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**THE EFFECT OF CHRONIC EXPOSURE TO ENDURANCE EXERCISE ON
THE SKELETAL MUSCLE OF DISTANCE RUNNERS**

By

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TERMS AND DEFINITIONS

The following list contains useful terms and definitions for concepts presented in this thesis.

- **Adaptation** – A change in structure, function or form that improves an organism's chance of survival within a given environment.
- **ATI** – Acquired Training Intolerance. A symptom used to describe athletes who are unable to tolerate endurance training loads to which they were previously accustomed. This is the more recent redefinition of the Fatigued Athlete Myopathic Syndrome (FAMS).
- **Co-stressor** – A stressor that acts in combination with a primary stressor.
- **Chronic fatigue syndrome** – An illness characterised by persistent, debilitating fatigue and accompanied by rheumatologic, cognitive and infectious-appearing symptoms.
- **Chronological age** – The age of a person as measured from birth to a given date.
- **Exercise-associated fatigue** – Symptoms of fatigue which develop during or following exercise training or racing.
- **Exercise intolerance** – An unexpected decline in performance and an inability to tolerate previous levels of training; often accompanied by exercise-associated fatigue and in some cases skeletal muscle symptoms including excessive delayed onset muscle soreness, stiffness, tenderness and skeletal muscle cramps.
- **FAMS** – Fatigued Athlete Myopathic Syndrome. The traditional name for the condition used to describe exercise-intolerant athletes with a large accumulated training volume and chronic exercise-associated fatigue. In some cases the athletes present with the skeletal muscle symptoms mentioned above and biopsies show that they may have myopathic abnormalities within their skeletal muscle. They do not suffer from any known disease, chronic fatigue syndrome or overtraining syndrome.

- **Maladaptation** – Poor or inadequate adaptation.
- **Masters runner** – A person of advanced chronological age, regardless of experience in the sport of distance running.
- **Myopathy** – A disorder of muscle tissue or muscles.
- **Novice runner** – A person new to and inexperienced in the sport of distance running, irrespective of chronological age.
- **Over-reaching** – The process of hard training which forms part of a planned programme to stimulate adaptation and, when combined with periods of rest, permits the normal physiological response of full super-compensation.
- **Overtraining** – The process of extensive training without adequate rest that leads to the overtraining syndrome.
- **Overtraining syndrome** – A symptom complex (emotional, behavioural, and physical) characterised by non-adaptation to training, decreased physical performance and chronic fatigue following high volume and / or high intensity training and inadequate recovery. The symptoms persist for weeks to months, are not reversed with adequate rest, and cannot be attributed to an identifiable medical cause,
- **Pathology** – The anatomical and physiological deviations from the normal that constitute disease or characterise a particular disease.
- **Sarcolemma** – Muscle cell membrane
- **Sarcoplasm** – The cytoplasm of a muscle cell
- **Stress** – A state that occurs when a physical, chemical or emotional factor perturbs the equilibrium or homeostasis of a system.
- **Stressor** – A stimulus that causes stress.
- **Veteran runner** – An experienced runner that has participated in the sport of distance running for several years, irrespective of chronological age.

ABBREVIATIONS

ALT	Alanine aminotransferase
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
ATH	Athlete group
ATI	Acquired Training Intolerance
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
ARF	Acute renal failure
AST	Aspartate aminotransferase
BMI	Body mass index
BUN	Blood urea nitrogen
CK	Creatine kinase
CK-MB	Creatine kinase-myocardial band
CPT	Carnitine palmitoyltransferase
CT	Contact time
DNA	Deoxyribonucleic acid
DOMS	Delayed onset muscle soreness
EAMC	Exercise-associated muscle cramps
ECG	Echocardiogram
EIA	Exercise-induced asthma
EMG	Electromyography
ER	Exertional rhabdomyolysis
FAMS	Fatigued Athlete Myopathic Syndrome
FT	Flight time
HE	Haematoxylin & Eosin
HR	Heart rate

HS	Heel strike
iEMG	Integrated electromyography
LBM	Lean body mass
LTV	Lean thigh volume
MDA	Malondialdehyde
MG	Medial gastrocnemius
MHC	Myosin heavy chain
NM	Neuromuscular
NOV	Novice runner
PB	Personal best
PTRS	Peak treadmill running speed
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPE	Rating of perceived exertion
S7	Sum of seven skinfolds
SDH	Succinate dehydrogenase
SED	Sedentary group
SF	Stride frequency
SL	Stride length
TO	Toe off
TRF	Terminal restriction fragment
VE	Ventilation rate
VET	Veteran runner
VL	Vastus lateralis
VM	Vastus medialis
VO₂	Volume of oxygen inspired
VO₂ max	Maximum volume of oxygen inspired

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VE	Ventilation rate
VET	Veteran runner
VL	Vastus lateralis
VM	Vastus medialis
VO₂	Volume of oxygen inspired
VO₂ max	Maximum volume of oxygen inspired

ABSTRACT**OBJECTIVE**

Endurance running is a sport that has grown tremendously over the last few decades with more recreational athletes participating in races than ever before. As a consequence, more people are undergoing large volumes of endurance training and racing. While moderate levels of exercise undoubtedly have numerous health benefits, the effects of chronic exposure to endurance running on the performance, physiological, neuromuscular and skeletal muscle characteristics of runners is an area less well explored.

Endurance running is a significant stressor to the human body since each bout of training or racing induces a stress response within the body. Hans Selye's model of stress response describes the change in resistance of an organism to a stressor over time. By way of analogy, runners who are able to manage the stress of endurance running appropriately build up resistance to the stressor and are able to participate in the sport of endurance running for a number of years. Chronic exposure to this stressor, however, may ultimately lower runners' resistance to endurance exercise, leaving them more vulnerable to maladaptations. This may explain why some athletes develop intolerance to exercise, which may manifest as an exercise-associated fatigue accompanied by a sudden, inexplicable decline in performance and an inability to sustain previous volumes of training.

Age-group world records for endurance races show a clear decline in performance with age. It is likely that masters runners may experience an intolerance to exercise primarily due to the age-related changes in their bodies, and specifically of those systems and organs most utilised during running. A more disturbing phenomenon, however, is that of younger runners becoming exercise intolerant. One study described exercise intolerance in athletes who were only 40 ± 10 years old. The muscle of these athletes had greater levels of markers of structural pathology and

showed evidence of having undergone more regeneration compared to age- and mileage-matched apparently healthy athletes. The authors attributed their intolerance to exercise to their large volumes of endurance training and racing which in turn compromised their skeletal muscle.

Therefore, the objective of this thesis was to examine the effects of chronic exposure to endurance running on the athlete, with particular emphasis on skeletal muscle. More specifically, the aims were:

- (i) To examine the longitudinal changes in performance of four age groups of runners who completed ten annual 56 km ultra-endurance marathon races.
- (ii) To compare the physiological and neuromuscular characteristics of veteran and novice runners while running.
- (iii) To compare the responses of veteran and novice runners to exercise-induced muscle damage.
- (iv) To compare the levels of structural pathology and the regenerative capacity of the skeletal muscle of veteran runners and sedentary individuals.
- (v) To examine cases of athletes suffering from intolerance to exercise.

