were significant increases in plasma K, muscle Na⁺-K⁺ pump content and the total muscle Ca²⁺ content. The authors also documented an increase in plasma levels of muscle specific enzymes, creatine kinase (CK) and lactate dehydrogenase (LDH), after the race as well as several days afterwards. This indicated a significant level of muscle membrane leakage. The findings of the increased Ca²⁺ content and membrane leakage supports the theory that Ca²⁺ plays a part in the initiation of the degenerative process of muscle after prolonged or severe exercise⁽⁵²⁾.

The majority of the literature shows that the z-disc is the most vulnerable structure to exercise induced damage, especially eccentric exercise. The evidence is obtained from muscle biopsies and electron microscopic examinations⁽⁴³⁾. Further damage or disruption has been shown in the sarcolemma, t-tubules, myofibrils and the cytoskeletal system. Predominantly type II muscle fibres are affected⁽⁵³⁾.

These physiological changes, as a result of exercise induced muscle damage, contribute to the decrease in a number of measures of physical performance. A good understanding of the underlying mechanism of exercise induced muscle damage is important to understand the decrease in these performance parameters.

2.2.2 NEUROMUSCULAR EFFECTS OF ENDURANCE RUNNING

Numerous studies have shown that endurance running performance after a marathon may be reduced as a result of the loss in muscle strength and a reduction in work capacity. Avela et al⁽⁵⁴⁾ documented a 30% reduction in ankle extensor strength and rate of force development after a marathon.

A full recovery of ankle extensor strength was reported by day two after the marathon and by day four post-race a full recovery was seen in the rate of force development⁽⁵⁴⁾.

The acute fatigue effects of long distance endurance exercise on isometric strength have also been reported. After a 42.2 km marathon a 26% reduction in isometric strength of the knee extensors was reported⁽⁵⁵⁾ as well as a 30% reduction in isometric strength of the knee extensors after a 65km ultramarathon⁽⁵⁶⁾.

Sherman et al⁽⁵⁷⁾ studied the effect of a 42.2 km footrace (marathon) on leg extensor strength and work capacity. A leg extensor fatigue test was implemented and measurements were taken before the marathon and 15 -20 min after as well as on days one, three, five and seven after the marathon. A reduction in work capacity of 47% was reported for immediately after the race⁽⁵⁷⁾.

Chambers et al⁽⁴⁵⁾ studied the time course of recovery of vertical jump height after a 90 km ultramarathon. The test is an indirect way to measure components of fitness related to running performance. The vertical jump test is used to measure muscle power as well as the stretch shortening cycle (SSC) of the quadriceps extensor muscles. Komi⁽⁴⁴⁾ described the SSC as combination of concentric and eccentric muscle actions and provides an indication of the muscle's ability to use stored elastic energy. The squat jump, counter movement jump and drop jump were measured on three occasions before the race and repeated on the day of the race and on days one to five, as well as on days 11, 18, 25 and 32 after the race. The vertical jump was significantly reduced immediately after the race as well as the squat jump for up to 18 days after the race, indicating a reduction in muscle power after an endurance race⁽⁴⁵⁾.

2.2.3 SUMMARY OF MUSCLE DAMAGE AND ENDURANCE RUNNING

Collectively, these studies demonstrate that endurance running results in ultrastructural muscle damage^(3, 4, 58) as well as neuromuscular adaptations^(45, 57, 59). These adaptations may be due to endurance running training and competition and may be associated with a decrease in running performance. There seems to be a large inter-individual variation in the ultrastructural muscle damage seen with endurance running^(3, 4, 58).

Endurance training changes the enzymatic, biochemical and morphological characteristics of skeletal muscle^(60, 61). These adaptations may improve muscle efficiency and may therefore improve endurance running performance. The following section will review the adaptations of skeletal muscle to endurance training.

2.3 ADAPTATIONS OF SKELETAL MUSCLE TO ENDURANCE TRAINING

Endurance exercise leads to a vast variety of responses in skeletal muscle. These include metabolic and morphological responses /adaptations that minimise cellular disruptions during subsequent training sessions⁽⁶²⁾. These adaptations can be divided into chronic and acute responses after endurance training and can influence the performance of athletes⁽⁶²⁾. Hawley⁽⁶²⁾ summarises that the key components of training is the volume/duration , intensity and frequency. The combination of these three elements is termed the training stimulus and can either improve (fitness) or decrease (fatigue) performance⁽⁶³⁾. The adaptations in skeletal muscle as a result of endurance training that is long term is known as the chronic adaptations and the cellular adaptations that happens in response to a single session is known as the acute adaptations⁽⁶⁴⁾. The acute responses of skeletal muscle to exercise include the mitogen-activated protein kinase signalling pathway (MAPK-pathway) and the adenosine monophosphate activated protein kinase (AMP- activated protein kinase) pathway⁽⁶⁴⁾.

2.3.1 PROTEIN KINASE PATHWAYS

The possible mechanisms that can assist with the training induced adaptations are increased blood flow⁽⁶⁾ that leads to a higher influx of hormonal factors to the muscle as well as the activation of receptor-mediator signalling, the release of autocrine and paracrine factors that will stimulate cell surface receptors and this will set in motion signalling cascades⁽⁶⁵⁾ and either concentric or eccentric muscle contraction. Local and systemic factors, such as energy depletion (ATP, glycogen), increased muscle lactate concentration, decreased muscle and blood pH and impaired oxygen flux can also produce signal transduction in skeletal muscle. The responses induced by exercise are numerous and it is likely that these responses depend on the interaction and integration of more than one response at a time. Most of the disruptions to cellular homeostasis happens during the exercise session, but there are several responses of signalling pathways post-exercise that might be significant for the activation of numerous stress-activated protein kinases^(66, 67) and the up-regulation of gene transcription^(68, 69).

Mitogen-activated protein kinase (MAPK) has been marked as one of the proposed mechanisms that are involved in the regulation of the numerous exercise induced responses in skeletal muscle^(70, 71).

These protein kinases are mediators of the stress response to exercise and can be intracellular messengers that connects muscle activation to adaptation⁽⁷²⁻⁷⁴⁾. Exercise also activates cytoplasmic proteins which are then translocated to the nucleus. These nuclear proteins can also be phosphorylated by MAPK^(20, 74).

The AMP- activated protein kinase has also been recognised as a possible candidate for signal transducing and is involved in the transcription regulation through gene regulating processes⁽⁷⁵⁾. It is reported that AMPK is involved in the regulation of metabolic responses and chronic adaptations to exercise. It is also involved in the increase in GLUT-4 translocation after exercise⁽⁷⁶⁾.

2.3.2 MORPHOLOGICAL ADAPTATIONS

The chronic responses of skeletal muscle to endurance exercise include morphological adaptations such as change in muscle fibre type composition. It has been reported that endurance athletes have a higher proportion of type 1 muscle fibres^(60, 77). Type 1 muscle fibres has a higher capillary density as well as a higher oxidative potential⁽⁷⁸⁾.

Coyle et al⁽⁷⁹⁾ documented that the amount of type 1 fibres in skeletal muscle is related to the number of years of training. Endurance training also increases the capillary supply to the skeletal muscle and there is an association with an increase in the activity of enzymes of the mitochondrial electron transport chain as well as reports of an increase in mitochondrial protein concentration⁽⁸⁰⁾.

As a result of endurance training there is a reduction of glycolytic flux in skeletal muscle⁽⁸¹⁾ and this causes a decrease in the maximal activity of glycolytic enzymes⁽⁸²⁾. These changes help the oxidation of fat based fuels over carbohydrate fuels. Endurance trained athletes deplete their glycogen stores at a slower rate during submaximal exercise in comparison to untrained individuals⁽⁸¹⁾. It also reduces the production, uptake and oxidative capacity of plasma glucose during moderate and intense exercise to compensate for lower rate of carbohydrate utilisation and there is a proportional increase in fat oxidation. This is seen by the reduced respiratory exchange rate at the same and relative exercise intensity⁽⁸³⁾.

This shift in substrate selection is due to improved respiratory control sensitivity that arises from the increased mitochondrial density, but there are other factors involved as well, such as greater supply of fat due to an increase in intramuscular triglyceride concentration and possibly other morphological adaptations such as increased recruitment of active muscle mass⁽⁸⁴⁻⁸⁶⁾. In endurance athletes there is a higher capacity to transport lactate across the

sarcolemma. There is also a positive correlation between the exercise intensity and the lactate transport values⁽⁸⁷⁾. It is also reported that endurance athletes with a larger proportion of type 1 fibres have a higher amount of lactate transporters^(87, 88).

In conclusion, endurance exercise produces various skeletal muscle adaptations. These adaptations help to reduce the cellular disturbances during subsequent exercise sessions. It also produces a shift to a greater dependency on fat as a fuel. All these adaptations result in an improved performance capacity^(62, 89).

2.3.3 NEUROMUSCULAR RESPONSES AND ADAPTATIONS TO ENDURANCE

TRAINING

The neuromuscular responses to endurance training are usually less than the responses seen after strength training exercises. It is important to note however, that the magnitude of fatigue remains dependent on the intensity and/or duration of the exercise. Following strength training, there is a large decrement in strength and neural activation; however, there is only a small-scale decrement in strength and neural activation following an endurance exercise session like running⁽⁹⁰⁾. Nicol et al⁽⁵⁵⁾ showed that marathon running induced a significant amount of fatigue. Endurance running requires a smaller amount of force production compared to strength exercise, and even with maximal uphill running, athletes were not able to produce maximal muscle activation⁽⁹¹⁾. Running, however, involves strenuous repetitive force production implementing the stretch – shortening cycle⁽⁴⁴⁾.

The maximal voluntary contraction (MVC) of the lower leg decreased by 15%, following a 5 km time trial⁽⁹²⁾. Millet et al⁽⁹³⁾ reported a 24% decrement in MVC, following 30 km of high intensity trail running and a 22 % decrease in MVC was observed after simulated marathon running⁽⁵⁵⁾. It appears that there is a strong correlation between strength loss and a decrease in voluntary muscle activation⁽⁹³⁾, but the average decrease in MVC is not necessarily related to a decrement in running velocity during a running trial^(90, 92).

Furthermore, it has been reported that the activity of the leg extensor muscles, specifically during the braking phase, is more than that recorded during a maximal voluntary contraction⁽⁹⁴⁾. While a decrement in force production and muscle activation are often observed after endurance running, trained middle and long distance runners who completed a 20 to 40 minute intensive running session, showed an increase in jump and half-squat performance, although muscle activation was observed to decrease⁽⁹⁵⁾.

In novice runners, it appears that 16 weeks of moderate marathon training induced specific muscle adaptations that would support endurance performance including the size of the muscle, contractile properties and fibre type distribution, that depends on the muscle examined⁽⁹⁶⁾. As part of this study muscle biopsies were obtained from the soleus and vastus lateralis muscles before, after 13 weeks and after three weeks of taper. Single muscle fibre size, contractile function (strength, speed and power) and oxidative enzyme activity (citrate synthase) were measured. Fibre type distribution was also measured before and after the 16 week intervention. There was an increase in vastus lateralis MHC I fibre diameter from the start of the study to 13 weeks after and power was reduced. There were no changes in any of the soleus parameters. The most important finding was that modest marathon training induced very specific skeletal muscle adaptations that would likely support the ability to perform 42.2 km of continuous running⁽⁹⁶⁾.

When comparing sprint runners to distance runners, it appears that distance running elicits or favours lean muscles with shorter fascicle lengths and smaller pennation angles compared to those observed in sprinters⁽⁹⁷⁾. Furthermore, it has been reported that trained strength athletes and sprinters are able to produce a greater maximal force as well as a greater maximal force per unit of cross-sectional area than endurance athletes⁽⁹⁸⁾.

Hakkinen et al⁽⁹⁸⁾ reported on the effects of combined strength and endurance training programme, compared to a strength training programme alone on functional and structural neuromuscular adaptations. Both groups had similar improvements in the one-repetition maximum load, maximum isometric force, maximum integrated electromyography of the vastus lateralis muscle, cross-sectional area of the quadriceps femoris muscle and mean fibre areas of type I, IIa, IIb muscle fibres. An improvement in the rate of force development was only demonstrated in the strength-training group. It was suggested that the increase in power development was partially assisted by a more rapid voluntary neural activation of the trained muscles⁽⁹⁸⁾.

Cycling is a popular and practical endurance training mode to utilise in endurance training studies. Saunders et al⁽⁹⁹⁾ documented the effects of endurance training in cyclists. There was a reduction in quadriceps muscle activity associated with endurance training, potentially due to a decreased oxygen demand of a bout of high-intensity submaximal exercise. The underlying mechanism for the weakening of end-exercise muscle activity was unclear. It was suggested that the increase in muscle mitochondrial content and oxidative capacity could be associated with a decreased reliance on anaerobic energy supply. This

extended the time to fatigue the muscle fibres. Therefore, less additional muscle fibres were recruited to replace the already fatigued fibres and so decreasing the end-exercise active muscle and oxygen consumption⁽⁹⁹⁾.

Furthermore, Widrick et al⁽¹⁰⁰⁾ compared the force-velocity and power-velocity properties of single muscle fibres between endurance-trained and sedentary participants. There was a significant reduction in type I and type IIa muscle fibre diameter, peak power output and absolute force production in the endurance-trained group, compared to the sedentary group. In addition the endurance-trained groups had significantly higher maximum shortening velocities. It was proposed that, although the ability to produce force was reduced in the endurance-trained group, the increased shortening velocity caused the muscle fibres to keep a higher level of force production. Thus, there was an increased contribution of type I muscle fibres to the total power output, and a decreased reliance on the type IIa muscle fibres⁽¹⁰⁰⁾.

It appears that muscle adaptations are specific to the force and velocity of movements, rather than the actual movement patterns or exercises used. One can therefore postulate that athletes involved in different types of training (running vs cycling) will have different muscular adaptations⁽¹⁰¹⁾. Furthermore, Blazevich et al⁽¹⁰¹⁾ reported an increase in fascicle length that was associated with changes in the force and velocity characteristics of the muscle. It was also established that the changes in muscle architecture (fibre hypertrophy, increased fascicle angle and length) are associated with an increase in muscle mass and the force-generating ability of the muscle⁽¹⁰¹⁾.

2.4 SUMMARY OF THE LITERATURE: ADAPTATIONS TO ENDURANCE TRAINING

Endurance training is therefore associated with morphological and neuromuscular adaptations. These adaptations are mainly to reduce the extent of cellular disturbances during subsequent training bouts. In addition, the effects of regular exercise training are associated with chronic adaptations of skeletal muscle such as increased capillary density, increased mitochondrial enzyme activity and an increased dependency on fat as a fuel. All these adaptations are associated with an improvement in endurance performance⁽⁶²⁾.

However, an increase in exercise intensity or duration or unaccustomed exercise is commonly associated with exercise-induced muscle damage.

One of the primary factors identified responsible for the development of exercise-induced muscle damage is lengthening muscle actions. This will be discussed further in the following section.

2.5 LENGTHENING (ECCENTRIC) MUSCLE ACTIONS

It is well-documented that lengthening (eccentric) exercise is the main factor involved in exercise induced muscle damage^(33, 102). Faulkner⁽²⁾ described three different types of contractions that can occur when skeletal muscle are activated and contract. When the force developed by the muscle is greater than the load on the muscle, it will lead to a shortening (concentric) contraction. If the force developed by the muscle is equal to the load on the muscle an isometric contraction results. When the load on the muscle is greater than the force developed by the muscle, the result will be a lengthening (eccentric) contraction.

Asmussen⁽¹⁰³⁾ reported in 1956 that participants who performed shortening muscle actions (positive work) had an inclination to fatigue more quickly than those participants who performed a lengthening muscle action (negative work). He also documented that only those subjects who performed the lengthening contractions experienced delayed onset muscle soreness (DOMS). Asmussen⁽¹⁰³⁾ concluded that the specific mechanical tension that is linked to negative work was probably the cause for the discrepancies in symptoms between positive and negative work. Other studies have shown that high numbers of shortening (concentric) or isometric contractions can result in a moderate increase in indirect markers of muscle damage^(104, 105), but the majority of the evidence supports Asmussen's theory that lengthening muscle actions are the primary source of muscle damage during exercise^(106, 107).

Early studies indicate that lengthening (eccentric) muscle contractions lead to greater muscle damage than isometric or concentric contractions^(33, 102, 108, 109). Therefore eccentric actions in various forms have been implemented in studies to experimentally induce muscle damage⁽²⁵⁾. Submaximal and maximal voluntary contractions as well as electrically stimulated contractions have been implemented. Studies used eccentric exercises of isolated muscle groups, such as elbow flexors or knee extensors or exercises that are associated with eccentric contractions such as downhill running or stair descends. Some of these studies used protocols that are rather artificial in order to produce exercise induced muscle damage such as resisting the action of a weight or lever^(35, 36, 110), as in eccentric cycling. Concentric and eccentric contractions were also combined in some protocols, with

the emphasis on the eccentric action, such as bench stepping, downhill running and drop jumps⁽¹¹¹⁻¹¹³⁾.

Lengthening (eccentric) muscle contractions have special features which can possibly indicate why these type of actions result in muscle damage⁽¹¹⁴⁾. In vitro studies showed that in the force-velocity relationship of maximally contracted muscle, the force produced by an eccentric contraction is 1.5 -1.9 times more than the isometric force^(115, 116). In human studies the torque-velocity relationship differs due to neural inhibition^(117, 118). Motor unit activation is also less for maximal eccentric contraction and fewer motor units are required for a specific force⁽¹¹⁴⁾. Thus, one theory is that the combination of high force and less fibre recruitment puts more mechanical strain on the structures involved and can lead to muscle damage⁽¹¹⁴⁾.

The mechanism of force generation during a lengthening action entails the cross-bridges being detached mechanically rather than undergoing the detachment process involving ATP splitting⁽¹¹⁹⁾. It is also documented that the compliant part of the individual cross bridges is also stretched further during eccentric actions and there is a positive correlation between length and damage, since eccentric muscle actions performed at a long muscle length leads to greater evidence of muscle damage⁽⁹⁾. It is well-documented that eccentric exercise induced muscle damage is initiated by mechanical factors⁽²⁸⁾. The amount of force produced and the magnitude of strain appear to be factors determining muscle damage. This theory is straight forward when taking into account the loading profile and the range of motion associated with high force eccentric actions, e.g. plyometrics and resistance training. When investigating muscle exercise induced muscle damage from prolonged and lower intensity activities such as long distance running, there appears to be other factors involved, such as metabolic depletion, calcium influx, the generating of reactive oxygen species and the musculotendinous stiffness regulation⁽⁴⁴⁾.

The initial process of muscle damage involves disrupted sarcomeres, damage to the excitation-contraction coupling system. During these stages the symptoms of exercise-induced muscle damage are experienced. The symptoms include soreness, swelling and stiffness and are collectively referred to as delayed onset of muscle soreness⁽⁴³⁾.

2.6 DELAYED ONSET MUSCLE SORENESS

Muscle soreness is one of the most commonly used markers of muscle damage in human studies⁽¹²⁰⁾. However it shares a poor relationship with histological evidence of muscle damage and measures of muscle function⁽¹²¹⁾.

It has been well-documented that there is a characteristic time course of muscle soreness. Exercised muscle appears to be pain free for approximately eight hours after exercise, then soreness increases. Muscle soreness peaks at 24 to 48 hours after exercise and all discomfort usually subsides within 96 hours^(10, 33, 109, 122), hence the term 'delayed onset muscle soreness'. The sensation of soreness includes muscle tenderness, pain on palpation and mechanical stiffness in the muscle. This leads to pain when the muscle is passively stretched or activated^(10, 122).

The degree of soreness is different from one type of exercise to another and is dependent on the amount of damage induced⁽¹⁷⁾. Exercise such as downhill running or isokinetic eccentric knee extension produced less muscle damage and soreness value of four or five on a scale of one (no soreness) to ten (very sore). Maximal eccentric exercise of the elbow flexors produced values of seven to eight. The differences in soreness values are consistent with the differences in prolonged force loss and increase in plasma CK activity. The maximal eccentric contraction of the elbow flexors produced greater and longer periods of force loss and higher plasma CK activity. There is a difference in the extent of soreness between downhill running and high force eccentric exercise, but the time course remains similar⁽¹⁷⁾.

Friden et al⁽¹²³⁾ investigated muscle fibre size and intramuscular pressure after eccentric exercise. Participants performed an eccentric exercise of the tibialis anterior muscle, and muscle biopsies were taken. Muscle biopsies taken after 48 hours showed larger muscle fibres and greater intramuscular pressure. It is thus possible that the soreness is a result from swelling and pressure in the muscle. Friden et al⁽¹²³⁾ also documented that the larger the increase in muscle fibre area, the longer it took for the tissue fluid pressure to subside. Crenshaw⁽¹²⁴⁾ confirmed fibre swelling and increased intramuscular pressure in knee extensor muscle after eccentric exercised induced muscle damage.

The presence of swelling is indicated after eccentric exercise⁽³⁴⁾, but MRI changes indicating muscle oedema are not coincident in time with muscle soreness⁽²⁴⁾. Clarkson et al⁽¹¹²⁾ showed that after eccentric elbow flexion, swelling started gradually after about 48 hours and peaked up to ten days after exercise, indicating that soreness peaks long before peak swelling occurs. It was also documented that swelling was located in the muscle for about five days after exercise and then transferred to the subcutaneous area⁽¹¹²⁾.

Histamines, bradykinins and prostaglandins have been indicated as being involved in the production of muscle soreness. When muscle tissue is damaged, the noxious chemicals are released and activate type III and type IV nerve endings. These nerve endings transport messages of pain from the muscle to the central nervous system⁽¹²⁵⁾. There is however no direct evidence for this theory and needs to be investigated further⁽¹⁷⁾.

Other symptoms associated with DOMS are strength loss, pain, muscle tenderness, stiffness and swelling⁽¹²⁶⁾. McHugh et al⁽¹²⁷⁾ documented a loss of strength immediately after exercise or within the first 48 hours, and full recovery generally taking more than five days. Pain and tenderness peaked between one to three days after exercise, subsiding within approximately seven days. Stiffness and swelling peaked three to four days after exercise and resolved within ten days⁽¹²⁷⁾.

2.7 MECHANISMS UNDERLYING EXERCISE INDUCED MUSCLE DAMAGE AND DELAYED ONSET MUSCLE SORENESS

Armstrong et al⁽¹²⁸⁾ suggested that the muscle injury sequence can be divided into two stages: the initial event which initiates the injury process followed by the loss of Ca²⁺ homeostasis and the start of the Ca²⁺ overload phase. Calcium buffering and translocation mechanisms regulate the cytosolic Ca²⁺ levels within muscle cells. After muscle injury these mechanisms are overwhelmed and various intrinsic pathways are triggered. These pathways include the activation of Ca²⁺-dependent proteolytic and phospholipolytic pathways. These pathways break down structural and contractile myofibre proteins and the myofibre membrane⁽¹²⁹⁾. Following these initial phases is a phagocytic phase, during which the inflammatory response allows the removal of damaged tissue and the regenerative phase during which damaged fibres repair⁽¹³⁰⁾. There are two theories explaining the initial injury event, the mechanical stress and the metabolic stress model⁽¹²⁸⁾.

2.7.1 MECHANICAL STRESS MODEL

The primary argument in support of this model is the fact that exercise induced muscle damage is more prominent following lengthening (eccentric) exercise. As discussed in Section 2.5, lengthening exercise have been shown to deliver a higher amount of force and a lower amount of recruitment of motor units in comparison to shortening (concentric) and isometric exercises⁽¹¹⁴⁾. McCully et al⁽²²⁾ theorised that the number of attached cross-bridges decreases as the muscle fibre length increase during lengthening actions. This would lead to an increase in force per cross-bridge and would expose the contractile proteins to failure. These findings imply that during a lengthening action the mechanical stress per fibre is higher than during a shortening action. The repetition of these lengthening actions may impose enough mechanical stress on the muscle to elicit failure in some fibres⁽¹³⁰⁾.

2.7.2 METABOLIC STRESS MODEL

In this model, it is suggested that the initial events in exercise induced muscle damage is caused by metabolic deficiencies within the working muscle or that these deficiencies predispose the muscle fibre to mechanical stress⁽¹³⁰⁾. During physical activity, metabolic flux is increased to match the rate of adenosine triphosphate (ATP) synthesis to the rate of ATP hydrolysis. This is achieved through the glycolytic and oxidative metabolic pathways⁽¹³¹⁾. During muscle activity there is usually a decrease in the concentrations of high energy phosphates⁽¹³¹⁾. There is the possibility that the ATP concentrations could drop so low that muscle damage can occur. Krstrup et al⁽¹³²⁾ supports this theory by reporting that during a football match approximately 50% of the muscle fibres were either almost or completely depleted of glycogen. Warhol et al⁽⁴⁾ also showed that in marathon runners the muscle damage was focal and restricted to fibres almost completely depleted of glycogen.

Armstrong⁽¹²⁸⁾ considered that the decrease action of Ca^{2+} - adenosine triphosphatase (ATPase) in the sarcoplasmic reticulum or sarcolemma may be a mechanism for metabolic muscle injury. This would compromise the removal of Ca^{2+} , which in turn would cause a rise in the level of cytosolic Ca^{2+} concentrations. This would result in a cascade of metabolic events that lead to muscle fibre degeneration⁽¹²⁸⁾.

It has been suggested that metabolic muscle damage is the result of ischaemia or hypoxia during exercise of a prolonged nature⁽¹³³⁾. It is considered that ischaemia causes changes in ion concentrations, metabolic waste accumulation and ATP deficiency. These changes result in muscle damage⁽²⁵⁾.

