Muscle damage and inflammation following a three-day trail run

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Declaration

I, Emmerentia C. Denissen, student number 210553532, declare that the work on which this project is based is original and my own work (except where acknowledgements indicate to the contrary) and that neither the whole work nor part thereof has been, is presently or is to be submitted for another degree at this or any other university.

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Thesis Abstract

Introduction

The physiological effects of single and multiday road running races have been studied extensively and include the occurrence of rhabdomyolysis, reflected by significantly increased urinary myoglobin (uMb), as well as increased concentrations of serum creatine phosphokinase (CPK), high sensitivity C-reactive protein (hsCRP), cortisol and cardiac troponin-T (cTnT), dehydration and compromised renal function. Furthermore, in hyperthermic athletes, a positive relationship has been noted between hyperthermia, muscle damage, dehydration and pacing. The physiological effects of a multiday trail run of similar duration to single day road races, however, are unknown.

The side-effects of the use of statin medication for hypercholesterolaemia include muscle fatigue, cramping and increased muscle damage. These have been found to be aggravated in endurance athletes and it has been reported that females, especially when being medicated from a young age, are more susceptible to these side-effects.

Objectives

1. To investigate the effect of a three-day trail run on systemic and urinary markers of muscle damage and inflammation in recreational runners and to establish the association of dehydration and hyperthermia with these markers.
2. To observe the effect of the three day trail run on systemic and urinary markers of muscle damage and inflammation on an additional hypercholesterolaemic female athlete using statin medication in combination with a lipid uptake inhibitor.

Method

Firstly, an observational cohort study was conducted on 19 recreational male (n=6) and female (n=13) athletes during a 95km trail run over three days.

Pre-and post-stage and 24 and 72 h post-race concentrations of serum CPK, hsCRP, cortisol, cTnT, and osmolality (sOsm) as well as uMb, changes in body mass, delayed onset muscle

soreness (DOMS) and thigh circumference (TC) were measured. Continuous recordings of heart rate (HR) and intestinal temperature (T_{intest}) were made throughout each stage.

In addition, a case report is included on one trained female endurance athlete currently being treated for familial hypercholesterolaemia with 20 mg Aspavor and 10 mg Ezetrol daily and not included in the above cohort, to investigate the degree of muscle damage and inflammation she experienced as a result of participation in the three-day event.

Results:

Heart rate ranged between 77 and 83% age-predicted-maximum (APmax) and T_{intest} between 36.1 and 40.2 ºC during the three stages. Significant rises in mean serum CPK, hsCRP, sOsm and blood neutrophil count reached peak concentrations of 1 488U/L, 8.91mg/l, 298mosm/L and 10.21 10^9/L ($p \leq 0.001$), respectively. No evidence of elevations in uMb and cTnT were detected. The stage-induced increments in DOMS correlated positively with CPK, r=0.71; 95% CI [0.62, 0.78]. TC decreased significantly post $S1_{post}$ and $S2_{post}$ ($p \leq 0.05$) and a maximum mean body mass loss of 3.09% (±1.04%) occurred during S2. There was no significant difference between non-steroidal anti-inflammatory drug (NSAID) users and non-users in terms of serum CPK, hsCRP, cortisol, post race DOMS scores, running times, TC or sOsm ($p>0.05$). The post-pre change in sOsm during each stage correlated inversely with the changes in % body mass, r = -0.36, 95% CI [-0.57,-0.094] and the pooled data examining the relationship between the change of sOsm and change in serum CPK for the three stages (n=57), revealed an insignificant positive correlation (r= 0.034, 95% CI [-0.228, 0.291].

The maximum T_{intest} ranged between 38.3 º C and 40.2 º C and only exceeded 40º C in two of the 12 athletes monitored. The relationship between the change in T_{intest} and serum CPK was insignificant ($p>0.05$) for the 11 individuals from whom complete sets of data were available (r= 0.24, 95% CI [-0.42, 0.734].

In the hypercholesterolaemic athlete, the maximum serum CPK (665U/L), hsCRP (1.9mg/Ll) and cortisol (845nmol/L) concentrations corresponded with undetected uMb despite a maximum body mass loss of 4.5%

Conclusion:

Three consecutive days of 95km trail running resulted in low markers of muscle damage and inflammation, when compared to results obtained in previous single day road races of similar duration despite the maintenance of a heart rate above 77% APmax, T_{intest} rising above 39^{o} C and mean body mass decrement of >2.0%. The unchanged concentrations of serum cTnT and uMb confirmed the low values of the markers of muscle damage and inflammation. An insignificant positive correlation between muscle damage and dehydration was noted.

Furthermore the daily use of 0.4 mg/kg Atorvastatin in combination with 10mg Ezetrol did not result in the subject experiencing subjective myalgia, cramps, fatigue or increased markers of muscle damage following her participation in the trail run.

Table of Contents

	Page
DECLARATION	ii
ACKNOWLEDGEMENTS	iii
THESIS ABSTRACT	iv
TABLE OF CONTENTS	vii
LIST OF ABBREVIATIONS	x
CHAPTER ONE: INTRODUCTION	
1.1 INTRODUCTION	1
1.2 AIMS AND OBJECTIVES OF STUDY	3
1.3 HYPOTHESES	3
1.4 SCOPE OF THE STUDY	4
CHAPER TWO: REVIEW OF RELATED LITERATURE	
2.1 MECHANICAL DAMAGE	6
2.2 PRESENTATION	7
2.3 HISTOLOGICAL APPEARANCE OF DAMAGED MUSCLE CELLS	9
2.4 SYSTEMIC MARKERS OF CELL DAMAGE IN SKELETAL AND CARDIAC MUSCLE	10
2.4.1 Creatine Phosphokinase	10
2.4.2 Alpha-Actin	11
2.4.3 Lactate Dehydrogenase	11
2.4.4 Plasma Fatty Acid Binding Protein	11
2.4.5 Troponin	12
2.5 INFLAMMATORY RESPONSE	12
2.5.1 Neutrophils, macrophages and cytokines	12
2.5.2 C-Reactive Protein	13
2.5.3 Cortisol	13
2.6 FACTORS AFFECTING MUSCLE DAMAGE AND INFLAMMATION	14
2.6.1 Repeated Bouts	14
2.6.2 Dehydration, hyperthermia and muscle damage	15

2.6.3 Anti-inflammatory drugs 16
2.6.4 Lipid lowering medication 17
2.6.5 Genetics 19
2.6.6 Chromosome telomere erosion 20
2.6.7 Oxidative stress 20
2.6.8 Age 21
2.6.9 Gender 21
2.6.10 Other 22
2.7 CONCLUSION 23

CHAPTER THREE: MANUSCRIPT 1 24
LOW MARKERS OF MUSCLE DAMAGE AND INFLAMMATION FOLLOWING A THREE-DAY TRAIL RUN
Abstract 25
Introduction 26
Method 27
Results 32
Discussion 37
Conclusion 40
References 41
Acknowledgements 43

CHAPTER FOUR: MANUSCRIPT 2 45
USE OF THE STATIN, ASPAVOR, IN COMBINATION WITH EZETROL, IN A HYPERCHOLESTEROLAEMIC ENDURANCE ATHLETE COMPLETING A 95KM MULTI-DAY TRAIL RUN
Abstract 46
Introduction 47
Case Description 47
Results 48
Discussion 51
Acknowledgements 54
References 55

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS 57
5.1 CONCLUSION 57
5.2 RECOMMENDATIONS 58

REFERENCES 60

APPENDICES 68
APPENDIX A: LETTER OF ACCEPTANCE OF PUBLICATION 69
APPENDIX B: EXAMPLE OF FEEDBACK GIVEN TO EACH SUBJECT 70
APPENDIX C: SUBJECT INFORMATION SHEET 79
APPENDIX D: INFORMED CONSENT FORM 82
APPENDIX E: PRE RACE QUESTIONNAIRE 84
APPENDIX F: POST RACE DOMS QUESTIONNAIRE 88

List of Abbreviations

ALT	Alanine aminotransferase
APmax	Age predicted maximum
AST	Aspartate aminotransferase
BMI	Body mass index
BP	Blood pressure
Bpm	Beats per minute
Ca^{2+}	Calcium
cAMP	Cyclic Adenosine Monophosphate
CPK	Creatine Phosphokinase
cTnT	Cardiac troponin T
DNA	Deoxyribonucleic acid
DOMS	Delayed onset muscle soreness
FABP	Plasma fatty acid binding protein
FDA	United States Food And Drug Administration
HB	Haemoglobin
HCT	Haematocrit
HMG-CoA	3-hydroxy-3-methylglutaryl co-enzyme A
HR	Heart rate
hsCRP	High sensitivity C-Reactive Protein
HSP	Heat shock protein
IL-1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin-6
IL-10	Interleukin-10
LDL	Low density lipoprotein
LDH	Lactate dehydrogenase
NSAID	Non-steroidal anti-inflammatory drugs
RBC	Red blood cell
ROS	Reactive oxygen species
S1	Stage 1
S2	Stage 2

S3	Stage 3
sOsm	Serum osmolality
TC	Thigh circumference
T_{intest}	Intestinal temperature
uMb	Urinary myoglobin
UOsm	Urinary osmolality
USG	Urinary specific gravity
24PR	24 hours post race
72PR	72 hours post race

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Multiday ultra distance events are becoming increasingly popular among professional and amateur athletes and may encompass running, cycling and/or other disciplines. Various studies have been published on the physiological changes that occur during single and multiday endurance road running and cycling events (Knechtle *et al*., 2008; Skenderi *et al.*, 2006; Nieman *et al.*, 2005), but few addressed the changes that occur during multiday endurance trail running races, which have been found to be different to single day ultra distance or marathon foot races (Millet *et al*., 2011).

Running as an activity involves eccentric contraction of a large muscle mass including the hip and knee extensor muscles and muscles of the anterior and posterior tibial compartments during every stride (Eston *et al*., 1995). This eccentric component is accentuated whilst running downhill when the muscle is forced to lengthen while contracting (Proske and Morgan, 2001; Eston *et al*., 1995).

Clarkson and Hubal (2002) attribute the muscle damage to an increased contractile load per unit, which occurs as fewer motor units are recruited. This damage results in changes in the excitation-contraction (E-C) coupling, inflammation, swelling, decreased mobility and delayed onset muscle soreness (DOMS), which would increase in relation to the duration of the event (Clarkson and Hubal, 2002). After eccentric exercise there is a presence of sarcoplasmic enzymes and proteins, including creatine phosphokinase (CPK) in the serum and myoglobin in the urine, when there is leakage of these enzymes and proteins through the damaged membranes of muscle cells into the circulation (Martinez-Amat *et al*., 2005; Clarkson and Hubal, 2002). These findings are, however, not always reflective of structural muscle damage, as demonstrated by histological studies (Clarkson and Hubal, 2002; Lieber and Friden, 2002) due to the specificity of serum CPK that might originate from cardiac muscle damage or might

be reduced by other kinases, such as mitochondrial CPK and CPK-immunoglobulin complexes (Martinez-Amat *et al*., 2005). In order to differentiate between skeletal and cardiac muscle damage, the concentration of cardiac troponin-T (cTnT) in the serum has been used as a reliable marker of cardiac, but not skeletal muscle damage (Martinez-Amat *et al*., 2005).

Increased levels of markers of skeletal muscle damage in the urine including the presence of urinary myoglobin (uMb), have been shown to reflect asymptomatic exertional rhabdomyolysis that has been found to occur during prolonged endurance exercise (Knechtle *et al.*, 2008; Skenderi *et al.*, 2006; Clarkson and Hubal, 2002) and possibly be related to inflammation, DOMS and skeletal and cardiac muscle damage (Skenderi *et al.*, 2006; Clarkson and Hubal, 2002). Knechtle *et al*.(2008) suggest that runners in a 1 200km multiday road running event experienced decreased skeletal muscle mass and increased total body water due to rhabdomyolysis and impaired renal function.

The effect of muscle damage on inflammatory response is evident in the circulation as concentrations of neutrophils and the hepatic acute - phase protein, C-reactive protein (CRP) are raised (Robson-Ansley *et al*., 2009, Nieman *et al*., 2005). Muscle damage during prolonged exercise is associated with an acute phase response, reflected by changes in pro-inflammatory cytokine concentrations which, are in turn, correlated significantly with serum CRP (Robson-Ansley *et al*., 2009, Nieman *et al*., 2005).

The use of non-steroidal anti-inflammatory drugs (NSAID) has been found to affect the inflammatory response, but not skeletal muscle damage during exercise (Nieman *et al.*, 2005). It would therefore be interesting to compare the results of subgroups of athletes who used NSAID to non-users to investigate the relationship between the use of NSAID and markers of muscle damage and inflammation.

Extensive research has also been conducted on the effects of exercise-induced dehydration and the physiological effects thereof (Casa *et al.*, 2010; Stearns *et al.*, 2009; Cleary *et al.*, 2006; Maresh *et al.*, 2004; Cheuvront and Haymes, 2001). Recent studies have found that the concomitant occurrence of dehydration, hyperthermia, increased metabolic rate and the

environment augments muscle damage (Cleary *et al.*, 2005), but it is not known to what extent this would be affected by three consecutive days of trail running.

The use of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors or statins, the primary lipid-lowering medication currently prescribed for hypercholesterolaemia has been associated with muscle cramping, myalgia, fatigue and in rare cases, rhabdomyoloysis and death (Di Stasi *et al*., 2010; Thompson *et al.,* 2010; Seachrist *et al.,* 2005, Pasternak *et al*., 2002; Evans and Rees, 2002). It is, however, not known how the simultaneous intake of ezetemibe, a lipid uptake inhibitor, affects the augmented muscle damage, which has been found to occur when hypercholesterolaemic athletes on statin medication compete in endurance running events. A case study of an athlete who uses these two medications concurrently, was therefore included to investigate her reaction to multiday ultra-distance racing.

1.2 AIMS AND OBJECTIVES OF THE STUDY

This observational cohort study firstly set out to determine the effect of a multiday trail running race over 95km on systemic markers of muscle damage and inflammation using blood neutrophil and serum CPK, hsCRP and cortisol concentrations, thigh circumference (TC), DOMS and uMb as markers of muscle damage and inflammation.

Secondly, the effect of increased intestinal temperature (T_{intest}) and of dehydration, measured by serum osmolality (sOsm) and body mass changes, was also monitored to determine the relationship between T_{intest}, dehydration and muscle damage and inflammation.

Thirdly, the effect of statin medication, at a dosage of 0.4mg/kg, combined with a lipid uptake inhibitor, on the systemic and urinary markers of muscle damage was investigated in a case study.

1.3 HYPOTHESES

During trail running the environmental demands, challenging terrain underfoot and steep ascends and descents are added stressors to contend with while running at least three hours per day (Millet *et al*., 2011). It is hypothesized that, due to these added stressors, the eccentric component of skeletal muscle contraction during trail running will be exaggerated causing increases in systemic and urinary markers of muscle damage and inflammation, DOMS and TC, which will be augmented by dehydration and hyperthermia and reflected in a change in anthropometric values (Casa *et al.*, 2010; Cleary *et al.*, 2005, Sawka *et al.*, 1998).

It is furthermore hypothesized that the hypercholesterolaemic athlete included in the case study will experience muscle cramps and fatigue, which will be reflected in elevated systemic and urinary markers of muscle damage and augmented by dehydration.

1.4 SCOPE OF THE STUDY

This observational cohort field study was conducted during and after a three day trail run over 95km. It was restricted to a convenience sample of twenty-one apparently healthy subjects of which nineteen (6 males, 13 females) completed all three stages of the race and 15 runners (4 males, 11 females) completed all within and post- race assessments.

Pre-race assessments included the recording of body mass and height, TC and four-site skinfold for the determination of % body fat. Self-reported data regarding the medical and training status, hydration and food intake and use of NSAIDS and chemical stimulants was obtained from questionnaires.

During the race, urine and blood sampling were done before and after every stage and at 24 and 72PR (post-race) and uMb, sOsm, cortisol, CPK, hsCRP, cTnT and blood neutrophil concentrations were determined. These results were correlated with sOsm and body mass changes as markers of dehydration, and $T_{intest,}$ TC reflecting inflammation, were also measured and subjects were requested to complete a DOMS questionnaire before and after each stage of

the race as well as for the five days following the race to record subjective perceptions of post-race muscle soreness.