METHODS

Study 1

This study examined the longitudinal changes in performance of runners competing in an annual 56 km ultra-marathon. The race times of runners who were either 20 (n = 20), 30 (n = 62), 40 (n = 97) or 50 (n = 15) years old for their first race were analysed over their ten year race span.

Study 2

The physiological and neuromuscular characteristics of veteran (experienced) and novice (inexperienced) runners were assessed both while running at a set-paced sub-maximal effort and during a 5km time trial. Seven veteran runners (mean age: 40 ± 8 years, mean years of running: 15.1 ± 7.3 years, mean accumulated running distance: $45\,285 \pm 56\,154$ km) were compared to six novice runners (mean age: 29 ± 7 years, mean years of running 2.6 ± 1.7 , mean accumulated running distance: $3\,275 \pm 2\,278$ km). During the sub-maximal effort, heart rate, rating of perceived exertion, oxygen uptake, ventilation rate, stride contact time, flight time, frequency and length, as well as pre-activation and ground contact phase electromyography activity in the vastus lateralis, vastus medialis and medial gastrocnemius muscles were measured. Except for the respiratory measurements, these parameters were also recorded during the 5 km time trial.

Study 3

The same veteran and novice runners then underwent a single bout of exercise designed to induce muscle damage. The extent of their sustained damage, as well as their recovery time courses, were assessed by measuring serum creatine kinase activity, muscle power and subjective muscle pain before, immediately after and during the week following the insult. To compare the effect of exercise-induced muscle damage on the running capacity of the veteran and novice groups, the same physiological, neuromuscular and performance characteristics in the sub-maximal run and the 5 km time trial described for the study above were assessed before and one week after the bout of damaging exercise.

Study 4

To determine the molecular effects of several years of endurance running on skeletal muscle, biopsies of the vastus lateralis muscle from 19 veteran endurance runners (mean age: 43 ± 7 years) were compared to those of 19 sedentary individuals (mean age: 39 ± 10 years). The runners had been involved in endurance running for approximately 15 years, during which time they had covered about 50 000 km in training and racing. Laboratory analysis determined the morphology, degree of structural pathology and telomere lengths of the muscle samples.

Study 5

Finally, five veteran runners who suffered from exercise intolerance were examined as case studies. Their running training, racing and medical histories as well as the circumstances surrounding the onset of their intolerance to exercise were explored. The runners were evaluated clinically and special investigations such as analysis of the biopsies of their vastus lateralis muscle were assessed as described for the veteran runners in the preceding study.

RESULTS AND DISCUSSION

Study 1

The pattern of change in ultra-endurance race speed over a ten year period was similar for all four age groups of runners who completed their first race at either 20, 30, 40 or 50 years of age. The extent of change in performance, however, was greater in runners younger than 40 years of age than in runners older than 40 years. The veteran runners of a given age, i.e. those who had competed in ultra-endurance races over a span of ten years, ran at a similar speed to novice runners of the same age. The most striking finding of this study was that despite similar current performance times, and irrespective of age, the novice runners would almost certainly improve their performance, while the veteran runners were more likely to continue slowing down.

Study 2

The main finding was that in comparison to the novice runners, the vastus lateralis and vastus medialis muscles of the veteran runners showed less electromyography activity during the pre-activation phase of the stride at steady state of the sub-maximal run. The novice runners had a longer ground contact component of the stride during the warm-up phase of the sub-maximal run. During the time trial, however, the two groups were indistinct with respect to physiological and neuromuscular variables measured in this study. The difference in pre-activation phase electromyography activity between the veteran and novice runners at a sub-

maximal pace suggests that the quadriceps muscles of the veteran runners may have a decreased capacity for shock absorption. However, since there were no differences between the groups during the time trial, it is not clear why, regardless of age, veteran runners are almost certainly beyond achieving personal best performance times, yet novice runners are still likely to be able to improve their endurance performance.

Study 3

The downhill run caused a similar extent of sarcolemmal damage in the muscle of both the veteran and the novice runners and there was no difference between their recovery time courses. One week after the insult, the physiological and neuromuscular characteristics measured during the sub-maximal and maximal running tests were not different from the values measured before the downhill protocol in both the veteran and novice runners. Therefore, it seemed unlikely that a vulnerability to, or incomplete recovery from, exercise-induced muscle damage could explain the decline in performance observed in veteran runners who have had a high exposure to running over the years.

Study 4

The minimum, mean and maximum telomere lengths measured in the vastus lateralis muscle of the runners were similar to those of the respective values of the sedentary individuals. However, minimum telomere length (a proxy for the regenerative capacity of skeletal muscle) measured in the muscle of the runners was correlated to their years in distance running and their hours spent training. Together these findings suggest that while exposure to endurance running may increase the demand for repair and regeneration in muscle, the regenerative capacity of the muscle of veteran runners is no more compromised than that of sedentary individuals. This study also showed that the muscle of endurance trained individuals was distinct from the sedentary population in terms of markers of structural pathology, as the muscle of the runners had greater levels of internal nuclei and subsarcolemmal mitochondrial aggregations. However, since the levels of these markers were not associated with dysfunction of the muscle of the veteran runners, they were not deemed to be pathological in nature.

Study 5

Examination of the five veteran athletes that presented with exercise intolerance showed that the triggers for, and the manifestation of, exercise intolerance for each case were unique. The most important lesson was that a high exposure to endurance running alone does not necessarily cause athletes of varying ages to become intolerant to exercise. Instead, the presence of co-stressors such as viral infection, behaviour, or underlying myopathy, in conjunction with exposure to endurance running, were associated with the development of exercise intolerance. Furthermore, the skeletal muscles of such cases do not necessarily have structural pathology or shortened telomeres.

CONCLUSION

In conclusion, the data from this thesis showed that an apparently healthy endurance runner can, under normal circumstances, tolerate a high exposure to endurance training and racing with no obvious ill effects. Furthermore, the skeletal muscle is a robust organ with a large capacity to tolerate a chronic exposure to endurance running. While the stress of aging might explain the decline in performance experienced by masters runners, it does not explain the similar decline observed in younger runners. Similarly, since athletes with varying degrees of exposure to running may present with exercise intolerance, a high exposure to this stressor cannot be the singular cause of the reduced performance capacity of veteran athletes. Instead, the presence of co-stressors in combination with the stress of endurance running appears to be a far better predictor of a runner's likelihood of developing exercise intolerance. This modifies previous thinking, which attributed maladaptations within the system of the endurance runner to a high exposure to endurance exercise.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION AND SCOPE OF THE THESIS

Participation in ultra-endurance running has grown tremendously in South Africa since 1921 when 34 runners participated in the inaugural 90 km Comrades Marathon in KwaZulu Natal. In contrast, 12 066 runners stood at the start line of this same race in 2006. In the past, ultra-distances were the domain of professional sports people, whereas today more amateur athletes are accumulating large distances of running training and racing over a number of years. Preparation for such races requires dedicated training particularly in the form of road running where individuals cover between 60 and 200 km per week. While regular physical activity has been shown to have numerous health benefits (108, 114, 170, 184, 194, 211), there is less research exploring the effect that many years of high volume endurance running might have on the human body. How much running can the body tolerate and is there a point at which the cumulative stress of endurance running begins to outweigh its positive adaptations?