Ebbeling and Clarkson⁽¹³⁴⁾ examined marathon running and reported muscle damage and suggested that the presence of muscle damage may be a result of reduced metabolic clearance. However, marathon running has a substantial amount of stretch shortening cycles and therefore also a substantial amount of lengthening muscle contractions. Schwane⁽¹³⁵⁾ suggested that the muscle damage is mainly due to mechanical stress from the lengthening muscle actions and that ischaemia may only increase the damage. It has been reported that uphill running has a higher metabolic cost and little muscle damage when compared to downhill running. Downhill running showed a lower rate of metabolic cost but a high incidence of muscle damage⁽⁵⁾.

While the pathophysiological mechanism of exercise induced muscle damage still seems unclear, the effects of the condition is well documented. In the following section these consequences will be reviewed.

2.8 EFFECTS OF EXERCISE INDUCED MUSCLE DAMAGE

2.8.1 SKELETAL MUSCLE

2.8.1.1 Muscle fibre damage

2.8.1.1.1 Histological and ultrastructural changes

In skeletal muscle the contractile filaments are kept as a highly organised structure by specialised proteins. This specific order allows for generation of rapid and synchronised movement as well as force in a specific direction⁽¹³⁶⁾. When muscle damage occurs one of the significant morphological features is the disruption and Z-disk streaming observed in muscle samples⁽²⁸⁾.

Friden et al⁽¹³⁷⁾ investigated participants that performed repeated stair descents. Muscle biopsies were taken from the soleus muscle two and seven days later. The biopsy analysis showed myofibrillar disturbances as well as damage to the sarcolemma, T-tubules, cytoskeleton and Z-line streaming. In the follow-up study by Friden et al⁽¹³⁸⁾ the biopsies showed evidence of focal disturbances, as well as changes in the ultrastructural integrity. These changes were observed as Z-line streaming, Z-lines out of order, a loss of thick myofilaments as well as a loss in mitochondria. It also showed a disturbance in the arrangement of filaments in the A-band. Type II muscle fibres were the main fibres involved⁽¹³⁷⁾.

Newham et al⁽¹⁰²⁾ showed that there was greater damage shown in the biopsies taken 24 to 48 hours after the exercise than in the biopsies taken immediately after exercise. Jones et al

⁽¹²¹⁾also showed that the muscle damage appeared to worsen in the immediate days after the exercise and then were gradually repaired within two to three weeks post exercise.

The characteristic “smearing” of the sarcomeric structure is noticeable after eccentric contractions and it has been suggested that the loss in force production and the symptoms of delayed onset of muscle soreness may be attributed to structural and /or contractile protein degradation ⁽²⁷⁾. In animal studies where animals were subjected to eccentric exercise, a marked loss of the structural protein desmin was recorded within minutes of exercise onset⁽¹³⁹⁾. There has also been reported loss of desmin in muscle fibres following eccentric exercise in human studies⁽¹⁴⁰⁾. On the other hand, Yu et al ⁽¹⁴¹⁾ documented increases in desmin staining of muscle biopsy samples, after an eccentric cycling exercise. Yu et al ⁽¹⁴¹⁾ suggested that the increase in or upregulation of desmin may be an indicator of muscle regeneration and differentiation. Other studies supported this theory subsequently⁽¹⁴²⁾. In contrast to the accepted opinion that sarcomere disruption is indicative of muscle damage, Yu et al ⁽¹⁴²⁾, suggested that it may be an indicator of muscle adaptation and regeneration. Therefore it may be useful as model of muscle adaptive processes.

Numerous studies (both animal and humans), have been implemented in the study of exercise induced muscle damage and specifically eccentric exercise induced muscle damage. Discrepancies have been reported between animal and human literature. These discrepancies include the degree of myofibrillar disruption ⁽¹⁴²⁾ and the inflammatory response ⁽¹⁴³⁾ both of which seems to be more intense in animal models. Cramer et al ⁽¹⁴⁴⁾ reported that these differences may be the result of discrepancies between voluntary and stimulated contraction protocols. Human studies rely on voluntary contractions whereas animal studies implement eccentric contraction by means of electrical stimulation. Cramer et al ⁽¹⁴⁴⁾ showed that participants who performed voluntary eccentric (lengthening) contractions had significantly less Z-line disruptions, showed no signs of myofibre disturbances and had an insignificant inflammatory response in comparison to participants who received electrical stimulation during lengthening actions.

Lauritzen et al ⁽¹⁴⁵⁾ analysed muscle samples of five human subjects who experienced a greater than normal loss of muscle strength after 70 eccentric contractions of the biceps brachii. Widespread Z-line disruptions, significant myofibre disruption as well as necrosis were documented, challenging the belief that such extensive muscle damage and inflammation only occur in animal models.

The study also differed from other studies in that it analysed muscle tissue from the upper limb rather than the lower limb (the vastus lateralis muscle is commonly used). The more severe pathological observations may be attributed to this as muscles of the upper limb are not used consistently in an eccentric manner compared with muscles from the lower limb⁽¹⁴⁵⁾.

Hyldahl and Hubal⁽²⁷⁾ suggested that the muscle response to lengthening actions functions on a continuum, where minor changes lead to a cell signalling mediated adaptive response and a greater or more intense stimuli appears to increase the chance of a more severe inflammatory response. The extent of this response is further mitigated by the stimulus novelty, the specific muscle group exercised, the mode of muscle activation and other modifying aspects, such as genetic variation⁽²⁷⁾.

2.8.1.1.2 Extracellular matrix damage

Abrahams⁽¹⁴⁶⁾ proposed that the muscle soreness following unaccustomed or novel exercise may be due to damage of muscle connective tissue elements, known as the extracellular matrix (ECM). The extracellular matrix is an amalgamation of collagen proteins that compromise one to ten percent of skeletal muscle. Its function is to support skeletal muscle structure, while providing a passive component of force⁽¹⁴⁷⁾. Furthermore the extracellular matrix transmits forces laterally between adjacent muscle fibres and fascicles through an intricate network of shear links. A network of membrane-spanning integrin, dystroglycan, and proteoglycan complexes facilitates the mechanical link between the ECM scaffold and the muscle fibre cytoskeleton. These complexes form the basis of force transmission from the outside to the inside of the cell and vice versa⁽¹⁴⁸⁾. Stauber et al⁽³⁰⁾ investigated the changes in the extracellular matrix with participants that performed eccentric exercises of the elbow flexors. Muscle biopsies of the biceps brachii were taken 48 hours afterwards. These showed mast cell degranulation in the perimysial area, near the blood vessels. Mononuclear cells were observed in both the perimysial and endomysial areas. Stauber et al⁽³⁰⁾ documented that the extracellular matrix pulled away from the fibres into the interstitial space indicating possible damage or disturbance. Also present in the perimysial and endomysial areas were fibrinogen and albumin. These are normally found in capillaries and these results indicated possible damage to the capillaries.

Stauber et al⁽³⁰⁾ further developed the idea that extracellular matrix damage occurs with eccentric exercise through this study.

Evidence from both human and animal studies show increases in collagen expression and higher enzymatic activity of collagen-degrading proteins after eccentric actions⁽¹⁴⁹⁾. The augmentation and timing of collagen degradation and synthesis may reflect a coordinated remodelling process, which is indicative of widespread extracellular matrix disruption⁽²⁷⁾. Mackey et al⁽¹⁴⁹⁾ documented that 100 eccentric contractions of knee extensors induced increased levels of the extracellular matrix, remodelling enzyme matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs) in serum for up to 14 days following the exercise protocol. This was then followed by an increased collagen IV staining 22 days post exercise.

Friden and Lieber⁽²⁸⁾ summarised the different types and locations of cellular damage or disturbances in the following table.

Table 2.1 (adapted from Friden and Lieber, 2001) : Evidence of exercise induced muscle structural changes in muscle fibres⁽²⁸⁾

Site of fibre lesion	Reference
Primary or secondary sarcolemmal disruption	Jenkins, 1988 ⁽¹⁵⁰⁾ , Armstrong, 1990 ⁽¹³³⁾ , Duan et al, 1990 ⁽¹⁵¹⁾
Swelling or disruption of the sarcotubular system	Friden and Lieber, 1996 ⁽¹⁵²⁾ , Armstrong 1990 ⁽¹³³⁾ , Willems et al, 1999 ⁽¹⁵³⁾
Distortion of the the myofibrils' contractile components	Armstrong et al 1983 ⁽⁵⁾ , Newham et al 1983 ⁽¹⁰²⁾ , Friden et al 1984 ⁽¹⁵⁴⁾ Friden et al 1988
Cytoskeletal damage	Friden et al 1984 ⁽¹⁵⁴⁾ , Lieber et al 1996 ⁽¹³⁹⁾ , Friden and Lieber 1998 ⁽⁵³⁾
Extracellular myofibre matrix abnormalities	Stauber 1989 ⁽³⁰⁾

2.8.2 NEUROMUSCULAR EFFECTS

Warren et al⁽¹²⁰⁾ suggested that the measures of muscle function provide the best way to evaluate the extent and time course of exercise induced muscle damage. The functional impairments such as reductions in strength and power are immediate and prolonged. It may also very well be the most important indicator of muscle damage when investigating athletic performance after exercise induced muscle damage.

2.8.2.1 Force loss

According to the review done by Warren et al⁽¹²⁰⁾, measures of isometric strength are the most commonly used method to evaluate muscle function after eccentric exercise.

The method entails the participants to perform a maximal voluntary contraction (MVC) of a specific muscle group for two to five seconds to determine muscle strength. The majority of studies documented a reduction in isometric strength immediately after exercise and that the recovery process is gradual and prolonged. The magnitude and time course of strength loss appear to be dependent on the training history of the muscle group. Elbow flexors appeared to have the longest lasting strength loss in comparison to the strength loss of the muscle of the lower limb ^(14, 34, 122).

2.8.2.2 Muscle soreness

Muscle soreness is evident hours after the performance of the exercise induced muscle damaging protocol and peaks at 24 to 48 hours post-exercise ⁽¹⁷⁾. Delayed onset muscle soreness (DOMS) has been discussed in Section 2.6 (page 17).

2.8.3 SUMMARY: EFFECTS OF EXERCISE INDUCED MUSCLE DAMAGE

From the literature it is evident that exercise induced muscle damage has effects over a broad spectrum, ranging from ultrastructural damage, to neuromuscular effects such as muscle soreness and force loss. Furthermore, it is clear that there are a variety of ways to investigate exercise induced muscle damage and that there are also varied methods and indicators implemented. Biochemical markers of muscle damage are one of these methods and will be discussed in the following section and the muscle protein, creatine kinase will be discussed in detail as an indirect marker of muscle damage ⁽¹⁵⁵⁾.

2.9 BIOCHEMICAL INDICES OF EXERCISE INDUCED MUSCLE DAMAGE

2.9.1 MEMBRANE LEAKAGE

Unaccustomed or exercise of prolonged duration results in increased muscle membrane permeability and subsequent appearance of muscle-specific proteins in circulation. Though the mechanisms that drive increased membrane permeability are still debated, the appearance and resolution of muscle proteins has been documented extensively ⁽²⁷⁾.

The structural damage in the muscle cell is accompanied by the leakage of muscle proteins such as creatine kinase out of the cell and into the circulation. Various studies have implemented creatine kinase as an indirect measure of muscle damage ⁽³⁷⁾.

Other biochemical markers that have been used include the muscle enzymes lactate dehydrogenase, aspartate aminotransferase and carbonic anhydrase isoenzyme ^(39, 42).

Muscle proteins such as myoglobin, heart fatty acid binding protein, troponin and myosin heavy chain have also been used as indicators of muscle damage⁽⁴¹⁾.

2.9.2 CREATINE KINASE AS INDICATOR OF MUSCLE DAMAGE

2.9.2.1 Background

Creatine kinase is a compact enzyme that is found in the cytosol as well as the mitochondria of tissues where there is a high energy demand. It is composed of two polypeptide subunits and there are two types of subunits: M (muscle type) and B (brain type). These subunits are responsible for the formation of three tissue – specific isoenzymes: CK – MB (cardiac muscle), CK – MM (skeletal muscle), and CK –BB (brain). The ratio of subunits differs with muscle type and the ratio for skeletal muscle is 98% MM and 2% MB. In the mitochondria two specific forms of mitochondrial CK (Mt – CK) can be found: a nonsarcomeric type and a sarcomeric type. The sarcomeric Mt – CK is expressed in cardiac and skeletal muscle⁽¹⁵⁶⁾.

CK also occurs as a macroenzyme and catalyses the reversible phosphorylation of creatine to phosphocreatine and of ADP to ATP⁽¹⁵⁷⁾. It forms the centre of the phosphocreatine circuit. In this circuit, the cytosol enzymes are coupled to glycolysis and produce ATP for muscle activity. Mitochondrial creatine kinase (Mt –CK) is linked to the electron transport chain and can use mitochondrial ATP to regenerate phosphocreatine, which returns to the cytosol to resupply cytosolic PCr. This system is important for the production and maintenance of energy supply⁽¹⁵⁸⁾. It therefore explains why skeletal muscle has high levels of CK.

The significance of raised levels of CK in serum following physical activity has been discussed extensively in the literature. However, it is still unclear why low to moderate intensity exercise should result in a release of CK into blood serum. Another confusing issue is the fact that resistance training allows for the greatest release of CK, but at the same time it is the best method for muscle hypertrophy. Myofibrillar CK – MM is bound to the M-line of the sarcoplasmic reticulum of myofibrils. It is also found in the space of the I-band sarcomeres and provides support for muscle energy requirements⁽¹⁵⁹⁾.

The question then arises; if the enzyme is usually confined to the muscle cell, why does raised levels of CK after a period of exercise represent a degree of muscle damage and loss of muscle cell integrity? It is possible that there may perhaps be another molecular reason that is not related to muscle damage, but rather a temporal disturbance or disruption of muscle processes⁽¹⁶⁰⁾.

2.9.2.2 Creatine kinase as marker of exercise-induced muscle damage

Creatine kinase has been the most popular method to use, as the magnitude of the increase in CK levels is so much greater than the other proteins. It is also relatively inexpensive and easy method of testing. The variations in the magnitude of creatine kinase levels may be influenced by gender, type of muscle actions, the intensity and duration of exercise, the training state of the participants, genetic predisposition as well as the distribution of muscle fibre types in skeletal muscle ⁽¹⁷⁾.

Two types of exercise are being utilised to investigate muscle damage, downhill running and high force muscle contractions^(161, 162). However, these two types of exercise have very different CK responses. After downhill running CK levels peak after 12 -24 hours, whereas with high force muscle actions, CK levels increase after about 48 hours and peaks about four to six days after exercise. This difference in CK response is well documented, but the underlying reasons for this needs to be further investigated⁽¹⁶⁰⁾.

There is a large variability in CK response to exercise and it does not seem to be linked to sex, muscle mass or activity level of participants. However, it does remain clear that plasma CK activity is an indirect measure of muscle damage ⁽¹⁷⁾.

2.9.3 SUMMARY OF THE LITERATURE: INDICES OF EXERCISE-INDUCED MUSCLE DAMAGE AND ENDURANCE RUNNING

It is therefore evident that although plasma CK activity is a common indicator of exercise-induced muscle damage, it still remains an indirect marker of muscle damage. The measurement of other muscle proteins, such as myosin heavy chain, also provides an indirect indication of exercise-induced muscle damage. It may be suggested that plasma CK should be considered as only one of the indirect indicators of exercise-induced muscle damage and that it should be measured in conjunction with changes in skeletal muscle function following muscle damage and the symptoms of delayed onset of muscle soreness, to provide an indirect description of exercise-induced muscle damage ⁽¹⁷⁾.

As described earlier muscle damage is usually induced by maximal and submaximal lengthening exercises, but it is also evident with exercises that contains a high volume of stretch shortening cycles, due to the involvement of lengthening contractions per se or as a result of a metabolic accumulation that may lead to muscle stress and impairment of the muscle fibres. Distance running involves high volume of stretch shortening cycles. The following section will briefly discuss stretch shortening cycles.

2.10 THE STRETCH SHORTENING CYCLE

During movement, lengthening (eccentric) contractions rarely occur in isolation. Muscle function occurs in a sequence where an active lengthening (eccentric) contraction is followed by an active shortening (concentric) contraction. This phenomenon is known as the stretch shortening cycle (SSC)⁽⁴⁴⁾. This phenomenon comes into play when the body is subjected to impact or stretch by external forces such as gravity and daily functional activities. The SSC is also involved in most sporting activities such as running, jumping, throwing and weightlifting^(25, 44, 163).

The purpose of the SSC is to improve performance during the last shortening (concentric) contraction when compared with the performance of an isolated shortening (concentric) contraction⁽⁴⁴⁾. Four mechanisms underlying performance enhancement have been identified. These contributors are: the time available to develop the force, storage and re-utilisation of elastic energy, the potentiation of the contractile machinery and the contribution of reflexes^(164, 165). Lengthening (eccentric) contractions are an integral part of the SSC. Muscle damage is a common occurrence during activities that involves the SSC. Distance running is one of these activities and it is estimated that approximately 10 000 SSC muscle actions occur during a marathon race⁽¹⁶⁶⁾. It is also documented that running downhill increases the contribution of lengthening (eccentric) actions to performance and is responsible for more muscle damage than uphill running⁽¹¹⁾.

The symptoms of muscle damage are usually observed immediately and a few days after the running bout. A summary of endurance events (with a high volume of SSC) and the symptoms of muscle damage as well as biochemical indicators of muscle damage are shown in Table 2.2.

Table 2.2: Summary of endurance running events and markers of muscle damage.

	Study design	Sample size (n)	Participants	Endurance event	Indicators of EIMD	Biochemical indicators of EIMD	Time of assessment	Significant results
Nie et al⁽¹⁶⁷⁾	Descriptive cross sectional LOE: III	12 male	Adolescent distance runners 16.2 ± 0.6 years	21 km		CK LDH AST ALT HBD	At rest, two, four and 24 hours after event	↑CK ↑LDH ↑AST ↑HBD
Denissen et al⁽¹⁶⁸⁾	Observational, cohort study LOE: III	19 participants (6 male, 13 female)	Recreational runners Age ≤ 50 years Average training = 60 km.wk ⁻¹	Staged, 3 day trail run	DOMS TC	CPK	Pre and post-stages, 24 h and 72 h post-race	↑CPK ↑DOMS ↓TC
Millet et al⁽⁵⁶⁾	Descriptive cross sectional study LOE: III	9 male	Healthy, endurance athletes 41.6 ± 5.9 years	65 km ultramarathon	MVC MVC-activation		Week pre-race, immediately post-race	↓MVC ↓MVC-activation
Gatterer et al⁽¹⁶⁹⁾	Descriptive cross sectional study LOE: III	16 male	41 ± 8 years	305 km in 8 stages	Jump performance	CK	Pre-race and post-stage	↑CK ↓jump performance
Kobayashi et al⁽¹⁷⁰⁾	Descriptive cross sectional study LOE: III	15 male	Male, recreational runners	42.2 km marathon		CK LDH	Pre and post-race, serially during recovery period of three weeks	↑CK ↑LDH

Table 2.2: Summary of endurance running events and markers of muscle damage.

	Study design	Sample size (n)	Participants	Endurance event	Indicators of EIMD	Biochemical indicators of EIMD	Time of assessment	Significant results
Lippi et al ⁽¹⁷¹⁾	Descriptive cross sectional study LOE: III	15 male	Healthy, trained runners	21.1 km		CK AST LDH Myoglobin	Pre-race, immediately post – race, three , six, and 24 hours post-race	↑AST ↑CK ↑LDH ↑Myoglobin
Kim et al ⁽¹⁷²⁾	Descriptive, cross sectional study LOE: III	54 male	Trained, ultra-marathon runners 45.7 ± 5.1 years	200km		Plasma CPK LDH AST ALT	Pre-race, mid-race and post-race	↑ CPK ↑ LDH ↑ AST ↑ALT
Kim et al ⁽¹⁷³⁾	Descriptive, comparative cross sectional study LOE: II	20 male (10 marathon, 10 ultra-marathon)	Experienced marathon runners mean age of 50 years Experienced ultra-marathon runners mean age of 52 years	42.2km 200 km		Plasma CPK	One to two hours pre-race, 10km, 20km, 30km and at end Six to ten hours post-race, 100km and 200km	↑ plasma CPK (x3) ↑ plasma CPK (X35)
Millet et al ⁽¹⁷⁴⁾	Descriptive, cross sectional study LOE: III	22 male	Experienced ultra-marathon runners	166 km mountain ultramarathon	MVC, MVC activation	CK Myoglobin CRP	Pre-race, immediately post-race and two, five, nine and 16 days	↓MVC Alteration in MVC activation ↑CK ↑myoglobin ↑C –reactive protein

Table 2.2: Summary of endurance running events and markers of muscle damage.

	Study design	Sample size (n)	Participants	Endurance event	Indicators of EIMD	Biochemical indicators of EIMD	Time of assessment	Significant results
							post-race	
Del Coso et al ⁽¹⁷⁵⁾	Descriptive, cross sectional study LOE: III	40 participants (34 males and 6 females)	Healthy experienced marathon runners 41 ± 8 years	42.2 km marathon	Jump height and power output	CK LDH myoglobin	One to three days pre-race and immediately post-race	↑ CK ↑LDH ↑Myoglobin ↓jump power output and height
Del Coso et al ⁽¹⁷⁶⁾	Descriptive cross sectional study LOE: III	25 participants	Trained triathletes 36 ± 7 years	Half-ironman distance triathlon (1.9 km swim, 90 km cycle, 21.1 km run)	Jump height and power output	CK myoglobin	One to three days pre-race and immediately post-race	↓jump height ↓ leg power output ↓ myoglobin ↓ CK
Suzuki et al ⁽¹⁷⁷⁾	Descriptive, cross sectional study LOE: III	9 males	Well-trained triathletes 34 ± 5 years	Ironman triathlon race	Muscle strength muscle soreness jump height	CK ALT LDH	Two days pre-race, within 30 min post-race and 14 – 20 hours post-race	↓muscle strength ↓jump height ↑muscle soreness ↑ALT ↑CK ↑LDH

Key: CK – Creatine kinase; LDH – Lactate dehydrogenase; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; HBD – Hydroxybutyrate dehydrogenase; CPK – Creatine phosphokinase; hsCRP – high sensitivity C-reactive protein; TC – Thigh circumference; DOMS – Delayed onset of muscle soreness; MVC – Maximal voluntary contraction

2.11 EFFECT OF EXERCISE-INDUCED MUSCLE DAMAGE ON ENDURANCE PERFORMANCE

It is well-established that exercise induced muscle damage is associated with prolonged reductions in muscle strength^(28, 43). In more recent studies it has been shown that measures of athletic performance requiring muscle power are negatively influenced by exercise induced muscle damage^(8, 178, 179). The effect of exercise-induced muscle damage on running performance remains unclear⁽¹⁸⁰⁾.

Marcora and Bossia⁽¹⁸⁰⁾ investigated the effect of exercise induced muscle damage on endurance running performance in moderately trained adult endurance runners. The experimental group performed 100 drop jumps to induce sufficient exercise induced muscle damage. Delayed onset muscles soreness (DOMS), plasma creatine kinase activity, mid-thigh circumference and knee extensors strength were the direct markers of muscle damage measured. Participants performed a standardised constant speed run at submaximal intensity and ten minutes later a 30 minute time trial. Participants returned 48 hours later for the final post-test visit. There were significant changes in muscle soreness, plasma creatine kinase activity and knee extensor strength between the experimental and control groups. Furthermore, the results showed a mean four percent difference in endurance running performance between the experimental group and the control group. Participants showed a reduction in running speed, with no change in perceived exertion. In addition, it was shown that there was a trend for increased rate of perceived exertion, which correlated with decreased time trial performance. It was concluded that exercise induced muscle damage does have an impact on running performance and that this effect seems to be mediated by alterations in the sense of effort⁽¹⁸⁰⁾.

Twist and Eston⁽¹⁷⁹⁾ investigated the effect of exercise induced muscle damage on perceived exertion and cycling endurance performance. Seven recreational athletes performed two submaximal fixed-load exercise bouts followed by a five minute time trial before, 48 hours and 168 hours following 100 counter-movement jumps. The measurements of VO_{2max} , heart rate, respiratory exchange rate (RER) and blood lactate concentration remained unchanged during the fixed-load exercise bouts following the 100 jumps. However, the rate of ventilation and the ventilator equivalent ratio for oxygen increased at 48 hours following the jump exercises. Rate of perceived exertion (RPE) values were higher at 48 hours as well as the ratio of RPE: HR and RPE: VO_{2max} . In the time trial, mean VO_{2max} , peak power output, mean power output, distance covered and post-exercise blood lactate were lower at 48 hours. These results indicate that the ventilator equivalent

for oxygen and perceived exertion at submaximal work rate are increased 48 hours following lengthening (eccentric) muscle contractions. Furthermore, the study has shown that exercise induced muscle damage increases the rate of perceived exertion and impairs performance during a five minute all-out effort time trial.

These studies illustrate that there is an increase in physiological response to endurance exercise when muscle damage is present and that exercise induced muscle damage can affect running performance. Further research is needed to understand the peripheral and central mechanisms relating to perceived exertion, exercise induced muscle damage and endurance running performance.

2.12 EXERCISE INDUCED MUSCLE DAMAGE AND THE REPEATED BOUT EFFECT

It is well established that a single bout of lengthening muscle actions (eccentric) or unfamiliar exercise can cause symptoms of muscle damage, such as loss of force, pain and muscle tenderness. It is also established that a repeated bout of the same or similar exercise results in markedly reduced symptoms of muscle damage than the initial bout⁽¹⁸¹⁾. This phenomenon is referred to as the repeated bout effect⁽¹²⁾. The repeated bout effect has been demonstrated in both animal and human studies. Furthermore, it has been shown to last for several weeks and possibly up to six months^(182, 183).