CHAPTER TWO

REVIEW OF THE RELATED LITERATURE

EXERCISE INDUCED MUSCLE DAMAGE

2.1 MECHANICAL DAMAGE

Acute bouts of prolonged running, involving eccentric contractions, generate high levels of strain on the muscle because fewer motor units are recruited, putting more strain on those motor units (Friden and Lieber, 2001). Clarkson and Hubal (2002) propose that this results in a higher load per unit which eventually may lead to injury. Lieber and Friden (2002) suggest that the initial damage to muscle is mechanical in nature, based on sarcomere strain. The subsequent damage occurs due to inflammation and changes in the E-C coupling in the muscle (Clarkson and Hubal, 2002).

With regard to mechanical damage, Proske and Morgan (2001) suggest that sarcomere disruption occurs at the sites of the weakest half-sarcomeres, which lengthen uncontrollably until the actin/myosin cross bridges are forcibly detached (Morgan, 1990) when the actin and myosin filaments slide past each other, according to the Huxley model (Huxley, 1974). With repetition of the eccentric contraction, more of the next-weakest sarcomeres are strained and lengthened (Proske and Morgan, 2001). These stretched sarcomeres are distributed randomly along the muscle fibre. When the muscle relaxes, the overstretched sarcomeres may re-integrate with the undamaged fibres and resume function (Lieber and Friden, 2002). However, with repeated eccentric contraction during exercise, weaker sarcomeres lengthen and become disrupted. This leads to an extension in the optimum length of the muscle and at greater muscle lengths, membrane damage and tearing of the T-tubules occur, leading to inactivation of some sarcomeres (Proske and Morgan, 2001). At this stage, before the necrosis of fibres, a fall in muscle tension would be reversible by the administration of caffeine which, in animal models, causes the release of calcium (Ca^{2+}) from the sarcoplasmic reticulum and subsequent

contraction of the muscle (Proske and Morgan, 2001). The continued eccentric contraction leads to an increased number of disrupted sarcomeres, an increased local contraction and in return the passive tension in the muscle increases (Clarkson and Hubal, 2002). With extensive damage and extensive local contraction, parts of, or the whole fibre dies and an irreversible secondary delayed fall in tension occurs about 24 hours after exercise which is not reversible with administration of caffeine due to the extensive damage that has occurred (Proske and Morgan, 2001). Thereafter breakdown products of tissue necrosis cause a local inflammatory reaction, leading to oedema and soreness (Proske and Morgan, 2001).

According to Huxley (1957), in his sliding filament theory of muscle contraction, the myosin filament head attaches to a single actin binding site, flexes and contraction of the fibre occurs in millions of sarcomeres in a single muscle. The Japanese scientist, Toshio Yanagida, proposed that the actin and myosin are involved in a series of loose couplings (Yanagida, 2007) which explains eccentric contraction, but does however not explain the damage that takes place when the actin-myosin bond is forcibly detached, according to Huxley's model.

2.2 PRESENTATION

Generally, there is a loss of strength of the affected muscle and a drop in active tension due to the mechanical damage and tissue necrosis (McHugh, 2003; Proske and Morgan, 2001), swelling, soreness and an increase in passive tension (or stiffness) leading to a reduced range of motion. Proske and Morgan (2001) postulate that stiffness occurs due to the local contraction and increased release of Ca^{2+} following the membrane damage. Low frequency fatigue, the ability to generate force at a lower frequency, lasting up to a week post-exercise, has also been described (Clarkson and Hubal, 2002).

DOMS, which has been reported to be unique to eccentric exercise, sets in several hours after eccentric exercise and peaks 24 – 72 hours later (Lambert and Dennis, 1994). Swelling and soreness appearing within 6 hours after exercise are ascribed to the local inflammatory response (Proske and Morgan, 2001) and to noxious chemicals such as histamines, bradykinins and prostaglandins that are released (Clarkson and Hubal, 2002). These chemicals sensitize the

Group IV afferent sensory nociceptors and the muscle becomes tender to touch, stretch and contraction. An accumulation of fluid in the muscle fibre causes increased pressure (Clarkson and Hubal, 2002) with swelling that may last up to 10 days post exercise.

Some research questions whether inflammation is a cause or consequence of muscle damage. When comparing the time course of changes in the inflammatory response and the development of DOMS, Malm *et al.* (2004) found no correlation between these variables and concluded that DOMS is a result of muscle adaptation or of the activation of leukocytes that are present in the epimysium before exercise (Malm *et al.*, 2004). Other studies, using radio-labeling of neutrophils, found that neutrophil infiltration into damaged muscle increased up to 24 hours post-exercise, coinciding with DOMS and a secondary decrease in eccentric torque (Raastad *et al.*, 2003).

The elevated calcium levels also trigger the release of endogenous proteases such as calpain (Feasson *et al*., 2002; Friden and Lieber, 2001), causing further muscle damage. Calpain does not affect actin and myosin, but has an affinity for desmin and alpha-actinin (Feasson *et al*., 2002; Friden and Lieber, 2001) contributing to the damage at the intermediate filament sections.

The loss of the structural protein desmin, which links adjacent Z-disks (Bennett *et al.,* 2005), has been noted in fibres that show signs of damage (Friden and Lieber, 2001). Desmin also helps to maintain proper alignment of sarcomeres within and between myofibrils (Boriek *et al*., 2001) Immunostaining of animal tissue has shown that muscle fibres lose "staining" for desmin within minutes after the initiation of eccentric contractions. In addition, the number of fibres that lack desmin increase with time following an eccentric exercise bout and those fibres that lose desmin staining demonstrate accumulation of plasma fibronectin, indicating a loss of membrane integrity in these fibres (Boriek *et al*., 2001).

Dystrophin is a large cytoskeletal protein associated within the muscle sarcolemma and is thought to help maintain the integrity of the membrane during repeated mechanical loading that muscle cells experience through everyday contractions (Blake *et al.*, 2002; Hawke and Garry,

2001). Within six hours following eccentric exercise, dystrophin staining has been reported to be completely missing in some fibres, with accompanying loss of desmin and another structural protein, alpha-actinin (Hawke and Garry, 2001). To compound the loss of membrane stability, other members of the complex of proteins associated with dystrophin, specifically beta-spectrin and alpha-sarcoglycan, show signs of early damage following eccentric contractions (Blake *et al.*, 2002). The number of affected fibres has been shown to increase for up to 2 days after exercise, indicating a progression of damage over time. It has been suggested that this rapid loss of membrane-stabilizing function may render the muscle fibres fragile and more susceptible to damage by further contractions (Hawke and Garry, 2001).

The loss of titin, a component in the A-band that plays an important part in the storage and use of elastic energy (Donovan, 2004) and links the myosin filament to the Z-disk, is a further possibility due to the overstretching of one half-sarcomere (Proske and Morgan, 2001) and the contraction of the other half.

2.3 HISTOLOGICAL APPEARANCE OF DAMAGED MUSCLE CELLS

The morphological findings indicate variable muscle fibre size (Grobler *et al.*, 2004; Friden and Lieber, 2001), hyper contraction of the fibres, loss of the A-band, distortion of the A-band and I-band alignment (Friden and Lieber, 2001), Z-disk streaming in mild damage and Z-disk smearing in severe cases, with dispersion of Z-disk materials into the adjacent sarcomeres (Clarkson and Hubal, 2002; Friden and Lieber, 2001). In the damaged areas focal loss of the Z-disk may occur as well as loss of lateral registration of myofilaments (Martinez-Amat *et al.*, 2005). Enlarged mitochondria, sub-sarcolemmal mitochondria (Grobler *et al.*, 2004) and lipid and glycogen accumulations are also present in the damaged areas.

According to Lieber and Friden (2002) and Friden and Lieber, (2001), mainly type II fibres are affected because of their short fibre length and higher tension developed and their lack of oxidative capacity. Proske and Morgan (2001), however, found that both fibre-types experience damage after eccentric exercise confirmed by a shift in the length-tension relation of both types, although the shift in Type 1 fibres was less. The difference in the shift in

length-tension relation of the two fibre types was not statistically significant (Proske and Morgan, 2001).

Activated satellite cells aid in muscle regeneration by proliferating and fusing to each other or existing damaged myofibres to form new fibres (Hawke and Garry, 2001), remodeling the damaged areas. Factors affecting satellite cell activity include muscle fibre type, age and the secretion of multiple growth factors, a timeous immune response, neurotransmitters and neurotrophic factors (Hawke and Garry, 2001). Without macrophage activity and their subsequent cytokine factor secretion, no muscle regeneration occurs (Hawke and Garry, 2001)

2.4 SYSTEMIC MARKERS OF CELL DAMAGE IN SKELETAL AND CARDIAC MUSCLE

Haematological findings after eccentric exercise include increased concentrations of the following muscle proteins in serum: CPK, alpha-actin, myoglobin and troponin (Martinez-Amat *et al.*, 2005), CRP, lactate dehydrogenase (Lambert and Dennis, 1994) aspartate aminotransferase and carbonic anhydrase iso-enzyme II (Clarkson and Hubal, 2002). The use of muscle proteins as indicators of muscle damage, however, reflect the difference between what is released from the tissue and cleared from the blood and can have large inter-subject variability in the response (Clarkson and Hubal, 2002).

2.4.1 Creatine Phosphokinase (CPK): Most researchers determine the extent of damage by using serum CPK concentration because of the low cost of the assay and because the increase in CPK concentration is high in comparison to that of other proteins (Clarkson and Hubal, 2002). It also is used clinically to diagnose myositis and rhabdomyolysis and to predict renal failure (Clarkson *et al.*, 2006). Exertional rhabdomyolysis may occur after strenuous exercise when serum CPK concentration is >500U/L and urine dipstick is positive for Mb and blood without haematuria (Knechtle *et al.*, 2008; Skenderi *et al.*, 2006; Clarkson *et al.*, 2006). Renal failure has been associated with serum CPK concentrations > 20 000U/L, although Clarkson *et al.* (2006) found that marked CPK and Mb elevations alone, are not sufficient to result in renal failure in healthy athletes in response to exercise. Other factors including underlying disease,

environmental heat stress, sickle cell trait or drug use also contribute to this condition (Clarkson *et al.*, 2006).

CPK often shows a large standard deviation and it should be taken into consideration that it is affected by other kinases such mitochondrial CPK, CPK-immunoglobulin complexes and CPK derived from cardiac muscle (Martinez-Amat *et al.*, 2005). According to Knechtle and Kohler (2007), a significant decrease of skeletal muscle mass has been noted during a running race over 338km within five days, although the biggest change in muscle mass occurred after the first stage.

2.4.2 Alpha(α)-actin: A major constituent of the contractile apparatus in skeletal muscle is α-actin which, when it leaks into the serum, has been found to be a reliable marker of muscle damage and to have a high diagnostic specificity especially within the first few hours after exercise (Martinez-Amat *et al.*, 2005). The use of α-actin as a marker of skeletal muscle damage has a high sensitivity (63-100%), represent more than 20% of all muscle cell proteins, is detected within 1 hour after the onset of muscle damage and can be detected in the serum for up to 72 hours after its release, indicating greater stability over time (Martinez-Amat *et al.*, 2005).

2.4.3 Lactate dehydrogenase (LDH): LDH is a commonly used indicator of exercise induced muscle damage. It reaches peak concentrations within six hours post exercise and returns to pre-exercise levels within 48 hours after exercise (Maughan *et al*., 1989). However, elevations of serum LDH concentrations occur after any tissue damage, including cardiac muscle damage, hence the specific iso-enzyme must be measured. Furthermore, large intra- and inter-individual differences of serum LDH changes have been reported, reducing its specificity and sensitivity as a marker of exercise induced skeletal muscle damage (Martinez-Amat *et al.*, 2005).

2.4.4 Plasma fatty acid binding protein (FABP): FABP and myoglobin have been found to increase and decrease more rapidly than CPK inferring that it is possibly more useful than CPK for the early detection and monitoring of exercise-induced muscle damage (Sorichter *et al*., 1998). Due to its rapid plasma clearance, it also is more suited to the assessment of recurrent

injury and the separate monitoring of skeletal muscle injury during repeated exercise bouts (Sorichter *et al*., 1998). Pelsers *et al.* (2005), however, recommend that the clinical application of using FABP will require further commercialization of automated and rapid assays.

2.4.5 Troponin: The effect of prolonged strenuous exercise on markers of cardiac muscle damage has been studied extensively. Troponin markers are used as reliable markers of cardiac muscle damage and can be used to differentiate between cardiac and skeletal muscle damage (Martinez-Amat *et al.*, 2005).

The troponin subunits, cardiac troponin I (cTcI) and cTnT have been found to increase transiently during and immediately after exercise, returning to normal in healthy athletes within 3 days (Frassl *et al.,* 2008; Middleton *et al*., 2008). According to Leers *et al*. (2006) these increases could be due to myocardial stress, which is confirmed by Middleton *et al.* (2008) who state that the reversible cardiomyocyte membrane damage during exercise occurs due to an increased myocardial oxygen demand and cardiac troponin turnover in all athletes. The cardiomyocyte damage might be linked to tachy-arrhythmias and sudden cardiac death, especially when associated with prolonged increased cTnT results above 0.05µg/l, the acute myocardial infarction cut-off (Middleton *et al*., 2008; Leers *et al*., 2006).

2.5 INFLAMMATORY RESPONSE

Inflammation occurs in response to prolonged intense exercise (Clarkson and Hubal, 2002; Lieber and Friden, 2002; Friden and Lieber, 2001; Hawke and Garry, 2001) and is associated with the invasion of neutrophils and macrophages into the damaged fibres within 6 hours (Peake *et al.,* 2005). These leukocytes secrete reactive oxygen and nitrogen species, cytokine factors and proteolytic enzymes that cause an increase in satellite cell proliferation and differentiation (Friden and Lieber, 2001; Hawke and Garry, 2001) and tissue remodeling may occur (Clarkson and Hubal, 2002; Feasson *et al.*, 2002; Friden and Lieber, 2001).

2.5.1 Neutrophils, macrophages and cytokines: Neutrophils remain present in the damaged muscle up to 24 hours post exercise while macrophages are present from 24 hours to 14 days

after exercise and produce pro-inflammatory cytokines including interleukin (IL)-1β and tumour necrosis factor (TNF)-α that initiate the breakdown of damaged muscle tissue (Peake *et al.*, 2005).

The systemic response to inflammation, on the other hand, rapidly becomes anti-inflammatory as plasma levels of IL-6, IL-10, IL-1ra and soluble TNF-α receptors rise in direct proportion to the intensity and duration of exercise (Nieman *et al.*, 2005; Peake *et al.*, 2005). The release of the pro-inflammatory cytokines into the circulation is inhibited by IL-6, which stimulates the production of the anti-inflammatory cytokines as well as the anti-inflammatory hormone, cortisol (Peters *et al.*, 2001).

In the160-km Western States Endurance Run event Nieman *et al.* (2005) tested the relationship between plasma CPK, DOMS and various plasma cytokines. These researchers found that muscle damage, post race DOMS and IL-6, IL-10, IL-1ra, granulocyte colony-stimulating factor (G-CSF) and macrophage inflammatory protein 1β (MIP-1β) were positively correlated. The increase in the cytokines was greatest for IL-6 (125-fold), corresponding with a 112-fold increase in CPK (Nieman *et al.*, 2005).

2.5.2 C-reactive protein (CRP): Hepatocyte production of CRP during prolonged exercise is also activated by raised plasma IL-6 concentrations (Robson-Ansley, 2008) and a training induced reduction in serum CRP concentration has been confirmed following a prolonged period (Kohut and Senchina, 2004). However, only a small increase in CRP concentration is required to induce the release of IL-10 and IL-1ra by circulating monocytes and inhibits the synthesis of pro-inflammatory cytokines in tissue macrophages (Peters, 2004). A strong correlation between serum CRP and IL-6 concentrations has been confirmed (Robson-Ansley, 2008; Nieman *et al.*, 2005; Peters *et al.*, 2001).