Derman et al. (48) identified a group of endurance athletes experiencing intolerance to exercise which was inappropriate for their age. Common to these athletes was a history of a high volume of training, a current inability to tolerate previous levels of training, a decline in performance, exercise-associated fatigue and accompanying skeletal muscle symptoms including soreness, tenderness, stiffness and exercise-associated cramps. Despite being evaluated by a range of medical experts, no formal diagnosis could explain the exercise intolerance and symptoms experienced by these athletes (48). What was discovered however, were abnormalities within their skeletal muscle such as greater levels of markers of structural pathology (75) and shorter telomeres, an indirect measure of replicative history of the satellite cells, compared to asymptomatic, age-matched athletes (33). This body of research

indicated that following a high exposure to endurance exercise, some athletes become intolerant to former levels of training and racing. Furthermore, abnormalities within the skeletal of these athletes appeared to be associated with their condition. What was not clear, however, was whether exposure to endurance running was the only stressor that led to their intolerance, or whether compounding factors (co-stressors) either contributed to or exacerbated the stress of running.

Therefore, the objective of this thesis is to examine the effects of chronic exposure to endurance running on the athlete, with particular emphasis on skeletal muscle. In preparation for the exploration and discussion of this topic in the subsequent experimental chapters, the review of literature focuses on the organ of study: skeletal muscle, the system: the human endurance runner, the stressor: endurance running, and selected co-stressors: aging, acute infection, metabolic myopathies and behaviour of the athlete.

1.2 THE ORGAN: SKELETAL MUSCLE

1.2.1 Overview of structure, properties, function and plasticity

The structure, properties, function and plasticity of skeletal muscle will be reviewed briefly in this section as a detailed discussion of these topics is beyond the scope of the thesis.

1.2.1.1 Skeletal muscle structure

In brief, skeletal muscle is made up of highly differentiated, elongated, multinucleated cells called muscle fibres or myofibres. These elongated cells in turn consist of parallel bundles of myofibrils which appear striated under the microscope due to their serially arranged sarcomeres containing the contractile proteins actin and myosin (16). Each myofibre is innervated by a single branch of a motor neuron, so that each motor neuron together with the myofibres that its branches innervate forms the basic contractile unit of skeletal muscle known as the motor unit. Myofibres are bundled together and surrounded by a connective tissue sheath called the perimysium. A number of these bundles are in turn grouped together by the epimysium to make up the entire muscle. The connective tissue framework within each muscle combines the contractile myofibres into a functional unit, in which the contraction of the myofibres is transformed into movement via myotendinous junctions at their ends which attach the myofibres to tendons, and in turn to bone (25) (Figure 1.1).

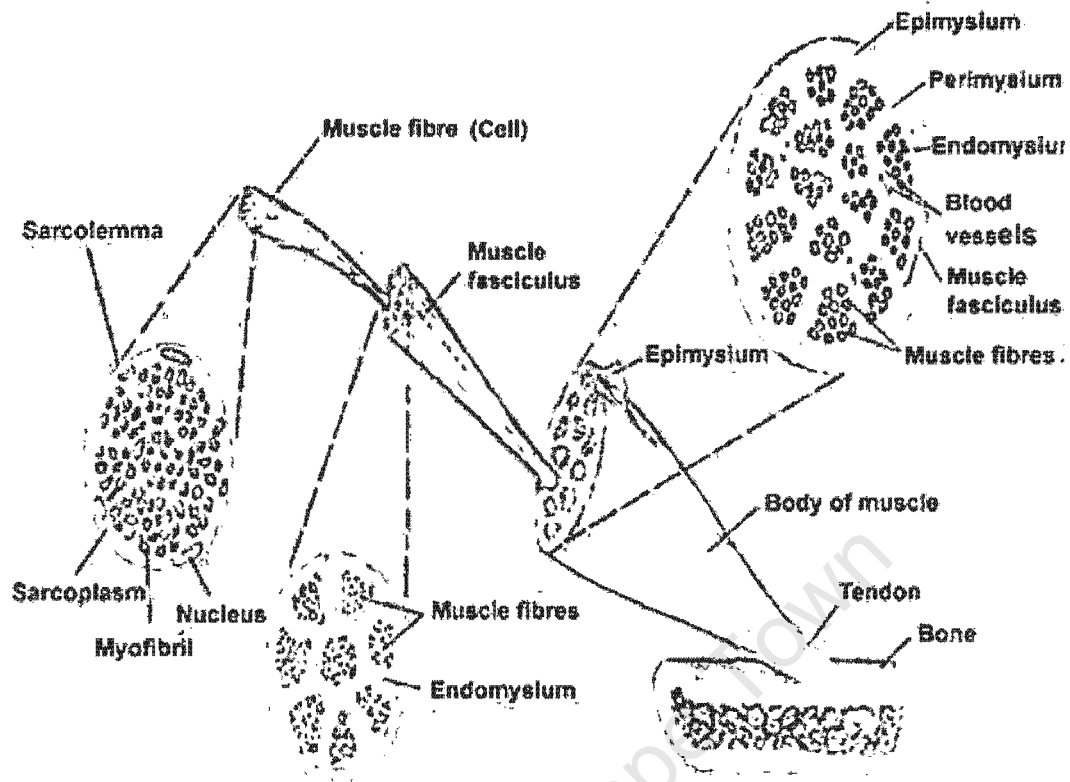


Figure 1.1 The hierarchical structure of skeletal muscle (modified from the public domain: <http://faculty.etsu.edu/currie/images/muscle12.jpg>)

1.2.1.2 Skeletal muscle properties

Mature skeletal muscle is composed of a mixture of myofibres with different physiological and biochemical properties. Although contraction is initiated at a neuronal level, the contractile properties of the muscle are mainly determined by the molecular differences in the primary calcium-dependent proteins involved in the electron coupling process. The natures of the fibres, which range from the slow-contracting fatigue-resistant type 1 fibres to the fast-contracting non-fatigue-resistant type 2 fibres, also affect contractile property. In addition, properties of the innervating nerve, including firing frequency, contribute to differences in the contraction speed of single fibres (25, 63). Contractile properties are important as they ultimately determine the phenotype of a given muscle. For example, the muscle fibres making up slow motor units have small cross-sectional areas, very few myofibres per motor unit, a low shortening velocity and contain many mitochondria which make them well suited to performing tasks requiring low force or power and

high precision, and that are of an endurance nature. The fast motor units are the antithesis and used for high force or power tasks of shorter duration (16).