It is also evident that the initial bout of lengthening muscle actions does not have to cause considerable damage to produce a protective effect^(7, 10, 183). Lengthening muscle actions between the ranges of two to ten maximal contractions of the elbow flexors have been associated with a protective adaptation for subsequent bouts of maximal contractions^(7, 183).

However, it has been established that the initial bout should consist of close to maximal contractions to develop the protective adaptation. Nosaka and Newton⁽¹⁸⁴⁾ established that eight weeks of eccentric training at submaximal level (50% of one repetition maximum) was not enough to develop a protective effect during the subsequent bout of maximal eccentric exercise. This may have implications relating to submaximal exercise intensities associated with endurance running training.

It has been established that the time course of the repeated bout effect can last for between several weeks and six months⁽¹⁸³⁾. Byrnes et al⁽¹⁸⁵⁾ reported a reduction in muscle soreness and smaller increases in plasma creatine kinase activity and myoglobin concentrations when a second bout of downhill running was repeated up to six weeks, but not nine weeks, after the first bout of 30-minute downhill running. Nosaka et al⁽¹⁸³⁾ investigated the time course of the repeated bout effect following

two bouts of eccentric exercise of the non-dominant elbow flexors, separated by either six, nine, or twelve months. A faster recovery in maximal isometric force was evident after a second bout performed at six or nine months. Furthermore, reduced soreness and smaller increases in swelling, plasma creatine kinase activity, and T2 relaxation times of magnetic resonance images were observed after the second exercise bout at six months, compared to the responses after the initial bout of exercise. There were no significant differences in range of motion between the repeated bouts of exercise and the 12-month group showed no repeated bout effect. These results suggested that the repeated bout effect lasts at least six months, but appears to be lost between nine and twelve months following the initial bout of exercise.

Nosaka et al ⁽¹⁸³⁾ suggested that the differences between the two studies may be associated with the different exercise protocols developed to induce muscle damage, that is downhill running compared to high-force muscle lengthening exercises of the elbow flexors, or to the differences in the extent of muscle damage induced by the different exercise protocols ^(183, 185). Byrnes et al ⁽¹⁸⁵⁾ reported significantly lower plasma creatine kinase levels, compared to the levels reported by Nosaka et al ⁽¹⁸³⁾. Both studies also reported a large variability in the extent of the symptoms of muscle damage after the exercise protocols.

Furthermore, it appears that the repeated bout effect is specific to the exercised muscle groups. There is no evidence to suggest a cross-transfer of the protective effect to contralateral muscle groups that have not been subjected to an initial bout of damaging exercise ⁽¹⁷⁾. In addition, Eston et al ⁽¹¹⁾ showed protective adaptations of the repeated bout effect during a downhill run that followed a prior bout of maximal isokinetic eccentric exercise. This demonstrates that the protective effect is not changed by variations in the mechanism of eccentric exercise.

The conditions required for inducing the protective adaptations of the repeated bout effect are relatively well understood; however the underlying mechanism of the repeated bout effect is unclear. Several theories have been suggested to explain this phenomenon, and these can be summarised as the neural, mechanical and cellular theories ⁽¹²⁶⁾.

2.12.1 NEURAL THEORY

Lengthening muscle contractions require less motor unit activation for a given muscle force⁽¹⁸⁶⁾ and involve preferential recruitment of type II muscle fibres⁽¹¹⁴⁾, compared to shortening muscle contractions. The “neural theory” suggests that an initial bout of muscle damaging exercise is followed by a more efficient recruitment of motor units, an increased recruitment of type I or slow-twitch muscle fibres, activation of a larger motor unit pool and a better distribution of workload among the active muscle fibres, increased motor unit synchronisation⁽¹⁸¹⁾.

Warren et al⁽¹⁸⁷⁾ investigated changes in motor unit activation between repeated bouts using surface electromyography (EMG) in humans. In theory, an increase in the amplitude of the EMG signal relative to torque production in the repeated bout would suggest a redistribution of contractile stress among a greater number of muscle fibres. This effect is evident in eccentric strength training⁽⁴⁴⁾. McHugh et al⁽¹²⁶⁾ and Warren et al⁽¹⁸⁷⁾ showed no change in the EMG amplitude between repeated exercise bouts in the hamstring or tibialis anterior muscles respectively. There was, however, a reduction in the median frequency in the repeated bout for the tibialis anterior muscle, suggesting either an increased recruitment of type I muscle fibres (slow-twitch) or an increased motor unit synchronisation.

Although Warren et al⁽¹⁸⁷⁾ provide evidence of a neural adaptation to a single bout of eccentric exercise; it is also evident that the repeated bout effect can occur independently of a neural adaptation^(188, 189). The repeated bout effect has also been demonstrated with electrical stimulation of rat tibialis anterior muscle⁽¹⁸⁸⁾ and human elbow flexors⁽¹⁸⁹⁾, indicating the possibility of a peripheral component to the repeated bout effect⁽¹⁸⁹⁾. In humans, the initial bout of electrically stimulated eccentric contractions resulted in strength loss, decreased range of motion, swelling, increased plasma creatine kinase activity and increased muscle soreness. Following a similar repeated bout two weeks later, there was a significantly lower response in the markers of muscle damage. It was theorised that there is minimal involvement of the central nervous system and that peripheral adaptations play a more important role in the repeated bout effect⁽¹⁸⁹⁾. These theories, however, requires further investigation.

2.12.2 MECHANICAL THEORY

The “mechanical theory” suggests that exercise induced muscle damage is associated with a mechanical disruption of myofibrils⁽¹⁸¹⁾. It is therefore proposed that an adaptation serving to protect against muscle damage (repeated bout effect) may alter the mechanical properties of the musculoskeletal system. These adaptations may occur in the whole muscle, at muscle fibre level and at the level of the cytoskeleton and the myofibril⁽¹⁸¹⁾.

An increase in both passive and dynamic muscle stiffness following eccentric training of human elbow flexors⁽¹⁹⁰⁾ and rat triceps brachii muscles⁽¹⁹¹⁾ have been reported. This effect has been associated with either increased tendon stiffness or increased cross-bridge stiffness⁽¹⁹⁰⁾ and more recently to adaptations in the cytoskeletal proteins responsible for maintaining the alignment and structure of the sarcomere⁽¹⁹¹⁾.

Barash et al⁽¹⁹²⁾ demonstrated increased desmin content between three to seven days after muscle damaging contractions in rat muscle. It was theorised that the increase in desmin may have been associated with remodelling of the intermediate filament system, in order to provide mechanical reinforcement against excessive loading of the sarcomere. In contradiction to this finding, Sam et al⁽¹⁹³⁾ reported a reduction in myofibrillar disruption in mouse muscle lacking desmin, compared to normal mouse muscle.

There are also contradictory findings related to changes in passive stiffness following eccentric exercise. Lapiere et al⁽¹⁹⁴⁾ theorised that an increase in passive muscle stiffness secondary to increased intramuscular connective tissue, might protect muscle from damage caused by lengthening muscle actions. In contrast, there is evidence suggesting that passive muscle stiffness may increase the susceptibility to muscle damage^(126, 181). In addition, an acute increase in muscle stiffness has been demonstrated in the elbow flexors and plantar flexors following exercise induced muscle damage^(34, 43). Further investigation is needed into the changes in passive stiffness associated with the repeated bout effect.

2.12.3 CELLULAR THEORY

Potential cellular mechanisms for the repeated bout effect include possible adaptations of the contractile mechanism of the muscle, as well as possible adaptations in the inflammatory response to exercise-induced muscle damage⁽¹⁸¹⁾.

Morgan⁽¹⁹⁵⁾ theorised that the repair process after an initial bout of eccentric exercise may result in an increase in the number of sarcomeres in series and therefore reducing strain during a repeated bout of exercise. Furthermore, indirect evidence of longitudinal addition of sarcomeres in humans was demonstrated following a bout of lengthening hamstring contractions⁽¹⁹⁶⁾. A rightward shift was noted in the length-tension relationship following recovery from the initial bout, this was attributed to the longitudinal addition of sarcomeres.

The theory of the longitudinal addition of sarcomeres to explain the repeated bout effect is most attractive. While there is experimental evidence supporting this theory⁽¹⁹⁶⁾, there seems to be conflicting evidence as well. It has been reported that the length-tension relationship returned to

normal within five hours in toad sartorius muscle and within two days in human triceps surae muscles respectively^(197, 198). Furthermore, submaximal eccentric training resulted in the longitudinal addition of sarcomeres in rats⁽¹⁹⁹⁾, but in humans submaximal training did not elicit a protective effect from subsequent maximal contractions of the elbow flexors⁽¹⁸⁹⁾.

It is also estimated from animal models that impairments of excitation-contraction coupling may account for 50% to 75% of strength loss that occurs in the first five days following exercise-induced muscle damage⁽¹⁸⁷⁾. This estimation is however based on electrically stimulated maximal contractions in an animal model and little is known about the effects in human skeletal muscle in relation to voluntary contractions. It is theorised that an adaptation in the excitation-coupling system may provide a mechanism for the reduced strength loss seen following a repeated bout of exercise.

In contrast, studies on the repeated bout effect in humans do not support an adaptation related to excitation-contraction coupling. Ingalls et al demonstrated that impairment of excitation-contraction coupling is greatest immediately post-eccentric exercise, accounting for 75% of the force loss. However, strength loss immediately following eccentric exercise has been demonstrated to be similar between initial and repeated bouts⁽¹⁰²⁾. Only on subsequent days, reduced strength loss was seen with a repeated bout. It goes to reason that if the repeated bout effect was due to an adaptation in excitation-contraction coupling reduced strength loss should be seen immediately following the repeated bout as well as on subsequent days⁽¹⁸¹⁾.

In addition, a reduction in the symptoms of muscle damage in a repeated bout may be attributed to adaptation of the inflammatory response. Pizza et al⁽²⁰⁰⁾ observed decreased neutrophil and monocyte activation following a repeated bout of eccentric exercise. It is theorised that a blunted inflammatory response to a repeated bout of exercise may be a potential adaptation to avoid excessive mechanical disruption of myofibrils⁽²⁰⁰⁾.

2.12.4 SUMMARY OF THE LITERATURE: THE REPEATED BOUT EFFECT

Currently there is little agreement in the literature regarding the mechanisms of the repeated bout effect. It is clear that one theory cannot explain the multitude of observations of the repeated bout effect. The repeated bout effect may occur as a result of the interaction between the neural, cellular and mechanical mechanisms that are dependent on the changes associated with the initial bout of exercise that causes muscle damage. The repeated bout effect is an integral factor to consider when studying the adaptations to endurance exercise and running performance^(17, 26, 155, 201).

2.13 SUMMARY OF THE LITERATURE: EXERCISE-INDUCED MUSCLE DAMAGE

Exercise induced muscle damage is associated with strenuous or unaccustomed exercise. It is particularly associated with activities involving lengthening (eccentric) muscle contractions. The term “eccentric” refers to the lengthening of a contracting muscle as well as highlighting the imbalance between the external strength put on the muscle and the strength produced by the muscle itself⁽²⁾. This scenario is common during downhill running, the activation of an antagonist muscle group or when muscles are exposed to high levels of repeated or unaccustomed activity. These situations are closely linked to muscle damage⁽²⁰²⁾.

Distance running imposes severe stress on the body and it is well documented that muscle damage occurs during distance running^(3, 4, 172-174). Indirect indicators of muscle damage include prolonged losses in muscle strength, alterations in range of motion, increased levels of muscle proteins in the blood, such as creatine kinase, and delayed onset muscle soreness^(155, 163).

Muscle biopsies are used to investigate exercise induced muscle damage directly by analysing the histological and biochemical components, but the invasive nature of muscle biopsies limits repeated measurements. Indirect investigations that include blood measurements of the activity of muscle enzymes such as creatine kinase (CK) also do not provide any quantitative or qualitative information about specific muscle damages⁽¹⁶⁾.

A non-invasive alternative to both these methods of investigating exercise induced muscle damage is magnetic resonance imaging (MRI). Magnetic resonance imaging allows the advantage of tissue visualisation and gives the opportunity to distinguish changes in each muscle specifically. It is also a non-invasive quantitative measurement of changes in muscle to identify and diagnose the clinical effects of exercise⁽²⁰³⁾. The following section will discuss the use of MRI as a direct indicator of exercise induced muscle damage.

2.14 MAGNETIC RESONANCE IMAGING AS INDICATOR OF MUSCLE DAMAGE

Direct measurements of muscle damage include muscle biopsies and magnetic resonance imaging. Muscle biopsies are invasive and can be problematic in the sense that a very small sample of muscle is used to determine the extent of damage in an entire muscle. This may lead to overestimation or underestimation of muscle damage as a localised area is being investigated⁽¹⁷⁾.

2.14.1 MAGNETIC RESONANCE IMAGING (MRI)

Magnetic resonance imaging is a valuable measurement instrument to gain understanding of what is taking place in the entire muscle⁽²⁰⁴⁾. It is also a sensitive tool to indicate physiological changes that occur in muscles activated during exercise, as it provides a detailed analysis of the anatomy of the muscle^(19, 205, 206).

Bouts of unaccustomed exercise or high intensity as well as prolonged exercise has been proven to cause muscle damage⁽⁵⁾. Exercise induced muscle damage leads to muscle soreness, an increase in inflammatory mediators⁽³⁹⁾ and mitochondrial swelling⁽⁵⁾. Other factors associated with exercise induced muscle damage include, increases in muscle dysfunction that leads to decrease in exercise performance or capacity, such as reductions force output^(8, 155, 207) or time to exhaustion⁽²⁰⁸⁾.

A number of studies have utilised magnetic resonance imaging (MRI) to investigate the underlying mechanisms of exercise induced muscle damage, as well as to provide an indication of the severity of the damage⁽²⁰³⁾. Methods that have been used to determine this includes alterations in the transverse (T2) relaxation time⁽²⁰⁹⁾, following exercise, calculation of muscle volume^(23, 210-212) and the measurement of the cross sectional area of the muscle⁽²¹³⁾. A summary of studies using MRI methods to evaluate exercise induced muscle damage is shown in Table 2.3.

Table 2.3: Summary of studies using MRI methods to evaluate exercise induced muscle damage.

	Study design	Participants	Muscles assessed	EIMD protocol	Indirect indices of EIMD	MRI Analyses	Time of assessments	Significant findings
Sorichter et al (37)	Descriptive cross sectional study LOE: II	10 healthy young males	Quadriceps	7 x 10 eccentric contractions of quadriceps femoris at 110° and at an intensity =150% of MVC	plasma CK activity MHC	T2 Signal intensities	Before and on days 1 and 4 after exercise	↑CK in participants with negative signal change; ↑CK, ↑MHC in participants with positive signal change
Larsen et al (214)	Descriptive, comparative cross-sectional study LOE: II	8 healthy females, doing regular physical activity	Quadriceps Adductors	Two step exercise bouts (30 min) separated by 8 weeks	Blood samples: CK and LDH Muscle strength: MVC of knee extensors Muscle soreness: VAS quadriceps and adductors	T2 times CSA	Immediately before, after and up to nine days after each bout	↑ T2 time in AM, peaked 3 days after bout 1; ↑ plasma CK activity, peaked day 3 after bout 1 and 2; Correlation between plasma CK and T2
Kubota et al (215)	Descriptive, cross sectional study LOE: II	12 healthy young males (23.7 ± 1.8 years)	Hamstrings (Biceps femoris, Semitendinosus, Semimebranosus)	Eccentric hamstring exercise, 5 sets of 10 reps MRI – anatomical CSA T2 values	Blood samples: plasma CK activity Muscle strength: MVC of knee flexors Muscle soreness: VAS hamstrings	T2 times CSA	Before, immediately after, and on days 1, 2, 3, and 7 after exercise	↓ MVC; ↑ plasma CK; ↑ muscle soreness; ↑CSA ST on day 3; ↑T2 time BF and ST, immediately after; Differences in T2 times between proximal and distal segments of ST

Table 2.3 Summary of studies using MRI methods to evaluate exercise induced muscle damage (continued).

	Study design	Participants	Muscles assessed	EIMD protocol	Indirect indices of EIMD	MRI Analyses	Time of assessments	Significant findings
Foley et al ⁽²⁰⁴⁾	Descriptive, comparative cross sectional study LOE: III	6 young adult males, aged 21 – 25 years	Biceps	2 bouts of eccentric biceps curls separated by 8 weeks	plasma CK activity muscle soreness	Muscle volume T2 times	Immediately before and after each bout; at days 1, 2, 4, 7, 14, 21,56 after bout one and at days 2, 4, 7, and 14 after bout two	↑ flexor muscle swelling; ↑ Total volume of imaged arm region after bout 1, returned to pre exercise values within 2 weeks; Exercised flexor compartment swelled by over 40%, after 2 weeks it reverted to a volume 10% smaller than that before exercise and maintained this volume loss through 8 weeks; ↑ in resting muscle T2 immediately post exercise after both bouts; T2 peaked 7 days after bout 1 and remained elevated at 56 days; T2 peaked earlier after

bout2 (2-4 days)

Table 2.3: Summary of studies using MRI methods to evaluate exercise induced muscle damage (continued).

	Study design	Participants	Muscles assessed	EIMD protocol	Indirect indices of EIMD	MRI Analyses	Time of assessments	Significant findings
Mendiguchia et al ⁽²¹⁶⁾	Descriptive, cross sectional study LOE: III	8 male national level soccer referees	Hamstrings (Biceps femoris, semitendinosus, semimebranosus)	Nordic hamstring curls, 5 sets of 8 repetitions		CSA T2 times	Before, within 3 minutes after and 72 hours after exercise	↑ CSA of BF shorthad after the exercise and on day 3; ↑ in T2 values after exercise
Hudelmaier et al ⁽²¹⁾	Comparative, cross sectional study LOE: II	41 untrained females	Quadriceps, Hamstrings, Adductors and Sartorius	Strength training group: 3 times a week for 60 min Endurance group: 60 min cycling Control group: once a week relaxation exercises		CSA Muscle volume	Before and after 12 week intervention	Strength training :↑ in Muscle volume of extensors, flexors and adductors, Endurance training: ↑ in muscle volume of extensors and sartorius Strength training: ↑ in mean anatomical CSA
Serrao et al ⁽²¹⁷⁾	Descriptive, cross sectional study LOE: III	10 healthy females (21.9 ± 1.5 years)	Quadriceps	4 x 15 maximal eccentric contractions of knee extensors MRI T2 Value	MVC Plasma CK activity	T2 times	Before and after exercise	↓ MVC up to 4 days after exercise; ↑ CK activity on day 2 after exercise; ↑ in signal intensity, day 2 and 7

Table 2.3: Summary of studies using MRI methods to evaluate exercise induced muscle damage (continued).

	Study design	Participants	Muscles assessed	EIMD protocol	Indirect indices of EIMD	MRI Analyses	Time of assessments	Significant findings
Fulford et al ⁽²⁰³⁾	Descriptive, cross sectional study LOE: III	17 healthy participants (8 males, 9 females)	Quadriceps	100 Smith squats, performed as 10 sets of 10 repetitions	Muscle soreness Plasma CK activity MVC	Muscle volume CSA T2 times	Before and 24 hours after exercise	↑ Muscle soreness; ↑CK activity; ↓ Isokinetic peak torque; Significant ↑ in T2 values within VI, VL, VM; Significant ↑ in muscle volume; Significant ↑ in CSA

Key :EIMD – exercise induced muscle damage; MRI – magnetic resonance imaging; MVC – maximal voluntary contraction; CK – creatine kinase; MHC – myosin heavy chain; T2 – transverse relaxation time; VAS – visual analogue scale; CSA – cross sectional area; AM – Adductor magnus; ST – Semitendinosus; BF – Biceps femoris; VI – Vastus intermedius; VL – Vastus lateralis; VM – Vastus medialis

The purpose of this section of this review is to give an overview of the MRI methods used to localise and quantify muscle damage and changes in muscle morphology. The areas that will be focused on are transverse relaxation times (T2), muscle volume (MV) and cross sectional area (CSA) as well as the relationship to other indicators of muscle damage such as plasma creatine kinase activity and muscle soreness.

2.14.1.1 Transverse (T2) relaxation times

The signal of MRI images is produced from the magnetic activity of hydrogen nuclei in tissue water and fat molecules and depends on the sequence parameters selected. These parameters are repetition time (TR), echo time (TE) and flip angle. When the pulse sequence parameters are varied, the images obtained will show differences in the spin-lattice (T1) or in the spin-spin (T2) relaxation times. It is well-documented that exercise produces changes in water distribution, both intracellularly and intercellularly in muscle cells. This shift in water distribution causes an increase in nuclear magnetic transverse (spin-spin) relaxation time (T2) of muscle. The transverse (T2) relaxation time is reported as a quantitative index of muscle activation and can be used to non-invasively monitor the changes in the amount and distribution of water in skeletal muscle after exercise^(19, 216, 218).

The T2 relaxation time is a fundamental property of MRI and it reflects the rate at which the generated MR signal decays. It can be widely altered by a number of physiological changes. Although the main mechanism behind these changes are uncertain, it seems apparent that these changes can in part be associated with osmotic changes, which can potentially indicate oedema and/or inflammation⁽²⁰³⁾.

Previous studies reported an increase in the T2 value after eccentric exercise^(19, 206, 219, 220). Shellock et al⁽²⁰⁹⁾ examined signal intensity (T2 relaxation time) changes after eccentric exercise and documented a prolonged change in T2 relaxation times after exercise. This was a unique finding as previous studies found short lasting T2 relaxation time increases. These studies however implemented concentric exercises to cause fatigue and did not result in muscle damage⁽¹⁷⁾.

Mair et al⁽²²¹⁾ investigated the relationship between changes in MRI and other indicators of muscle damage (strength loss and CK activity, myoglobin concentration and myosin heavy chain fragment concentration). Eccentric contractions of the knee extensors were utilised to cause muscle damage and T2 relaxation times were measured at three, six, and nine days after exercise. The T2 relaxation times illustrated peak increases at six days after exercise, which corresponded with the peak CK activity, but not with peak soreness development.

The authors concluded that strength was improving and muscle soreness subsiding while the T2 relaxation times (signal intensity) was increasing.

It is also reported that the ultrastructural damage assessed from MRI guided biopsies correlated significantly with the increase signal intensity 48 hours after downhill running⁽²²²⁾. Foley et al⁽²⁰⁴⁾ studied the time course of MRI measurement (muscle volume and T2 relaxation times) after eccentric exercises and compared these to pain measurements and plasma CK activity. The authors found that pain and muscle volume peaked at 48 hours after exercise, which was then followed by an increase in CK activity and an increase in T2 values. Larson et al⁽²¹⁴⁾ documented an increase in T2 relaxation times in the adductor magnus muscle in young woman after a step exercise was used to elicit muscle damage. Plasma creatine kinase activity and T2 times peaked at three days post-exercise.

In an animal study, Marqueste et al⁽¹⁶⁾ reported that after downhill running, T2 relaxation times and plasma CK activity levels increased and recovered quickly after a single bout of exercise. When a second bout was performed four days after the initial bout, the changes in T2 values and plasma CK activity did not recover and endurance time throughout additional exercise sessions was significantly reduced. When there was a longer rest period (seven days) between exercise bouts, the endurance time of additional exercise sessions was significantly longer and the changes in T2 values, plasma CK activity and muscle oedema slowly and completely reversed. The authors found significant correlations between T2 relaxation times and plasma CK activity.

From the studies above it is clear that the changes in T2 relaxation times after high intensity or prolonged exercises are evident long after the other direct and indirect markers of muscle damage had been resolved. Shellock et al⁽²⁰⁹⁾ documented that after 75 days there were still elevated T2 relaxation times after eccentric exercise of the elbow flexors. Nosaka and Clarkson⁽²²³⁾ reported an increase in T2 relaxation times up to 31 days after exercise in some participants. Fulford et al⁽²⁰³⁾ used various magnetic resonance techniques to measure muscle damage 24 hours after eccentric exercise and reported that only the T2 values and muscle volume as well as cross sectional area gave statistically significant changes 24h after exercise. The origin of increases in T2 values remain uncertain, most likely due to various components that can attribute to these changes such as oedema, inflammatory responses and direct muscle fibre damage. It has been suggested that the increase in T2 values may be due to osmotic fluid shifts⁽²²⁴⁾. It is theorised that there is an osmotic fluid shift into the intracellular space due to a build-up of inorganic phosphate and lactate. Another theory is that there might be an increase in extracellular fluid as a result of an accumulation of degraded protein components^(17, 209, 225).

In summary, although there is uncertainty about the actual underlying mechanism of the increase in T2 values, it has been shown to be a reliable indicator of muscle damage⁽²⁰³⁾.

2.14.1.2 MRI and muscle volume

When taking into account that oedema and/or inflammation are considered as possible underlying mechanisms for changes in T2 values, it might be reasoned that muscle volume can accompany these T2 relaxation time changes. These changes in muscle volume may well be detectable within MRI images. Magnetic resonance imaging has widely been used to assess limb and muscle volumes⁽²¹²⁾ as well as oedema⁽²²⁶⁾. Very few studies have investigated exercise induced muscle damage by means of calculating muscle volume.

Foley et al⁽²⁰⁴⁾ used MRI measurements to investigate muscle damage and the adaptation of muscle after eccentric exercise. The authors showed that pain and muscle volume peaked 48 hours after exercise, which was then only followed by an increase in plasma CK activity and an increase in T2 relaxation times. Fourteen days after exercise muscle volume had decreased to below baseline by 7% to 10%. Eight weeks later this decrease was still present. The authors suggested that this loss in muscle volume showed a loss in stress susceptible fibres that were damaged beyond repair by the exercise⁽²⁰⁴⁾.