2.5.3 Cortisol: Cortisol is a glucocorticoid, secreted by the adrenal cortex and regulated by adrenocorticotrophic hormone (ACTH) from the anterior pituitary (Borer, 2003). In response to dehydration, stress, increased core temperature and reduced glucose levels, corticotrophin-releasing factor (CRF) is released by the hypothalamus and stimulates the secretion of ACTH

(Borer, 2003). It is essential to life, regulating carbohydrate, lipid and protein metabolism, assisting during stress and it has a minor effect on the kidneys during fluid regulation (Borer, 2003). Serum cortisol concentration is often elevated in times of physical or mental stress during which it increases the glucose available to the muscles by stimulating the breakdown of glycogen. It also acts as a powerful anti-inflammatory hormone (Borer, 2003). Cortisol secretion in the body normally follows a diurnal pattern, being highest between 5 – 10am and lowest between 8pm – 4am. In the case of athletes running a race, blood cortisol concentration may also be elevated in the morning due to anticipation, but do tend to increase further during prolonged exercise due to physiological stress (Borer, 2003).

2.6 FACTORS AFFECTING MUSCLE DAMAGE AND INFLAMMATION

2.6.1 Repeated Bouts: After a single bout of unaccustomed eccentric exercise, a repeated bout (RB) of the same exercise results in reduced symptoms of muscle damage compared to the initial bout and has been referred to as the repeated bout effect (McHugh, 2003). This effect is reported to last up to 6 months when there has been no intervening exercise between bouts (Clarkson and Hubal, 2002). Even if the eccentric exercise is repeated within 2-6 days after the first bout, before the muscle was fully recovered, recovery is not delayed (Clarkson and Hubal, 2002).

Various mechanisms have been postulated to explain the RB (McHugh, 2003), including inflammatory adaptation, which is confirmed by Peake *et al.* (2005) who found that there is a 10 – 45% decrease in circulating neutrophil numbers after the repeated bout and a moderate attenuation in leukocyte receptor expression. This corresponds with changes in serum myoglobin concentration and serum CPK activity (McHugh, 2003), which reflect a dramatic increase after the first bout, but only a small increase after the RB (Clarkson and Hubal, 2002). Peake *et al.* (2005) suggest that there is a decreased receptor expression after a RB which appears to be a secondary response to a reduced degree of muscle damage. According to Clarkson and Hubal (2002), the increase in serum CPK concentration after a RB is less than expected. They suggest that an increased clearance of CPK from the blood after the RB is activated by the increase in serum CPK concentration from the first bout.

Other theories explaining the RB effect state that neural, mechanical or cellular adaptations occur (Radom-Aizik *et al.*, 2009; Mahoney *et al.*, 2008; Martinez-Amat *et al.*, 2005; McHugh, 2003). According to McHugh (2003) the neural adaptation might be reflected by the increased recruitment patterns during the RB, although no difference was noted in the EMG/unit torque during the RB (Clarkson and Hubal, 2002). McHugh (2003) attributes mechanical adaptations to increased desmin content during repair, causing increased dynamic and passive muscle stiffness with eccentric training. On the cellular level, it is postulated that an increase in the sarcomere numbers in series and a decrease in the tendon length (Radom-Aizik *et al.*, 2009; Clarkson and Hubal, 2002) within muscle fibres occur in response to the destruction of the weakest fibres during the initial bout. These fibres are repaired, with an increase in sarcomere numbers in series and increased resistance to damage, which results in less damage during the RB (Clarkson and Hubal, 2002).

The strength loss which occurs after exercise may be due to impaired E-C coupling according to McHugh (2003), who found that strength loss is similar immediately after the initial and RB of exercise but with less impairment after the RB on subsequent days.

Proske and Morgan (2001) also report that the muscle spindles and tendon organs are damaged during severe eccentric exercise, increasing the resting activity of the muscle. This rise in passive tension is a simple, non-invasive indicator of muscle damage and these researchers suggest that it can be used to determine the extent of damage instead of measuring the tension-deficit, or the shift in the length-tension relationship.

2.6.2 Dehydration, hyperthermia and muscle damage: The hydration status of athletes has been shown to detrimentally affect their pacing, physiologic function and thermoregulatory abilities (Casa *et al.*, 2010; Stearns *et al.*, 2009; Cleary *et al*., 2005). According to Casa *et al.* (2010), at a decrement of 2% body mass loss, increases in T_{intest} of 0.22^{o}C and heart rate of 6 beats per minute (bpm) were recorded for every additional 1% of body mass lost, which results in increased core body temperatures, heart rates, perception of effort and an altered anticipatory regulation of pace when attempting to maintain a predetermined pace. This has been suggested

to lead to higher levels of muscle damage, heat stroke, rhabdomyolysis, renal impairment and even death (Casa *et al.,* 2010; Knechtle *et al.,* 2008).

Casa *et al.* (2010) add that dehydrated athletes might have higher body core temperatures than euhydrated athletes, who can continue to perform at a higher intensity. This is contrary to earlier findings (Cheuvront and Haymes, 2001; Noakes *et al.*, 1991) stating that the metabolic rate and not dehydration affects T_{intest}, although Cheuvront and Haymes (2001) added that metabolic rate, hydration status and environment all contribute to increased T_{intest}, but are influenced by individual differences in fluid intake and racing strategies.

According to Cleary *et al.* (2005), skeletal muscle damage, as indirectly reflected by DOMS, was augmented in hyperthermic dehydrated athletes due to the elevated deep muscle temperature, which resulted in increased susceptibility of muscle fibres to damage and increased degradation and denaturation of structural and functional proteins. However, dehydration alone did not augment DOMS (Cleary *et al.,* 2005).

When excessive skeletal mass loss, dehydration and haemolysis occur which could result in impaired renal function, rhabdomyolysis and myoglobinuria (Knechtle *et al.,* 2008). Clarkson *et al.* (2006), however found that despite exercise induced CPK and myoglobin elevations, renal impairment does not always occur. Even when indicators of muscle and liver damage were extremely elevated after a 246km continuous running race, and exertional rhabdomyolysis occurred, the rhabdomyolysis was asymptomatic (Skenderi *et al.*, 2006).

2.6.3 Anti-inflammatory drugs: Friden and Lieber (2001) and Lieber and Friden (2002) investigated the administration of NSAID after eccentric exercise and reported a short term benefit of pain relief, but a long term detrimental effect on muscle adaptation, inhibiting protein synthesis by suppressing the inflammatory reaction. This is contrary to Nieman *et al.* (2005), who found that NSAID users did not have reduced race times, muscle damage or DOMS, but had higher post race plasma levels of IL-6, IL-8, G-SCF, MIP-1β and monocyte chemotactic protein 1. Paulsen *et al.* (2009), however, indicated that NSAID inhibited prostaglandin synthesis and affected neutrophil adhesion, activation and production of ROS by

reducing intracellular cyclic adenosine monophosphate (cAMP), promoting chemoattractant binding and inhibiting changes in membrane fluidity.

2.6.4 Lipid lowering medication: Three-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors or statins, are the primary lipid-lowering medication currently prescribed for hypercholesterolaemia, to reduce the amount of low-density lipoprotein (LDL) cholesterol (Thompson *et al.,* 2010; Seachrist *et al.,* 2005). Statins specifically inhibit the rate-limiting enzyme HMG-CoA reductase in the liver, thus effectively reducing endogenous plasma cholesterol levels (Seachrist *et al.,* 2005; Evans and Rees, 2002). According to the latest U.S. Food And Drug Administration Safety Announcement (FDA Drug Safety Communication, 06-08-2011), the FDA recommends limiting the use of the highest approved dose of simvastatin (80mg per day) (Seachrist *et al.,* 2005; Pasternak *et al*., 2002) due to the increased risk of muscle damage with muscle cramping, myalgia, fatigue and in rare cases, rhabdomyoloysis and death (Di Stasi *et al*., 2010; Thompson *et al.,* 2010; Seachrist *et al.,* 2005, Pasternak *et al*., 2002; Evans and Rees, 2002).

Potential mechanisms to which side effects of statins in skeletal muscle have been attributed include:

i. intracellular depletion of essential metabolites and destabilization of cell membranes, resulting in increased cytotoxicity, affecting the maintenance of skeletal muscle architecture (Di Stasi *et al*., 2010; Seachrist *et al.,* 2005),
ii. the reduced production of isoprenoids such as ubiquinone, which participates in electron transport during oxidative phosphorylation in mitochondria (Di Stasi *et al*., 2010; Thompson *et al.,* 2010),
iii. impaired signal transduction and structural protein formation and regulation (Di Stasi *et al*., 2010; Urso *et al*., 2005),
iv. alterations in Ca^{2+} shuttling such that Ca^{2+} leaking from the mitochondria directly increases cytosolic Ca^{2+} and impairs sarcoplasmic reticulum calcium cycling (Thompson *et al.,* 2010) and
v. interactions with the cytochrome P-450 hepatic enzyme system (Evans and Rees, 2002).

Atorvastatin and simvastatin are lipophilic statins which are absorbed via first-pass metabolism into gastro-intestinal and liver cells and which promote ease of diffusion through passive transport across the bilipid skeletal muscle cell membrane, causing augmented toxic effects on skeletal muscle at a dosage of >80mg.day (Di Stasi *et al*., 2010; Pasternak *et al*., 2002). During absorption, enzyme inhibitors such as calcium channel blockers, fibrates, anti-fungals and grapefruit juice can increase statin toxicity by competing with the statins (Di Stasi *et al*., 2010; Seachrist *et al*., 2005; Evans and Rees, 2002; Pasternak *et al*., 2002).

At 0.5mg/kg^{-1} Seachrist *et al*. (2005) demonstrated changes to the morphology of mitochondria in skeletal muscle without concurrent CPK elevation or histological changes to the myofibre. These mitochondrial alterations occurred to a greater extent in Type II glycolytic fibres that have a lower content of mitochondria, than in Type I muscle fibres which are oxidative with a high content of mitochondria (Seachrist *et al*., 2005), which suggest the presence of an impaired oxidative metabolism. They concluded that toxicity occurred at ≥1mg/kg/day with significant increases in serum CPK, ALT, AST, plasma lactate concentrations and lactic acidosis and that this dosage might promote statin-induced muscle mass loss, cramping, fatigue, rhabdomyolysis and even death (Di Stasi *et al*., 2010; Seachrist *et al*., 2005; Pasternak *et al*., 2002). Seachrist *et al.* (2005) reported that 78 % of professional athletes with familial hypercholesterolaemia could not tolerate statin therapy due to muscular problems. Predisposing risk factors include being female, the presence of renal or hepatic disease, advancing age and the use of concurrent medications (Di Stasi *et al*., 2010; Seachrist *et al.,* 2005; Pasternak *et al*., 2002). There is also the possible implication of statins altering the response of muscle to exercise by affecting the action of the ubiquitin proteasome pathway, protein folding and catabolism, thus disrupting the balance between protein degradation and repair (Urso *et al.*, 2005).

It has been found (Riesen *et al.*, 2002) that therapy with various statins decreases serum CRP concentrations even after 4 weeks of therapy, which shows a benefit for early statin treatment in acute coronary events and other chronic inflammatory conditions (Thompson *et al.,* 2010; Seachrist *et al.,*2005). Thus the number of patients taking statins is expected to increase

despite the reported lack of clinical data concerning the direct effects of statins on skeletal muscle function (Thompson *et al.,* 2010).

The use of another drug, Ezetimibe (Ezetrol), a lipid uptake inhibitor, has also been associated with muscle cramping, myalgia, fatigue and in rare cases, rhabdomyoloysis and death (Di Stasi *et al*. 2010; Thompson *et al.,* 2010; Seachrist *et al.,*2005, Ballantyne *et al.,* 2003; Pasternak *et al*., 2002; Evans and Rees, 2002). Ezetimibe selectively inhibits the intestinal absorption of dietary and biliary cholesterol into the serum, reducing the delivery of intestinal cholesterol to the liver. It also attenuates the hepatic cholesterol stores and increases cholesterol clearance from the blood (Ballantyne *et al.,* 2003). The co-administration of Ezetimibe and Atorvastatin has been found to reduce LDL-cholesterol and CRP more than Atorvastatin alone (Ballantyne *et al.,* 2003). Although Ezetimibe is well tolerated, related adverse events include diarrhea, elevated skeletal muscle damage and hepatic dysfunction (Ballantyne *et al.,* 2003). Co-administration with Atorvastatin might increase the risk of myopathy and rhabdomyolysis (Ballantyne *et al.,* 2003).

2.6.5 Genetics: Genetic predisposition might be the most interesting determinant of muscle damage, recovery and performance. Being a relatively new field of study, already it has found that some individuals, those with single nucleotide polymorphisms in the IGF-II gene (Devaney *et al.*, 2007), are more susceptible to muscle damage and thus less likely to excel or participate in sport. Genetics also determine the cardiovascular, explosive power and maximum oxygen uptake abilities of an individual (Radom-Aizik *et al.*, 2009; Seene *et al.*, 2009; Mahoney *et al.*, 2008; Virtanen and Takahashi, 2008; Coffey and Hawley, 2007). Furthermore, several changes in gene expression occur after exercise (Radom-Aizik *et al.*, 2009; Seene *et al.*, 2009; Mahoney *et al.*, 2008; Virtanen and Takahashi, 2008; Coffey and Hawley, 2007) and analyzing these changes may be revolutionary to exercise physiology with regard to selecting training programs, means of recovery or the most appropriate sport for each individual (Virtanen and Takahashi, 2008). There are genes that determine endurance and strength (angiotensin-converting enzyme gene, actinin 3, growth hormone/IGF regulators); genes regulating muscle fibre type and metabolism, regulating oxygen delivery, oxygen

carrying capacity in the blood and many more (Radom-Aizik *et al.*, 2009; Mahoney *et al.*, 2008; Goldspink, 2003).

2.6.6 Chromosome telomere erosion: Chromosome telomere erosion might contribute to muscle and immune dysfunction, morbidity and mortality (Simpson and Guy, 2009). Telomeres are deoxyribonucleic acid (DNA) nucleo-protein complexes that cap the ends of chromosomes, promoting their stability. These telomeres shorten progressively with each round of cell division and are not fully replicated unless counteracted by elongation by telomerase, thus affecting muscle function (Puterman *et al.*, 2010). Excessive telomere erosion presumably occurs due to repeated exposure to pathogens and/or oxidative stress (Simpson and Guy, 2009).

2.6.7 Oxidative stress: Part of the response in muscle during eccentric exercise is the increase of free radical activity (Grobler *et al.*, 2004; Feasson *et al*., 2002; McArdle *et al*., 2002) that might initiate the adaptive response in muscle, stimulating the production of anti-oxidant enzymes and various proteins, including the important heat shock proteins. After stress the amount of intracellular heat shock proteins (HSP) increase (McArdle *et al*., 2002; Hawke and Gary, 2001) where they are associated with repair and homoeostasis by ensuring the correct folding and functioning of new proteins. The increased number of HSP also provides protection against more damage (McArdle *et al*., 2002). The use of anti-oxidant supplements like Vitamin C (Jackson *et al.*, 1999) and tart cherry juice (Connolly *et al*., 2007) has been found to improve this reaction.

The production of anti-oxidant enzymes such as superoxide dismutase and catalase is enhanced by regular endurance exercise (Peters *et al*., 2009; Radak *et al*., 2005; Bruunsgaard and Pedersen, 2000). Reactive oxygen species (ROS) have effector functions in cellular metabolism, signaling and host defense (Bruunsgaard and Pedersen, 2000). A decreased generation may cause signaling deviations and increased concentrations might cause ROS-mediated damage to cellular components, thus contributing to immune system dysfunction, chronic inflammation and autoimmunity (Peters *et al*., 2009). In contrast to this, moderate exercise might attenuate the severity of oxidative stress by increasing the production of

endocrine hormones, which might reduce the accumulation of autoreactive immune cells and increase cell death (Bruunsgaard and Pedersen, 2000).

According to the hormesis-theory, low concentrations of ROS may have a stimulating, beneficial effect. McArdle *et al*. (2001) found that a single bout of intense exercise causes an increased production of ROS which leads to oxidative damage to lipids, DNA and proteins. Regular moderate exercise, however, might stimulate tolerable transient increased ROS production, might change signaling pathways or cause mild molecular damage and thus induce adaptive responses that protect against subsequent stressors (Radak *et al*., 2004). To support this theory, Radak *et al*. (2004) quote studies that demonstrated that exercise upregulates the antioxidant system and stimulates the oxidative repair system possibly by increasing the activity of the proteasome complex. The proteasome complex is involved in the reduction of oxidatively modified proteins, resulting in a faster turnover of proteins and a reduced post translational period, thus providing a mechanism for damaged proteins to be replaced by intact, efficient proteins (Radak *et al*., 2004).