1.2.1.3 Skeletal muscle function

Skeletal muscle is a large organ constituting approximately 40 % of total body mass in healthy sedentary males, approximately 30 % in females (104), and making up to 50 % of body mass in some athletes. Skeletal muscles are primarily involved in locomotion, posture and breathing (25) but this important metabolic organ also plays a large role in the energy balance of the human body (63).

1.2.1.4 Skeletal muscle plasticity

Stimuli such as exercise or denervation affect skeletal muscle plasticity. Adaptations include modifications of cellular (for example mitochondria and myofibrils) and extracellular (for example capillaries, nerves and connective tissue) components, altered gene or protein expression and even changes at the levels of transcription, translation, protein translocation, assembly, degradation and recycling. Together these adaptations contribute to changes in the contractile function of the muscle in response to the initial stimulus (63).

1.2.2 Damage to skeletal muscle

Skeletal muscle sustains injury through a variety of mechanisms including disease, exposure to agents that are toxic to muscle, direct trauma, ischemia, exposure to extreme temperatures and through the muscles own contractions (16). This review will focus on contraction initiated injury as this is the predominant type sustained during endurance exercise.

1.2.2.1 The mechanism of exercise-induced muscle damage

It is apparent that acute bouts of either resistance (147), sprint (106) or endurance (201) exercise result in damage to skeletal muscle. The types of injury sustained

include sarcolemmal disruption, swelling or disruption of the sarcotubular system, distortion of the myofibrils contractile components, cytoskeletal damage and extracellular myofibre matrix abnormalities. In particular, levels of muscle damage are greater after activities predominated by the muscle lengthening under tension (eccentric contractions) compared to activities predominated by either shortening (concentric) or static (isometric) contractions (65). Endurance running involves repetitive cycles of the muscle shortening and lengthening. While the forces generated during running are not necessarily large, it is likely that their repetitive nature leads to muscle damage.

Three factors have been hypothesised to explain how exercise causes damage to muscle: mechanical disruption to the myofibre, disturbances in intra-cellular calcium homeostasis and / or the inflammatory response (29, 54, 59). Injury through mechanical disruption to the myofibre is thought to occur when individual sarcomeres are stretched excessively, presumably damaging the structural components within or between the sarcomeres (16). Electron microscopy supports this as disrupted sarcomeres, Z-line streaming, t-tubule damage and myofilament disruption are commonly observed in skeletal muscle immediately after the damaging activity (30). Myofibrillar damage is visible immediately following exercise, and increases in severity one to three days after the damaging insult (65) before repairing two to three weeks later (29). The injury may be focal in nature, involving only a few neighbouring sarcomeres, or more widespread spanning an entire fibre (16). The ensuing cascade of events leads to the infiltration of the damaged area by inflammatory cells, usually three to four days after the insult (30) and phagocytic cells remove debris such as disrupted myofilaments, various cytosolic structures and the damaged sarcolemma. In only the most severe cases is the complete myofibre broken down, leaving the empty basal lamina in tact (16). Mechanical disruption to myofibres may explain muscle damage during eccentric contractions where the forces developed may be large enough to cause physical shearing of either the myofilaments or membranes. However, whether the forces developed during concentric contractions are sufficient for mechanical disruption is under debate (59).

Mechanical factors may cause the initial damage, but they do not explain why the muscle injury worsens in the days following the damaging bout of exercise. A disrupted calcium homeostasis within the muscle cell, or mediators of the inflammatory response may, however, account for this delayed increase in damage (29). Disruption of the sarcolemma of the myofibres is common following a bout of damaging exercise. This increases the muscle cells permeability, explaining the presence of molecules which are usually sequestered in the cytosol, including creatine kinase, in the plasma. One explanation for the loss of membrane integrity following muscle injury might be an excessive increase in cytosolic calcium ions. It has been suggested that an altered calcium homeostasis within the cell following injury to either the myofibre sarcolemma or the sarcoplasmic reticulum leads to an increase in calcium dependent proteolysis that would initiate tissue degeneration (25, 29, 59). Alternatively, damage to skeletal muscle is known to initiate an inflammatory response, similar to that observed in reaction to an infection, to set in motion clearing of damaged tissue and signal subsequent repair and growth (59). Neutrophils which infiltrate damaged muscle within hours of injury have been associated with an increase in calcium-stimulated cysteine protease which may increase protein degradation. Macrophages may produce reactive oxygen species (ROS) thereby compounding existing muscle damage, explaining the delayed increase in ultra-structural damage some days after the initial insult. Macrophages also give rise to cytokines which may exacerbate damage by enhancing ROS production and the release of other proteolytic enzymes (29).

1.2.2.2 Exercise-induced muscle damage following endurance exercise

The standard marathon has routinely been used to determine the type and extent of damage sustained by the skeletal muscle of trained runners during a single bout of endurance exercise. Analysis of blood, urine and muscle samples from runners provide a broad range of tools to assess exercise-induced muscle damage. The haematology and biochemistry parameters of 37 runners were measured before, as well as four and 24 hours after, a standard marathon. Evidence that the race elicited severe muscle damage included markers such as elevated levels of myoglobin, creatine kinase (CK) activity, creatine kinase-myocardial band (CK-MB) and aspartate aminotransferase (AST). Many other blood biochemistry parameters were also altered following the marathon. For example, the concentration of serum

glucose, total protein, albumin, uric acid, calcium, phosphorus, blood urea nitrogen (BUN), creatinine and bilirubin were also elevated four hours after the race, as were the activities of alkaline phosphatase and alanine aminotransferase (ALT). The levels of magnesium, chloride, carbon dioxide and globulin were all lower than normal values at this time. Twenty four hours later myoglobin, CK, CK-MB, AST, ALT, uric acid, BUN, creatinine and bilirubin remained elevated and levels of sodium, potassium, chloride, carbon dioxide and cholesterol had fallen to below pre-race levels. Complete blood count results indicated significant leukocytosis (increased white blood cells), increased levels of circulating neutrophils, haemoglobin and a higher platelet count, while the levels of lymphocytes, eosinophils, haematocrit and red blood cell count decreased after the marathon. The authors suggest that the blood count results reflected an inflammatory response, most likely caused by tissue damage (112).

Another study showed that serum myoglobin, creatine kinase and malondialdehyde (MDA) levels in trained runners were elevated after a 21.1km run. The authors suggested that the increased MDA levels indicated free-radical induced cell membrane damage as opposed to mechanical damage (72). This is plausible since exhaustive endurance exercise is known to increase oxygen consumption, thereby stressing the anti-oxidant system to reduce elevated levels of reactive oxygen species (ROS). Should the levels of ROS become too high during physical activity, exercise-induced oxidative stress increases and so too may tissue damage, which may be associated with cell dysfunction related to energy production and excitation-contraction coupling (95).