Fulford et al⁽²⁰³⁾ showed a significant difference in muscle volume 24 hours after exercise induced muscle damage. This further supports the suggestion that general oedema and muscle swelling might contribute in part to T2 relaxation times changes. It also shows that muscle volume can be a reliable indicator of muscle damage⁽²⁰³⁾.

2.14.1.3 MRI and cross sectional area

Takahashi et al⁽²¹³⁾ investigated the cross sectional areas of specific muscle groups and reported an increase in the vastus lateralis, vastus Intermedius and vastus medialis by 5% to 7%, 24 hours after exercise. Rectus femoris, however showed a decrease of two percent in area at the same time. It was also documented that the increase in T2 relaxation times paralleled the changes in cross sectional area. Fulford et al⁽²⁰³⁾ also showed significant differences for cross sectional area in the quadriceps muscle.

2.14.1.4 Localisation of muscle damage

One of the strengths of MRI is that it can provide spatially localised information, something that other measurements of muscle damage such as exercise performance and CK activity cannot do⁽²⁰³⁾. Magnetic resonance images allow us to compare different muscles as well as different regions within the same muscle.

Prolonged changes of T2 values have been documented in certain muscles of the thigh complex, but not all of them. Takahashi et al⁽²¹³⁾ showed prolonged changes of T2 values in vastus lateralis, vastus intermedius and vastus medialis but not in rectus femoris. Furthermore, Le Blanc et al^(23, 211) reported variability among sections of the same muscle. In support of this, Kubota et al⁽²²⁰⁾ showed significant changes in T2 values of biceps femoris and semitendinosus immediately after exercise, but T2 values of only semitendinosus increased significantly on the third day. There were also significant differences of T2 values between proximal and distal regions in the semitendinosus muscle.

2.14.2 SUMMARY OF MRI AS INDICATOR OF EXERCISE INDUCED MUSCLE DAMAGE

Magnetic resonance imaging has a unique property to assess which muscle has been damaged following exercise⁽¹⁵⁵⁾ and has an extremely sensitive method to display the physiological changes that can occur in muscles activated during exercise. It provides a detailed anatomical analysis of the associated soft tissues⁽²¹⁶⁾. Magnetic activity of hydrogen nuclei in tissue, water and fat molecules give rise to the MRI signal and this signal is also dependant on various sequence parameters such as: repetition time (TR), echo time (TE) and flip angle. When the pulse sequence parameters are varied, the acquired images can show differences in the spin-lattice (T1) or in the T2 (spin-spin) relaxation times⁽²¹⁶⁾. The transverse relaxation time (T2) measurements can provide direct information regarding exercise induced muscle damage⁽¹⁶⁾. Exercise produces changes in both intracellular and intercellular distribution of water in muscle cells. This shift in water distribution produces an increase in T2 relaxation times. The T2 relaxation time is used as a quantitative index of muscle activation and is a non-invasive measurement tool of the changes in the amount of water and the distribution of water in skeletal muscle after strenuous exercise^(19, 20). Shellock et al⁽²²⁵⁾ showed that T2 increases after eccentric training and can remain elevated for a period of up to three months. A direct relationship between T2 increase and CK blood levels has also been reported^(23, 211). This again indicates that T2 changes can be used to illustrate damage severity.

Detailed information about muscle morphology can also be obtained by calculating the cross sectional areas (CSA) and muscle volume after exercise. The assessment of muscle morphology is useful to evaluate how training (such as distance running), aging and immobilisation can affect muscle function⁽²¹⁾.

In conclusion, MRI has the potential to provide valuable information about exercise induced muscle damage as well as the potential to provide insight into the underlying mechanisms and to provide detailed and localised information. This can mean greater precision in determining the site of muscle damage.

CHAPTER 3: A COMPARISON OF MUSCLE SORENESS, MORPHOLOGY, T2 CHANGES AND RUNNING PERFORMANCE FOLLOWING AN ULTRAMARATHON RACE

3.1 INTRODUCTION

It is well-established that distance running causes muscle damage^(3, 4). The assessment of indirect indicators of exercise induced muscle damage, such as plasma CK activity^(10, 13, 14) and muscle soreness⁽¹⁵⁾, only allows a limited interpretation of the extent of muscle damage and do not accurately reflect the extent of muscle damage⁽¹⁵⁵⁾. Magnetic resonance imaging is a non-invasive technique that may provide valuable insight into the degree of exercise induced muscle damage. Specifically, there may be changes in transverse (T2) relaxation times^(19, 20) and morphological measurements, such as muscle volume and peak cross sectional area, following exercise⁽²¹⁾. However, there is a lack of evidence regarding changes in both T2 relaxation times and muscle morphology after endurance running.

Therefore, the overall aim of this study was to investigate changes in T2 relaxation times and muscle morphology in endurance runners after a 90 km ultramarathon race. The specific objectives of this dissertation have been described in Section 1.2.2 (page 2).

3.2 METHODS

3.2.1 OVERVIEW OF RESEARCH DESIGN

This was a descriptive, correlational study that involved secondary analysis of previously collected data. The original study investigated changes in running economy, running kinematics, neural regulation, exercise performance and glucose oxidative capacity after a 90 km ultra-marathon (HREC REF: 136/2005)(Appendix VI). Magnetic resonance imaging (MRI) was conducted as part of the original study, but the MRI scans were not analysed. These MRI scans were analysed as part of this sub-study. The new analyses performed for this study as well as the data from the original study that were re-analysed are shown in Table 3.1.

Table 3.1: Summary of data analysed for this sub-study.

Sub-study (secondary data analysis)	Original Study (Note: all data were re-analysed as part of the sub-study)
Analysis of MRI scans – muscle volume	Muscle pain
Analysis of MRI scans – peak cross sectional area	Plasma creatine kinase (CK) activity
Analysis of MRI scans – T2 relaxation times	Heart rate (HR)
	Rate of perceived exertion (RPE)
	Running speed

The methods for the original study will be described first, followed by the methods of the sub-study.

3.2.2 ORIGINAL STUDY

3.2.2.1 Research design and recruitment

The original study had a quasi-experimental design. Male endurance runners were recruited through radio and newspaper advertisements and through advertisements placed at running clubs in the Western Cape. The experimental group consisted out of runners that participated in the 2005 Comrades marathon (a road race that is run between Durban and Pietermaritzburg, South Africa). The control group was made up out of endurance runners that did not participate in the 2005 Comrades marathon.

3.2.2.2 Participants

3.2.2.2.1 Inclusion criteria

Healthy male runners over 30 years of age, with a minimum training mileage of 60 km per week and who have previously completed an ultramarathon race of over 50 km in distance were included in the study. Eleven participants who took part in the 2005 Comrades marathon formed the experimental group and eleven participants who did not take part in the 2005 Comrades marathon formed the control group.

3.2.2.2.2 Exclusion criteria

Participants who had used any medication or analgesic within twelve weeks before testing were excluded from the study. Furthermore any participants with a history of lower limb bone stress injuries or a history of muscle pathology were also excluded as well as participants with any relevant medical or surgical history. Any participants that presented with a viral infection within twelve weeks prior to testing were also excluded.

Participants who had any of the following were also excluded from the study as these factors would be affected by the strong magnetic field with magnetic resonance imaging:

- Cardiac pacemaker or defibrillator
- Insulin or infusion pump
- Cochlear, otologic, or ear implant
- Any implant held in place by a magnet
- Tissue expanders (plastic surgery)
- Implanted catheter, clamp, clips, valves, or other metal
- Shrapnel or metal fragments

Furthermore, participants were requested to avoid any medication and strenuous training and racing other than the ultramarathon for the duration of the study. Participants were instructed to maintain the same diet and training regime for 24 hours prior to testing. To facilitate adherence with instructions participants completed a training logbook for the duration of the study. In addition participants were questioned about their compliance with instructions prior to each laboratory test. Testing occurred at a similar time (to within an hour) for each participant for the duration of the study.

3.2.2.2.3 Sample size determination

Data used from a previous study measuring submaximal oxygen consumption and glucose oxidation rates ⁽²²⁷⁾ was used to ensure that the sample size would provide sufficient statistical power. These parameters were selected to determine the required sample size, as both parameters have the greatest degree of standard deviation of all parameters to be measured in this study. With statistical significance accepted as $p < 0.05$, groups of 11, 14 and 17 participants would provide 80%, 90% and 95% statistical power for submaximal oxygen consumption respectively. Due to budgetary constraints for MRI testing, 22 participants were recruited for this study. Eleven participants formed the experimental group, and 11 participants formed the control group.

3.2.2.3 Measurement instruments

3.2.2.3.1 Descriptive information

3.2.2.3.1 (a) Informed consent form

All participants were required to complete the informed consent form (Appendix I) prior to their involvement in the research study. All relevant information relating to the study and the physical

testing that occurred was included on the informed consent form. The risks and benefits to the participants were described and the participants' right to withdraw from the study at any time was discussed.

3.2.2.3.1(b) Questionnaires

All participants completed a questionnaire regarding their medical and training history (Appendix II) as well as a physical activity questionnaire (Appendix III). Participants were required to provide information about the types of sport that they participated in, the number of sessions per week as well as the duration of the each session.

3.2.2.3.1(c) Anthropometry measurements

All participants had their body composition assessed during the familiarisation sessions four weeks prior to the ultra-marathon. Body mass (kg) was recorded, as well as stature (cm). Body fat was expressed as the sum of seven skinfolds (biceps, triceps, subscapular, suprailiac, calf, thigh and abdomen) as described by Ross and Marfell Jones⁽²²⁸⁾ and as a percentage of body mass as well⁽²²⁹⁾. Harpenden skinfold callipers (Baty International, West Sussex, United Kingdom) were used to measure skinfold thickness. The validity of these tests has previously been established^(228, 229).

3.2.2.3.2 Maximal effort treadmill test

Two weeks before the Comrades marathon, preliminary tests were performed on all participants. A maximal treadmill test was conducted two weeks before the ultramarathon race. Maximum oxygen consumption (VO_{2max}), peak treadmill running speed (PTRS), and maximal heart rate (HR_{max}) were determined. The maximal test was performed on a treadmill (Quinton Instruments, Seattle, WA, USA) with the elevation set at 1%, to reproduce the energy cost of running outdoors on a flat surface⁽²³⁰⁾.

Participants warmed up before the maximal test. The timing and intensity of the warm-up was specific for each individual, and was maintained for the duration of the study. The starting speed of the test was set to 10 km.h⁻¹. This was maintained for two minutes, after which it was increased by 0.5 km.h⁻¹ every 30 seconds until the participants were unable to maintain the speed of the treadmill. Participants wore a mouthpiece and nose clip for the duration of the maximal test and the incremental phase of the maximal test was approximately 10 minutes.

Oxygen consumption (VO_{2max}) was determined every 15 seconds. The expired air passed through a computer attached to an Oxycon Alpha automated gas analyser (Jaeger/Mijnhardt, Groningen, The Netherlands). Before every test the gas analyser was calibrated with a Hans Rudolph 5530 L syringe

using room air, as well as with an on-line CO₂:N₂ gas mixture of known composition. Heart rate was documented (Polar Vantage XL, Polar Electro, Kempele, Finland) every five seconds.

Maximum oxygen consumption was defined as the oxygen consumption that coincided with volitional fatigue⁽²³¹⁾. Peak treadmill speed was defined as the highest speed that the runner could maintain for a period of thirty seconds prior to fatigue. Maximum heart rate was recorded as the highest heart rate during the last 30 seconds of the treadmill test.

3.2.2.3.3 Measurement of running performance

3.2.2.3.3(a) Five kilometre time trial

A five kilometre time trial was completed by all participants on a 140 metre indoor track.

Participants were required to run “as fast as possible” and standardised verbal encouragement was given during the run. At every kilometre, split times and distance covered was conveyed to the participants. Participants were required to indicate their rate of perceived exertion at every kilometre split. A modified Borg scale was used for the rate of perceived exertion⁽²³²⁾. Heart rate was recorded (Polar Vantage, XL, Polar Electro, Kempele, Finland) at five second intervals throughout the time trial. The total time for the five kilometre time was recorded as well as the time after each kilometre. Running speed was calculated from the total time.

3.2.2.3.4 Measurement of muscle damage

3.2.2.3.4(a) Muscle pain

A multidimensional visual analogue pain scale was used to assess muscle pain before the five kilometre time trial. Participants were required to rate the pain in the quadriceps and hamstrings muscle groups according to “*general pain at rest*”, “*pain during activities of daily living*”, “*pain during passive stretch*”, and “*pain when pressure was applied to the mid-belly of the muscle*”.

Digital pressure was applied to the mid-belly of the muscle until moderate tissue resistance was felt, to determine pressure pain. Participants rated the pain in each of the categories for each muscle by drawing a vertical line on a ten centimetre (10 cm) pain rating scale. “No pain” was represented by 0 cm and “maximal pain” was represented by 10 cm. The distance along the pain rating scale to the vertical line drawn by the participant was measured in millimetres (mm) and the pain score for each category was recorded. Cleather et al⁽²³³⁾ determined that multidimensional pain scales provide a cleared description of delayed onset of muscle soreness (DOMS) and can assist in distinguishing the pain associated with delayed onset of muscle soreness from other sources of pain.

3.2.2.3.4(b) Plasma creatine kinase activity

A five millilitre (5 ml) blood sample was obtained from each participants' antecubital vein before the five kilometre time trial for the analysis of plasma creatine kinase (CK) activity. Blood samples were collected into pre-chilled tubes containing lithium heparin. All samples were kept on ice until centrifugation.

The blood samples were centrifuged at 2000 x g for ten minutes at 4 °C upon completion of the time trial test. Samples were stored at -20 °C until the analysis of plasma creatine kinase activity. Plasma CK activity was measured by spectrophotometric (Beckman DU-62, Beckman Instruments, Fullerton, CA) enzymatic assays (CK-NAC activated, Boehringer Mannheim Automated Analysis for BM/Hitachi Systems 704, Meylan, France).

3.2.2.3.4(c) Magnetic resonance imaging

Participants underwent magnetic resonance imaging of the upper thigh after completion of the five kilometre time trial. These scans were taken by a radiologist at the Radiology department at the Sports Science Centre, Cape Town. Bilateral cross sectional images were acquired at mid-thigh level (10 cm above the knee joint line, marked with an indelible ('permanent') marker) with participants in the supine position. Scans were done with a 0.2 –tesla scanner (E-scan; Esaote, Genoa, Italy). The imaging protocol was as follow: repetition time (TR) = 3240 ms, echo time (TE) = 28 ms, slice thickness = 6 mm.

3.2.2.4 Ultramarathon race

Participants in the experimental group completed a 90 km ultramarathon race during the study period. Participants took part in the "down run" of the Comrades marathon. A race profile of the "down" run is included in Appendix IV). Participants ran from Pietermaritzburg to Durban. During the race, heart rate was recorded (Polar Vantage XL, Polar Electro, Kempele, Finland) at one-minute intervals. Race heart rate data were averaged and expressed as a percentage of maximum heart rate to provide an indication of exercise intensity during the ultra-marathon race.

3.2.2.5 Acute tests

Muscle pain measurements and blood samples, for the analysis of plasma CK activity were collected for one day before, and daily for seven days after the ultra-marathon race, using methods described in Sections 3.2.2.3.4(a) and 3.2.2.3.4(b) respectively. Tests were performed in both the experimental

and the control groups in an attempt to quantify acute changes associated with muscle damage induced by the 90 km race.

3.2.2.6 Testing procedure

Three weeks before the ultramarathon race, participants had their first visit to the laboratory. During this visit participants gave written informed consent after being informed about the demands of the study. Questionnaires were completed and anthropometrical assessments were conducted. Participants were also familiarised with the laboratory equipment and testing protocols that would be used during the trial to reduce error associated with participants performing unaccustomed exercise. Two weeks before the ultramarathon race a maximal treadmill test was performed. Maximum oxygen consumption (VO_{2max}), peak treadmill running speed (PTRS), and maximal heart rate (HR_{max}) were determined.

A five kilometre time trial run was performed seven days before the race and ten days after the race. Muscle pain measurements and plasma CK activity were recorded before and after the time trial. Heart rate (HR), rate of perceived exertion (RPE) and running speed were also determined. Magnetic resonance images were taken on the same days as the five kilometre time trial run. Muscle pain measurements and plasma CK activity were measured for one day before and for seven days after the ultramarathon race. The testing procedure is summarised in Figure 3.1.

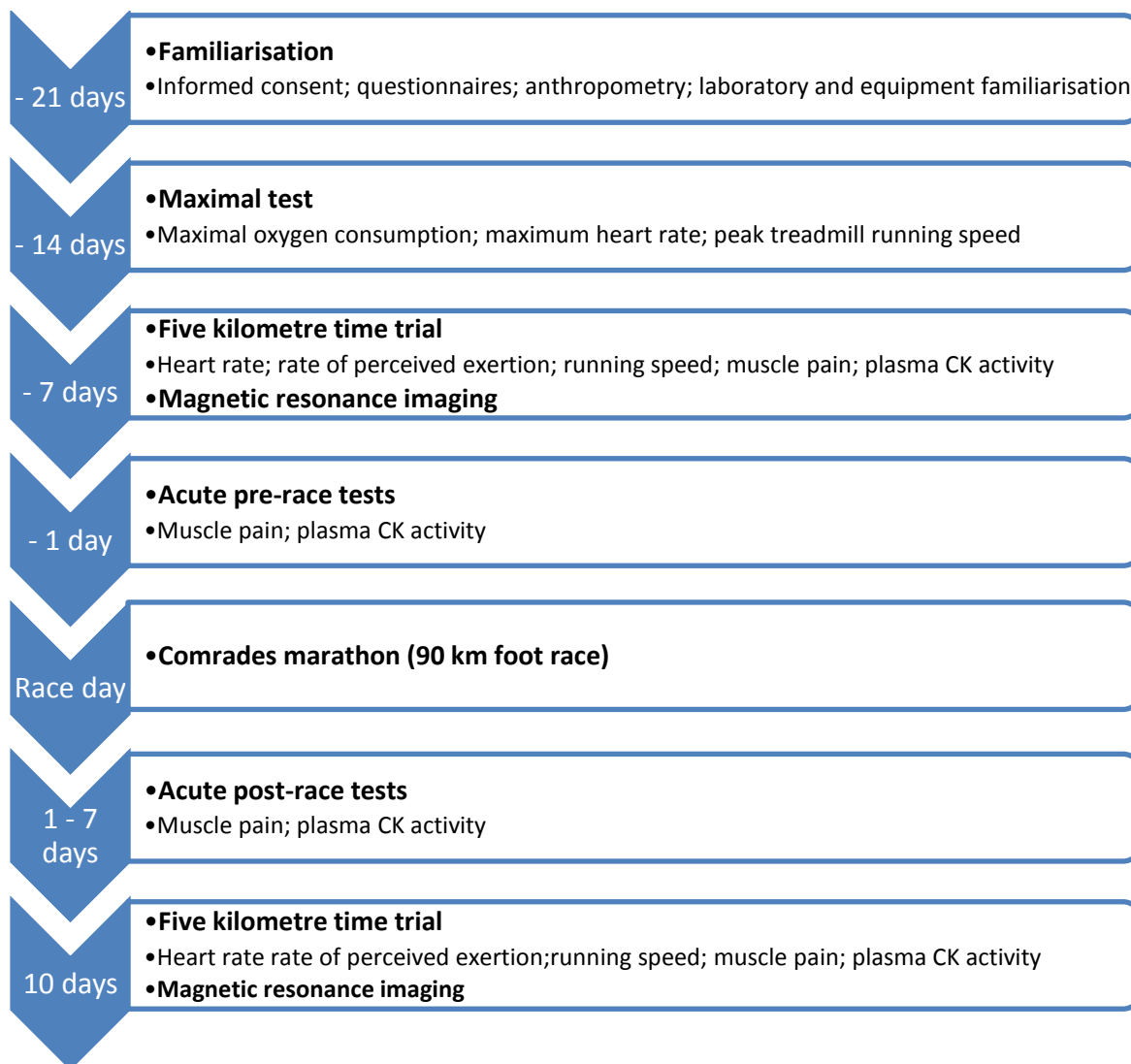


Figure 3.1: Testing procedures for the original study.

3.2.3 SUB-STUDY (SECONDARY DATA ANALYSIS)

3.2.3.1 Research design and recruitment

This was a descriptive, correlational study that involved secondary analysis of previously collected data (Section 3.2.2). Magnetic resonance imaging was conducted during the original study, but the MRI scans were not analysed. Therefore, these MRI scans were analysed as part of the sub-study. The investigator was blinded to which group the participants were assigned to until all analyses of the MRI scans were completed. No new participants were recruited for the sub-study.

3.2.3.2 Participants

No new participants were recruited for the study. Therefore the inclusion and exclusion criteria were the same as for the original study, which have been described in Section 3.2.2.2.

3.2.3.2.1 Sample size determination

A sample size of 22 participants was pre-determined by the original study criteria. However, the sample size calculation for the original study was based on power calculations for the primary outcome measures of the original study, namely submaximal oxygen consumption. Therefore, a sample size calculation was performed for the sub-study, to determine whether the study was adequately powered. Data from a previous study that investigated changes in muscle volume (measured with MRI) were used to ensure that the sample size would provide sufficient statistical power⁽²¹⁾. The mean change in thigh extensor muscle volume was selected to determine the required sample size, as this will be one of the main outcome measures for this study, and may have the greatest degree of variance of all the parameters to be measured in this study. Required sample size for quadriceps muscle volume was calculated using a smallest meaningful difference of 10 cm³, and a typical error of 5 cm³. With statistical significance accepted as $p < 0.05$, groups of 9, 12, and 14 participants would provide 80%, 90% and 95% statistical power for quadriceps muscle volume respectively. Therefore, with experimental and control group sizes of 11 participants respectively, this study had between 80% to 90% statistical power.

3.2.3.3 Measurement instruments

3.2.3.3.1 Descriptive information

3.2.3.3.1(a) Informed consent

All participants completed an informed consent form (Appendix I) prior to their involvement in the original study.

3.2.3.3.1(b) Questionnaires

The raw data of the completed questionnaires on the medical and training history as well as physical activity of participants were provided to the investigator (Appendices II and III).

3.2.3.3.1(c) Anthropometry measurements

The raw data of the body composition measurements done during the original study were provided to the investigator. Body mass (kg), stature and body fat (expressed as the sum of seven skinfolds and as a percentage of body mass) was presented for both groups.

3.2.3.3.2 Measurement of running performance

3.2.3.3.2(a) Five kilometre time trial

The raw data of the five kilometre time trial were provided to the investigator. Measurements for rate of perceived exertion (RPE), heart rate (HR) and running times were provided. Averages for the rate of perceived exertion (RPE), heart rate (HR), and running time and speed were calculated for both groups as part of the MRI sub-study.

3.2.3.3.3 Measurement of muscle damage

3.2.3.3.3(a) Muscle pain measurements

The raw data for muscle pain measured before and after the five kilometre time trial as well as the muscle pain data one day before and seven days after the ultramarathon race were provided to the investigator. Muscle pain measurements for the quadriceps muscle groups and hamstrings muscle groups were included for analysis as these were the muscle groups of interest in the MRI sub-study. The methods for the muscle pain measurements during the original study are described in Section 3.2.2.3.4(a).

3.2.3.3.3(b) Plasma creatine kinase activity

The raw data of the plasma CK activity levels before and after the five kilometre time trial were provided to the investigator. The measurements of plasma CK activity one day before and seven days after the ultramarathon race were also provided. The methods used for plasma CK activity in the original study are described in Section 3.2.2.3.4(b).

3.2.3.3.4 Magnetic resonance imaging

The magnetic resonance images of the upper thigh were taken after the five kilometre time trial. These images were T2-weighted images. The images are from a multi-echo sequence with two echoes. The images were converted from dicom format to nifty (nii) format for the images to be opened in other programmes. The programme SPM (<http://www.fil.ion.ucl.ac.uk/spm/>) was used to convert the images.

The contrast in the T2-weighted images depends on the time between the magnetic resonance excitation pulse (90°) and the 180° refocussing pulse (which produces the echo). The concept of multiple echoes is illustrated in Figure 3.2. The top row shows the pulse and the bottom row the signal, which is measured at each of the vertical dotted lines to produce the image.

Therefore the contrast in the first and second echo images is different because the delay between the 90° pulse and the 180° pulse is different. The rate of signal decay will depend on the T₂ relaxation time of the tissue being imaged.

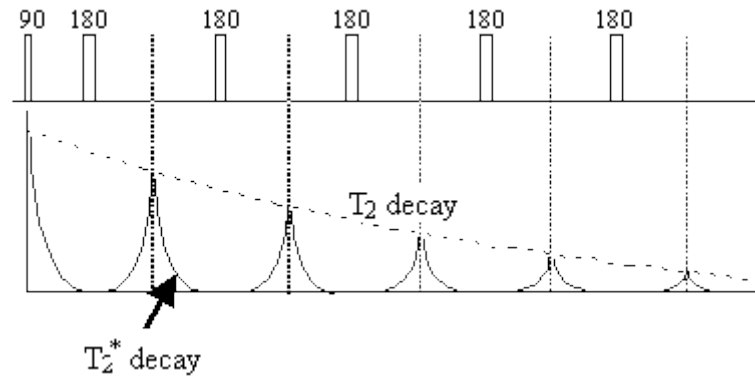


Figure 3.2: Concept of multiple echoes. The top row indicates the pulse and the bottom row the signal, which is measured at each of the vertical dotted lines to produce the image.

The magnetic resonance images were acquired during the original study (section 3.2.2) with participants in the supine position. Bilateral cross sectional images of the mid-thigh (10 cm above the knee joint line, marked with an indelible ('permanent') marker) with a 0.2 – tesla magnet MRI scanner (E –scan 2000, Esaota, Genoa, Italy) were acquired. The imaging protocol was: repetition time (TR) = 3240 ms, echo time (TE) = 28 ms and slice thickness = 6 mm. For each image, between 18 and 20 slices were obtained.