2.6.8 Age: Various studies have shown that exercise reduces the chronic low grade systemic inflammation that occurs after 35 years of age and that it attenuates the effects of sarcopenia (Simpson and Guy, 2009; Peake *et al.*, 2005). The elderly with increased muscle mass were reported to have an increased number of NK cells, thus an improved resistance against various infections, and an improved efficacy to vaccination, further improving resistance to infectious diseases (Simpson and Guy, 2009; Krause, 2003). According to Peake *et al.* (2005) ageing generally impairs the leukocyte mobilization and migration into skeletal muscle which impairs tissue remodeling. Long term moderate exercise stimulates the secretion of various anti-inflammatory cytokines, such as IL-6, which inhibits the secretion of pro-inflammatory cytokines and delays the decrease in neutrophil numbers (Peake *et al*., 2005; Krause, 2003)

2.6.9 Gender: According to Peake *et al.* (2005) there are no general differences in the muscle damage after exercise between males and females. It has, however, been reported that males display higher levels of serum CPK due to their larger muscle mass, although it was found that the recovery period of males and females of the same age and training ability should be similar

after unaccustomed exercise (Clarkson and Hubal, 2002). Ronkainen *et al*. (2009) suggest that oestrogen offers protection to muscle damage, although further studies are recommended, examining the difference in male and female membrane permeability which could be reflected by the increased oedema that was noted in the muscle fibres of men after resistance training (Roth *et al.*, 2000). Regarding the inflammatory response, Peake *et al.* (2005) mention that females show increased concentrations of neutrophil infiltration 24 hours after eccentric exercise and oestrogen augmented macrophage infiltration after the RB.

2.6.10 Other: Various methods have been examined to prevent or treat DOMS, with inconclusive results. Stretching before and after eccentric exercise seems to have no effect (Herbert and de Noronha, 2007) neither did ice water immersion (Sellwood *et al.*, 2007), nor hyperbaric oxygen therapy (Boriek *et al.*, 2001). Vibratory massage and massage have been reported to have some positive effects (Zainuddin *et al.*, 2005; Pietzsch, 2004) although timing of the massage therapy after eccentric exercise appears to be crucial and should occur during the inflammatory stage (Zainuddin *et al.*, 2005; Pietzsch, 2004). Many athletes use ice water immersion, compression garments and hyperbaric therapy and it has been found that whole body cryotherapy (three minutes at -110^{o}C) immediately after exercise enhanced muscular recovery by restricting the inflammatory process (Pournot *et al.*, 2011). The use of the omega-3 polyunsaturated fatty acids, docosahexaenoic and eicosapenetaenoic acid have been found to induce anti-inflammatory effects through altering the cyclooxygenase 2 and lipoxygenase 5 pathways by suppressing the production of prostaglandins and leukotrienes that modulate the production of pro-inflammatory cytokines (Tartibian *et al.*, 2009).

The protective effect of oestrogen on muscle damage has been supported in the study by Roth *et al*. (2000), proving that older females experience more muscle damage than younger females after eccentric exercise. Ronkainen et al. (2009) showed that females on hormone replacement therapy have better mobility, increased muscle strength and decreased body fat content.

2.7 CONCLUSION

Despite a substantial amount of published research providing evidence of histologic changes and systemic markers of skeletal muscle damage and inflammation following single stage road- and trail running events, and the factors which affect this, little, if any evidence, is available on skeletal muscle damage following multi-day trail running. Although Knechtle *et al.* (2008) found that the eccentric component of multi-day road running lead to the accumulation of total body water and the loss of skeletal muscle mass due to skeletal muscle damage, which caused rhabdomyolysis and impaired renal functioning, the accumulative effect of multiday trail running has not been investigated. This exposes a gap in the literature, which was investigated in this study.

CHAPTER THREE

SCIENTIFIC ARTICLE ACCEPTED FOR PUBLICATION

Low markers of muscle damage and inflammation following a three–day trail run

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Abstract

Objectives: To investigate the effect of a three-day trail run on markers of muscle damage and inflammation in recreational runners.

Method: An observational cohort study was conducted on 19 recreational male (n=6) and female (n=13) athletes during a 95km trail run consisting of three stages, covering 29.3, 37.9 and 27.8 km with peak elevation gains and losses of 1226 and 1231m, respectively.

Main Outcome Measures: Pre-and post-stage and 24 and 72 h post-race concentrations of serum creatine phosphokinase (CPK), high sensitivity C-reactive Protein (hsCRP), cortisol, cardiac Troponin T (cTnT), and osmolality (sOsm) as well as urinary myoglobin (uMb), changes in body mass, delayed onset muscle soreness (DOMS) and thigh circumference (TC) were measured. Continuous recordings of heart rate (HR) and intestinal temperature (T_{intest}) were made throughout each stage.

Results: Heart rate ranged between 77 and 83% age-predicted-maximum (APmax) and T_{intest} between 36.1 and 40.2 ºC during the three stages. Significant rises in mean serum CPK, hsCRP, sOsm and blood neutrophil count reached peak concentrations of 1488U/L, 8.91mg/l, 298mosm/L and 10.21 10^9/L ($p \leq 0.001$), respectively. No evidence of elevations in uMb and cTnT were detected. The stage-induced increments in DOMS correlated positively with CPK, r=0.71; 95% CI [0.62, 0.78], TC decreased significantly post $S1_{post}$ and $S2_{post}$ ($p \leq 0.05$) and a maximum mean body mass loss of 3.09% (±1.04%) occurred during S2.

Conclusion: Three consecutive days of 95km trail running resulted in modest increases in markers of muscle damage and inflammation, despite the maintenance of a heart rate above 77% APmax, T_{intest} rising above 39^{o} C and mean body mass decrement of >2.0%.

Introduction

Trail running events are becoming increasingly popular with amateur athletes.[1] These are generally regarded as more strenuous than road running due to the nature of the trails, which can involve diverse challenges including single track paths on steep ascends and descends in mountains, crossing rivers and running along grasslands and through forests.[2] Although physiological response to single-day trail running has been assessed,[1-4] the accumulative effects of multi-day trail running on markers of muscle damage and inflammation have not yet been reported to date.

Prolonged endurance exercise causes muscle damage that initiates an inflammatory response and subsequent remodeling of muscle.[5] The extent of this damage is augmented by increases in exercise intensity, the eccentric component of contraction[6-8] heat stress index and dehydration.[3] The greater contractile load per unit in muscles of the lower limb, as they contract eccentrically during downhill running,[8] has been associated with increased mechanical damage to the muscle fibres, resulting in muscle membrane leakage and elevated concentrations of circulating muscle enzymes and proteins.[9] Systemic markers of inflammation also rise[5,7] and swelling, decreased mobility and delayed onset muscle soreness (DOMS) are common.[5,6] The presence of myoglobin in the urine has been reported in severe cases.[5]

Although the direct cause and effect relationship between dehydration and hyperthermia is currently contentious[10], it has been reported that these augment exercise-induced muscle damage,[3,4] detrimentally affect performance and pacing during trail running and increase post-exercise DOMS.[3,4,11] Cleary *et al.*[11] reported an association between dehydration and hyperthermia and attributed an increase in muscle damage to the increased degradation of muscle proteins with elevated deep muscle temperature.

The aims of the study were therefore to determine effects of a multiday trail run on the markers of muscle damage and inflammation in experienced recreational runners, measuring serum and urinary levels of selected skeletal muscle, cardiac and hepatic proteins in association with changes in red and white blood cell and serum cortisol concentrations before and after every stage and at 24hours post-race (24PR) and 72hours post-race (72PR). A further aim was to

assess the possible effect of dehydration and hyperthermia on the markers of muscle damage and inflammation.

It was hypothesised that the three consecutive days of trail running would result in elevations of systemic and urinary markers of skeletal muscle damage and inflammation that are higher than previously reported during road running events of similar duration, and that the muscle damage and inflammation would be augmented by hyperthermia and dehydration.

Method

Ethical Clearance

This eight-day observational cohort study took place during a 3-day trail run and for 5 days following completion of the Three Cranes Trail Run, at Karkloof, KwaZulu-Natal, South Africa on 25 – 27 Feb 2011.

Following approval by the relevant institutional research ethics committee (Ref No: BF 210/010), subjects gave written consent after having been informed of the experimental procedures.

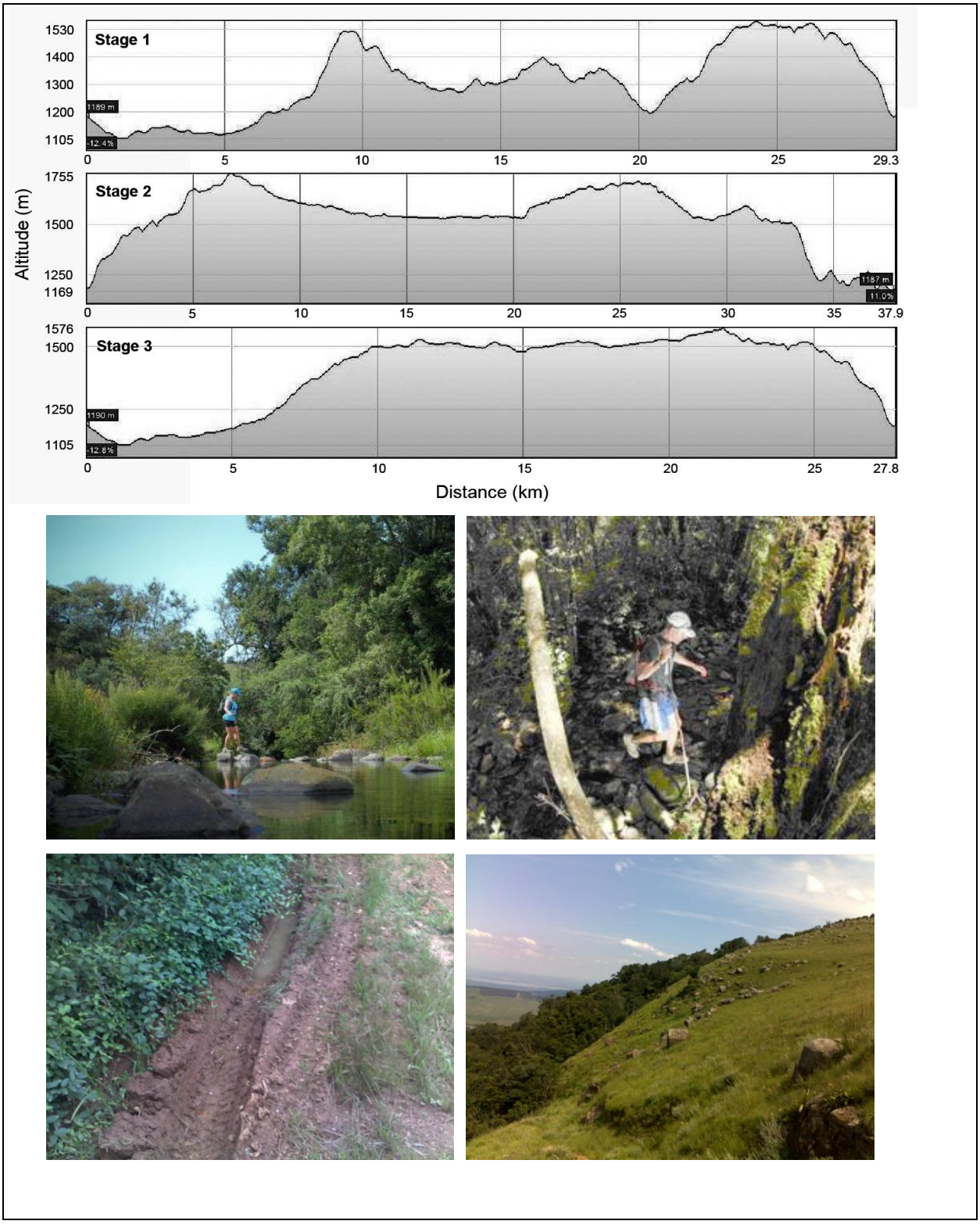
Subjects

Twenty-one apparently healthy recreational athletes, who met the inclusion criteria (age: ≤ 50 and an average training distance of 60km per week) and did not use chemical stimulants, were accepted into the study. Nineteen (6 males, 13 females) completed all three stages of the race and 15 runners (4 males, 11 females) completed all within and post- race assessments. Twelve athletes used NSAID's during the race.

Setting

The Three Cranes Trail run, over 3 days and a total distance of 95km, was divided into 3 consecutive stages comprising 29.3, 37.9 and 27.8 km, starting and finishing each day at the same base camp. Athletes were accommodated in a race village and full catering was

Figure 1: The topographical features of the three stages and selected images of the running terrain



provided for the duration of the race, including at the aid stations along the route. The routes consisted of gravel and forestry roads, narrow rocky mountain footpaths and grassy jeeptrack. Elevation gains reached 1020, 1226 and 680m, while elevation losses were recorded at 1021, 1231 and 687m during S1 (Stage 1), S2 (Stage 2) and S3 (Stage 3) respectively (Table 1). The topographical features of the three stages, as well as selected images of the running terrain, are presented in Figure 1.

Table 1: Elevation changes (m) and ambient temperature ranges during the three stages of the trail run

	Elevation Gain (m)	**Elevation Loss (m)**	**Ambient Temperature range (º C)**
Day 1	1020	1021	11.5 - 21.7
Day 2	1226	1231	12.4 - 22.8
Day 3	680	678	12.1 - 21.2
Total	**2926**	**2930**	

Baseline measurements

Following race registration the afternoon before the race, basic anthropometric measurements were recorded, including body mass (kg), stature (cm) in bathing suits without shoes, thigh circumference (TC) (measured 15 cm above the superior border of the patella) and four-site skinfold (suprailiac, subscapular, biceps and triceps) for the determination of % body fat.[12] A pre-race questionnaire detailing the athletes' running and racing experience, training terrain and health status was also completed.

Daily protocol

Pre stage: The subjects presented themselves to a designated testing area 30-90 minutes before the start of the stage, handing in a first early morning urine sample. TC was measured, venous blood sampling was conducted in the seated position and resting heart rate (HR) and blood pressure (BP) were recorded after a 3-5 minute period of relaxation. A simple pre-stage questionnaire including rating of the degree of muscle soreness they were experiencing was completed and the subjects were asked to keep a record of their fluid intake and urine output

during the stage. After breakfast and final voiding of bladders, body mass (measured in running attire without shoes), was taken within 5 minutes prior to the start of the event.

Within-stage: Environmental conditions and temperature were supplied on the hour by a meteorological station located 9.5 km from the base camp. Heart rate was recorded using a polar HR monitor (Polar Electro OY, Finland) at five-minute intervals and % age predicted max (APmax) was determined according to the formula, 220-age.[14]

A subsample of 12 athletes volunteered to ingest the *Cor-Temp* disposable tablets, containing temperature sensors (HQ Inc, Palmetto, FL), at least three hours prior to the start of each stage. The HR and intestinal temperature (T_{intest}) data are part of a more detailed study focussing on the relationship between $T_{intest,}$ HR and hydration status.[13]

Post stage: The subjects proceeded directly to the designated testing area where BP, mass and TC were measured within 3-5 minutes, blood and urine samples were taken and a short DOMS and post-stage questionnaire, providing details regarding the use of non-steroidal anti-inflammatory drugs (NSAIDs) and muscle soreness, were completed. In available athletes (n=10), a further measurement of TC was taken four hours after completion of S1 and S2.

The same protocol was followed pre- and post stage on the three days of the race.

Post-race: At 24PR and 72PR, participants presented for further blood/urine sampling, BP, HR and anthropometric measurements. They were also requested to complete a DOMS questionnaire for the five days following the race, using a five-point Likert scale and return this together with a general post-race questionnaire, following completion of the study.

Haematological analysis and anthropometric measurements

All measurements were carried out by the same researcher for all subjects and on all occasions. Venous blood samples were drawn from the antecubital fossa, with subjects in the seated position, within 5 - 15 min of completing the stage. Blood samples for the assessment of full blood count (FBC) and serum osmolality (sOsm) and urine samples were stored at 4ºC and transported to a commercial Pathology Laboratory. Complete blood counts were measured on

an Advia-120 Hematology Analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) and included erythrocyte indices and differential leukocyte counts. Plasma volume (PV) changes were determined from pre- and post-exercise haematocrit and haemoglobin concentrations according to the method of Dill and Costill.[15] All concentration dependant, post stage blood cell parameters (leukocyte counts, cortisol, CPK and hsCRP values) were then adjusted for percentage change in PV. Both urine and serum osmolality were measured by freezing point depression, using a Kyoto Daiichi osmostat, OM 6020 (Japan). Urine samples were also assessed for myoglobin (uMb) and specific gravity using the refractive index method on a Beyer Test Strip.