A number of studies have examined skeletal muscle biopsies of runners after a marathon to assess exercise-induced muscle damage. For example, Hikida et al. showed that the medial gastrocnemius muscle of experienced runners immediately on completion of a marathon contained free erythrocytes and mitochondria in the extra-cellular spaces, crystalline inclusions within the mitochondria, Z-line streaming, empty basal lamina tubes and evidence of rhabdomyolysis (86). The authors concluded that other than these observations, the muscle of the runners immediately post-marathon appeared normal. During the week following the marathon, however, the abnormalities increased, being the worst between one and

three days later. The most common findings were sarcolemma disruption, degenerating or disorganized myofibrils and the presence of satellite cells, which the authors interpreted as signs of necrosis. The disrupted sarcolemmas were often associated with contracture knots (a shortened sarcolemma), swollen mitochondria and large gaps in the muscle fibres. They also found free mitochondria and myofibrils in the extracellular space and focal disruption or extensive streaming of the Z-line. Most of the samples contained erythrocytes, both extra-vascularly and within the fibres, and all samples contained leukocytes including neutrophils, lymphocytes, monocytes and mast cells. Alongside the muscle fibres were macrophages with large vacuoles containing cellular debris. Satellite cells, which were present at all stages during the week post-marathon, but were most prevalent in the latter part of the week, did not appear to be involved in the regenerative activities. Necrotic cells were identified as those with basal lamina tubes, and were found in many of the samples up to five days after the marathon (86).

Warhol et al. also studied biopsies of the gastrocnemius muscle of veteran runners following a standard marathon (201). Immediately after the race, the biopsies showed injury to the sarcoplasmic units in all the runners, but the severity varied between the individuals. There was also significant myofibrillar lysis and disappearance of the sarcoplasmic reticulum. Mitochondrial damage was evidenced by the dissolution of cristae and loss of the mitochondrial matrix and these mitochondrial changes appeared to be independent of any myofibrillar changes. Endothelial cells were also damaged and the focal damage observed in the individual fibres was indicated by empty myotubes and interstitial collagen, suggesting fibre degeneration. There were no signs of cellular inflammation in any of the samples, indicating that acute necrosis had not occurred. The biopsies also revealed that the skeletal muscle of endurance runners had abundant glycogen stores and morphologically abnormal mitochondria (201).

One week after the run, the muscle of the runners showed signs of repair, most fibres had replenished their glycogen stores and satellite cells and interstitial cells resembling fibroblasts were also noted. Most of the damage was repaired within four weeks of the marathon when biopsies showed that few damaged myofibrils remained, glycogen stores were fully replenished and numerous mitochondria were

present. Interestingly, there were still internal nuclei, suggesting that regeneration was still occurring. Eight to ten weeks after the run, biopsies showed the injury to be resolved. There were however, satellite cells still present, which appeared to be metabolically active with numerous polysomes and the development of endoplasmic reticulum. The presence of rough endoplasmic reticulum and Golgi complexes suggested that the muscle fibres were regenerating. Abnormalities of mitochondrial size and shape, however, persisted (201).

Warhol et al. proposed that the focal damage seen in the sarcomeric units of runners after a standard marathon may be related to the metabolic stress of the event, since restoration of the mitochondrial architecture and repair of the sarcomeric damage corresponds with repletion of the glycogen stores (201). Hikida et al. suggested that mechanical trauma, inhibition of adenosine triphosphatase (ATP) synthesis and changes in the sarcolemma properties, which may result in ionic imbalances within the cell, may all contribute to the damaging effects as a consequence of the stress of a marathon. In particular, an increase in intra-cellular calcium levels may be responsible for necrosis as it is thought to activate intracellular neural protease, which may damage the Z-line. Alternatively, a change in the level of potassium concentration may lead to necrosis (86).

In addition to the muscle damage induced by racing, Kuipers et al. observed low levels of pathology suggestive of ongoing muscle damage in the gastrocnemius muscle of runners training for a marathon (116). The frequency of these markers of structural pathology increased with the distance covered during training and not the intensity of training. Importantly, exercise-induced muscle damage sustained through endurance running is thought to be transient (116), reversible with rest (201) and presumably contributes to the adaptation to running (178). In fact, repetitive bouts of exercise-induced muscle damage have been shown to have a protective effect on subsequent bouts (126).

From the perspective of endurance runners, the development of exercise-induced muscle damage may manifest as delayed onset muscle soreness 24 to 48 hours following exercise, peaking between 24 and 72 hours later and resolving within five

to seven days following the initial insult (54). In addition to the muscle soreness, runners may experience a temporary decrease in strength (54) and altered neuromuscular characteristics such as a reduced capacity to produce maximum force as well as a slower rate of force development, indicative of reduced muscle stiffness (139) and less efficient contractile mechanisms (140) following a marathon.

1.2.2.3 Exertional rhabdomyolysis

Extreme levels of exertion that stress muscle to the point of large increases in serum enzymes, rhabdomyolysis, myoglobinuria and acute renal failure (ARF) can be life threatening (107, 124). While training provides some degree of resistance to rhabdomyolysis, exertional rhabdomyolysis is common and there is no known myopathy which increases the likelihood of its occurrence (107). Briefly, the integrity of the muscle cell membrane is compromised, so that the contents of the cell leak into the plasma (202). Biochemical findings in cases of acute rhabdomyolysis include the presence of the haem pigment in the urine, elevation of serum creatine kinase or aldolase, hyperkalaemia, hypocalcaemia, hyperphosphataemia, hyperuricaemia, hypoalbuminuria, a high creatinine:BUN ratio and disseminated intravascular coagulation (107). Excessive release of myoglobin from muscle cells into circulation, which is filtered in the kidney and excreted in the urine, leads to a high risk of acute renal failure since the passage of myoglobin through the kidney is thought to be directly toxic to the renal tubule (6). These serious biochemical complications, along with acute respiratory distress syndrome may appear in the 24 hour period after the event (107). The most sensitive plasma indicator of rhabdomyolysis is elevated creatine kinase activity. Should this enzyme be elevated more than five times above the normal level, skeletal muscle rhabdomyolysis is indicated, in the absence of brain or cardiac infarction (202). The clinical features of rhabdomyolysis include acute muscle necrosis, swollen, tender muscle and limb weakness, and may be accompanied by fever, leukocytosis, myoglobinuria and ARF (202).

Although there are many possible causes of rhabdomyolysis, there are two common events which occur in all aetiologies. The first is direct injury to the sarcolemma and the second is failure of energy supply within the muscle cell. Both events lead to a rise in the intracellular calcium levels. A rise in intracellular calcium is considered to

be caused by a disruption of the calcium and sodium ion fluxes across the sarcolemma and the sarcoplasmic reticulum. The major places where the calcium flux may be disrupted are malfunctioning sodium / potassium – adenosine triphosphatase (ATPase) pumps in the sarcolemma, calcium-ATPase pumps in the sarcoplasmic reticulum, ATP depletion, a malfunctioning ryanodine receptor located in the sarcoplasmic reticulum, or an error in the cytoplasmic binding loop on the dihydropyridine-sensitive voltage-dependent calcium channel in the T-tubule in the sarcolemma. Activation of calcium-dependent neutral proteases and phospholipases result in destruction of myofibrillar, cytoskeletal and membrane proteins, and lysosomal digestion of muscle fibre contents occurs. These enzymes have high metabolic requirements, which accelerate the consumption of ATP. The destruction of the myofibrillar network speeds up the disintegration of the muscle fibres. To date, two therapeutic modalities have been shown to have a protective effect against exercise-induced and hyperthermic muscle damage. Nifedipine blocks influx of calcium across the T-tubules, and dantrolene impedes calcium release from the sarcoplasmic reticulum (202). In summary, skeletal muscle is therefore vulnerable to minor levels of damage as induced by acute bouts of exercise through to extreme disruption that is life-threatening in cases of exertional rhabdomyolysis. That this organ is well equipped to recover from and adapt to these insults, will be discussed in the next section.