3.2.3.3.4(a) Muscle morphology analysis

The magnetic resonance images were analysed with the software programme ITK-SNAP (www.itksnap.org.com) by a single investigator. The investigator was blinded to which group the participants were assigned to until the analysis was completed.

The morphological analysis consisted out of the digital segmentation and reconstruction of the muscles, from the MRI scans, by tracing the contours of the selected muscle groups in each of the axial slices. The contours were grouped and used to build participant-specific triangle based mesh surface models of each muscle with the ITK-SNAP software programme. Examples of the MRI slice, the segmentation and the triangle based mesh are seen in Figure 3.3.

The muscle groups selected on the MRI scans for segmentation were rectus femoris, combined quadriceps muscle group (vastus lateralis, vastus medialis and vastus intermedius) and combined hamstrings muscle group (biceps femoris, semimembranosus and semitendinosus). The muscles

were segmented as individual entities in each slice, excluding bones, blood vessels and connective tissue. An area of interest was determined for each muscle group to segment, as the muscles in the proximal and distal slices to this area were difficult to identify. Seven slices were selected for segmentation of the rectus femoris and combined quadriceps muscle group (left side: slices 5 – 11 and right side: slices 10 – 16). Six slices were selected for the segmentation of the hamstrings muscle group (left side: slices 5 - 10 and right side: slices 10 - 15).

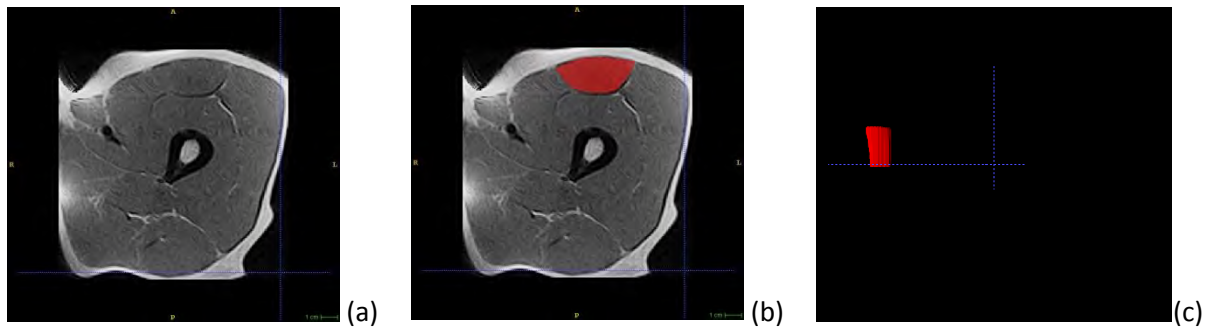


Figure 3.3: (a) Example of left upper thigh MRI slice 5 before segmentation; (b) Example of left rectus femoris segmented in slice 5; (c) Example of three dimensional triangle based mesh surface model after completed segmentation of slices 5 – 11.

Muscle volume

Following the segmentation of the individual muscle groups, muscle volume was determined by the numerical integration of the segmented voxels with the ITK-SNAP software programme^(21, 203, 234). Values were given as cubic millimetre (mm³). Separate segmentation and volume measurements were carried out for both legs, with the values reported an average of the two⁽²⁰³⁾.

Peak cross sectional area

Peak cross sectional area were calculated from the segmented images, by calculating the number of voxels within a particular segmented section. The maximum voxels across all slices were then multiplied by 0.781*0.781 (voxel size) to determine the peak cross sectional area in mm².

3.2.3.3.4(b) Transverse relaxation times (T2)

The transverse relaxation time (T2) reflects the rate at which the generated magnetic resonance signal decays. The two echo images (Figure 3.2) were used to calculate a map of the T2 relaxation time of the tissue. In Figure 3.2 it can be seen that the signal at each echo marks out a T2 decay envelope (the dotted line). An exponential of the form $S = S_0 \exp - TE/T_2$ can be fitted to the signal value (S) measured at two or more echo times (TE) to get a measure of the T2 relaxation time (in ms). This was done with the programme Matlab. This allowed for comparisons to be made between the images taken at different times. The segmented regions were transferred to the T2

maps in the ITK-SNAP software programme and the mean T2 relaxation time (ms) were calculated within specific regions. An example of a T2 map and a T2 map with the transferred segmentation is shown in Figure 3.4.

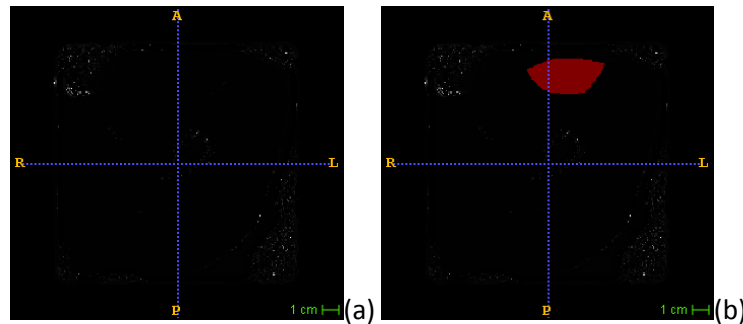


Figure 3.4: (a) Example of T2 map; (b) Example of T2 map with transferred segmentation.

3.2.3.4 Feasibility study

A feasibility study was performed to determine the intra-tester reliability of segmentation of the MRI scans and the calculation of muscle volume (Appendix V). The magnetic resonance images of three participants were used for this pilot study. The ITK-SNAP software programme (www.itksnap.org.com) was used for the analysis. The analysis consisted out of the segmentation of the rectus femoris muscle of the left upper thigh. The area of interest for segmentation was selected as in section 3.2.3.3.4.1. Measurements were taken on two separate occasions by the same investigator. The images used in the feasibility study also formed part of the MRI sub-study. The intra-tester reliability was assessed (Appendix V). Intra-tester reliability was high (Cronbach's $\alpha = 0.99$). The intra-tester reliability was only done for the segmentation and calculation for muscle volume as the same segmentation were applied to the T2 maps to determine T2 relaxation times.

3.2.4 DATA ANALYSIS

The magnetic resonance images were transferred to a personal computer and the ITK-SNAP software was downloaded onto the same computer. The MRI scans were analysed by a single investigator who was blinded to which groups the participants were assigned. Images that were not of good quality were excluded from specific analysis of muscle volume, peak cross-sectional area or T2 relaxation times respectively. Segmentation of the rectus femoris, combined quadriceps muscle group and combined hamstrings muscle group were done. Thereafter muscle volume and peak cross sectional area was determined with the software as well as the T2 relaxation times.

Muscle volume and peak cross sectional area data were normalised. The relative changes in muscle volume and peak cross sectional area post-race were expressed as a percentage of the pre-race values. Absolute change values (pre-race measurement – post-race measurement) were also calculated and a negative value indicated that the post-race value was higher than the pre-race value and a positive value indicated that the pre-race value was higher than the post-race value. Absolute change values were determined for T2 relaxation times.

The raw data from the original study were entered into an Excel Spreadsheet (Microsoft Corporation, Redmond, USA). Average values for muscle pain, heart rate, rate of perceived exertion, time trial time and running speed were calculated for all participants before and after the ultramarathon race. Absolute change values (Δ) were also calculated for average muscle pain, plasma CK activity, average heart rate, and average rate of perceived exertion, average time trial time and average running speed. A negative delta value indicated that the post-race value was higher than the pre-race value and a positive delta value indicated that the pre-race value was higher than the post-race value.

3.2.5 STATISTICAL ANALYSES

Statistical analyses were performed using Statistica software (Statsoft, Inc. 2004) STATISTICA (Data analysis software system, version 14, www.statsoft.com). During the feasibility study, the typical error of measurement and intra-class coefficient were used to assess intra-tester reliability. All data were assessed for normality using the Shapiro Wilkes test.

Differences in descriptive characteristics (age, body mass, height, percentage body fat) between the experimental and control groups were assessed using an independent t-test. Differences in training and racing history between the experimental and control groups were assessed using an independent t-test. Differences in, average heart rate, average rate of perceived exertion and average running speed between groups were assessed using an independent t-test. Statistical significance for the two main effects of group and time, and the interaction (group x time) for plasma CK activity were assessed using a two-way analysis of variance (ANOVA) with repeated measures. Tukey's *post hoc* comparisons were performed where necessary. A Mann-Whitney U test was used to assess differences in the pain scores between groups. A Friedman's ANOVA and Kendall's concordance was used to assess differences in the pain scores within groups over time.

Differences in muscle volume, peak cross sectional area, and T2 relaxation times between groups were assessed using an independent t-test. Effect sizes were assessed for muscle volume and peak

cross sectional area before and after the race in the experimental and control groups. The effect size (d) was calculated using a spreadsheet downloaded from www.work-learning.com/effect_sizes.htm. The following criteria were used: Cohen's d: d = 0.2-0.49 (small effect size); d = 0.5-0.79 (medium effect size); d = 0.8 or above (large effect size).

A Pearson's product moment correlation coefficient determined the relationships between variables of muscle volume, peak cross sectional area, T2 relaxation times and performance as well as pain and plasma CK activity. All data are presented as the mean \pm standard deviation, and 95% confidence intervals (95% CI). Statistical significance was accepted as $p < 0.05$.

3.2.6 ETHICAL CONSIDERATIONS

The original study and the sub-study were performed in accordance with the principles of the Declaration of Helsinki (Fortaleza, Brazil, 2013). Both studies were approved by the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee (HREC REF 136/2005 and HREC REF 931/2014 respectively) (Appendix VI).

During the original study all participants were informed about the purpose of the study, the testing to be undertaken, the possible risks related to the trial, and their right to withdraw from the study at any stage. Participants were provided with full, adequate and understandable oral and written explanations of the testing procedures, including all possible risks, involved in the study. All participants were required to provide written informed consent (Appendix I) before being allowed to take part in the study. Participants were given the right to withdraw from the study at any time without reason or prejudice. In addition, all participants consented to the MRI scans and analysis of these scans during the informed consent process for the original study. All data were kept confidential. All data were coded, and contained no personal identifying information. The magnetic resonance images were provided on CD's. The scans were transferred to a personal computer and files were password protected. The original scans are stored in a locked cupboard in the study supervisor's office at the University of Cape Town.

3.2.6.1 Risks to participants

There were no potential risks to the participants in the original study that may be associated with anthropometrical measurement such as mass, stature and skinfold measurement. Treadmill running was associated with a risk of the participant falling, and therefore possible injury to the participant. In the original study all participants underwent a thorough familiarisation process. All due care were

taken to ensure that the participant was both familiar and confident with treadmill running. In addition, a medical practitioner was present for all maximal effort treadmill tests. Participants were also excluded on the basis of medical or surgical history, any musculoskeletal injury, and any medication use or viral infection within the 12 weeks that preceded the study.

All participants were required to warm-up before the treadmill tests and the five kilometre time trial. The warm-up was monitored to reduce the risk of any musculoskeletal injury.

Ultramarathon racing is associated with risks inherent to endurance exercise. Running the Comrades marathon in itself did not present any additional risks to the experimental group. In addition, all participants selected for the original study had previous ultramarathon running experience and were well-trained. Participants were also provided with a heart rate monitor for the duration of the Comrades marathon, and were instructed on how to use the heart rate monitor during competition. In addition, participants were to be excluded from the study, and advised to avoid running the Comrades marathon if they developed a viral infection during the study.

Blood samples were drawn for the analysis of plasma creatine kinase activity. A trained phlebotomist performed the procedure and a medical practitioner was present during the testing procedures. Sterile equipment was used and good clinical practice guidelines strictly adhered to. Participants with cardiac pacemakers or metal implants were excluded from the study on the basis of medical history, or history of musculoskeletal injury. There are no other potential physical or physiological risks to participants that can be associated with magnetic resonance imaging. There were no additional risks to participants during the sub-study, as it involved secondary data analysis only. No new participants were recruited and no new physical assessments were performed.

3.2.6.2 Benefits to participants

Participants received financial compensation (R300) to cover any costs incurred during participation in the original study. Participants also received a full summary of their individual results, as well as the overall findings from the original study.

3.3 RESULTS

3.3.1 DESCRIPTIVE CHARACTERISTICS

The descriptive characteristics of the participants are shown in Table 3.2. There were no significant differences between the groups for any of the descriptive variables.

Table 3.2: Descriptive characteristics of participants in the experimental and control groups. Data are expressed as mean \pm standard deviation.

	Experimental group (n =11)	Control group (n = 11)	95% CI	t-value	P
Age (years)	42 \pm 6	38 \pm 6	[-0.87,9.96]	1.75	0.1
Mass (kg)	77.4 \pm 4.9	76.9 \pm 12.3	[-7.71,8.79]	0.14	0.89
Height (cm)	176.8 \pm 7.0	179.0 \pm 8.1	[-8.78,4.64]	-0.64	0.52
Sum of skinfolds (mm)	70.6 \pm 13.6	71.1 \pm 27.1	[-19.56,18.61]	-0.05	0.96
% Body fat	20.6 \pm 3.2	18.9 \pm 3.6	[-1.29,4.74]	1.20	0.24
Lean body mass (kg)	61.4 \pm 3.5	62.2 \pm 9.2	[-6.99,5.40]	-0.27	0.79

3.3.2 TRAINING AND RACING HISTORY

The training and racing history of participants are shown in Table 3.3. There was a significant difference between the two groups in pre-competition training distance (km.wk⁻¹) during the three months before the race. The experimental group had a significantly higher pre-competition training distance compared to the control group (p = 0.01) in the three months before the ultramarathon. There were no other significant differences between groups for the other training and racing history variables.

Table 3.3: Training and racing history of participants in the experimental and control groups. Data are expressed as mean \pm standard deviation.

	Experimental group (n = 11)	Control group (n = 11)	95% CI	t-value	p
Total number of years running	13.9 \pm 7.3	12.6 \pm 7.1	[-5.13,7.67]	0.41	0.68
Average training distance (km.wk ⁻¹)	51.4 \pm 14.7	42.3 \pm 14.9	[-4.06, 22.24]	1.44	0.16
Pre-competition training distance (km.wk ⁻¹)*	75.9 \pm 7.4	63.2 \pm 13.3	[3.18, 22.28]	2.78	0.01
Number of standard marathons (42.2 km)	21.4 \pm 10.2	13.0 \pm 17.5	[-4.38,21.11]	1.36	0.18
Personal best 10 km time (min)	40.8 \pm 3.5	40.1 \pm 5.3	[-3.37, 4.69]	0.34	0.74
Personal best 10 km speed (m.s ⁻¹)	4.1 \pm 0.3	4.2 \pm 0.5	[-0.50, 0.29]	-0.55	0.59
Personal best 42.2 km (min)	201.4 \pm 18.9	200.9 \pm 24.2	[-18.85, 19.76]	0.05	0.96
Personal best 42.2 km speed (m.s ⁻¹)	3.5 \pm 0.3	3.5 \pm 0.4	[-0.36, 0.31]	-0.16	0.87

* p < 0.05

3.3.3 ULTRAMARATHON RACE

The participants in the experimental group completed the 90 km race in 602.5 \pm 90.9 minutes. The average intensity (% HR_{max}) during the race was 80 \pm 2%. The experimental group displayed a relatively wide range of finishing times for the ultramarathon race.

3.3.4 MUSCLE PAIN

Subjective pain scores of general pain at rest and pain during daily activities are shown in Figure 3.5. Quadriceps and hamstrings general pain scores were significantly higher on day one post-race (p < 0.05) in the experimental group, compared to the control group. Quadriceps pain during daily activities scores were significantly higher on days one (p = 0.0006), two (p = 0.004) and three (p = 0.02) post-race in the experimental group, compared to the control group.

Subjective pain scores of stretch pain and pressure pain are shown in Figure 3.6. Quadriceps stretch pain scores were significantly higher in the experimental group compared to the control group in the quadriceps on days one ($p = 0.004$) and two ($p = 0.04$) post-race in the experimental group compared to the control group. Quadriceps pressure pain scores were also significantly higher on days one ($p = 0.002$) two ($p = 0.01$) post-race in the experimental group compared to the control group.

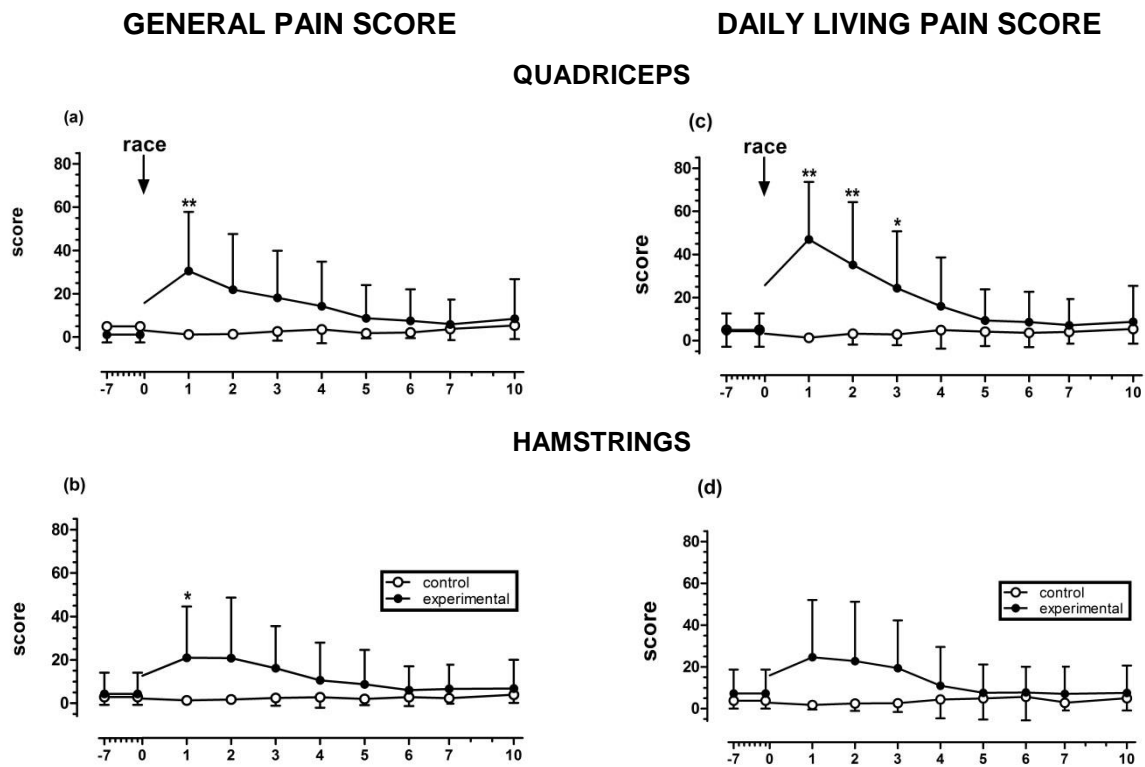


Figure 3.5: Pain scores of participants in the experimental (-●-) (n = 11) and control (-○-) (n = 11) groups. General pain scores in the (a) quadriceps and (b) hamstrings; and daily living pain scores in the (c) quadriceps and (d) hamstrings. Tests were conducted at seven and one days before the race, daily for seven days after the race, and at 10 days after the race. Data are expressed as mean ±SD.

Significant differences:

General pain:

Quadriceps: ** experimental group day 1 vs control group day 1 ($p = 0.002$)

Hamstrings: * experimental group day 1 vs control group day 1 ($p = 0.04$)

Daily living pain:

Quadriceps: ** experimental group day 1 vs control group day 1 ($p = 0.0006$)

** experimental group day 2 vs control group day 2 ($p = 0.004$)

* experimental group day 3 vs control group day 3 ($p = 0.02$)

Stretch pain:

Quadriceps: ** experimental group day 1 vs control group day 1 ($p = 0.004$)

* experimental group day 1 vs control group day 2 ($p = 0.01$)

Pressure pain:

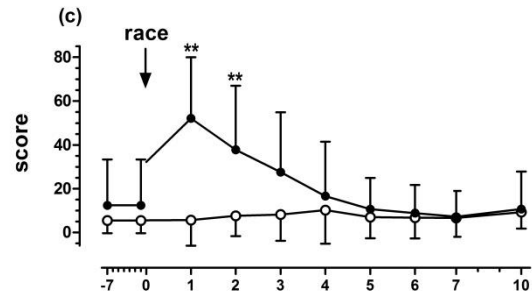
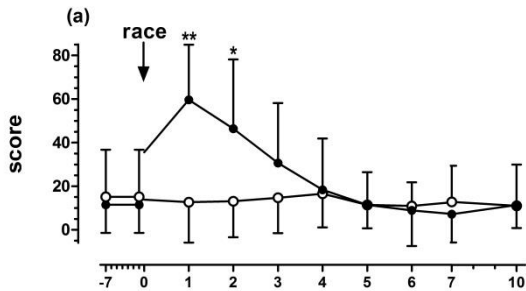
Quadriceps: ** experimental group day 1 vs control day 1 ($p = 0.0002$)

** experimental group day 2 vs control group day 2 ($p = 0.01$)

STRETCH PAIN SCORE

PRESSURE PAIN SCORE

QUADRICEPS



HAMSTRINGS

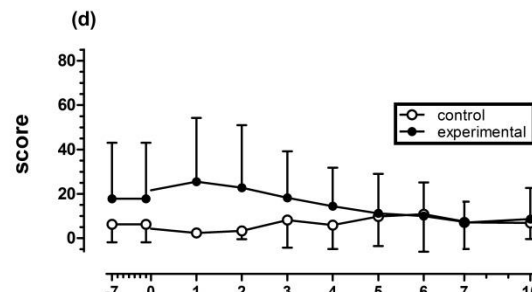
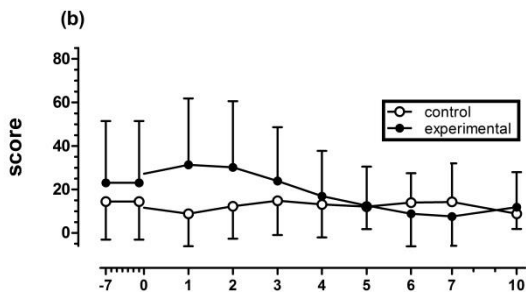


Figure 3.6: Pain scores of participants in the experimental (-●-) ($n = 11$) and control (-○-) ($n = 11$) groups. Stretch pain scores in the (a) quadriceps and (b) hamstrings; and pressure pain scores in the (c) quadriceps and (d) hamstrings. Tests were conducted at seven and one days before the race, daily for seven days after the race, and at 10 days after the race. Data are expressed as mean \pm SD.

Significant differences:

Stretch pain:

Quadriceps: ** experimental group day 1 vs control group day 1 ($p = 0.004$)

* experimental group day 1 vs control group day 2 ($p = 0.01$)

Pressure pain:

Quadriceps: ** experimental group day 1 vs control day 1 ($p = 0.0002$)

** experimental group day 2 vs control group day 2 ($p = 0.01$)

3.3.5 PLASMA CREATINE KINASE ACTIVITY

There was a significant interaction between groups over time for plasma CK activity ($F_{(9,180)} = 16.55$; $p < 0.0009$) (Figure 3.6). The plasma CK activity was significantly higher in the experimental group on days 1, 2, 3, and 4 ($p < 0.006$) after the ultramarathon. From day five onwards, and for the duration of the study thereafter, there were no differences between groups (Figure 3.7).

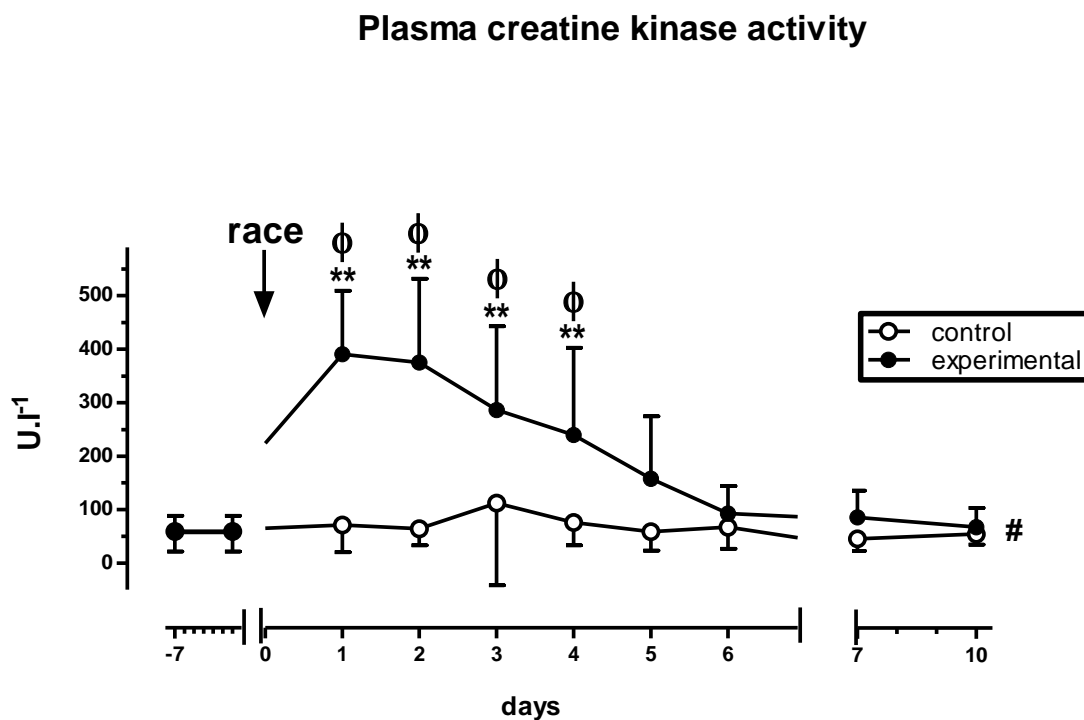


Figure 3.7: Plasma creatine kinase activity (U.l⁻¹) of participants in the experimental (-●-)(n = 11) and control (-○-)(n = 11) groups. Tests were conducted at seven and one days before the race, daily for seven days after the race, and at 10 days after the race. Data are expressed as mean ± standard deviation.