Further aliquots of serum, separated by centrifugation @ 3000rpm and stored in dry ice were transferred to an -80 º ultrafreezer or transported to a commercial Pathology Laboratory for analysis of creatine phosphokinase (CPK), cortisol, cardiac troponin T (cTnT) and high sensitivity C-reactive protein (hsCRP) concentrations.

Statistical analyses

Data are presented as mean ± standard deviation (SD). The significance of the accumulative time-dependent stage-induced changes from pre-race ($S1_{pre}$) to post race ($S1_{post}$, $S2_{post}$, $S3_{post}$), as well as recovery rates were assessed comparing $S2_{pre}$, $S3_{pre}$, 24PR and 72PR to baseline ($S1_{pre}$,), $S2_{pre}$ and $S3_{pre}$ were assessed for the entire group using repeated measures one way analysis of variance. The time point of the significant differences was confirmed using a Tukey *post hoc* analysis.

Comparisons between NSAID users and non-users were conducted using independent student's *t* tests. Pearson's product moment co-efficient of correlation, with a confidence interval (CI) of 95%, was used to test the relationship between the changes in measured outcomes including CPK, neutrophil concentrations, hsCRP and serum cortisol.

All statistical calculations were performed using SPSS, version 18 (SPSS Inc., Chicago, USA). Level of significance was set at $p \leq 0.05$.

Results

Environmental Conditions

Temperature recorded on the hour during the three stages of the race ranged from 11.5º C to 22.8 º C (Table 1). It did not rain, maximum windspeed recorded was 2.8 m/s and the relative humidity ranged from 54- 97%.

Subjects

As is shown in Tables 2 and 3, athletes ranged from 25 to 50 years of age, their weekly training distance averaged 65.9 ±20.1km per week for 12.4 years (range 2 – 27 years) and they presented without abnormalities in their vital signs. Of the 19 subjects, 12 used NSAIDs, including aspirin, ibuprofen and diclofenac.

Table 2. Mean ± SD baseline physical characteristics of subjects (n =19)

Variable	Mean ±SD
Age (years)	39.3 ±7
Height (cm)	169.0 ±10
Mass (kg)	65.8 ±12
% body fat	21.7 ±4
Resting heart rate (bpm)	56.9 ±5
Systolic Blood Pressure (mmHg)	124.7 ±7
Diastolic Blood Pressure(mmHg)	81.8 ±7

Of the 21 subjects who initially agreed to participate in the study, one subject (male) withdrew after S1 due to an ankle injury and another (female) after S2 due to medical reasons. The baseline physical characteristics of the remaining 19 subjects are provided in Table 2. Four subjects were however unable to provide blood samples at 24PR and 72PR.

Table 3 Training status and performance characteristics of athletes (n=19).

Characteristics	Mean ± SD	Range
Running experience		
Number of years	12.4±8.1	2 – 27
Number of competitive endurance events	136.3±55.6	18 – 500
Weekly Training Distance (kilometres per week)	65.9±20.1	12.5 – 105
Number of days per week on different training terrains		
Hills	1.4±0.8	1-4
Off Road incl. forest /trail/beach	1.7±1.5	0-6
Road	3.9±1.3	0-5
Race Time (hour:minute:second)		
Stage 1	4:04:31±25:54	3:06:06 – 5:22:48
Stage 2	5:39:12±25:31	4:14:56 – 7:46:27
Stage 3	3:14:15±21:06	2:38:38 – 6:51:50
Mean as %APmax*		
Stage 1	83±8.8	71-112
Stage 2	78±7.8	55-105
Stage 3	77±8.1	63-105

Data presented as mean **(**± SD**)** and range; *age-predicted maximum (220-age)

Intensity of Effort

The mean ±SD and range, of time spent completing each stage and average HR on the run, are given in Table 3. Total average running time of the athletes was 12h57±2h51.

Markers of muscle damage and inflammation

As shown in Table 4, these included a significant increase in circulating neutrophil concentrations ($p \leq 0.001$) which peaked at 10.21±1.54 10^9/L at $S1_{post}$, serum CPK and hsCRP which peaked at $S3_{post}$ at 1488±1053U/L ($p \leq 0.001$) and 8.91±6.63mg/L ($p \leq 0.001$), respectively. cTnT and uMb were undetected in all samples throughout the three day event.

An exercise-induced increase in serum cortisol concentration was only detected following $S2_{post}$. TC decreased significantly from 54.1±4.4cm at $S1_{pre}$ to 51.8±3.9cm at $S1_{post}$ ($p \leq 0.001$) and returned to the pre-race measurement of 54.1 ±4.0cm at 24PR. DOMS ranged from 4.8±1.6, 5.6±1.8 and 5.1±1.1 at $S1_{post}$, $S2_{post}$ and $S3_{post,}$ respectively, and decreased to 1.73 ±1.3 at 24PR.

Significant positive correlations were evident between blood neutrophil concentrations and serum CPK, r = 0.27, 95% CI [0.11, 0.41], serum CPK and hsCRP concentrations, r=0.50, 95% CI [0.29, 0.66] and DOMS and CPK, r=0.71, 95% CI [0.62, 0.78].

Dehydration, intestinal temperature (T_{intest}), HR and muscle damage

The mean % body mass loss for the entire group (n=19) during the three stages was 2.9±0.7, 3.1±0.8 and 1.9 ±0.9, while the mean sOsm (n=19) increased from 288.9±4.8 to 293.7±5.7 (p=0.003), 288.4±6.4 to 295.6±6.0 (p=0.003) and 292.2±4.1.to 295.0±5.6 (p=0.006) mOsm/kg, during S1, S2 and S3, respectively. When the pooled data for each stage were compared (n=51), the paired post-pre changes in sOsm correlated inversely with the changes in % body mass, r = -0.36, 95% CI [-0.57,-0.094].

The pooled data examining the relationship between the change of sOsm and change in serum CPK for the three stages (n=57), revealed an insignificant positive correlation (r= 0.034, 95% CI [-0.228, 0.291].

The maximum T_{intest} ranged between 38.3 º C and 40.2 º C and only exceeded 40º C in two of the 12 athletes monitored (Table 5). The relationship between change in T_{intest} and serum CPK was insignificant ($p>0.05$) for the 11 individuals from whom complete sets of data were available (r= 0.24, 95% CI [-0.42, 0.734].

Table 4: Mean±SD white and red blood cell indices and markers of muscle damage and inflammatory response before and after every stage and at 24PR and 72PR.

Variable	Stage 1		Stage 2		Stage 3		24PR	72PR
	Pre	Post	Pre	Post	Pre	Post		
Red blood cells (10^12/L)	4.7 ±0.5	4.7 ±0.5	4.5 ±0.4	4.6 ±0.5	4.4 ±0.4	4.4 ±0.4	4.1 ±0.4	4.3 ±0.4
Haemoglobin (g/dL)	14.4 ±1.3	14.3 ±1.2	13.7 ±1.0	14.1 ±1.2	13.2 ±0.9	13.3 ±1.1	12.7 ±0.9	13.2 ±1.2
Hematocrit (%)	42.4 ±3.9	42.1 ±3.3	40.2 ±2.9	41.1 ±3.2	39.9 ±2.7	39.5 ±2.9	38.1 ±2.5	40.5 ±3.4
White blood cells (10^9/L)	6.0 1.2	12.8* ±1.7	6.5 ±1.3	12.6** ±2.0	7.1 ±1.3	9.7*** ±2.2	6.7 ±1.2	6.5 ±1.5
Neutrophils (10^9/L)	2.9 ±0.7	10.2* ±1.5	3.4 ±1.0	9.7** ±1.9	3.7 ±1.0	7.3*** ±2.1	3.6 ±1.0	4.2 ±1.4
Lymphocytes (10^9/L)	2.1 ±0.5	1.4 ±0.5	2.2 ±0.6	1.73 ±0.6	2.4 ±0.7	1.5 ±0.4	2.1 ±0.6	1.6 ±0.5
CPK (U/L)	116.5 ±54.6	275.4* ±105.9	419.8 ±212.6	971.6** ±534.2	953.7 ±579.3	1488*** ±1053	595.6* ±361.4	201.9* ±111.3
hsCRP (mg/L)	0.7 ±0.5	-	-	-	-	8.9* ±6.6	6.6* ±6.2	2.0* ±1.7
Cortisol (nmol/L)	759.1 ±154.8	779.1 ±233.3	729.2 ±134.1	934.9** ±216.9	646.8 ±112.4	583.2 ±213.2	-	-
uMb (mcg/mL)	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
cTnT (µg/L)	-	-	-	-	<0.01	<0.01	<0.01	-
Thigh circum (cm)	54.1 ±4.4	51.8* ±3.9	54.1 ±4.6	53.3 ±4.6	53.4 ±4.4	53.8 ±4.2	54.1 ±3.9	53.5 ±3.8

*vs. S1pre, p≤0.001; ** vs. S2 pre, p≤0.001; ***vs S3 pre, p≤0.001

uMb, urinary myoglobin; cTnT, cardiac Troponin T; nd: not detected

Table 5: Individual T_{intest} , and associated HR, changes in hydration status and peak serum CPK concentration (n=12)

Subject Number	**Max** T_{intest}	**Min** T_{intest}	**Mean** T_{intest}	**Max HR (bpm)**	**Mean HR**	**Max% APmax HR***	**Mean% APmax HR** *	**ΔsOsm (mOsm /kg)**	**ΔBody Mass %**	**Max serum CPK (U/L)**
1	39.2	36.6	38.5	176	167	100	95	-3	-3.4	#
2	38.7	37.2	38.3	161	148	94	87	-6	-2.9	772
3	39.4	37.3	38.7	164	153	88	82	13	-2.7	1562
4	39.6	36.8	38.7	193	144	112	83	-6	-4.1	1057
5	39.2	35.2	37.4	168	154	95	87	10	-2.8	1523
6	39.8	36.1	37.7	168	160	92	87	7	-3.1	4478
7	38.7	36.9	38.2	152	140	89	82	11	-3.0	772
8	39.2	37.3	38.1	180	159	95	72	-23	-2.3	1198
9	40.2	37.1	38.0	181	144	102	81	-5	-3.3	1076
10	38.9	37.4	38.4	156	150	84	81	5	-3.2	1089
11	38.3	35.8	37.4	146	135	84	78	6	-2.8	1057
12	40.1	37.3	38.5	171	149	90	79	7	-2.3	1151
Mean	**39.3**	**36.8**	**38.2**	**168**	**150.3**	**93.8**	**82.8**	**4.17**	**2.99**	**1430**
SD	**0.6**	**0.7**	**0.4**	**13.4**	**9.0**	**8.0**	**5.8**	**9.42**	**0.49**	**1042**

T_{intest}: intestinal temperature; Max: maximum; Min: minimum; HR: heart rate; bpm: beats per minute; SD: standard deviation; *****age-predicted maximum heart rate[14]; CPK: creatine phosphokinase; #subject withdrew after completing S1

Users of NSAIDs

The 12 athletes who used NSAIDs had maximum serum CPK and hsCRP concentrations of 1332±943.5U/L and 8.58±6.7mg/L at $S3_{post}$ and the non-users 1754±1251.3U/L and 9.47±7.0mg/L, with no significant difference between the groups (p=0.456; 0.788). The neutrophil count reached a maximum of 9.95± 2.1 and 9.75±0.4 10^9/L, respectively, for users and non-users (p=0.82). There was also no significant difference between NSAID users and non-users in terms of serum cortisol, post race DOMS scores, running times, TC or sOsm (p>0.05).

Discussion

Evidence of muscle damage and inflammation

The results of the present study indicate that very little muscle damage and inflammation occurred during three days of trail running despite athletes running for a total average of 12h57 at an average HR of 77 – 83% APmax (Table 3). The serum CPK concentration which increased progressively to reach peak concentrations at $S3_{post}$, indicated only a mild accumulative effect of muscle damage during the race, which rejects the original hypothesis. Furthermore, the changes in neutrophil count, serum cortisol and hsCRP concentrations and DOMS also confirm low levels of inflammation and a rapid recovery. Most athletes in our study had no muscle soreness at 72PR, which correlated with the CPK concentration that had dropped close to the clinical upper limit of normal[16] by 72PR. The consistently low release of muscle proteins into the bloodstream in all 19 subjects, which was also not accompanied by elevation in cTnT and uMb in this study, confirms a profile of low degrees of muscle damage. Further evidence is the fact that TC was not significantly elevated at any post-stage or post-race time-point, but was reduced after S1 ($p \leq 0.001$), confirming previous findings of reduced swelling and a post-race decrease in muscle mass.[17]

The low systemic markers of muscle damage and inflammation, when compared to previous findings following the Comrades Marathon[17] confirm the findings of Millet *et al.*[1] who, in their study on the neuromuscular consequences of extreme running in a 166km mountain ultra-marathon, reported that post race serum concentrations of CPK, hsCRP and neutrophils were lower than those measured after a road race with similar finishing times.[1] These researchers attributed their findings of low concentrations of systemic markers of muscle damage and inflammation to the relatively soft underfoot surfaces and to the athletes frequently being forced to walk, jump, and climb due to the technical demands of the terrain.

During extensive exercise-induced muscle damage myoglobin may be released into the urine and be indicative of exertional rhabdomyolysis and possible risk of renal failure.[8] Clarkson,[9] however, reported that exertional muscle damage in healthy athletes can cause profound serum

CPK elevations without renal impairment. In our study the absence of uMb was confirmed by the relatively low increases in systemic neutrophil, serum CPK and hsCRP concentrations.

In this study, we suspect that although the primary factor which reduced the amount of repetitive and eccentric unidirectional stress encountered during the race, was most probably the underfoot surfaces the majority of which were primarily soft, large fluctuations in the pace of running and varied muscle recruitment patterns over the different terrains, may also have played a role.

The positive correlation found between DOMS scores and CPK concentrations supports the findings of Nieman et al.[2] who, in their study on 60 participants in the 160 km one-day Western States Endurance Trail Run in the Sierra Nevada Mountains in northern California, showed that there were significant associations between CPK, muscle soreness and the cytokines, interleukin (IL)-6, IL-10, IL-1ra (receptor antagonist), granulocyte colony-stimulating factor and macrophage inflammatory protein 1β.

Systemic markers of cardiac damage

The effect of prolonged strenuous exercise on systemic cardiac markers of damage has been studied extensively[19-21] with evidence of transient elevations during and immediately after exercise, which return to normal within 3 days in healthy athletes.[19,20] These temporary elevations have been hypothesised to be due to myocardial stress and reversible cardiomyocyte membrane damage.[19, 20] Exercise is known to cause an increased myocardial oxygen demand and cardiac troponin turnover in all athletes,[19] which might be linked to tachyarrhythmias and sudden cardiac death, when associated with prolonged increases (> 3 days) in cTnT concentrations above 0.05μg/l.[19] At no stage during our study were increased cTnT concentrations however measured. This supports the attenuated increase in serum CPK concentration and absent uMb values as well as the lower concentration of serum cortisol despite maintenance of an intensity of effort which fluctuated from 63 – 112% APmax. It is possible that serum cTnT also did not increase due to the variation in HR (60 – 220bpm) that occurred during this race, which may have stimulated the cardiac muscle at irregular intervals and possibly reduced myocardial stress by permitting periods of recovery.

Users of NSAIDs

Both NSAID users and non-users were included in this study following recent findings that although markers of muscle inflammation are changed by NSAID usage, degree of muscle damage is unaffected.[22,23] Nieman *et al*.[2] reported that NSAID users did not have reduced race times, muscle damage or DOMS, while Friden and Lieber[6] reported that administration of NSAID after eccentric exercise resulted in a short term benefit of pain relief, but a long term detrimental effect on muscle adaptation, inhibiting protein synthesis by suppressing the inflammatory reaction. Paulsen *et al.*[23] also indicated that although NSAID inhibited prostaglandin synthesis and local and systemic responses, it did not affect actual markers of muscle damage. In this study, there was however no statistical difference in the measured markers of muscle damage or inflammatory response between NSAID users and non-users.