1.2.3 Repair and regeneration of skeletal muscle

1.2.3.1 Overview

Adult skeletal muscle is a post-mitotic, stable tissue that easily copes with the wear and tear induced by everyday living. However, this robust organ also has the ability to repair and regenerate very effectively in response to severe injury or damage induced by direct trauma. Depending on the severity of the injury, either necrosis occurs or the muscle fibre is repaired (25). Minor disruptions to the thick or thin filaments of skeletal muscle only require the resynthesis of the involved molecules. More severe damage, however, requires regeneration of the disrupted section of the fibre, or even of the entire cell. In cases in which regeneration of a muscle fibre is

required, satellite cells (refer to section 1.2.3.2) are activated and drawn into the regeneration process (16).

Skeletal muscle repair is composed of two phases: a degeneration phase followed by a regeneration phase. The degeneration phase is characterised by the activation of inflammatory and myogenic cells. Cytokines from inflammatory cells and growth factors are released from the extracellular matrix or from the injured muscle fibres (212). Within one to six hours after damage, neutrophils invade the injured site, while macrophages take over as the dominant inflammatory cell about 48 hours post injury. Therefore the main histologically observable characteristics in muscle during the degeneration phase are fibre necrosis and the appearance of non-muscle mononucleate cells (25).

The regeneration phase is characterized by activation and proliferation of satellite cells so that either damaged myofibres can be repaired or new muscle fibres can be formed. Following fusion of the new myogenic cells, the newly formed fibres increase in size and the myonuclei move to the periphery of the cell. Signs of regeneration can be visualised using histological staining. Cross-sections of skeletal muscle might show the appearance of smaller fibres and fibres with centrally located nuclei. Splitting or branching fibres, possibly due to the incomplete fusion of regenerating fibres within the same basal lamina, would be seen in longitudinal sections (25).

1.2.3.2 The role of satellite cells

Since skeletal muscle is a post-mitotic tissue, specialized muscle pre-cursor cells called satellite cells are required for muscle fibre repair (161) and for the replacement of any myonuclei lost following damage (9). These mononucleate cells reside beneath the basal lamina of the muscle fibre in a state of quiescence (129) characterized by a cessation of mitosis and loss of organelles (167). During the regeneration phase, quiescent satellite cells all along the fibre are activated to enter the cell cycle. Activation of satellite cells requires the up-regulation of muscle transcription factors and muscle specific genes. This is communicated intrinsically through cell-to-cell and cell-to-matrix interactions, and extrinsically by extracellular

secreted factors. The actual stimulus or stimuli for the activation of satellite cells may be a consequence of the muscle injury itself where growth factors are released from the injured muscle cell (212). Alternatively, molecules released from inflammatory cells, invading macrophages, soluble factors from connective tissue and extract from the injured fibres have also been proposed as possible stimuli (25, 212). Since a detailed discussion of the signalling events leading to the activation of quiescent satellite cells is beyond the scope of this thesis, the reader is referred to a thorough review of this topic (80).

Activated satellite cells can migrate in both directions across the basal lamina and often travel considerable distances to the site of injury (9). Satellite cells then proliferate to increase the number of myogenic cells available for repair (25). Following the proliferation phase some of the satellite cells become terminally differentiated. Differentiation of satellite cells is most likely controlled by a group of myogenic factors including MyoD and myogenin, which function as transcription factors to activate muscle-specific gene expression, either directly, or indirectly. The final decision of whether a myogenic cell divides or differentiates may be determined by a balance between growth and differentiation signals (212). Differentiated satellite cells then fuse either to damaged muscle fibres for repair, or to each other to form new fibres, often characterized by central nuclei, which grow to resemble original muscle fibres. Other satellite cells, however, leave the cell cycle before differentiating, return to the quiescent state and are restored under the basal lamina (129), presumably to replenish the pool of satellite cells to be used for subsequent rounds of regeneration (25) (Figure 1.2).

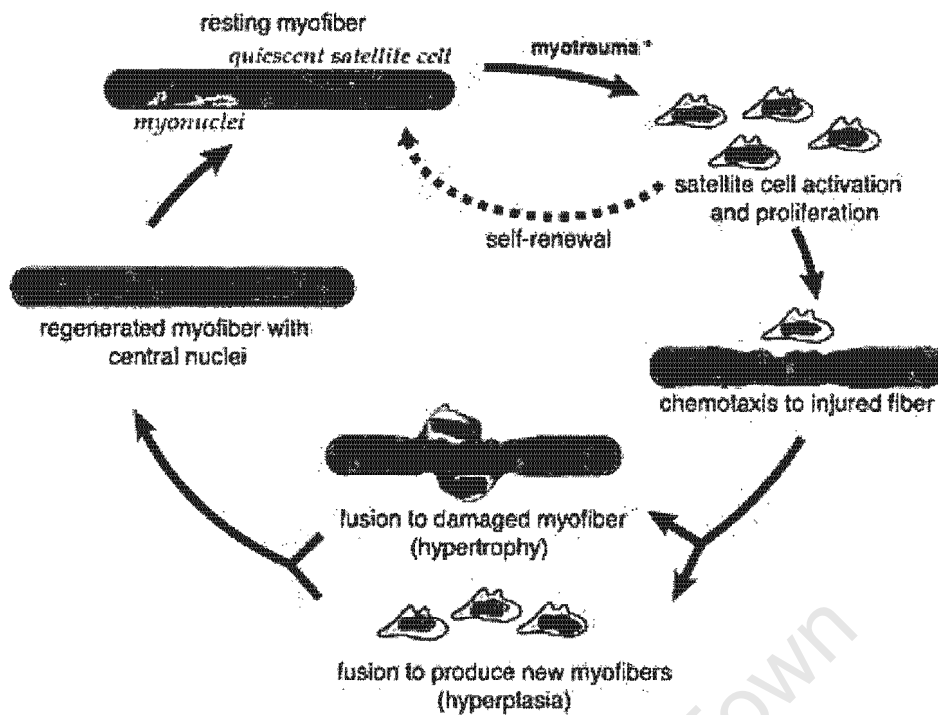


Figure 1.2 The role of satellite cells in the repair and regeneration of muscle fibres in response to injury. Following injury to the muscle fibre, satellite cells are activated and proliferate to increase the pool of satellite cells available for repair. Some of the newly formed satellite cells return to the beneath the basal lamina and become quiescent in a self-renewal process while others become terminally differentiated. The differentiated satellite cells then move to the area of damage, and depending on the nature of the injury, either fuse to the damaged fibre, or align and fuse to each other to form a new cell. The regenerated fibre contains central myonuclei, which later move to the periphery of the cell. (Diagram used with permission of the publishers of Hawke T. J. and Gary, D. J., JAP, 91:534 – 551, 2001)

It is possible that satellite cells are not the only source of myonuclei during muscle repair. Progenitor cells from bone marrow and muscle adult stem cells have been shown to differentiate into muscle cells *in vitro* and to contribute to muscle regeneration *in vivo*. However, this is likely an unusual occurrence brought on by specific cellular and environmental conditions. Alternatively, regenerating myofibres may gain new myonuclei through the recycling of existing myonuclei from severely injured muscle fibres (25).