Significant differences:

- # interaction of group x time ($p < 0.00009$)
- ** experimental days 1, 2, and 3 vs experimental days -7, -1, 4, 5, 6, 7, and 10 ($p < 0.006$)
- ** experimental day 4 vs experimental days -7, -1, 6, 7, and 10 ($p < 0.003$)
- φ experimental days 1, 2, and 3 vs control days -7, -1, 1, 2, 3, 4, 5, 6, 7 and 10 ($p < 0.003$)
- φ experimental day 4 vs control days -7, -1, 1, 2, 4, 5, 6, 7, and 10 ($p < 0.003$)

3.3.6 PERFORMANCE

There were no significant differences in the average five kilometre time trial time between groups or pre-post the ultramarathon race. Pre-race 5 km time trial times were 21.5 ± 1.5 minutes and 21.0 ± 2.3 minutes for the experimental and control groups respectively. Post-race time trial times were 21.5 ± 1.6 minutes and 20.9 ± 2.3 minutes for the experimental and control groups respectively (Table 3.4). The pre-race and post-race 5 km time trial times remained relatively unchanged between the experimental and control groups.

There were no significant differences in the average running speed between groups or pre-post the ultramarathon race. Pre-race average running speeds were $3.9 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ and $4.0 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ for the experimental and control groups respectively. Post-race average running speeds were $3.9 \pm 0.3 \text{ m}\cdot\text{s}^{-1}$ and $4.0 \pm 0.4 \text{ m}\cdot\text{s}^{-1}$ for the experimental and control groups respectively (Table 3.4). The pre-race and post-race average running speeds remained unchanged between the experimental and control groups.

3.3.7 RATE OF PERCEIVED EXERTION (RPE)

There were no significant differences in the average rate of perceived exertion (RPE) between groups or pre-post the ultramarathon race. The average pre-race RPE values were 15.0 ± 1.2 and 15.6 ± 1.0 for the experimental and control group respectively. The average post-race RPE values were 15.6 ± 1.8 and 15.7 ± 2.1 for the experimental and control group respectively (Table 3.4).

3.3.8 HEART RATE

There were no significant differences in the average heart rate (HR) between groups. The average pre-race heart rate values were $171 \pm 11 \text{ b}\cdot\text{min}^{-1}$ and $172 \pm 8 \text{ b}\cdot\text{min}^{-1}$ for the experimental and control group respectively. The average post-race heart rate values were $174 \pm 9 \text{ b}\cdot\text{min}^{-1}$ and $173 \pm 8 \text{ b}\cdot\text{min}^{-1}$ for the experimental and control group respectively (Table 3.4).

Table 3.4: Average values for 5 km time trial times, running speed, rate of perceived exertion and heart rate of experimental (n = 11) and control (n =11) groups before and after the ultra-marathon race. Tests were performed 7 days before and 10 days after the ultra-marathon race. Data are expressed as mean \pm standard deviation

	Experimental group (n =11)		Control group (n =11)	
	Pre-race	Post-race	Pre-race	Post-race
Average 5 km time (min)	21.5 \pm 1.5	21.5 \pm 1.6	21.0 \pm 2.3	20.9 \pm 2.3
Average speed (m.s⁻¹)	3.9 \pm 0.3	3.9 \pm 0.3	4.0 \pm 0.4	4.0 \pm 0.4
Average RPE	15 \pm 1.2	15.6 \pm 1.0	15.6 \pm 1.8	15.7 \pm 2.1
Average heart rate (b.min⁻¹)	171 \pm 11	174 \pm 9	172 \pm 8	173 \pm 8

3.3.9 MUSCLE VOLUME

Muscle volume post-race were normalised as a percentage of the pre-race muscle volume to account for variations in limb size between participants. A value less than 100% indicates that the post-race muscle volume was less than the pre-race muscle volume. A value more than 100% indicates that the post-race muscle volume was more than the pre-race muscle volume. The percentage change (relative change) in muscle volume was also calculated. Data for muscle volume are presented as the combined average of the left and right limb values. The changes in normalised muscle volume, percentage change and effect sizes between groups are shown in Table 3.5.

There were significant differences in normalised muscle volume values and percentage change in muscle volume of the combined hamstrings group between the experimental and control groups, with muscle volume values being significantly lower in the experimental group compared to the control group ($p = 0.003$). There were no other significant differences between groups in normalised muscle volume or percentage change in muscle volume for the rectus femoris muscle or the combined quadriceps muscle group respectively.

The effect sizes for normalised muscle volume and percentage change were medium in the rectus femoris muscle ($d = 0.49$). The effect sizes for normalised muscle volume and percentage change were small in the combined quadriceps muscle group ($d = 0.2$). The effect sizes for normalised muscle volume and percentage change were large in the hamstrings muscle group ($d = 1.42$).

Table 3.5: Changes in muscle volume in the experimental and control groups. Data are expressed as mean ± standard deviation.

	Experimental group			Control group			95% CI	t-value	p	Effect size (d)
	Valid N	Normalised muscle volume	% change	Valid N	Normalised muscle volume	% change				
Rectus femoris	8	100.19 ± 10.82	0.19 ± 10.82	10	92.94 ± 16.28	-7.06 ± 16.28	[-7.0, 21.48]	1.08	0.3	0.49
Combined quadriceps	10	100.95 ± 5.47	0.95 ± 5.47	11	101.99 ± 4.56	1.99 ± 4.56	[-5.6, 3.54]	-0.47	0.64	0.20
Combined hamstrings*	10	96.96 ± 4.47	-3.04 ± 4.47	11	104.11 ± 5.12	4.11 ± 5.12	[-11.6, -2.73]	-3.39	0.003	1.42

Key: *Normalised muscle volume* = $\left(\frac{\text{post-race volume}}{\text{pre-race volume}}\right) \times 100\%$; *% Change* = $\left(\frac{\text{post-race volume} - \text{pre-race volume}}{\text{pre-race volume}}\right) \times 100\%$; **Effect size:** Cohen's d: d = 0.2-0.49 (small effect size); d = 0.5-0.79 (medium effect size); d = 0.8 or above (large effect size)

Significant differences: * Combined hamstrings normalised & percentage change muscle volume (p = 0.003)

3.3.10 PEAK CROSS SECTIONAL AREA (CSA)

Changes in peak cross sectional area (CSA) post-race were normalised as a percentage of the pre-race peak cross sectional area to account for variations in limb size between participants. The percentage change (relative change) was in peak CSA also calculated. A value more than 100% indicates that the post-race peak CSA of the muscle was more than the pre-race peak CSA. Data for peak cross sectional area are presented as the combined average of the left and right limb values. The changes in peak CSA are shown in Table 3.6.

The differences in normalised peak CSA and percentage change in peak CSA of the combined quadriceps group between the experimental group and the control group approached significance ($p = 0.05$), with experimental group peak CSA and percentage change in peak CSA tending to be higher than that of the control group. There were no other significant differences between groups in normalised peak CSA values and the percentage change in peak CSA for the rectus femoris muscle or the hamstrings muscle group respectively.

The effect sizes for normalised peak CSA and percentage change in peak CSA were medium in the rectus femoris muscle ($d = 0.7$) and the hamstrings muscle group ($d = 0.5$) respectively. The effect sizes for normalised peak CSA and percentage change in peak CSA were large in the combined quadriceps muscle group ($d = 0.9$).

Table 3.6 Changes peak cross sectional area in the experimental and control groups. Data are expressed as mean ± standard deviation.

	Experimental group			Control group			95% CI	t-value	p	Effect size (d)
	Valid N	Normalised peak CSA	% change	Valid N	Normalised peak CSA	% change				
Rectus femoris	9	120.55 ± 59.63	20.55 ± 59.63	10	87.23 ± 59.63	-12.77 ± 59.63	[-7.8, 74.36]	1.71	0.10	0.70
Combined quadriceps	10	101.52 ± 5.66	1.52 ± 5.66	11	94.92 ± 8.13	-5.08 ± 8.13	[0.1, 13.08]	2.14	0.05	0.90
Combined hamstrings	11	108.53 ± 35.90	8.53 ± 35.90	11	95.56 ± 8.51	-4.44 ± 8.51	[-10.2, 36.17]	1.17	0.30	0.50

Key: *Normalised peak CSA* = $\left(\frac{\text{post-race peak CSA}}{\text{pre-race peak CSA}}\right) \times 100\%$; *% Change* = $\left(\frac{\text{post-race peak CSA} - \text{pre-race peak CSA}}{\text{pre-race peak CSA}}\right) \times 100\%$; **Effect size:** Cohen's d: Cohen's d: d = 0.2-0.49 (small effect size); d = 0.5-0.79 (medium effect size); d = 0.8 or above (large effect size)

3.3.11 TRANSVERSE (T2) RELAXATION TIME

The absolute change in transverse (T2) relaxation times is shown in Table 3.7. Data for T2 relaxation times are presented as the combined average of the left and right limb values. There were no significant differences between groups in the T2 relaxation times of the rectus femoris muscle, the combined quadriceps muscle and hamstrings muscle groups respectively.

Table 3.7: Absolute changes in T2 relaxation times in the experimental and control groups. Data are expressed as mean \pm standard deviation.

	Experimental group		Control group		95 % CI	t-value	p
	Valid N	Δ T2 time	Valid N	Δ T2 time			
Rectus femoris	8	2.84 \pm 5.84	10	-4.67 \pm 8.41	[0.1; 14.96]	2.14	0.05
Combined quadriceps	10	0.23 \pm 8.81	11	-3.93 \pm 9.85	[-4.4;12.74]	1.01	0.32
Combined hamstrings	10	1.04 \pm 6.48	11	-3.86 \pm 9.13	[-2.4; 12.20]	1.4	0.18

Key: Δ = pre-race value – post-race value

3.3.12 CORRELATIONAL ANALYSES

3.3.12.1. Absolute change in muscle volume and absolute change in peak cross sectional area

There was a significant positive correlation between the absolute change in muscle volume and the absolute change in peak cross sectional area of the rectus femoris muscle for all groups ($r = 0.82$, $p < 0.0009$) as well as for the experimental group ($r = 0.88$, $p = 0.004$) and the control group ($r = 0.80$, $p = 0.005$) (Table 3.10). A positive correlation indicates that as the absolute change in muscle volume increases, the absolute change in peak cross sectional area increases.

There was a significant positive correlation between the absolute change in muscle volume and the absolute change in peak cross sectional area of the combined quadriceps muscle group for the experimental group ($r = 0.83$, $p = 0.003$). No significant correlations were found for all groups and the control group (Table 3.8) A positive correlation indicates that as the absolute change in muscle volume increases, the absolute change in peak cross sectional area increases.

There was a significant positive correlation between the absolute change in muscle volume and the absolute change in peak cross sectional area of the hamstrings muscle group for the experimental group ($r = 0.82$, $p = 0.004$). No significant correlations were found for either all groups or the control group (Table 3.8). A positive correlation indicates that as the absolute change in muscle volume increases the absolute change in peak cross sectional area increases in the hamstring muscle group.

3.3.12.2 Absolute change in muscle volume and absolute change in transverse relaxation (T2) times

There were no significant differences in relationships between the absolute change in muscle volume and the absolute change in transverse relaxation (T2) times in any of the muscle groups.

3.3.12.3 Absolute change in peak cross sectional area and absolute change in Transverse relaxation (T2) times

There was a significant negative correlation between the absolute change in peak cross sectional area and the absolute change in T2 relaxation time of the rectus femoris muscle for all groups ($r = 0.54$, $p = 0.02$). No significant correlations were found for either the experimental or control group (Table 3.9). A negative correlation indicates that as the absolute change in peak cross sectional area increases, the absolute change in T2 relaxation time decreases. No significant correlations were found between the absolute change in peak cross sectional area and the absolute change in T2

relaxation times of the quadriceps muscle as well as the hamstrings muscle for any of the groups.
(Table 3.9)

Table 3.8 Relationships between the absolute changes in MRI measurements: Muscle volume and peak cross sectional area and Transverse relaxation times. Note '+' indicates a positive correlation, and '-' indicates a negative correlation. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation			All groups			Experimental group			Control group		
			Relationship	R	P	Relationship	R	P	Relationship	r	P
Rectus femoris	Δ Volume	Δ peak CSA	+	0.82	0.0009	+	0.88	0.004	+	0.80	0.005
		Δ T2 times	-	0.34	0.16	-	0.53	0.18	-	0.18	0.63
Combined quadriceps	Δ Volume	Δ peak CSA	+	0.29	0.20	+	0.83	0.003	+	0.19	0.58
		Δ T2 times	+	0.10	0.66	-	0.05	0.90	+	0.19	0.60
Hamstrings	Δ Volume	Δ peak CSA	+	0.19	0.41	+	0.82	0.004	+	0.17	0.62
		Δ T2 times	+	0.17	0.45	-	0.13	0.71	+	0.05	0.89

Key: Δ = absolute change (pre – post); CSA = Cross sectional area; T2 times = Transverse relaxation times

Table 3.9 Relationships between the absolute changes in MRI measurements: Peak cross sectional area and transverse relaxation times (T2). Note '+' indicates a positive correlation, and '-' indicates a negative correlation. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation			All groups			Experimental group			Control group		
			Relationship	R	P	Relationship	R	P	Relationship	r	P
Rectus femoris	Δ peak CSA	Δ T2 times	-	0.54	0.02	-	0.48	0.23	-	0.43	0.22
Combined quadriceps	Δ peak CSA	Δ T2 times	-	0.41	0.06	-	0.07	0.84	-	0.49	0.12
Hamstrings	Δ peak CSA	Δ T2 times	-	0.43	0.053	-	0.16	0.66	-	0.50	0.12

Key: Δ = absolute change (pre – post); CSA = Cross sectional area; T2 times = Transverse relaxation times

3.3.12.4 Absolute change in MRI measurements and absolute change in plasma creatine kinase activity

3.3.12.4.1 Absolute change in T2 time and absolute plasma creatine kinase activity

There was a significant positive correlation between the absolute change in transverse relaxation time of the rectus femoris muscle and the absolute change in plasma creatine kinase activity for the experimental group ($r = 0.74$, $p = 0.04$). No significant correlations were found for all groups and the control group (Table 3.10). A positive correlation indicates that as the absolute change in T2 time increases, the absolute change in plasma creatine activity also increases.

There was also a significant positive correlation between the absolute change in transverse relaxation time of the combined quadriceps muscle and the absolute change in plasma creatine kinase activity for the experimental group ($r = 0.79$, $p = 0.006$). No significant correlations were found for all groups and the control group (Table 3.10). A positive correlation indicates that as the absolute change in T2 time increases, the absolute change in plasma creatine kinase activity also increases.

No significant relationships were found between the absolute change in transverse relaxation time of the hamstrings muscle and the absolute change in plasma creatine kinase activity for either all groups, the experimental group or the control group.

3.3.12.4.2 Absolute change in peak cross sectional area and absolute change in plasma creatine kinase activity

There were no significant differences in relationships between the absolute change in peak cross sectional area in any of the muscle groups and the absolute change in plasma creatine kinase activity for all groups, the experimental group as well as the control group.

3.3.12.4.3 Absolute change in muscle volume and absolute change in plasma creatine kinase activity

There were no significant differences in relationships between the absolute change in muscle volume in any of the muscle groups and the absolute change in plasma creatine kinase activity for all groups, the experimental group and the control group.

Table 3.10: Relationships between the absolute change of MRI measurements (muscle volume, peak cross sectional area and transverse relaxation time) and the absolute change in plasma creatine kinase activity. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Muscle group	Correlation		All groups			Experimental group			Control group		
			Relationship	R	P	Relationship	R	p	Relationship	r	P
Rectus femoris	Δ plasma CK activity	Δ T2 time	+	0.35	0.89	+	0.74	0.04	+	0.07	0.85
		Δ peak CSA	-	0.15	0.54	-	0.26	0.54	-	0.31	0.38
		Δ volume	+	0.09	0.73	-	0.22	0.60	+	0.07	0.84
Combined quadriceps	Δ plasma CK activity	Δ T2 time	+	0.13	0.57	+	0.79	0.006	+	0.007	0.98
		Δ peak CSA	-	0.22	0.33	-	0.12	0.74	-	0.52	0.10
		Δ volume	-	0.39	0.08	-	0.23	0.52	-	0.48	0.14
Hamstrings	Δ plasma CK activity	Δ T2 time	+	0.04	0.85	+	0.57	0.09	+	0.05	0.89
		Δ peak CSA	-	0.24	0.30	-	0.09	0.79	-	0.41	0.22
		Δ volume	+	0.01	0.97	-	0.18	0.61	+	0.47	0.14

Key: Δ = absolute change (pre – post); CSA = cross sectional area; CK = creatine kinase; T2 = transverse relaxation time

3.3.12.5 Relationships between the absolute change in MRI measurements and the absolute change in performance measurements

3.3.12.5.1 Absolute change in muscle volume and rate of perceived exertion

There was a significant positive correlation between the absolute change in muscle volume of the hamstrings muscle and the absolute change in the rate of perceived exertion (RPE) in the experimental group ($r = 0.67$, $p = 0.03$). No significant relationships were found for all groups or the control group (Table 3.11). A positive correlation indicates that as the absolute change in muscle volume increases, the absolute change in the rate of perceived exertion (RPE) increases.

There were no significant relationships between the absolute change in muscle volume and the rate of perceived exertion of the rectus femoris and combined quadriceps muscles for all groups, the experimental group and the control group.

3.3.12.5.2 Absolute change in muscle volume and absolute change in heart rate

There was a significant positive correlation between the absolute change in muscle volume of the hamstrings muscle and the absolute change in heart rate in the experimental group ($r = 0.70$, $p = 0.02$). No significant relationships were found for all groups or the control group (Table 3.11). A positive correlation indicates that as the absolute change in muscle volume increases, the absolute change in heart rate increases.

There were no significant relationships between the absolute change in muscle volume and the absolute change in heart rate of the rectus femoris and combined quadriceps muscles for all groups, the experimental group and the control group.

Furthermore, there were no significant differences in the relationships between any other variables of MRI measurement and performance measurements.

Other correlation analyses with no significant differences are shown in Appendix VII.

Table 3.11: Relationships between the absolute change in MRI measurements and the absolute change in performance measurements. Note '+' indicates a positive correlation, and '-' indicates a negative correlation. Significant relationships ($p < 0.05$) are highlighted in bold.

Muscle group	Correlation		All groups			Experimental group			Control group		
	MRI	Performance	Relationship	r	P	Relationship	R	p	Relationship	r	p
Rectus femoris	Δ Volume	Δ Speed	-	0.07	0.77	-	0.11	0.80	-	0.06	0.87
		Δ RPE	+	0.19	0.46	-	0.38	0.36	+	0.26	0.47
		Δ HR	+	0.17	0.50	-	0.34	0.41	+	0.58	0.08
	Δ peak CSA	Δ Speed	-	0.04	0.87	-	0.06	0.89	-	0.05	0.90
		Δ RPE	+	0.03	0.89	-	0.24	0.57	+	0.03	0.94
		Δ HR	+	0.19	0.44	-	0.23	0.57	+	0.38	0.28
	Δ T2 time	Δ Speed	-	0.02	0.99	-	0.39	0.34	+	0.32	0.37
		Δ HR	+	0.13	0.61	-	0.10	0.81	+	0.25	0.49
		Δ RPE	-	0.27	0.27	-	0.17	0.70	-	0.25	0.49
Combined quadriceps	Δ Volume	Δ Speed	-	0.18	0.44	-	0.46	0.18	+	0.24	0.48
		Δ RPE	+	0.35	0.12	+	0.11	0.76	+	0.53	0.10
		Δ HR	+	0.12	0.60	+	0.29	0.42	+	0.01	0.98
	Δ peak CSA	Δ speed	-	0.12	0.61	-	0.26	0.47	-	0.06	0.85
		Δ RPE	-	0.11	0.62	-	0.12	0.75	-	0.20	0.56
		Δ HR	+	0.05	0.84	+	0.06	0.88	-	0.26	0.44
	Δ T2 time	Δ speed	-	0.11	0.64	-	0.50	0.14	+	0.34	0.31
		Δ RPE	+	0.11	0.64	-	0.05	0.90	+	0.20	0.57
		Δ HR	-	0.37	0.10	-	0.42	0.23	-	0.22	0.51
Hamstrings	Δ Volume	Δ speed	+	0.04	0.87	-	0.11	0.76	+	0.23	0.49
		Δ RPE	+	0.01	0.96	+	0.67	0.03	-	0.03	0.93
		Δ HR	+	0.20	0.39	+	0.70	0.02	+	0.34	0.31
	Δ peak CSA	Δ speed	-	0.59	0.80	-	0.17	0.64	-	0.34	0.92
		Δ RPE	-	0.19	0.41	+	0.39	0.26	-	0.34	0.31
		Δ HR	+	0.29	0.20	+	0.46	0.18	+	0.09	0.80
	Δ T2 time	Δ speed	+	0.01	0.96	-	0.19	0.59	+	0.20	0.55
		Δ RPE	+	0.13	0.57	+	0.32	0.37	+	0.16	0.64
		Δ HR	-	0.20	0.39	+	0.15	0.68	-	0.37	0.27

Key: MRI = magnetic resonance imaging; Δ= absolute change (pre – post); peak CSA = peak cross sectional area; T2 = transverse relaxation time; RPE = rate of perceived exertion; HR = heart rate

3.3.13 SUMMARY OF RESULTS

In this study, muscle pain and plasma CK activity were significantly elevated in the experimental group compared to the control group for up to three days and four days after the race, respectively. Thereafter, muscle pain and plasma CK activity returned to pre-race values by day six after the race. Normalised muscle volume and relative change in muscle volume of the hamstrings muscle group were significantly lower in the experimental group compared to the control group ($p = 0.003$). Normalised peak CSA and relative change in peak CSA of the combined quadriceps group approached significance ($p = 0.05$), with values tending to be higher in the experimental group compared to the control group.

Significant correlations were reported between a number of measurements

- The absolute change in muscle volume of the rectus femoris muscle group was positively correlated with the absolute change in peak CSA in all groups ($p < 0.0009$), the experimental group ($p = 0.004$) and the control group ($p = 0.005$).
- The absolute change in muscle volume of the combined quadriceps muscle groups was positively correlated with the absolute change in peak CSA in the experimental group ($p = 0.003$).
- The absolute change in muscle volume of the hamstrings muscle group was positively correlated with the absolute change in peak CSA in the experimental group ($p = 0.004$).

As the absolute change in muscle volume increased, so did the absolute change in peak CSA.

- The absolute change in peak CSA of the rectus femoris muscle group was negatively correlated with the absolute change in T2 relaxation times ($p = 0.02$). As the absolute change in peak CSA increased the absolute change in T2 relaxation times decreased.
- The absolute change in T2 relaxation times of the rectus femoris group was positively correlated with the absolute change in plasma CK activity in the experimental group ($p = 0.04$).
- The absolute change in T2 relaxation times of the combined quadriceps groups was positively correlated with the absolute change in plasma CK activity in the experimental group ($p = 0.006$).

As the absolute change in T2 relaxation times increased, so too did the absolute change in plasma CK activity increase.

- The absolute change in muscle volume of the hamstrings muscle group was positively correlated with the absolute change in RPE ($p = 0.003$) as well as the absolute change in HR ($P = 0.02$) in the experimental group. As the absolute change in muscle volume increased, so too did the absolute change in RPE and HR.

3.4 DISCUSSION

3.4.1 PARTICIPANTS

3.4.1.1 Sample size

The total sample size of this study was 22 participants, 11 male endurance runners that participated in an ultramarathon race and 11 male endurance runners that did not participate in an ultramarathon race. Recent studies investigating exercise induced muscle damage with an MRI measurement component such as muscle volume, cross sectional area and T2 relaxation times had sample sizes of between six and 41 participants^(21, 37, 203, 204, 214, 216, 217, 220). Most of the studies investigated the thigh musculature and implemented exercise induced muscle damage protocols that targeted muscles such as the quadriceps and hamstrings^(21, 37, 203, 214, 216, 217). Other muscles that were also investigated were the biceps⁽²⁰⁴⁾, the adductors^(21, 214) and sartorius⁽²¹⁾. The magnetic resonance imaging measurements used, included muscle volume^(18, 21, 203, 204), anatomical cross sectional area^(21, 203, 216, 220, 235) and T2 relaxation times^(37, 203, 204, 214). Many of the studies had small sample sizes of between six and seventeen participants^(37, 203, 204, 214, 216, 217, 220) and used only one limb for investigation^(37, 204, 220), whereas other studies included measurements for both limbs^(21, 203, 214, 216). Where bilateral measurements were taken measurements were normalised to account for variations in limb sizes and values were given as an average. In this study bilateral assessments were done on all MRI scans of the participants and averaged for use in data analysis.

The physical activity level of the participants in the various studies differed and included sedentary participants to participants involved in regular physical activity. None of the studies investigated runners specifically. It is therefore difficult to ascertain whether the volume, peak cross sectional area and T2 relaxation time measurements are accurate in endurance runners, and make comparisons to other studies.

Only one study conducted a between group analysis⁽²¹⁾, whereas the other studies mainly focused on the before and after effects of a specific exercise induced muscle damaging protocol^(37, 203, 204, 214, 216, 217, 220). The exercise induced muscle damaging protocols also varied.