Dehydration, intestinal temperature (T_{intest}), HR and evidence of muscle damage

Although some athletes in our study experienced up to 4% body mass loss and others, on occasion, raced at a HR of more than 100% APmax (Table 5), these athletes did not present with clinical signs of dehydration, severe hyperthermia or increased muscle damage as reflected by changes in sOsm, T_{intest} > 40 º C or changes in serum CPK concentration, respectively.

As the statistically significant ($p \leq 0.05$) inverse correlation between % change in body mass and post-pre change in sOsm was low ($r=-0.365$), sOsm, widely reported golden marker of hydration status,[24] was used to quantitate changes in hydration status.

The correlation between hydration status and systemic markers of muscle damage, as reflected by stage– induced changes in sOsm and serum CPK concentrations, although statistically significant, was low. Hence it cannot be concluded from the 51 sets of paired data reported in this study, that hydration status has an overriding effect on systemic markers of muscle damage.

In the 12 individuals in whom continuous recordings of T_{intest} were recorded (Table 5), the correlation between race induced changes in T_{intest} and systemic markers of muscle damage, was also low and statistically insignificant. The data provided in this study, although, based on a relatively small sample size, therefore does not provide any support for the suggestion that rises in core body temperature, exacerbate muscle damage.

Conclusion

The relatively low post-race concentrations of systemic and urinary markers of muscle damage and inflammation,[5] when compared to those reported following road running events of similar duration,[16] are attributed to softer underfoot surfaces, large fluctuations in pace of running, and varied muscle recruitment patterns over the widely differing terrains.[1] The sporadic increases in intensity of effort, rises in T_{intest}, substantial body mass loss and increases in serum osmolality during the event, did not confirm previous suggestions[3,4,11] that thermal and hydration status is directly related to the degree of muscle damage.

It would be of interest to the investigate the impact of pre-race preparation on markers of muscle damage and inflammatory response found following this multi-day trail running event and to control the nutritional and fluid intake in future field work on multiday trail running.

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CHAPTER FOUR

CASE REPORT

Use of the statin, Aspavor, in combination with Ezetrol, in a hypercholesterolaemic endurance athlete completing a 95km multi-day trail run

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Abstract

Aim: To examine the effect of statin therapy, combined with a lipid uptake inhibitor, on markers of muscle damage and inflammation during trail running

Setting: Three Cranes Challenge Trail run, in Karkloof, KwaZulu-Natal, South Africa

Participant: A trained female endurance athlete currently being treated for familial hypercholesterolaemia with 20 mg Aspavor and 10 mg Ezetrol daily

Independent Variables: 3-day 95 km trail run

Main Outcome Measures: Changes in heart rate, body mass, blood neutrophil, serum creatine phosphokinase (CPK), high sensitivity C-Reactive Protein (hsCRP), cortisol, urinary myoglobin concentration (uMb), osmolality (UOsm) and specific gravity (USG)

Results: Maximum post stage serum CPK (665U/L), hsCRP (1.9mg/Ll) and cortisol (845nmol/L) concentrations corresponded with undetected uMb despite a maximum body mass loss of 4.5%

Conclusions: 0.4 mg/kg Atorvastatin daily in combination with 10mg Ezetrol did not result in subjective myalgia, cramps, fatigue or increased markers of muscle damage following participation in the trail run

Key Words: Muscle damage, trail running, combined statin and lipid uptake inhibitor usage

Introduction

The metabolic and cardiovascular benefits of regular prolonged exercise and the use of lipid lowering medication for patients with hypercholesterolaemia are undisputed (1). However, the use of 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors or statins, the primary lipid-lowering medication currently prescribed for hypercholesterolaemia, and Ezetrol, a lipid uptake inhibitor, have been associated with muscle cramping, myalgia, fatigue and in rare cases, rhabdomyoloysis and death (2,3,7,10,11). According to the latest U.S. Food And Drug Administration (FDA) Safety Announcement, the highest approved dose of simvastatin is limited to 80mg per day due to the risk of muscle damage (7,10).

Potential mechanisms to which side effects of chronic statin treatment in muscle have been attributed include intracellular depletion of essential metabolites and destabilization of cell membranes, resulting in (i) increased cytotoxicity (ii) the deficiency of isoprenoids which participate in electron transport during mitochondrial oxidative phosphorylation and (iii) alterations in calcium shuttling, causing calcium leakage from the mitochondria and impairment of sarcoplasmic reticulum calcium cycling and (iv) interactions with the hepatic cytochrome P-450 drug-metabolizing system (3, 7,10,11).

The purpose of this study is to investigate muscle damage and inflammation during a multiday trail run on an athlete using a statin and a lipid-uptake inhibitor. It is hypothesized that the muscle damage and inflammation of this athlete will be augmented above the mean of the study group (n=19) during the race and that she will experience fatigue and cramping which might be augmented by concomitant dehydration. Whether simultaneous intake of Ezetrol which has also been related to muscle damage, exacerbates this negative effect on muscle tissue in an endurance athlete, is however unknown.

Case Description

Written informed consent was obtained from a female provincial cross-country athlete, aged 42 years (height, 1.63cm; mass, 54.8kg; body mass index (BMI), 20.63kg/cm^2; % body fat, 25.3%; blood pressure (BP), 122/80 mmHg), who was monitored during the Three Cranes Challenge trail event. She was diagnosed with familial hypercholesterolaemia at 17 years of

age and her total cholesterol pre-medication was > 9mmol/l. Her current daily prescription includes 20 mg Aspavor (containing Atorvastatin) and 10 mg Ezetrol. Her training distance of an average of 60km/wk includes a high intensity session on hills, one on the track and one time trial per week. She has experienced chronic/recurring tendinosis of the right adductor muscles during the past 2 years.

The Three Cranes Challenge is a 3-day trail run in South Africa over 95 km, 29.3, 37.9 and 27.8 kilometers (km) during Stage 1 (S1), Stage 2 (S2) and Stage 3 (S3), with elevation gains of 1020, 1226 and 680m respectively. Ambient temperature and relative humidity ranged between 11.5 and 22.8 º C and 54 – 97%. The running surface varied from dual jeep track, gravel roads, grassland to single track through forests, over rocks, roots and streams.

Venous blood and a urine sample were taken immediately before and after each stage and at 24 and 72 hours post-race (PR). At these time points, body mass and thigh circumference (TC) were also measured. A subjective rating of delayed muscle soreness (DOMS) was recorded before and after each stage as well as on Days 1-5 following the event.

A commercial pathology laboratory conducted full blood counts on the venous blood samples, which were also analysed for serum creatine phosphokinase(CPK), high sensitivity C-reactive protein (hsCRP), cortisol and osmolality (sOsm). Urine was analysed for myoglobin (uMb) content as well as osmolality (UOsm) and specific gravity (USG). Heart Rate (HR) was recorded throughout each stage using a polar monitor (Polar Electro OY, Finland)

Results

As shown in Table 1, running speed averaged 7:41, 7:33 and 6.17 min/km and heart rate (HR), 131, 98 and 141 beats per minute (bpm), the equivalent of 74, 55 and 79% age predicted maximum (APmax)HR during the 3 stages respectively. A representation of her continuous HR recordings in relation to the topographical characteristics of the routes for the different stages, is presented in Figure 1.

Table 1: Race Performance and Mean, Maximum and Minimum Heart Rate Responses

Race Performance				**Heart rate**				
Stage	**Distance (km)**	**Time to completion (hh:mm:ss)**	**Mean Minutes per km**	**Min**	**Max**	**% AP Max***	**Mean**	**Mean % AP Max**
ONE	29.3	03:57:13	7:41	100	166	93	131	**74**
TWO	**37.9**	**05:07:43**	**7:33**	**60**	**162**	**91**	**98**	**55**
THREE	27.8	03:17:19	6:17	125	169	95	141	**79**

Max: maximum; Min: minimum; *APMax = Age Predicted maximum (220-age)

Figure 1: Continuous heart rate recordings and topographical characteristics of the routes

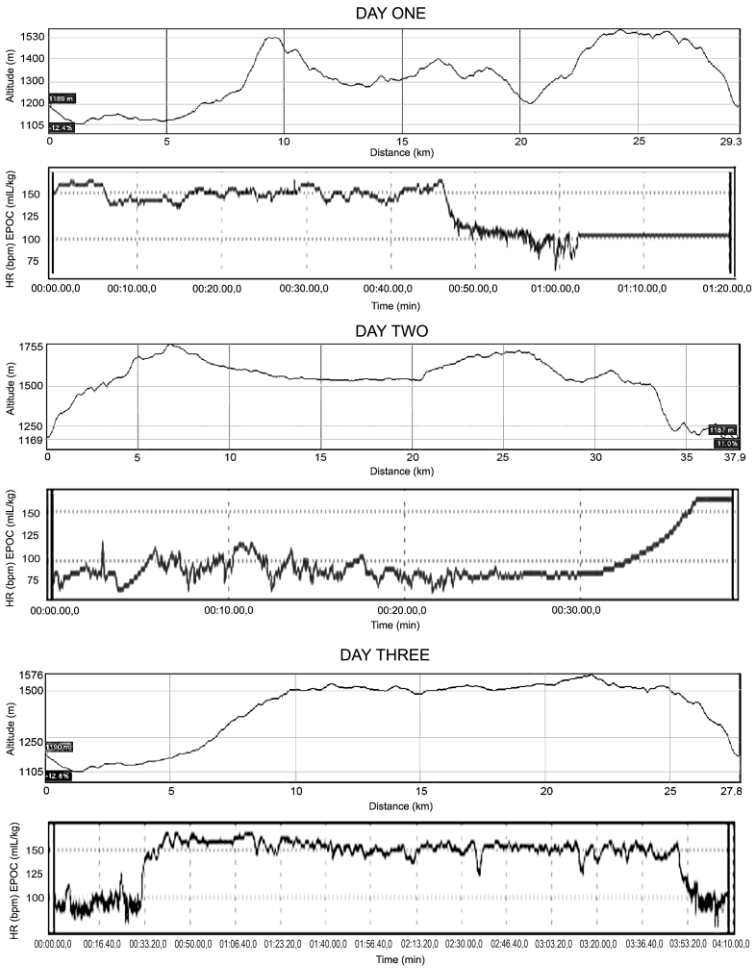

Table 2: Haematological analyses and markers of muscle damage, inflammation and hydration status

Variable	PreS1	PostS1	PreS2	PostS2	PreS3	PostS3	24h	72h	Reference range
RBC(10^12/l)	4.37	4.01	4.03	4.17	3.79	4.01	3.86	3.88	3.8 – 4.8
Hb (g/dl)	14.1	12.9	13.3	13.3	11.9	12.8	12.3	12.5	12 – 15
Hct (%)	42.8	39.1	39.2	40.9	37.5	39.9	38.4	40.4	36 – 46
Platelet (10^9/L)	236	238	225	245	207	246	187	208	150 -450
Leukocyte (10^9/L)	4.35	6.79	4.75	7.5	4.86	5.05	4.21	6.75	3.92– 9.88
Neutrophil (10^9/L)	1.83	5.20	2.02	5.79	2.25	5.05	1.85	4.64	2.00 – 7.50
Serum CPK (U/L)	134	215	269	478	470	665	391	140	39 – 308
Urinary myoglobin (mcg/ml)	ND*	ND*	ND*	ND*	ND*	ND*	ND*	ND*	**<1**
Serum hsCRP(mg/l)	0.2	-	-	-	-	1.9	1.4	0.6	<1.0
TC (cm)	49.5	47	49.5	49	49	49	49	49	
DOMS Score	0	4	4	7	5	5	2	0	
Serum Cortisol (nmol/l)	845	617	846	943	755	670	-	-	190 – 690
Serum Osmolality (mOsm/kg)	290	292	291	305	290	303	-	-	275 – 295
Urine Osmolality (mOsm/kg)	754	482	564	705	1005	802	-	-	300 – 800
USG	1.020	1.015	1.020	1.025	1.03	1.03	-	-	Dehydration > 1.025
% body mass loss	3.2		4.5		2.7				

RBC: Red blood cell; Hb: Haemoglobin; Hct: Haematocrit; Serum CPK: Serum Creatine phosphokinase; Serum hsCRP: Serum High sensitivity C-Reactive Protein; TC: Thigh circumference; DOMS: Delayed onset muscle soreness; USG: Urine specific gravity; *ND: not detected

Her red blood cell and platelet indices reflected a healthy profile. Race-induced elevations in leukocyte and neutrophil concentrations were modest, remaining within reference range (Table 2).

As shown in Table 2, pre-race serum CPK concentrations (134U/L) were within the reference range (39-308 U/L) and rose to a maximum of 665U/L following S3. No uMb was detected at any reading. Serum CRP concentration rose from 0.2 mg/l to 1.9 mg/l post race, although she reported DOMS ratings of 4/10, 7/10 and 5/10 respectively during the 3 days of racing and 2/10 for 24h and 48h PR. Early morning pre-race serum cortisol concentration (845 nmol/l) rose to maximum of 943 nmol/l following S2. Post-stage TC did not increase.

sOSm concentrations and USG were indicative of substantial dehydration after S2, during which she experienced nausea and an inability to ingest carbohydrates and fluids. The dehydration persisted until completion of the race and is confirmed by the body mass loss of 4.5% during S2 (Table 2).

Discussion

Statins have been found to be the most effective medications for reducing elevated blood concentrations of low density lipoprotein cholesterol and are currently being prescribed to more patients in greater dosages (11). Although statins have been shown to reduce chronic inflammation in patients with coronary artery diseases and cardiovascular related mortality (8), dose related ($\geq$ 1.0mg/kg) statin myotoxicity (7), has been found to have adverse effects on muscle which may affect performance in sport (2,3,7,10,11).

Seachrist *et al.* (10) reported that 78 % of professional athletes with familial hypercholesterolaemia could not tolerate statin therapy due to muscular problems and predisposing risk factors include being female, advancing age and the use of concurrent medications (2). It was therefore of interest to observe how this female athlete, aged 42 years, ingesting a dose of 20mg/52 kg Aspavor combined with 10 mg Ezetrol per day, would respond to participation in a three-day 95 km trail run.

Atorvastatin and simvastatin are lipophilic statins which promote ease of diffusion through passive transport across the bilipid cell membrane, causing augmented toxic effects on muscle at a dosage of >80mg/day (2,8). At 0.5mg/kg, Seachrist *et al*. (10) demonstrated changes to the morphology of mitochondria, which might sensitize muscle to injury, but without concurrent

changes to the myofibre. At doses of ≥1mg/kg/day significant alterations in the myofibre and increases in serum CPK, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and plasma lactate were reported which were greater in Type II glycolytic fibres than in Type I muscle fibres. They concluded that ≥1mg/kg/day might promote statin-induced muscle damage and subsequent muscle mass loss.

As athletes regularly present with CPK values in the "thousands" following ultradistance road running events (4,6), this subject's accumulative CPK concentrations following the three stages of the event indicate very little exercise-induced muscle damage. Her training status, a repeated bout effect and reduced muscle damage resulting from the inclusion of hill training in her program, might, together with genetic factors and the dosage of her medication (0.4 mg/kg), have contributed to her lower serum CPK concentrations (5).

According to her HR data (Figure 1, Table 1), the greatest effort was during S3 although during all three stages her HR sporadically reached a maximum of over 90% APmax. This indicates a moderate intensity of prolonged effort during S1 and S3. The reduced intensity during S2 (Figure 1, Table 1), was probably due to her reported nausea and vomiting and evident dehydration (Table 2), which could have reduced muscle damage, resulting in an attenuated serum CPK concentration and the absence of uMb throughout the race. This is also confirmed by her post-race concentration of serum hsCRP, 1.9 mg/l, which is indicative of only mild inflammation due to muscle damage, when compared to the mean concentration hsCRP in previous ultradistance events (6). The anti-inflammatory effect of Atorvastatin (8) is likely to be a factor responsible for this attenuation.

Her decreased thigh circumference after S1 (49.5cm – 47cm) may not be muscle mass loss due to statin therapy, but rather confirm previous findings (4) at the 2006 Isarrun, a 5-stage 338 km ultra- endurance run, where athletes experienced significant losses in skeletal muscle mass (particularly after S1), but not body mass during the race.

Although her pre stage serum cortisol concentrations were above the reference range (Table 2), it increased further only following S2, which confirms greater physiological stress, possibly

linked to her reported inability to ingest carbohydrates during this stage. However, it did not affect her muscle damage during that stage (Table 2). Post S1 and S3 serum cortisol concentrations indicated that she was running comfortably within her physiological range.