The potential for the repair and regeneration of skeletal muscle is affected by the number of available satellite cells, the proliferative capacity of these satellite cells and the speed of the response to the injury (162). Since satellite cells are mortal and have a finite capacity to proliferate, the major determinant of the ability of satellite cells to proliferate appears to be the proliferative history of the cells. This is simply the previous number of degeneration and regeneration cycles the cells have undergone as determined by growth, disease or injury (167). Extensive proliferation would eventually lead to a reduction in the mitotic reserve of the cells which are then said to reach replicative senescence (21).

Satellite cells isolated from infants have a greater proliferative capacity compared to those isolated from older individuals, with little difference existing between young and older adults. Decary et al. suggest that this is due to the fact that satellite cells need to divide frequently to maintain muscle growth during development thereby utilising a large proportion of their replicative capacity before reaching senescence (45). Satellite cells are activated less frequently once muscle cells have reached their adult size, explaining the relative stability of their remaining replicative capacity during adulthood. The primary reason for the activation of satellite cells in adult muscle is to repair muscle in response to injury (45). A gradual decrease in the number of skeletal muscle satellite cells has been observed with increasing age during adulthood. Furthermore, the satellite cells of the older individuals displayed different morphological characteristics including thinner myotubes and less well organized desmin filaments compared to the muscle of the younger individuals. Regardless of the age of the donor the pre-senescent cells were still able to fuse correctly, indicating that cell differentiation was not compromised with age. Once cell senescence was reached, however, they fused less effectively and more slowly (161).

While everyday wear and tear on adult skeletal muscle does not appear to impact the regenerative capacity of satellite cells, excessive demands may result in the premature senescence of satellite cells, and consequently, impaired muscle regeneration. This is illustrated in patients with Duchenne's muscular dystrophy where skeletal muscle undergoes excessive degeneration regeneration cycles compared to healthy age-matched individuals. The satellite cells of these patients

are constantly activated to initiate repair and therefore reach senescence prematurely. Senescent satellite cells are unable to proliferate and cannot aid regeneration. Therefore the greatly reduced proliferative capacity of the satellite cells of Duchenne's patients ultimately limits the ability of their muscle to regenerate (44, 161).

Since exercise is known to damage skeletal muscle, it is conceivable that this stressor may increase the demand on the replicative capacity of satellite cells. Both single bouts of exercise and regular exercise programmes have been shown to activate satellite cells rapidly (167). In fact the pool of satellite cells can be increased within four days of a single bout of exercise, presumably reflecting the response of satellite cells to exercise-induced muscle damage. When training is stopped, a gradual reduction in the number of satellite cells in the pool is observed (101). Cramer et al. showed that after a single bout of unaccustomed, high intensity exercise, satellite cell activation was indeed increased, but that this did not automatically lead to terminal differentiation (38). There was no necrosis in the muscle fibres of these subjects since their muscle had no signs of lesion; nor was there evidence for disruption to either desmin or dystrophin in the cell membrane. Yet there was still an increase in the number of satellite cells. There was also an increase in the expression of the membrane-bound protein fetal antigen 1, a marker of muscle cell regeneration in adults. The authors suggested that either a subsequent bout of exercise, or myofibre lesions are necessary to stimulate terminal differentiation of the activated satellite cells (38). Therefore while single bouts of exercise do activate satellite cells, this may not necessarily stress their future replicative capacity since they do not automatically move into the differentiation phase of the cell cycle after activation.

An alternative explanation for the altered proliferative capacity of satellite cells is that of oxidative stress. Endurance exercise is known to increase levels of oxidation in human skeletal muscle (95). Furthermore, the proliferation capacity of satellite cells may be reduced as a result of exposure to oxidative stress (160). In summary, the potential for skeletal muscle to regenerate depends, in part, on the number of available satellite cells, their remaining proliferative capacity, as well as the impact of environmental stressors (138, 160, 162). The regenerative capacity of skeletal

muscle can be determined indirectly through measuring the telomere lengths of the deoxyribonucleic acid (DNA) in satellite cells (162).

1.2.3.3 Skeletal muscle telomeres

Telomeres are simple repetitive non-coding DNA sequences $(TTAGGG)_n$ located at the tips of eukaryotic chromosomes and are between 5 – 20 kilobase pairs (kbp) in length in humans (160). They are important for chromosome structure and function, as they play a protective role in preventing aberrant recombination of DNA and degradation of the ends of chromosomes (138). Each time a cell divides a small portion of the telomeric sequence is lost since the enzyme DNA polymerase is unable to replicate the ends of linear DNA (45). Immortal cells are able to maintain their telomere length with the help of the enzyme telomerase, thereby preventing the cell from reaching senescence and giving it the ability to replicate indefinitely. Healthy mortal cells such as satellite cells, however, do not express telomerase and their telomeres shorten by approximately 75 - 155 base pairs (bp) of telomeric sequence with each cell division (45, 46). Once a telomere reaches a critical length, which appears to be tissue specific, it signals the senescence of the cell (21) so that it can no longer play a role in regeneration (105). Therefore telomere shortening is a mechanism that ultimately limits the capacity of a cell to divide (46). The mechanism by which telomere length signals cell senescence, however, is not clear (21).

Telomere length is regarded as a record of the division capacity and replicative age of human somatic cells such as satellite cells (46). To determine telomere length, terminal restriction fragments (TRFs) are isolated from the DNA of a given tissue using the restriction enzyme *Hinf1*. TRFs contain the telomeric region with several kilobase pairs of the TTAGGG repeat, as well as a subtelomeric region of the non-TTAGGG DNA (44) (Figure 1.3). The TRFs isolated from the DNA of a given tissue are then separated by molecular weight on an agarose gel and visualised as a band (Figure 1.4). The minimum, mean and maximum limits of the band are calculated from the gel using software to determine the density of the band.