Protocols such as eccentric contraction of the quadriceps⁽²¹⁷⁾, squats⁽²⁰³⁾, step down exercises⁽²¹⁴⁾, Nordic hamstring exercises⁽²¹⁶⁾ and biceps curls⁽²⁰⁴⁾ were implemented to induce muscle damage. Only one study investigated the effect of endurance exercise intervention on thigh muscle volume and anatomical cross sectional area⁽²¹⁾. The endurance exercise intervention consisted of a cycling session.

In this study a between group analysis was done to investigate the effect of an ultramarathon on changes in muscle volume, peak cross sectional area and T2 relaxation times in endurance runners. Again, since there is a shortage of studies investigating endurance runners specifically, it is difficult to compare the results to other studies.

3.4.1.2 Descriptive characteristics

There were no significant differences in the age, mass, stature, body fat percentage or lean body mass between participants of the experimental and control groups. There were no significant differences between groups in individual skinfold measurements, although previous studies have found changes in the lower limb skinfolds related to highly trained endurance runners, specifically ultramarathon runners⁽²³⁶⁾.

Studies investigating muscle morphology of the thigh muscles and the effects of exercise interventions have used sedentary to physical active female participants^(18, 21, 214), male participants^(37, 204, 216, 220) or a combination of male and female participants^(203, 205, 237). In a recent study investigating anthropometry and training in recreational endurance runners, only male participants were used. Furthermore, the body fat percentage and mass were slightly lower than the participants in this study, and the average age slightly greater⁽²³⁶⁾. This study also only included male participants. By including both female and male participants, the total changes across the running population may be observed, rather than sex specific changes.

3.4.1.3 Training and competition history

There were no significant differences between the experimental group and the control group in the number of years running, the average weekly training distance and the number of marathons completed. Furthermore, there were no significant differences between groups in the personal best times and speed for either a 10 km or a 42.2 km run. There was, however, a significant difference between the experimental group and the control group in the weekly training distance covered

three months prior to the ultramarathon. The experimental group had a significantly higher weekly training distance (75.9 km.wk⁻¹) compared to the control group (63.2 km.wk⁻¹).

Rust et al⁽²³⁶⁾ showed similar results with regards to the weekly training distance of ultramarathon runners compared to marathon runners. It is theorised that endurance athletes taking part in shorter distance endurance races such as marathons or Ironman triathlons base their training more on intensity whereas it is possible that ultramarathon athletes rather concentrate on high volume as part of their training^(236, 238).

3.4.2 EXERCISE INDUCED MUSCLE DAMAGE PROTOCOL AND MUSCLE PAIN

In the original study, taking part in an ultramarathon (Comrades 2005) caused muscle pain in the experimental group, consistent with the signs of delayed onset muscle soreness (DOMS). Delayed onset muscle soreness is characterised by the time course of the perception of discomfort or pain. The onset of muscle soreness starts approximately eight hours after exercise, and peaks at about 24 to 48 hours after exercise. The symptoms usually subside after seven to ten days after exercise^(10, 11, 25, 33, 109, 122, 201). Muscle pain in the quadriceps and hamstring muscle groups occurred within 24 hours after the ultramarathon race and returned to a pain free state after seven days after the race. These findings are supported by other studies investigating exercise induced muscle damage^(11, 25, 201).

Furthermore, the ultramarathon race included an increased amount of downhill running. Downhill running is associated with a greater magnitude of lengthening muscle actions compared with level running and therefore also associated with a greater degree of muscle damage⁽¹¹⁾. During downhill running, the extensor muscles of the knee (quadriceps femoris) perform a lengthening contraction during each stride to decelerate the centre of mass after the foot touches the ground⁽¹¹⁾. It is also evident that greater delayed onset of muscle soreness is produced in the gluteal muscles, the quadriceps, and the anterior and posterior tibial muscles during downhill running⁽¹¹⁾. In this study, muscle pain was measured in the quadriceps and hamstring muscle groups. The subjective muscle pain scores in the experimental group was significantly higher in the quadriceps on day one, two and three, whereas the pain score in the hamstrings muscle group was only significantly higher on day one. This can be explained by the biomechanics of downhill running and the greater amount of lengthening muscle actions required in the quadriceps femoris during downhill running.

In the studies implementing magnetic resonance imaging, various muscle damaging protocols were used, ranging from squats⁽²⁰³⁾, step down exercises⁽²¹⁴⁾, Nordic hamstring exercises⁽²¹⁶⁾ and biceps curls⁽²⁰⁴⁾. Only one study investigated the effect of endurance exercise intervention on thigh muscle volume and anatomical cross sectional area⁽²¹⁾. The endurance exercise intervention consisted out a cycling session. From the literature it is evident that lengthening (eccentric) muscle actions provide a greater amount of exercise induced muscle damage^(27, 33, 102). Although there are no specific MRI studies investigating exercise induced muscle damage induced specifically by endurance running and changes in muscle morphology, the large amount of lengthening muscle actions needed during the downhill phases of the ultramarathon can be regarded as sufficient to induce muscle damage.

Furthermore, it has been established that delayed onset muscle soreness does not accurately reflect the extent of muscle damage, as it has been documented that muscle function may remain impaired even after the dissipation of muscle soreness^(14, 15). It can therefore be theorised that the delayed onset of muscle soreness could have affected running performance in the experimental group, even though at the time of the five kilometre time trial test, participants were not experiencing any perceived discomfort. Marcoro and Bosio⁽¹⁸⁰⁾ reported that the risk of having a significant reduction in performance is higher in participants experiencing exercise induced muscle pain, than in participants not suffering from exercise induced muscle damage.

3.4.3 PLASMA CREATINE KINASE ACTIVITY

Plasma creatine kinase (CK) activity is a commonly used indicator of muscle damage⁽²³⁹⁾. In this study plasma CK activity was significantly higher in the experimental group on days one, two, three and four after the ultramarathon compared to the control group. These findings are consistent with other studies that also reflect a rapid increase in plasma CK activity from 24 hours after endurance events^(167, 169-171, 175, 177, 240, 241). It is well-documented that the two types of exercise predominantly used to investigate muscle damage are downhill running and high-force eccentric exercise protocols. After downhill running, plasma CK activity peaks about 12 to 24 hours after exercise, whereas with after high-force eccentric exercise, the increase only begins at 48 hours post-exercise⁽¹⁵⁵⁾. The underlying mechanism for the different responses however is unclear.

3.4.4 PERFORMANCE

There were no significant differences in the five kilometre time trial performance between the experimental and control groups. The post-race time trial was performed ten days after the ultramarathon and this may be a reason for no significant differences. Delayed onset muscle soreness has been found to reduce after seven days following the onset of pain, and by the day ten post-race, the pain levels of the participants have returned to pre-race values.

It is therefore possible that if the post-race time trial was performed earlier that there would be a decrease in the performance of the experimental group, seeing that exercise induced muscle damage negatively affects performance in humans⁽¹⁸⁰⁾.

3.4.5 HEART RATE

There were no significant differences in heart rate between the experimental and control groups. Chambers et al⁽⁴⁵⁾ documented a significant difference in heart rate response to steady-state exercise for up to 25 days after an ultramarathon. The tendency was for heart rate to be higher in the experimental group after the ultramarathon, but these findings should be interpreted with caution. The authors found large intra-subject variation for the measurements and this in combination with the relatively small sample size, increased the possibility of a type II error with the analysis⁽⁴⁵⁾. Additional research is necessary to investigate the heart rate response to exercise after an ultramarathon.

3.4.6 RATE OF PERCEIVED EXERTION

Although there were no significant differences in the rate of perceived exertion (RPE) between the experimental and control groups, it is of value to note that the pre-race RPE of the experimental group was slightly reduced. It is theorised that this may be due to the pre-race taper. Tapering consists of a systematic reduction in training load before an event and it is reported to have physiological and psychological benefits that may improve performance⁽⁹⁶⁾. It can possibly be deduced that the experimental group had a reduced pre-race RPE prior to the ultramarathon to avoid fatigue or even injury. Future studies should investigate daily RPE values during the taper period prior to determine whether there might be any training or performance benefits associated with low pre-event RPE scores.

3.4.7 MAGNETIC RESONANCE IMAGING

3.4.7.1 Muscle volume

There were significant differences in normalised muscle volume values and percentage change in muscle volume of the combined hamstrings group between the experimental and control groups, with muscle volume values being significantly lower in the experimental group compared to the control group in the post-race period.

There were no other significant differences between groups in normalised muscle volume or percentage change in muscle volume for the rectus femoris muscle or the combined quadriceps muscle group respectively, but for both these muscle groups there was an increase in muscle volume in the experimental group in the post-race period.

It is interesting to note the difference in muscle volume changes between the agonist and antagonistic muscle groups; there is an increase in muscle volume of the quadriceps, although not significant and a significant reduction in the antagonist or hamstring muscle group.

Hudelmaier et al⁽²¹⁾ documented an increase in muscle volume of the knee extensors and sartorius after 12 weeks of endurance cycling in untrained perimenopausal women. The knee flexors (hamstrings) and adductors showed small and inconsistent changes. Furthermore, it was reported that in the strength training group, there were significant increases in muscle volume of the extensors, flexors and adductors, with only sartorius showing borderline significance⁽²¹⁾. It is however, difficult to compare the results of this study with Hudelmaier et al⁽²¹⁾, as the endurance training component was a 12 week endurance cycling programme compared to running an ultramarathon that caused exercise induced muscle damage. Furthermore the participants are quite different with regards to sex and physical activity. This study investigated male endurance runners, whereas Hudelmaier et al⁽²¹⁾ investigated untrained perimenopausal women.

Hudelmaier et al⁽²¹⁾ concluded that MRI can be an effective method to monitor location-specific effects of exercise on muscle morphology. It is also important in the context of the design of scientific and clinical studies on muscle adaptation, where muscle morphology is measured as an imaging endpoint. This study supports the theory that MRI is an effective method to monitor effects of endurance running and exercise induced muscle damage on muscle morphology.

With the exception of a small amount of studies ⁽²¹⁾, MRI volume techniques have not been utilised to assess changes resulting from exercise induced muscle damage. Fulford et al ⁽²⁰³⁾ investigated muscle damage 24 hours after eccentric exercise in the quadriceps muscle group and reported a significant increase in percentage change in muscle volume of the quadriceps. This supports the theory of general oedema and muscle swelling being present after exercise induced muscle damage. Although this study did not find any significant changes in percentage change of the quadriceps, it did show an increase in percentage change which may support the theory of general oedema and muscle swelling.

There was however, a significant decrease in percentage change for muscle volume of the hamstrings muscle group indicating a loss of muscle volume in the experimental group after the ultramarathon. The reason for the loss in muscle volume remains unclear. Armstrong et al ⁽⁵⁾ theorised about the existence of a small population of “susceptible” or “vulnerable” fibres within a muscle compartment. These authors observed necrosis of 5% of fibres in muscles of rats eccentrically exercised by downhill running⁽⁵⁾. Another animal study reported apparently irreversible muscle fibre damage as well as muscle mass and protein content reduction 14 days after in vivo eccentric exercise ⁽²⁴²⁾.

Human studies also reported evidence of this phenomenon. Mair et al ⁽²²¹⁾ reported the appearance of myosin heavy chain fragments in the blood three to five days after peak appearance of the smaller, cytoplasmic CK protein. This was interpreted as evidence of irreparable muscle fibre damage. In another study, biopsies of human calf and biceps muscles after eccentric exercise showed proof of muscle fibre damage at 21 days, but not as early as seven days after exercise ⁽¹²¹⁾. Foley et al observed a volume loss of seven to ten percent in the biceps muscle during the period from two to eight weeks post-exercise, supporting the theory of Armstrong et al⁽⁵⁾.

The significant loss in hamstring muscle volume of the experimental group after the ultramarathon may be attributed to irreparable muscle damage. This finding may also provide insight into chronic hamstring injuries experienced by distance runners. Furthermore, a significant positive correlation was found between the absolute change in muscle volume of the hamstrings muscle group and the absolute change in rate of perceived exertion as well as heart rate. This needs to be further investigated, but this finding could indicate a link between irreparable muscle fibre damage and exercise performance. Further research is needed to investigate the effect of an ultramarathon on muscle morphology and also aimed at extending the time course the variables are measured over.

3.4.7.2 Cross sectional area

The differences in normalised peak CSA and percentage change in peak CSA of the combined quadriceps group between the experimental group and the control group approached significance ($p = 0.05$), with experimental group peak CSA and percentage change in peak CSA tending to be higher than that of the control group. There were no other significant differences between groups in normalised peak CSA values and the percentage change in peak CSA for the rectus femoris muscle or the hamstrings muscle group respectively.

It is interesting to note however, that directional changes within peak cross sectional area measurements (Table 3.6) and muscle volume measurements (Table 3.5) are not similar. If muscle volume and peak cross sectional area is a surrogate for changes in 'leg size', one would expect the directional changes to be similar in both measurements. This relationship does not seem to be the case. In the experimental group the relative change in hamstring volumes were significantly less after the ultramarathon (as discussed in Section 3.4.7.1), but when examining peak cross sectional area the relative change in peak cross sectional area of the hamstring muscle in the experimental group was higher than the control group. This is also the case with the normalised values.

Furthermore the directional changes differ between these two measurements. With normalised muscle volume of the hamstrings in the experimental group, the value is less than a 100% ($96.96 \pm 4.47\%$), indicating that the post-race muscle volume was less than the pre-race muscle volume. With regards to the normalised value of peak CSA of the hamstrings in the experimental group the value is 108.53 ± 35.90 , which is higher than 100%, indicating that the post-race peak CSA was higher than the pre-race peak CSA.

It is unclear why there is such a discrepancy, but it can possibly be attributed to peak cross sectional area being a more error prone technique. The standard deviations of the peak CSA measurements are very large in comparison to the muscle volume measurements. It is also possible that the method of calculating peak cross sectional area is not the most accurate way to investigate and one should rather utilise anatomical cross sectional area measurements or physiological cross sectional area. With this study it was not possible to measure these cross sectional area as there was not sufficient information available from the MRI images.

Various studies have utilised cross sectional area to investigate morphological changes in muscle. Anatomical cross sectional area (cross section through the entire muscle, perpendicular to the axis of the muscle, ACSA) and physiological cross sectional area (cross section perpendicular to the muscle fibre direction, PCSA) are the two common methods used⁽²¹⁾. Furthermore, studies have focused on the ACSA from a single slice of MRI, rather than muscle volume and other studies have investigated ACSA and PCSA as well as muscle volume to investigate changes in muscle morphology. It is still unclear however, which is the preferable measurement method⁽²¹⁾.

Previous studies investigating exercise induced muscle damage and cross sectional areas of the thigh musculature have reported an increase in cross sectional area of the vastus lateralis, vastus medialis, and vastus intermedius, but not the rectus femoris 24 hours after exercise⁽²⁰³⁾. In the present study normalised values of the combined quadriceps (VL, VM and VI) approached significance ($p=0.05$).

3.4.7.3 T2 relaxation time

There were no significant differences between groups in the absolute change in T2 relaxation times of the rectus femoris muscle, the combined quadriceps muscle and hamstrings muscle groups respectively.

Previous studies investigating muscle damage and T2 relaxation times have reported significant T2 increases in a range of muscle groups, following different exercise studies, including EIMD protocols. These have included, after downhill running in mice⁽¹⁶⁾, biceps curl⁽²⁰⁴⁾, short duration eccentric exercise⁽²³⁾, extended duration concentric leg contractions⁽²¹⁴⁾ and squats⁽²⁰³⁾. The T2 relaxation times in these studies were measured at different time intervals after exercise or the EIMD protocol. The time course varied but most included measurements before, immediately after and up to nine days after. Furthermore these studies were before and after studies, with no control group. This study measured the absolute change in T2 relaxation times and compared this between the two groups of endurance runners. Although there were no significant differences between the experimental and control groups, it is interesting to note that the absolute change in T2 relaxation times was lower in the experimental group compared to the control group.

Previous studies have reported that changes in T2 relaxation times reach a peak three to five days post-damage induction⁽²²⁵⁾.

Subsequently at the time of analysis within the current study, T2 times may have already reached the maximum amplitude. It is well-established that the T2 relaxation time of muscle in proton MR images increases during exercise, and returns to resting values within one hour after exercise. Changes in intracellular water chemistry are theorised to be the reason for this acute change in T2. Shellock et al⁽²²⁵⁾ observed a second T2 increase that developed gradually from day one to six after eccentric, but not after concentric or isometric exercises. Previous studies have described the time course and magnitude of the T2 increase as well as its relationship to other markers of muscle damage for the early post-exercise. The general conclusion in these studies was that the T2 increase reflected the presence of oedema (112). The origin of the increase in T2 values still remains unclear. It is hypothesized that there are multiple component influencing these T2 value increases such as oedema, inflammatory responses and direct muscle fibre damage⁽²⁰³⁾ Meyer and Prior suggested that the increased T2 values may be due to osmotically mediate fluid shifts into the intracellular space as a result of the build-up of inorganic phosphate and lactate. Another theory is the presence of increased extracellular water content due to the accumulation of degraded protein components⁽¹⁷⁾. However, the exact underlying mechanism causing T2 changes remains unclear, but studies have shown that it is a reliable indicator of muscle damage. Although not significant, the lower T2 values in the experimental group in this study could indicate that there may be other underlying mechanisms other than the presence of oedema, inflammatory processes and muscle fibre damage, involved in exercise induced muscle damage caused by running an ultramarathon.

Other studies investigating T2 relaxation times have investigated small groups of two to three participants over longer periods of time and reported increased T2 relaxation times at two to three months after a single bout of eccentric exercise. These findings indicate that the residual T2 increase cannot only be attributed to an increase in extracellular water, and may be a long lasting adaptation. Further research is needed over longer periods of time to investigate the underlying mechanisms for the T2 value changes as well as for the time course of these changes.

It is interesting that although there were no significant differences in T2 relaxation times between groups for any of the muscle groups investigated, absolute change in T2 relaxation times of the rectus femoris muscle group approached significance ($p = 0.05$). Fulford et al⁽²⁰³⁾ reported increased T2 relaxation times in vastus lateralis, vastus medialis and vastus intermedius, but not in rectus femoris.

The type of exercise protocol used to induce muscle damage may have a role in this difference. Fulford et al⁽²⁰³⁾ implemented a squat exercise as the damage-inducing exercise. All four muscles within the quadriceps group will be challenged during a squat, but they will not however be equally susceptible to the eccentric form of exercise that typically induces muscle damage. The rectus femoris is the only muscle within the quadriceps that crosses the hip, the other originate on the proximal femur. During knee flexion, as the body is lowered, the rectus femoris is not as strained as the VL, VI and VM, as the portion of its proximal end is not as extensively stretched⁽²⁰³⁾. The VL, VI, and VM on the other hand are working at relatively longer lengths, resulting in greater damage being induced⁽²⁰³⁾.

During running, the quadriceps and rectus femoris fire from late swing to midstance to prepare the limb for ground contact as well as to absorb the shock of that impact during the stance phase absorption⁽²⁴³⁾. The rectus femoris is the only muscle of the quadriceps that is active in midswing⁽²⁴³⁾. The particular ultramarathon in this study also included an extensive component of downhill running. During running the quadriceps muscles perform eccentric contractions during each stride to decelerate the centre of mass after the foot touches the ground⁽¹¹⁾. Furthermore, it has been reported that during downhill running the quadriceps muscle group works over a greater length, with more work at its longer length and therefore producing muscle damage⁽¹¹⁾. The difference seen in the T2 relaxation times of the rectus femoris muscle in the experimental group can possibly be due to a greater extent of muscle damage caused by running an ultramarathon with a large component of downhill running.

There was a significant positive correlation between the absolute change in T2 relaxation times of the rectus femoris and combined quadriceps muscle groups and the absolute change in plasma creatine kinase activity in the experimental group. This supports similar findings of positive correlations between T2 relaxation times and plasma creatine kinase activity following activities compromising a large eccentric component^(112, 162, 202, 222). It is theorised that changes in T2 relaxation times can quantitatively illustrate the extent of muscle damage in a non-invasive manner⁽¹⁶⁾.

Furthermore, the positive correlation may support the theory that MRI is useful in localising which muscle or part thereof has been damaged after the exercise and is leaking muscle proteins into the blood system. Takashi et al⁽²¹³⁾ reported T2 changes in VL, VI, and VM, but not in RF after an

eccentric exercise protocol designed to damage the quadriceps muscle. A variability in T2 changes among different sections of the same muscle has also been reported ⁽²³⁾.

This study shows T2 changes in the rectus femoris and combined quadriceps group of the experimental group, positively correlated with another marker of muscle damage. It can therefore be hypothesized that the greater extent of plasma CK leakage was due to disturbances of the sarcolemma of the rectus femoris and combined quadriceps muscle groups, more than the hamstring group. However, further studies are needed to investigate the changes in T2 relaxation times, the variances among different sections of the same muscle as well as the specific muscles damaged after endurance running. One of the limitations of this study was the number of slices that were viable to analyse, therefore not making it possible to further investigate the differences in T2 relaxation times between proximal and distal parts of the same muscle.

3.4.8 LIMITATIONS OF THIS STUDY

The analysis of the magnetic resonance images had some limitations. While the intra-rater reliability was high in this study, the results are different to previous studies investigating muscle morphology ^(21, 203, 204). One of the limitations appears to be the number of slices analysed and used to calculate muscle volume and peak cross sectional area. Only six or seven slices on the scans were of good enough quality to analyse, whereas previous studies have analysed between ten and twenty slices ^(21, 203). This could explain the difference in values found.

It is also difficult to compare the results of this study to previous studies, as the muscle damage inducing protocols varied. No studies implemented endurance running as a muscle damaging protocol, but rather focused on squats or step down exercises for the lower limb and bicep curls for the upper limb.

Unfortunately, due to resource constraints we were unable to take serial MRI images during the recovery period after the ultramarathon race, but we strongly recommend that this should be considered in future studies as MRI imaging becomes more accessible and affordable. In addition, we purposefully selected the post-race MRI time period to coincide with the time where the experimental group were no longer symptomatic, but where evidence suggested that there were likely to be underlying morphological changes associated with muscle damage. It is recognised that

the absence of MRI imaging during the acute, symptomatic period post-race limits the interpretation of the study findings, as we are unable to confirm the extent of inflammatory-type changes that may have been evident during the acute phase when both muscle pain and plasma CK activity were elevated.

The sample size for this study is another limitation. Some of the MRI tests images were excluded and thus reducing the sample size. Future studies should aim for larger sample sizes to ensure that the MRI analyses are adequately powered.

CHAPTER 4: SUMMARY AND CONCLUSION

It is evident that endurance running induces muscle damage^(3, 4). Commonly reported indirect indicators of exercise induced muscle damage, such as plasma CK activity^(10, 13, 14) and muscle soreness⁽¹⁵⁾, are most evident in the initial period after muscle damage, and do not accurately reflect the extent of muscle damage⁽¹⁷⁾. Magnetic resonance imaging is a non-invasive technique that may provide valuable insight into the degree of exercise induced muscle damage. There is some evidence to suggest that T2 relaxation times are increased following eccentric training⁽²²⁾, and that there are associations between changes in plasma CK activity and T2 relaxation times^(23, 24). However, there is a lack of evidence regarding changes in both T2 relaxation times and muscle morphology after endurance running.

Therefore, the overall aim of this study was to investigate changes in T2 relaxation times and muscle morphology in endurance runners after a 90 km ultramarathon race. Based on the evidence provided in this dissertation, the study objectives described in Section 1.2.2 (page XXX) may be answered as follows:

To determine the time course of recovery of muscle pain and plasma creatine kinase activity after a 90 km ultramarathon race

Muscle pain was significantly elevated for up to three days after the race; and plasma CK activity was significantly elevated for up to four days in runners who completed a 90 km ultramarathon. Thereafter, muscle pain and plasma CK activity returned to pre-race values by day six after the race. Participants also completed the ultramarathon race at an average intensity of approximately 80% of maximum heart rate. The ultramarathon race was therefore effective at inducing muscle damage in runners; however it is somewhat surprising that values returned to pre-race values relatively quickly after the race. This relatively quick recovery in indirect markers of muscle damage suggests that trained endurance runners may adapt relatively quickly to sustained stretch shortening cycle activity associated with ultra-endurance running.

To determine changes in 5 km time trial performance in an experimental group of endurance runners that took part in a 90 km ultramarathon race compared to a control group of endurance runners that did not take part in a 90 km ultramarathon race

There were no differences in 5 km time trial performance between the experimental group and control group, before or after the ultramarathon race. This suggests that 5 km time trial performance was unchanged in runners exposed to an ultramarathon race. This finding is in contrast to performance deficits that have been previously documented in response to exercise induced muscle damage. It is possible that the 5 km time trial might not have been sensitive enough to elicit performance changes in trained endurance runners, particularly as the performance test was conducted when participants' muscle pain and plasma CK activity levels had returned to pre-race values. Further studies are needed to assess changes in running performance after an ultramarathon race.

To determine changes in muscle morphology (volume and average cross sectional area) and T2 relaxation times of the quadriceps and hamstrings muscles in an experimental group of endurance runners that took part in a 90 km ultramarathon race and a control group of endurance runners that did not take part in a 90 km ultramarathon race

Normalised muscle volume and relative change in muscle volume of the hamstrings muscle group were significantly lower in the experimental group compared to the control group after the ultramarathon race. The reason for this loss of muscle volume is unclear. It is theorised that there is a reduction in muscle volume due to an irreparable damage of muscle fibres. Further studies are needed to investigate this theory.

Normalised peak CSA and relative change in peak CSA of the combined quadriceps group approached significance ($p = 0.05$), with values tending to be higher in the experimental group compared to the control group after the ultramarathon race.

The increase in peak CSA after an ultramarathon may be due to an increase in intercellular fluid and oedema as a response to the muscle damage induced by running a 90 km ultramarathon. It is interesting to note that the area that showed a potential increase in peak CSA was the quadriceps muscle, which is known to have an increased work load with distance running, due to the high volume of lengthening muscle actions.