Pre race sOsm indicates that she started the race adequately hydrated. In view of the reported effect of dehydration on exercise-induced muscle damage (4) it is interesting to note that despite dehydration occurring during S2 and S3, as indicated by the substantial increases in sOsm,UOsm and USG and a body mass loss of 4.5% during S2, there was no excessive increase in the markers of muscle damage and inflammation (Table2).

Although her pre-race RBC, Hb and HCT values reflect the absence of anaemia, the slight drop in RBC indices over the 3-day period may be due to transient osmotic stress, membrane lipid peroxidation caused by free radicals released by leucocytes, or even intramuscular destruction of RBC (9), which returned to normal within 72 hours post race. The anti-inflammatory effect of Atorvastatin (8) probably contributed to the attenuation of the typical increase in leukocyte count during intense prolonged exercise, indicating an increase in metabolic activity and activation of neutrophil release (4, 5, 6).

In conclusion we report that this subject, despite presenting with evidence of dehydration after S2 and S3, experienced no symptoms of statin- induced myalgia, fatigue or cramps during the 3-day trail run. The adverse effects of Atorvastatin, combined with simultaneous intake of Ezetrol, were not evident at 0.4mg/kg. Although the nausea experienced may have been an adverse effect, the fact that it only occurred during the more challenging strenuous S2, points to greater likelihood of exercise-induced gastric irritation.

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CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study set out to determine the effect of multiday trail running on systemic and urinary markers of muscle damage and inflammation and the relationship with factors affecting muscle damage and inflammation during this endurance event. The findings of this thesis reflect that

i. the eccentric component of skeletal muscle contraction, when compared to road running events of similar duration, was not augmented during the three days of trail running
ii. low markers of muscle damage and inflammation were recorded despite the maintenance of a mean intensity of effort of 77-84% APmax and the evidence of hyperthermia and dehydration, which have previously been found to augment exercise induced muscle damage and inflammation
iii. markers of rhabdomyolysis and cardiac muscle damage were absent
iv. the attenuated skeletal muscle damage and inflammation was confirmed by the small changes in DOMS and TC, which returned to baseline values by 72PR.

It is concluded that the reduced muscle damage and inflammation are attributed to the softer surface underfoot and the technical difficulty of the terrain, which forced athletes to employ varied muscle recruitment patterns and to reduce their pace while maintaining a moderately high level of intensity. It is therefore possible that trail running, although it involves a greater element of danger and hence greater predisposition to acute injuries, is less damaging than road running in terms of intrinsic exercise-induced skeletal muscle damage.

The systemic and urinary evidence of muscle damage and inflammation in the case study was also attenuated and, in contrast to our hypothesis, she did not experience either muscle cramps or excessive fatigue, despite the use of Atorvastatin combined with Ezetimibe, which have both

been associated with muscle cramping, myalgia, fatigue and in rare cases, rhabdomyoloysis and death.

It is thus suggested that the lower dosage of medication benefits this hypercholesterolaemic athlete, allowing her to participate in sport without experiencing the adverse events associated with higher dosages of the prescribed medication.

In conclusion it is however important to highlight the limitations of this study. These include

i. the use of a limited convenience sample from whom full compliance was obtained, which may not have been representative of the population,
ii. financial constraints limiting the number of tests performed and
iii. lack of more comprehensive monitoring of the activity levels of the subjects during the post-stage recovery period.

5.2 RECOMMENDATIONS FOR FUTURE RESEARCH

Directions for further research include

i. investigating the difference in skeletal muscle damage and inflammation between multi-day road and trail running, using the same athletes whilst controlling variables such as their intensity of effort, nutrition, hydration and the activities of the athletes during the events and replicating the elevations of the routes, to match road and trail running routes. This would include exploring the difference in the extent and duration of fatigue experienced after multiday trail running events compared to road running;
ii. investigating the hormonal control of fluid balance during multiday trail running events;
iii. investigating the changes in cardiac structure and function during multiday events and the permanency of these changes, followed over a 5-10 year period.

Further research investigating the effects of the administration of different dosages of Atorvastatin with or without the use of Ezetimibe in double blind randomly selected placebo-controlled studies is also suggested, enforcing strict control of above mentioned variables.

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APPENDICES

APPENDIX B

UNIVERSITY OF
KWAZULU-NATAL

Discipline of Physiology
School of Medical Sciences
Faculty of Health Sciences
2011-07-14

THREE-CRANES TRAIL RUN 2011
Study Title: Physiological assessment of participants in a three day trail run event: markers of muscle damage, hydration and immune status.

Dear PW,

Thank you very much for agreeing to take part in the above trial. Although the final research papers are not yet published, we are happy to provide you with your results.

Your Physical Characteristics

	Your value	**Predicted norm for active non-athlete of your age, gender and BMI**
Height (m)	**1.78**	
Mass (kg)	**80.1**	
BMI	**25.28**	**19 – 26**
Triceps skin fold (mm)	**17.0**	
Biceps skin fold (mm)	**8.0**	
Sub scapular skin fold (mm)	**9.67**	
Suprailliac skin fold (mm)	**12.13**	
Body Fat % (determined from triceps, biceps, suprailliac and sub scapular skin folds)	**20.8**	**11-17% (athletes 5 – 12%)**
Blood Pressure (mmHg)– Baseline	**127/70**	**120/80**
Heart Rate (bpm)– Baseline	**53**	**65**

Your BMI and resting blood pressure and heart rate fall within normal for a male athlete of your caliber, though your body fat % is higher than the norm. Enclosed are charts which you may find interesting for a comparison of body fat percentages of athletes in various disciplines.

Body Fat Percentage for The Average Population			
Age	**Up to 30**	**30-50**	**50+**
Females	14 - 21%	15-23%	16-25%
Males	9-15%	11-17%	12-19%

Average Body Fat Percentage of Athletes					
Sport	**Male**	**Female**	**Sport**	**Male**	**Female**
Baseball	12-15%	12-18%	**Rowing**	6-14%	12-18%
Basketball	6-12%	20-27%	**Shot Putters**	16-20%	20-28%
Body building	5-8%	10-15%	**Skiing (X country)**	7-12%	16-22%
Cycling	5-15%	15-20%	**Sprinters**	8-10%	12-20%
Football (Backs)	9-12%	No data	**Swimming**	9-12%	14-24%
Football (Linemen)	15-19%	No data	**Tennis**	12-16%	16-24%
Gymnastics	5-12%	10-16%	**Triathlon**	5-12%	10-15%
High/long Jumpers	7-12%	10-18%	**Volleyball**	11-14%	16-25%
Ice/field Hockey	8-15%	12-18%	**Weightlifters**	9-16%	No data
Racquetball	8-13%	15-22%	**Wrestlers**	5-16%	No data

Adapted From: Sports Fitness Advisor-Sports Training Tips for Athletic Peak Performance (www.sport-fitness-advisor.com)

Ambient weather conditions during the three stages

Stage	Ambient temperature range (° C)	Windspeed Range (m/s)	Relative Humidity %	Barometric Pressure (hPa)
1	11.5 - 21.7	0 - 2.3	63- 95	895.2 - 896.1
2	12.4 - 22.8	0 - 2.8	54- 97	896.5 - 897.7
3	12.1 - 21.2	0 - 2.3	64- 96	896.8 - 897.9

Elevation gains and losses

Running speed varied depending on topography of the routes. elevation gains reached 1020, 1226 and 680m, while elevation losses were recorded at 1021, 1231 and 687m during Stages One, Two and Three respectively.

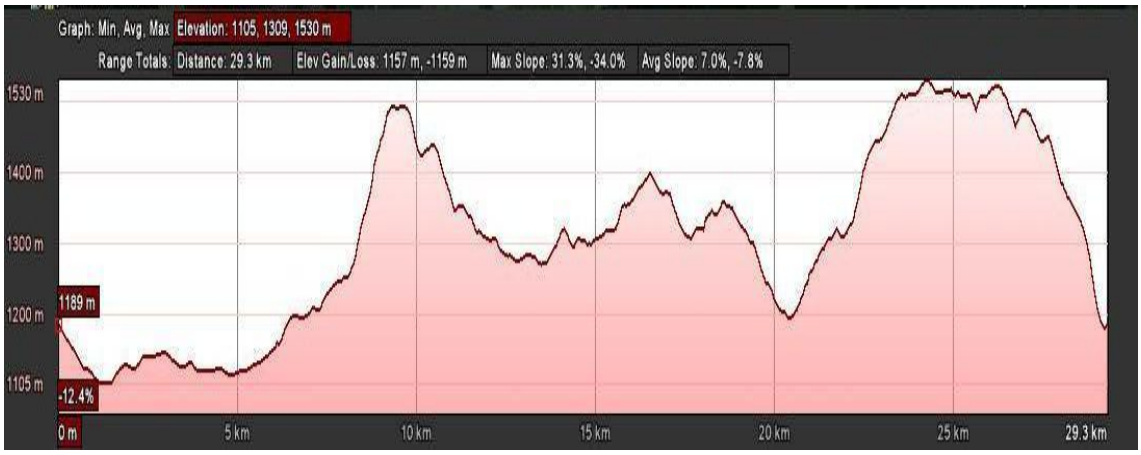
Running speed, heart rate and core body (intestinal) temperature

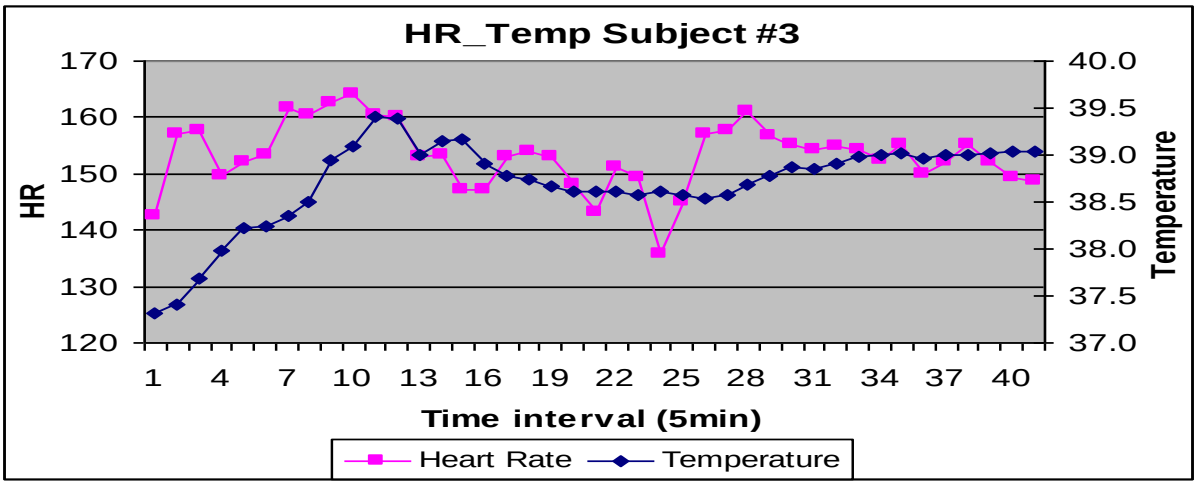
Your Running Speed

Stage completed	Distance of stage (km)	Time to completion (hh:mm:ss)	Minutes per km
ONE	32	03:25:14	6:41
TWO	42	04:23:47	6.28
THREE	30	02:38:38	4.96

Although the terrain was more technical during Stage 2, your average speed was higher than Stage 1 and increased even more to 4:96 min/km during Stage 3.

Topographical characteristics of Stage 1





As is shown above, your mean heart rates and temperate changes averaged over five-minute time-periods during Stage 1. In the first five minutes of the race, your heart rate already reached 145 bpm after and after which there were two substantial rises (over five minute intervals) to above 160 bpm with a mean of 153 bpm during the stage. You may find it

interesting to relate the data in the two above figures noting the correlations between elevation gain/loss and heart rate – temperature response.

Your Heart Rate changes during stage 1

	Age predicted (AP)Max	Max	% AP Max	Minimum	Mean	Mean as % AP max
Day 1	187	164	88	136	153	82
Day 2		165	88	107	149	80
Day 3		180	96	127	155	83

You maintained a high intensity heart rate of between 80 and 83% of age predicted maximum during all three stages, reflecting your experience in multiday trail running events.

Core Body Temperature changes during stage 1

	Maximum	Minimum	Mean	Median	Mode
DAY 1	39.4	37.3	38.7	38.8	39.0

Your maximum body temperature appears to have been reached about 55-60 minutes into the stage. This is relatively high, but well below the cut- off point of > 40.5 º C for heat related injury and heat stroke.

Blood/Urine Tests and Related Results

1. **Serum Creatine Phosphokinase and urinary myoglobin**

Serum CPK concentration is commonly used as a systemic marker of exercise-induced skeletal muscle damage. It leaks into the blood when the membrane surrounding muscle cells is damaged.

Your CPK concentration in the blood on the morning of the race was 269U/L which falls within the norm of 39-308 U/L showing that you started the race without evidence of muscle damage.

Serum CPK concentration is known to peak 24 hours post exercise. Before Stage 2 (Pre S2) your serum CPK concentration was 746U/L (reference range 39-308 U/L), indicating that some muscle damage occurred during stage 1. Your serum CPK concentration reached its maximum for this race, 1562U/L at the post S3 reading. As athletes present with values in the "thousands" following a road running event such as Comrades, your accumulative CPK values over the 3 days, however, only indicate mild exercise-induced muscle damage.

For example, you may be interested that following the 166km North Face Ultra Trail du Mont Blanc mountain marathon (2009) with only 9500m of positive and negative elevation change, mean CPK increases of over 13 000 IU/L were reported in the 11 runners assessed.

Remembering that training status and the inclusion of hill training in your programme results in a repeated bout training effect and reduced muscle damage, your result is very encouraging as you are showing far less muscle damage than these European athletes.

Most of the athletes who attended the 72 h post race testing session recorded serum CPK concentrations similar to pre-race levels, indicating no further leakage of this muscle enzyme into the blood. A pity that you missed these sampling stages.

Serum Creatine Phosphokinase (U/L)								
Pre S1	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**24h**	**72h**	**Reference range**
269	537	746	1193	1069	1562	-	-	39-308 U/L

Interestingly these results are confirmed by your urinary myoglobin levels which were within the normal range again reflecting the absence of severe muscle damage.

2. High Sensitivity C-Reactive Protein and other evidence of muscle inflammation

C-Reactive Protein is a systemic marker of inflammation and acute phase response to muscle damage. Although it often used as an indicator for chronic cardiovascular disease when raised in a resting state, we have used it as a marker of delayed inflammatory response to exercise-induced muscle damage in this study.

Your resting hs-CRP concentration was 0.8mg/l which represents both a low cardiovascular disease risk (according to American Heart Association guidelines) and indicates the absence of muscle inflammation pre-race. In the North Face Ultra Trail mountain marathon, mean concentration measured was 37.7 mg/l and in our lab we have recorded mean 24 hour post-race values of almost 40 mg/l in two studies completed on the Comrades downrun.

Hs-CRP (mg/l)				
Pre S1	**Post S3**	**24h**	**72h**	**Reference**
0.8	14.5	-	-	<1.0mg/l = low risk

Correlating with the above-mentioned indications of mild muscle damage incurred during the event, is your post S3 concentration shown in the above table.

It is a pity that you were not able to provide post-race blood samples. Most athletes recorded reduced values at 24 and 72 h post race, representing a normal reduction in inflammation post-exercise as a result of rest.

Thigh Circumference (cm):

Pre S1	Post S1	+4hours	Pre S2	Post S2	+ 4hours	Pre S3	Post S3
59	54.5	59	59	57	58	59	57.6

A study by Knechtle and Kohler (2007) conducted on the 2006 Isarrun, a 5-stage 338 km ultra- endurance run in Bavaria, Germany, revealed that athletes experienced significant losses in skeletal body mass (particularly after Stage 1 of the race), but not body mass during the race. Your decreased thigh circumference after Stage 1 therefore may well confirm these findings.

The return to pre-race circumference within 4 hours post race confirms an inflammatory response which follows mild degrees of swelling and accumulation of fluid within the interstitial spaces between the active muscles.

3. Serum Cortisol concentration

Cortisol is a hormone secreted by the adrenal glands and is often elevated in times of physical or mental stress. Cortisol increases the glucose available to the muscles by stimulating the breakdown of glycogen and also acts as a powerful anti-inflammatory hormone. Cortisol secretion in the body normally follows a diurnal pattern, being highest between 5 – 10am and lowest between 8pm – 4am. In the case of athletes running a race, blood cortisol concentrations may also be elevated in the morning due to anticipation or hyperglycaemia, but do tend to increase further during prolonged exercise.

Your cortisol result for the morning of the first day was 711nmol/l which is higher than the norm, probably due to anticipatory anxiety. Usually during a race the cortisol values increase due to physiological stress. As shown in the table below, the metabolic demands/ anti-inflammatory response appear to have been increased during Stage 2 and greatest during Stage 3, confirming your increased average heart rate and speed maintained during Stage 3.

Serum Cortisol concentration (nmol/l)						
Pre S1	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**Reference**
711	740	562	938	604	983	190 – 690nmol/l

4. Phadiatop and Immunoglobulin E

These blood tests are used to diagnose and monitor allergy reactions. The Phadiatop test shows whether you are allergic to a range of common inhalant allergens, including animal dander, pollen and mould. IgE concentrations represent the severity of the allergic reaction.

Your Phadiatop test result was 2.08 IU/ml. As the reference values for this test are 0.00-0.35 IU/ml, this is positive for inhaled allergens, signifying a degree of sensitivity to an airborne allergen.

However, your IgE concentrations during the race ranged between 42.8 and 62 IU/ml which are within the normal range (0.0-100.0 IU/ml), indicating that the specific allergen to which you are sensitive, was not present during the race.

Serum IgE concentration								
Pre S1	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**24h**	**72h**	**Reference range**
42.8	49.7	49.4	46.8	62	58.2	-	-	0 – 100 IU/ml

6. Full blood Count

The full blood count (also known as differential blood count) measures the concentrations of various blood cells (red blood cells, white blood cells and platelets) and may give an indication of general health status and point to the presence of inflammation, allergy or infection.

The full blood count is also used to diagnose anaemia by reflecting haemoglobin concentrations.

Tabulated below are your readings of red blood cell indices during and after the race:

Red blood cell (RBC) count (10^12/l)								
Pre S1	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**24h**	**72h**	**Reference range**
5.21	5.16	4.86	5.22	4.89	4.93	-	-	4.5 – 5.5 10^12/l

Haemoglobin (Hb) (g/dl)								
Pre S1	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**24h**	**72h**	**Reference range**
15.3	15.1	14.4	15.5	14.3	14.6	-	-	13 - 17g/dl

Haematocrit (Hct) (%)								
Pre S1	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**24h**	**72h**	**Reference range**
44.4	44.1	41.7	44.2	42.8	42.5	-	-	40 - 50%

Your initial/pre-race RBC, HB and Hct values reflect the absence of anaemia and a healthy red blood cell status for a male distance runner. Well done!

The slight drop during the race may be due to a possible shift of the fluid from the interstitial fluid into the blood. This is nothing to worry about as it corrected itself post race.

The slight drop in RBC, Hb and Hct concentration experienced during the latter part of the three-day race may also confirm a small degree of “foot strike haemolysis”, that is to be expected, given the amount of downhill work you were doing in this trail run.

At rest, your platelet count was 301 10^9/L and total white blood cell count was 7.58 10^9/L. These parameters, including your differential white blood cell count, were within normal limits

After every stage there was an increase in the white blood cell count, which is the expected normal physiological and inflammatory response to this type of exercise. Results are given in the table below.

White blood cell count (10^9/L)								
Pre S1	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**24h**	**72h**	**Reference range**
7.58	13.63	7.71	12.55	9.87	13.36	-	-	3.92 – 9.8810^9/L

With regard to the **URTI** symptoms that you experienced before and after the race, we can conclude from the results of the blood analyses conducted that

(1) there was no indication of allergic origin
(2) there was no indication of a viral infection
(3) the elevated CRP, WBC and neutrophil concentrations confirm the possibility of a mild inflammatory response to a bacterial infection

5. Hydration status changes: Serum and Urine Osmolality, Urine Specific Gravity and Body Mass

Serum osmolality is the golden marker of hydration status during endurance events.

The values shown in the table below indicate that you started the race slightly underhydrated with a more concentrated urine with higher osmolality than advisable. The serum osmolality increased after S1 indicating that you underhydrated during Stage 1. It returned to within normal values pre Stage 2 and 3, indicating adequate hydration overnight, although it again increased to levels above the laboratory reference range during Stage 2 and 3, reflecting a pattern of dehydration during the race, which might be detrimental to your performance.

Test	**Pre S1**	**Post S1**	**Pre S2**	**Post S2**	**Pre S3**	**Post S3**	**Reference range**
S Osmol	284	297	288	301	290	298	275 – 295mOsm/kg
U Osmol	1029	801	774	924	1176	990	300 – 800mOsm/kg
USG	1.025	1.03	1.02	1.03	1.025	1.03	Substantial Dehydration> 1.026

Urine osmolality and specific gravity values provide a somewhat delayed indication of hydration status following kidney filtration processes.

Interestingly, your urine osmolality was particularly high at the start of Stages 1and 3, indicating inadequate hydration. You therefore needed to drink more as failure to do so

resulted in the high values throughout the race and might have affected your running performance.

The above is confirmed by the changes in urine specific gravity (USG). A urine specific gravity of 1.010 is indicative of a well hydrated state. At no stage was your USG at that level.

The changes in body mass also reflect some degree of fluid loss, although recent research (Nolte, 2010) has, however, confirmed that changes in body mass cannot be used as accurate surrogate for changes in total body water during prolonged exercise.

Body mass (kg)								
Pre S1	**Post S1**	**% loss**	**Pre S2**	**Post S2**	**% loss**	**Pre S3**	**Post S3**	**%loss**
79.68	77.5	2.7	79.86	76.95	3.6	79.74	78.04	2.1

We do hope that these findings are of interest to you and would once again like to thank you for being such a reliable research subject during the 3 days of racing.

With very best wishes,
.

Prof E Peters-Futre
Research Director

Dr. Rentia Denissen
Dr. Anton de Waard
Mr Navin Singh
Masters in Medical Science:
Sports Medicine students

APPENDIX C

UNIVERSITY OF KWAZULU-NATAL

DEPARTMENT OF HUMAN PHYSIOLOGY
FACULTY OF HEALTH SCIENCES

SUBJECT INFORMATION FORM

Study title: Physiological assessment of runners participating in three-day trail run event, with particular emphasis on markers of muscle damage, hydration and immune status.

Dear Runner

Ethical Approval:
This study has received ethics approval from the University of KwaZulu-Natal, Nelson R Mandela School of Medicine, Research Ethics Committee (Reference No: BF210/010)

Study Aims:
The primary questions to be examined in this study are to include the following:

Whether amateur athletes competing in a multistage mountain trail run
(1) incur muscle damage
(2) are adequately, over or under hydrated and
(3) experience an increased incidence of Upper Respiratory Tract Infections due to airborne allergic responses

Who will qualify to participate in this study?
1. Healthy athletes (not in possession of CVS, renal, hepatic or endocrine dysfunction) between the ages of 25 and 50 years taking part in the 2010 3-Cranes Mountain Trail Run on 25-27 February 2010.
2. Residents in the greater eThekweni and Umzumduzi area.
3. Those willing to take part in the study and consent for all aspects of test e.g. regular blood tests, provision of saliva and urine samples, skin prick tests, weighing, filling in questionnaires, measuring urine output.

What will be expected from volunteers in this study?
You will be asked to:
1. Maintain your normal fluid use habits throughout the study.

2. Maintain your normal diet throughout the study.

Outline of tests:
Pre race Briefing – Day before race – REGISTRATION: **24 February 2011**
1. Complete a medical questionnaire and undergo a full medical exam if indicated.
2. Complete a pre-race questionnaire concerning your training experience as well as details about the fluids you consume when exercising
3. Assessment of height, mass and percentage body fat (to be estimated from skinfold thicknesses).

During race (before and after each stage)– Research station at Karkloof
1. Body mass measurement.
2. Three vials (approx 9-12 ml) of blood to be taken before and after the event.
3. Provide a urine sample
4. Complete a brief questionnaire.
5. Record all fluid consumed and all urine output during stage and overnight.
6. Wear a heart rate monitor throughout the race

24, 48 and 72 Hours after race - Exercise Laboratory, Physiology Department, E2 Building
1. Body mass measurement.
2. Three vials of blood (approx 9-12 ml) to be taken.
3 Provide a urine and saliva sample.
4. Complete a brief questionnaire .

How can you benefit from participation in this study?
Following the study, you will be given the results of your full blood counts and other biochemical/ laboratory tests which were run on your blood samples. As soon as the data has been collated, you will receive the study results.
A furthering of knowledge regarding your fluid balance during endurance exercise as well as hydration status will be gained from this work. This will allow you to optimize your fluid use in the future and improve your performance. You will be the first to be notified of the results of this study.

Will you be exposed to adverse effects of the study?
There will be minimal risks to you by being involved in the study. Some people find venipuncture (taking blood) uncomfortable. There is a very slight risk of complications from this procedure, mainly infection at the site of puncture or inflammation (swelling) of the vein used. In the unlikely event of a complication occurring, it is not likely to prevent you from completing the event. A n experienced qualified nurse will be taking the samples. She will do my best to prevent complications by ensuring procedures are performed according to the same standards the subjects would experience in a hospital environment. Both of the above mentioned complications are easily treated.

Can you withdraw from the study?

As participation is entirely voluntary, you may withdraw from the study at any time without penalty.

Will your individual results remain confidential?
Yes. The records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. The study is for degree purposes however the results of the study may be published. In all cases the participant's identity will remain confidential.

Financial compensation.
Any out-of-pocket expenses which you may incur as a result of your participation in this study (e.g. traveling expenses) will also be reimbursed by the research team.

Further queries?
Should you have any queries or wish to obtain further details regarding this study, please do not hesitate to contact us.

Prof Edith Peters-Futre – (031) 260-4237(w); 261-3869(h).
Study Director

Contact details of REC administrator and chair – for reporting of complaints / problems.

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
University of KwaZulu-Natal
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001, Durban, 4000
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Email: BREC@ukzn.ac.za
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APPENDIX D

DEPARTMENT OF PHYSIOLOGY

COLLEGE OF HEALTH SCIENCES

Physiological assessment of participants in a three-day trail run event: markers of muscle damage, hydration and immune status.

SUBJECT CONSENT FORM

I, …….. hereby agree to participate in a research study to be performed by Dr. Rentia Denissen, Dr Anton de Waard, Mr. Navin Runjit Singh and Professor Edith Peters-Futre in the Department of Human Physiology in the College of Health Sciences of the University of KwaZulu-Natal. I have been informed about the study by the principal investigator, Professor Peters-Futre.

I understand that the basic procedures to be carried out are to include:
1. Completion of URTI questionnaire for 14 days prior to the race
2. Assessment of height, mass and percentage body fat, heart rate, blood pressure and peak flow prior to registration on 24 February
3. Completing two questionnaires as well as providing a small vial of blood (2ml) to perform an allergy test at the pre-race briefing
4. Completion of a brief questionnaire, giving a urine and saliva sample and the taking of three vials of blood (a total of no more than 14ml of blood), twice daily (before and after each stage of the three-day event)
5. Completion of a post-stage questionnaire, giving a urine sample and three vials of blood 24, 48 and 72 hrs after the race finishes
6. Accurately measuring all fluid consumed and urine voided throughout the duration of the race, and recording the incidence of upper respiratory tract infection symptoms during the 2-week post-race period
7. Completion of a URTI questionnaire for 14 days following the race

The details of these procedures have been explained to me in full. I am aware that a certain level of discomfort may occur when the blood is taken and that this procedure may be accompanied by certain medical risks including infection and inflammation of the vein.

I understand that the study forms part of the Masters degrees of Drs Rentia Denissen and Anton de Waard and that the results may be published.

I understand that participation is entirely voluntary and that I may withdraw from the study at any time.

I may contact the principal investigator of the project, Professor Peters Futre at 073- 7597974 at any time if I have questions about the research or if I are injured as a result of the research.

_____ _____
Signature of Participant Date

_____ _____
Signature of Witness Date (Where applicable)

APPENDIX E

DEPARTMENT OF PHYSIOLOGY
FACULTY OF HEALTH SCIENCE

THREE-CRANES RESEARCH PROJECT, 2011

PRE-RACE QUESTIONNAIRE

Physiological assessment of participants in a three-day trail run event: markers of muscle damage.

Name of Subject:…….....Number……

SECTION A: PLEASE ANSWER ALL QUESTIONS.

1. Date of birth: _____ Age:_____

2. Address:

_____

3 Telephone numbers:

Home: _____/_____

Work: _____/_____

Cell: _____

4. Running club: _____

5. Occupation: _____

Please tick one of the boxes in each of the following questions:

6. How many hours do you generally run per week?

☐ < 5
☐ 6 - 10
☐ 11 - 15
☐ 16 – 20

7. How would you classify your running ability?

- ☐ WEEK-END WARRIOR (only run on weekends)
- ☐ SERIOUS AMATEUR (> 5 races per year)
- ☐ ELITE (regular top 10% finisher)
- ☐ PROFESSIONAL (paid to run)

8. Racing experience within last year?

- ☐ FUN RUNS
- ☐ < 5 x 21 km races) PER YEAR
- ☐ < 5 marathons (42km) PER YEAR
- ☐ > 2 ultra marathons (52 km or longer) PER YEAR
- ☐ PREVIOUS MULTISTAGE RACES e.g. Cape Odyssey, 3 Cranes
- ☐ OTHER -

Training details:

11. Average weekly running distance: _____km
12. Running history

    serious\social :_____

    age started running: _____(….yrs)

    level of training during these years _____

    total number of running races: _____

13. Describe the terrain used for training

    _____

    (take into consideration – number of days on flat; on hills, distance on hills, track, etc)

14. Other sports played in last 12 months (squash, rugby weights, etc)

    _____

15. List races / competitions over the last 6 months (all sports)

_____

16. List races planned for next 2 months (all sports)

_____

17. Personal best times in last 12 months:

10 km		56 km	
21.1 km		90 km	
42.2 km		Other- specify	

Dietary and fluid intake details

1. Are you on any specific diet? (Vegetarian, fish only, etc) yes □ no□. If yes, please specify:
_____
_____

2. Are you presently using any supplements? (Multivit, Calmag, etc) yes □ no □. If yes, please specify how much you are taking and how often:
_____
_____

3. Do you use water and/or a sports drink during the race?
_____

4. Please specify how much of each and how often:
_____
_____
_____
_____

THANK YOU FOR YOUR PARTICIPATION

_____ _____
Participant's Signature Date

_____ _____
Principal Investigator's Signature Date

SECTION B: FOR LABORATORY USE ONLY

Pre-event/Post-event:

Height (cm): _____ Mass (kg): _____

Thigh circumference (cm) _____

Skin – fold Measurements:

Biceps: _____ _____ _____ _____

Triceps: _____ _____ _____ _____

Suprailliac: _____ _____ _____ _____

Subscapular: _____ _____ _____ _____

Body fat percentage: _____

Resting heart rate: _____ /min _____/min

Resting blood pressure: _____

STUDY NUMBER:……

APPENDIX F

DEPARTMENT OF PHYSIOLOGY
FACULTY OF HEALTH SCIENCE

THREE-CRANES RESEARCH PROJECT, 2011

The effect of a multiday trail run on systemic markers of muscle damage

POST RACE QUESTIONNAIRE:
DELAYED ONSET MUSCLE SORENESS

Please rate muscle pain on a scale of 0 – 5 where
0 = no pain at all
5 = very severe/maximal

	QUADS	HAMSTRINGS	CALVES
DAY 1 (after run)			
DAY 2			
DAY 3			
DAY 4			
DAY 5			

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5th February, 2012,

Dear Emmerentia Denissen,
I have the pleasure to inform you that your paper "*Low markers of muscle damage and inflammation following a three–day trail run*" has been accepted for publication. The publishers will contact you within 3 weeks and ask you to check the proofs of your paper which will be published in issue 1, 2012. The PDF version of the paper will be available for downloading once the paper has been published.

Thank you for supporting the South African Journal of Sports Medicine.

regards

Professor Mike Lambert

South African Journal of Sport Medicine
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