To evaluate potential relationships between indicators of muscle damage (plasma creatine kinase activity and muscle pain measurements), morphological muscle changes, and T2 relaxation times in an experimental group of endurance runners that took part in a 90 km ultramarathon race and a control group of endurance runners that did not take part in a 90 km ultramarathon race.

There were no significant relationship between indicators of muscle damage and morphological muscle changes after the 90 km ultramarathon. A positive relationship was found between the absolute change in T2 values of the rectus femoris muscle and the absolute change in plasma CK activity in the experimental group compared to the control group. This correlation could indicate the existence of exercise induced muscle damage still evident ten days after an ultramarathon, as well as indicate which specific muscles are leaking proteins such as creatine kinase into the blood stream and thereby localising the exact area of muscle damage.

Based on the findings in this study, there are morphological changes evident in skeletal muscle after an ultramarathon. An interesting finding was the decrease in muscle volume in the hamstrings muscle group and an increase (approaching significance) in muscle volume of the quadriceps. It may be concluded that there may be a relationship between the agonistic and antagonistic muscle and the extent of muscle damage which needs to be further investigated. Also, a significant positive correlation was reported between the absolute change in muscle volume of the hamstrings muscle group and the absolute change in rate of perceived exertion as well as heart rate in the experimental group. It is theorised that the reduction in muscle volume may be attributed to irreparable muscle damage. Marcora and Bosa⁽¹⁸⁰⁾ reported that the negative effect of exercise induced muscle damage on running performance is mediated by an increased perception of effort. The significant correlation between the change in muscle volume and change in indicators of running performance may indicate that if there are less muscle fibres to recruit during exercise due to irreparable damage, that the perception of effort is also reduced. Furthermore, the significant positive correlation between the absolute change in T2 relaxation times and the absolute change in plasma CK activity supports the suggestion that MRI can be a useful tool to investigate exercise induced muscle damage and the extent thereof. However, further research is needed to investigate the time course of these changes as well as the relationship of these changes with other indicators of exercise induced muscle damage.

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APPENDICES

APPENDIX I: INFORMED CONSENT FORM

INFORMED CONSENT

The MRC/UCT Research Unit for Exercise Science and Sports Medicine will be conducting a study to investigate the relationship between running economy, running performance, glucose oxidative capacity, and neuromuscular function after a 90 km ultramarathon race. These factors may be related to the rate of recovery after the Comrades marathon. This study will improve our understanding of the underlying mechanisms of muscle damage associated with endurance exercise.

The research project will involve the following tests:

1. Body mass and stature measurements.
2. Anthropometric assessment of body composition involving the measurement of skinfold thicknesses using skinfold calipers, and limb diameter and length measurements to determine fat content and muscle mass.
3. A maximum effort treadmill test (to exhaustion) to determine maximum oxygen consumption (VO_{2max}), maximal heart rate (HR_{max}), and peak treadmill running speed. The anthropometric assessment and maximum effort treadmill test will take place three weeks before the Comrades marathon.
4. A 20-minute submaximal treadmill tests to determine submaximal oxygen consumption (a measure of running economy), heart rate (using a heart rate monitor), rate of perceived exertion, and blood glucose oxidation.
5. The measurement of blood glucose oxidation involves an intravenous injection of a low dose of radioactive glucose to measure blood glucose oxidation while running. All injections will be performed under sterile conditions and under the supervision of a qualified medical practitioner. The total radioactivity injected into the body will be 1.8 mrem, the unit in which radiation dose is measured. To put this in perspective, this is 1.1% of the radiation dose from a diagnostic bone scan. The health risk involved is a 0.000018% chance of developing leukaemia (1.8% of that from a bone scan)

6. Blood samples will be taken via a cannula inserted into the antecubital vein before and during each 20-minute submaximal treadmill test to measure plasma creatine kinase (an indicator of muscle damage) and blood glucose oxidation. Good clinical practice and sterile procedures will be strictly adhered to.
7. Muscle pain will be measured subjectively using a “rating of pain” scale.
8. Muscle recruitment is measured using sticky electrodes placed on the surface of the skin, which measure the electrical activity [electromyographic (EMG) activity] of the muscle.
9. A 1.4 km submaximal run and three 40 m sprint tests will be performed before a 5 km time trial run. The tests will be performed on an indoor running track. During the tests electromyographic (EMG) activity, ground contact time, heart rate (using a heart rate monitor), and rate of perceived exertion will be recorded.
10. EMG activity measures the amount of electrical activity in muscles. In this study, the EMG activity of the quadriceps (front thigh), hamstrings (back thigh) and calf muscles will be recorded during the 1.4 km submaximal run, the 40 m sprint tests, and the 5 km time trial run. This involves the placement of electrode on to the muscles so that EMG activity may be recorded.
11. Ground contact time will be measured during the 5 km time trial using an accelerometer that will be attached to the running shoe.
12. The 20-minute submaximal treadmill test, the 1.4 km submaximal run, the 40 m sprint tests, the 5 km time trial run, and the MRI tests will all be performed two weeks before the Comrades marathon, and will be repeated between 10-15 days after the Comrades marathon.
13. Subjects running the Comrades marathon will be required to wear a heart rate monitor for the duration of the race.
14. In addition, daily measurements of muscle pain, and blood samples, for the analysis of plasma creatine kinase activity, will be taken for 7 days after the Comrades marathon. This is to quantify the muscle damage experience after the ultramarathon. Sterile procedures and good clinical practice will be strictly adhered to.
15. Lastly, it will be requested that each subject keep a detailed logbook, and that all training during the testing procedure be recorded.

Time commitments

The following table summarises the time commitments required to participate in this study. The total time commitment necessary for this study from 4 weeks before the Comrades marathon until 15 days after the ultramarathon race is approximately 14 hours.

Date	Testing procedures	Number of visits/week	Time commitment
3 weeks before Comrades (23/05/2005 – 28/05/2005)	Familiarisation - informed consent, questionnaire, anthropometry, familiarisation with equipment and testing procedures Maximum effort treadmill test - VO_{2max} , HR_{max} , PTRS	1	1 hour
2 weeks before Comrades (30/05/2005 – 04/06/2005)	20 minute submaximal treadmill test - oxygen consumption, heart rate (HR), respiratory exchange ratio, blood glucose oxidation, rate of perceived exertion (RPE)	1	1 hour
	1.4 km submaximal run, 20 m sprint tests - EMG activity, RPE	1	1.5 hours
	5 km time trial run - EMG activity, ground contact time, HR, RPE		
Comrades marathon (16/06/2005)	Experimental group only		
1 week after Comrades (17/06/2005 – 23/06/2005)	Acute post-race testing – plasma creatine kinase and muscle pain	7	15 minutes
10-15 days after Comrades (26/06/2005 – 01/07/2005)	20 minute submaximal treadmill test - oxygen consumption, heart rate (HR), respiratory exchange ratio, blood glucose oxidation, rate of perceived exertion (RPE)	1	1 hour
	1.4 km submaximal run, 20 m sprint tests - EMG activity, RPE	1	1.5 hours
	5 km time trial run - EMG activity, ground contact time, HR, RPE		

Possible risks to subjects

There are no potential risks that may be associated with mass, stature, skinfold measurements, and muscle pain measurements. Treadmill running is associated with a risk of falling, and therefore possible injury. In this study, a thorough familiarisation process will be performed to ensure familiarity and confidence with treadmill running. In addition, a medical practitioner will be present for all maximal effort treadmill tests. A warm up will be performed before each running test to reduce the risk of musculoskeletal injury. The use of an accelerometer for the measurement of ground contact times provides no additional potential risks to the subjects.

The only potential risk associated with EMG activity measurements is an allergic reaction to the EMG electrodes. The skin will be carefully examined following removal of the electrodes on completion of testing. Any allergic reaction will be treated with topical cortisone cream, and referral to a medical practitioner for further management. In addition, subjects will be excluded from further testing.

Blood samples will be drawn for the analysis of plasma creatine kinase activity and blood glucose oxidation. As always when working with blood, potential risks include infection with blood borne diseases including hepatitis and HIV. In order to minimize these potential risks, a trained phlebotomist will perform the procedures, and a medical practitioner will be present during the testing procedures. Furthermore, sterile equipment will always be used for these procedures, and good clinical practice will be strictly adhered to.

The measurement of blood glucose oxidation involves an injection of a low dose of radioactive glucose into the blood stream. All injections will be performed under sterile conditions and under the supervision of a qualified medical practitioner. The total amount of radioactivity injected into the body will be 1.8 mrem, the unit in which radiation dose is measured. To put this in perspective, this is 1.1% of the radiation dose from a diagnostic bone scan. The health risk involved is a 0.000018% chance of developing leukaemia (1.8% of that from a bone scan). For additional safety, all radioactive materials will be sent for independent testing to confirm the absence of pyrogens (which cause an increase in body temperature).

Anticipated benefits to subjects

Subjects will receive financial compensation (R300) to cover any costs related to time, travelling expenses, and inconvenience, due to participation in this study. In addition, subjects will receive a full summary of their individual results, as well as the overall findings from this study. The individual results will include information regarding body composition measurements, peak treadmill running speed, maximum oxygen consumption, maximum heart rate and running economy, and training advice. Finally, the runners participating in the Comrades marathon will have the use of a heart rate monitor (Polar Vantage XL, Polar Electro, Kempele, Finland) for the duration of the race.

Privacy and confidentiality

All records and results generated within this study will be stored in a computer database in a secure facility, and in a manner that maintains subject confidentiality. All participants will remain anonymous in any ensuing publication.

Contact Information

Investigator Name	Telephone	Email
Theresa Burgess	(021) 406 6043	tburgess@uctgsh1.uct.ac.za
Mike Lambert (Supervisor)	(021) 650 4569 (021) 650 4567	mlambert@sports.uct.ac.za

I confirm that the exact procedures and possible complications of the above tests have been explained to me. I understand that I may ask questions at any time during the testing procedures. I realise that I am free to withdraw from the study without prejudice at any time, should I choose to do so. I have been informed that the personal information required by the researchers will be held in strict confidentiality. In addition, I know that the information derived from the testing procedures will remain confidential and will be revealed only as a number in statistical analyses.

I have carefully read this form. I understand the nature, purpose and procedure of this study. I agree to participate in this research project of the MRC/UCT Research Unit for Exercise Science and Sports Medicine.

Name (in full) of volunteer: _____

Signature of volunteer: _____

Name (in full) of witness: _____

Signature of witness: _____

Date: _____

APPENDIX II: MEDICAL AND TRAINING HISTORY QUESTIONNAIRE

QUESTIONNAIRE (MEDICAL AND TRAINING HISTORY)

Name: _____

Date of Birth: _____

Age: _____

Medical and Surgical History (last 2 years): _____

Present/previous injuries to lower limbs, pelvis or spine:

Medication: _____

Are you currently receiving any massage, soft tissue or physiotherapy treatment?

Y	N
---	---

If "yes", please state details of treatment: _____

In what year did you start running? _____

In what year did you run your first marathon? _____

In what year did you run your first ultra-marathon? _____

How many of the following races have you run in the past:

- i. Standard Marathons (42,2km) _____
- ii. Ultra-Marathons: Two Oceans (56km) _____
- iii. Ultra-Marathons: Other (< 80km) _____ Other (>80km) _____
- iv. Comrades: Up _____ Down _____

How many months a year do you run? _____

What is your average weekly distance when training for competition? _____

What is your average weekly distance when not training for competition? _____

What has been your average weekly training distance for the last three months? _____

Please complete the following table:

Event	PB		Most Recent Performance	
	Year	Time	Year	Time
5km				
10km				
21,1km				
42,2km				
Two Oceans				
Comrades Up				
Comrades Down				

Have you ever run on a treadmill before?

Y N

Are you able to visit the laboratory at the Sports Science Institute (Boundary Road, Newlands) for testing in the 3 weeks before the Comrades marathon, daily for 1 week after Comrades, and thereafter, for testing sessions between 10-15 days after the Comrades marathon?

Y	N
---	---

Are you planning to train at a reduced level after Comrades (at least for the duration of the study)?

Y	N
---	---

Signature: _____

Date: _____

Thank-you for your co-operation in completing this questionnaire.

APPENDIX III: PHYSICAL ACTIVITY QUESTIONNAIRE

PHYSICAL ACTIVITY QUESTIONNAIRE

We would like to find out about any physical activities that you participate in. Below the table is a list of different types of activity. Please list by number any type of sport that you regularly participate in.

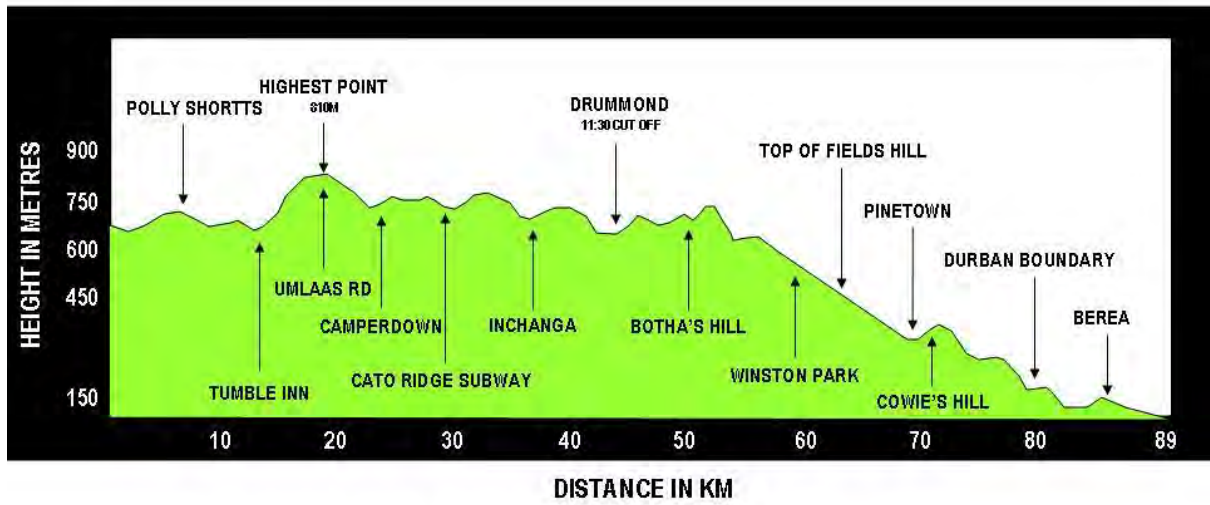
Please indicate the number of times per week and the duration of participation in these events:

Type of Sport	Months per Year (months/year)	Number of Sessions per Week	Duration of Each Session (hr:min)	Total Hours per Week (hrs/week)

Examples of sporting activities:

- | | |
|----------------------------------|---------------|
| 1. Jogging | 11. Rugby |
| 2. Aerobics/ Step | 12. Swimming |
| 3. Martial arts | 13. Cycling |
| 4. Volleyball | 14. Walking |
| 5. Strength/ Resistance Training | 15. Squash |
| 6. Hiking | 16. Tennis |
| 7. Golf | 17. Badminton |
| 8. Canoeing | 18. Netball |
| 9. Dancing | 19. Basketbal |
| 10. Skating | 20. Soccer |

APPENDIX IV: COMRADES RACE PROFILE



APPENDIX V: FEASIBILITY STUDY

RELIABILITY STUDY: INTRA-RATER RELIABILITY OF MUSCLE VOLUME MEASURED WITH ANALYSIS OF MAGNETIC RESONANCE IMAGES

V.I BACKGROUND

Intra-rater reliability is the ability of an examiner to accurately perform a specific testing method repeatedly, over a period of time.

V.II AIM

The aim of this reliability study was to determine the intra-rater reliability of a single rater for the measurement of muscle volume with analysis of magnetic resonance images (MRI).

V.III METHODS

V.III.I Participants

Three male participants participated in the study.

V.III.II Testing procedure

The MRI images of three male participants were analysed by the investigator. The computer programme ITK Snap (www.itksnap.org) was used for the analysis. The analysis consisted out of the segmentation of the rectus femoris muscle of the left upper thigh. A region of interest was selected to segment between the distal end of the femoral neck proximally and the proximal end of the rectus femoris tendon distally for the rectus femoris.

The proximal and distal slices were not of good quality and the muscles were difficult to identify. A region of 7 slices was selected for segmentation of the rectus femoris muscle. The muscle was digitally segmented and reconstructed as an individual entity in each slice. The contours were grouped and used to build patient – specific triangle based mesh surface models of each muscle with the ITK SNAP software.

The volume of the muscles was determined by the numerical integration of the segmented voxels in the software programme. Values were given as cubic millimetre (mm³).

Measurements were taken in on two separate occasions by the same investigator. Measurements were recorded on independent data collection sheets for the first and second occasion. The data were then collaborated into an Excel Spreadsheet(Microsoft Corporation, Redmond, USA).

V.IV STATISTICAL ANALYSES

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, ver. 22, SPSS Inc, Chicago, Illinois, USA). Correlations between data were determined using Cronbach's alpha where a perfect $\alpha = 1$. Intra-rater reliability was accepted as $\alpha \geq 0.7$.

All data were presented as mean \pm standard deviation.

V.V RESULTS

Table V.I shows the results. A Cronbach's $\alpha = 0.988$ was determined indicating a satisfactory intra-rater reliability.

Table V.I Results of intra-rater reliability study of measurement of muscle volume

Variable	Mean \pm SD	Cronbach's α
Measurement 1: Muscle volume RF (mm ³)	32013.7 \pm 6205.67	0.988
Measurement 2: Muscle volume RF (mm ³)	31962.7 \pm 6711.3	0.988

*Cronbach's $\alpha \geq 0.7$ indicates satisfactory intra – rater reliability.

V.VI SUMMARY AND CONCLUSION

Intra-rater reliability is expressed as Cronbach's α where a perfect score is alpha equal to 1. Acceptable intra-rater reliability is regarded as Cronbach's $\alpha \geq 0.7$. Therefore the results of the intra-rater reliability tested showed satisfactory reliability.

APPENDIX VI: ETHICS APPROVAL

UNIVERSITY OF CAPE TOWN



Faculty of Health Sciences
Research & Innovation
Research Ethics Committee
ES2 Room 23 Old Main Building Grootte Schuur
Hospital, Observatory, 7925
Queries : Lamees Emjedi
Tel : (021) 406-6492 Fax: 406-6411
E-mail : lemjedi@curie.uct.ac.za

10 May 2005

REC REF: 136/2005

Ms. T.L. Burgess
Human Biology

Dear Ms. Burgess

CHANGES IN RUNNING ECONOMY, NEURAL REGULATION, EXERCISE PERFORMANCE, GLUCOSE OXIDATIVE CAPACITY, AND MITOCHONDRIAL FUNCTION AFTER A 90KM ULTRAMARATHON

Thank you for submitting your study to the Research Ethics Committee for review.

Date Considered: 29 April 2005

Decision: Approved

- No insurance mentioned

Attached please find the list of members who attended the meeting
Please quote the REC REF in all your correspondence

Yours sincerely

PROF. T. ZABOW
CHAIRPERSON



UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee



Room E52-24 Old Main Building
Groota Schuur Hospital
Observatory 7925

Telephone [021] 406 6338 - Facsimile [021] 406 6411
Email: shuretta.thomas@uct.ac.za

Website: www.health.uct.ac.za/fhs/research/humanethics/forms

17 December 2014

HREC REF: 931/2014

Dr T Burgess
Health & Rehab
F45, OMB

Dear Dr Burgess

PROJECT TITLE: A COMPARISON OF MUSCLE DAMAGE, SORENESS, MORPHOLOGY, T2 CHANGES AND RUNNING PERFORMANCE FOLLOWING AN ULTRAMARATHON RACE

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th December 2015.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the student, Wanda van Niekerk will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

HREC 931/2014

FHS017: Annual Progress Report / Renewal

09 FEB 2016

Record Reviews/Audits/Collection of Biological
 Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
<input checked="" type="checkbox"/> Approved	Annual progress report	Approved until/next renewal date	30.1.2017
<input type="checkbox"/> Not approved	See attached comments		
Signature Chairperson of the HREC	Signed by candidate		Date Signed 10/2/16

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	8 th February 2016		
HREC REF Number	931/2014	Current Ethics Approval was granted until	
Protocol title	A COMPARISON OF MUSCLE DAMAGE, SORENESS, MORPHOLOGY, T2 CHANGES AND RUNNING PERFORMANCE FOLLOWING AN ULTRAMARATHON RACE		
Principal Investigator	Theresa Burgess		
Department / Office Internal Mail Address	Department of Health and Rehabilitation Sciences, F45 Old Main Building, Groote Schuur Hospital		
1.1 Does this protocol receive US Federal funding?			<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

2. Protocol status (tick ✓)

<input type="checkbox"/>	Research-related activities are ongoing
<input checked="" type="checkbox"/>	Data collection is complete, data analysis only
Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository	
N/A	

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval	24
Total number of records or specimens collected, reviewed or stored since last progress report	24
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

4. Signature

Signature of PI	Signed by candidate		Date	08/02/2016
-----------------	---------------------	--	------	------------

APPENDIX VII: ADDITIONAL RESULTS

Table VII.I: Relationship between running speed and other markers of running performance. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation	All groups			Experimental group			Control group		
	Relationship	r	P	Relationship	r	p	Relationship	r	p
Δ average running speed and Δ average RPE	+	0.14	0.53	-	0.03	0.94	+	0.36	0.28
Δ average running speed and Δ average HR	+	0.18	0.42	-	0.18	0.60	+	0.23	0.50

Key: Δ = pre-race value – post-race value; RPE – Rate of perceived exertion; HR – Heart rate

Table VII.II: Relationship between running speed and indirect markers of muscle damage. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation	All groups			Experimental group			Control group		
	Relationship	r	P	Relationship	r	p	Relationship	r	P
Δ average running speed and Δ average muscle pain	+	0.03	0.88	+	0.08	0.81	-	0.26	0.45
Δ average running speed and Δ average plasma CK activity	-	0.29	0.19	-	0.27	0.43	-	0.40	0.23

Key: Δ = pre-race value – post-race value; CK – Creatine Kinase

Table VII.II: Relationship between rate of perceived exertion (RPE) and other markers of performance. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation	All groups			Experimental group			Control group		
	Relationship	r	P	Relationship	r	p	Relationship	r	p
Δ average RPE and Δ average speed	+	0.14	0.53	-	0.28	0.94	+	0.36	0.28
Δ average RPE and Δ average HR	+	0.38	0.08	+	0.34	0.30	+	0.35	0.30

Key: Δ = pre-race value – post-race value; RPE – rate of perceived exertion; HR – heart rate

Table VII.IV: Relationship between rate of perceived exertion (RPE) and indirect indicators of muscle damage. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation	All groups			Experimental group			Control group		
	Relationship	r	P	Relationship	r	p	Relationship	r	p
Δ average RPE and Δ average muscle pain	+	0.23	0.31	+	0.41	0.21	+	0.22	0.53
Δ average RPE and Δ average plasma CK activity	-	0.79	0.73	-	0.03	0.94	-	0.22	0.51

Key: Δ = pre-race value – post-race value; RPE – rate of perceived exertion; CK – creatine kinase

Table VII .V: Relationship between heart rate (HR) and other markers of performance. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation	All groups			Experimental group			Control group		
	Relationship	r	P	Relationship	R	p	Relationship	r	P
Δ average HR and Δ average speed	+	0.18	0.42	+	0.18	0.60	+	0.23	0.50
Δ average HR and Δ average RPE	+	0.38	0.08	+	0.34	0.30	+	0.35	0.30

Key: Δ = pre-race value – post-race value; RPE – rate of perceived exertion; HR – heart rate

Table VII.VI: Relationship between heart rate (HR) and indirect indicators of muscle damage. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Correlation	All groups			Experimental group			Control group		
	Relationship	r	P	Relationship	r	p	Relationship	r	P
Δ average HR and Δ average muscle pain	-	0.08	0.71	-	0.01	0.99	+	0.01	0.97
Δ average HR and Δ average plasma CK activity	+	0.05	0.84	-	0.27	0.43	+	0.03	0.92

Key: Δ =pre-race value – post-race value; HR – heart rate; CK – creatine kinase

Table VII .VII: Relationships between the absolute change of MRI measurements (muscle volume, peak cross sectional area and transverse relaxation time) and the absolute change in muscle pain. Note '+' indicates a positive relationship, and '-' indicates a negative relationship. Significant relationships ($p < 0.05$) are highlighted in bold.

Muscle group	Correlation		All groups			Experimental group			Control group		
			Relationship	r	p	Relationship	r		Relationship	r	P
Rectus femoris	Δ muscle pain	Δ T2 time	+	0.17	0.89	-	0.25	0.56	+	0.25	0.49
		Δ peak CSA	-	0.05	0.85	+	0.18	0.66	+	0.14	0.69
		Δ volume	+	0.12	0.62	+	0.39	0.34	+	0.17	0.65
Combined quadriceps	Δ muscle pain	Δ T2 time	-	0.05	0.82	-	0.28	0.43	-	0.14	0.69
		Δ peak CSA	-	0.13	0.57	+	0.06	0.87	+	0.05	0.88
		Δ volume	+	0.15	0.52	+	0.08	0.82	+	0.28	0.41
Hamstrings	Δ muscle pain	Δ T2 time	+	0.06	0.80	-	0.19	0.61	+	0.18	0.60
		Δ peak CSA	-	0.02	0.93	+	0.09	0.80	+	0.03	0.93
		Δ volume	+	0.29	0.22	+	0.09	0.80	+	0.10	0.77

Key: Δ = absolute change (pre – post); CSA = cross sectional area; CK = creatine kinase; T2 = transverse relaxation time