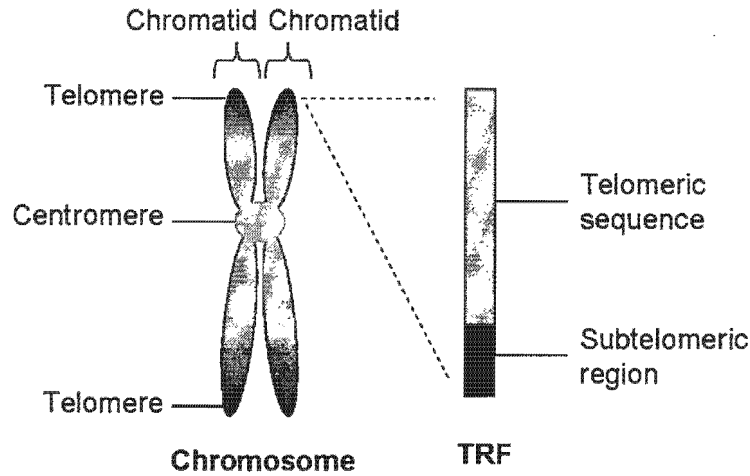


Figure 1.3 The terminal restriction fragment of a telomere. Telomeres are DNA repeat sequences $(TTAGGG)_n$ located at the tips of eukaryote chromosomes. The terminal restriction fragment (TRF) of DNA contains the non-coding but variable telomeric sequence as well as a constant subtelomeric region. TRFs are generated by digesting genomic DNA with the restriction enzyme *Hinf1* and used to estimate telomere length (44).

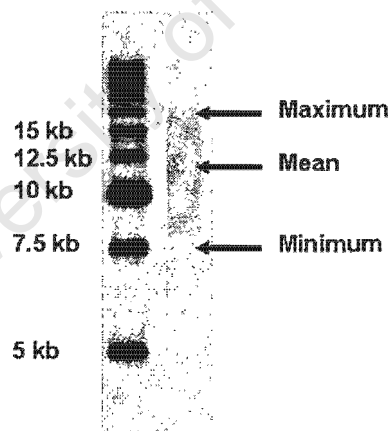


Figure 1.4 Representative autoradiogram displaying the band of terminal restriction fragments obtained from the DNA of a healthy sedentary individual. The left hand column contains molecular weight standards in kilobase pairs (kb) used as a scale of reference for the TRF sizes. The band in the column on the right contains the TRFs isolated from the DNA of one individual. The arrow pointing to the top end of the band represents the maximum TRF length measured for this individual and would correspond to the satellite cells having undergone the least replication. The arrow indicating the

mean TRF length is positioned where the majority of the fragments are found and represents the average amount of satellite cell replication having occurred. The minimum TRF length is shown by the arrow pointing to the smallest TRF fragments. These fragments would be from the satellite cells having undergone the most replication.

Mean telomere length is a good marker of the remaining replicative capacity in mitotic tissues such as liver and skin that undergo constant regeneration. This is not the case in post-mitotic tissue such as skeletal muscle since the majority of telomere shortening in satellite cells occurs during development when satellite cells proliferate constantly to generate new fibres for growth. During adulthood, satellite cells are primarily activated in response to muscle injury and the need for regeneration. Therefore mean telomere length of skeletal muscle is remarkably similar in young, adult and elderly humans, indicating that normal, uninjured muscle is very stable in terms of loss of DNA from early development through to old age (45). Since mean telomere length in healthy skeletal muscle represents the heterogeneity of the tissue, it is not a good marker of the replicative history of its satellite cells (162). Minimum telomere length measured in the skeletal muscle of healthy aging humans, however, decreases by approximately 13 bp per year. This suggests a continual, low level of turn-over in myonuclei. Therefore minimum telomere length, which corresponds to the satellite cell nuclei in skeletal muscle having undergone the most divisions (162), can be used as a measure of the regenerative history of satellite cells (44). A more simplistic view is that mean telomere length is indicative of the point at which development stopped, whereas minimum telomere length reflects the repair and regeneration that takes place from the end of development to old age.

In skeletal muscle, it is the telomeres of the satellite cells which are of interest, since it is their replicative capacity that determines the remaining regenerative potential of the muscle (162). The telomeres of satellite cells may shorten through the continuous degeneration and regeneration of muscle fibres, which requires the activation and proliferation of satellite cells, or through oxidative damage induced by stress. The muscular dystrophies, a group of disorders that are characterised by progressive weakness and wasting of the musculature, provide a model for telomere shortening associated with excessive degeneration regeneration cycles. Decary et

al. obtained biopsies from the quadriceps muscles of Duchenne's muscular dystrophy patients, from the deltoid muscle of patients suffering from limb girdle muscular dystrophy and from the quadriceps muscle of healthy controls (44). They confirmed that the muscle of the dystrophy patients was in a continuous state of regeneration. They also showed that the mean telomere lengths of all the dystrophy patients were significantly shorter than those of both the healthy age matched control group and even those of an aged population (44). This illustrates that increased telomere shortening occurs in situations where muscle undergoes extensive degeneration.

Oxidative damage has also been suggested as a mechanism for telomere shortening. For example, mothers looking after ill children were under chronic environmental stress and both their perceived stress and chronic stress levels were shown to be related to higher levels of oxidative stress and shorter leukocyte telomere length. That the activity of telomerase was also shown to be decreased in those individuals with higher levels of both perceived and chronic stress may provide a mechanism for the observed shortened telomeres (55).

The enzyme telomerase, a ribonucleoprotein, extends telomere lengths by adding TTAGGG repeats to the 3' end of the DNA strand (10, 73). *In vitro* experiments have shown that in cell strains without telomerase, telomeres shorten with each cell division and ultimately reach senescence. When telomerase was introduced to the cultured cells, mean telomere length remained constant while minimum telomere length was increased (79, 84) and cell senescence was avoided (74). Furthermore, telomerase has been shown to target cells with the shortest telomere lengths, presumably to restore telomere length to prevent cell dysfunction or death (84). It also seems that telomerase activity is associated with the cell proliferation status since it is more active in the cell cycling stages (and in immortal cells) and less active in quiescent differentiated cells (74)

It is usually only the mitotically active (germline or immortal) cells in a given tissue that express telomerase (74). For example, although telomerase is inactive in healthy mortal cells such as skin or breast tissue, it has been shown to be active in

tumours (immortal cells) of the same tissue (105). Although *in vitro* experiments have shown that the reconstitution of telomerase activity extends the replicative life span of the human skeletal muscle cell, (49, 210, 210), telomerase does not seem to be activated, or even present, in healthy mortal cells such as skeletal muscle *in vivo* (105). Therefore it seems unlikely that telomerase plays a role in extending telomere length in healthy skeletal muscle. This is in accordance with the observation that minimum telomere lengths measured in human skeletal muscle shorten with aging (162).

1.2.4 Normal and diseased skeletal muscle

The biopsy of skeletal muscle is a useful diagnostic tool to assess the structural, ultra-structural and enzymatic state of an individual's muscle. In particular, analysis of biopsies can be used to distinguish diseased and normal muscle by determining the level of markers of pathology present in the sample. Pathology is defined as the "anatomical and physiological deviations from the normal that constitute disease or characterize a particular disease". Histological and histochemical stains are routinely used to examine the structure or morphology of muscle cells. For example the histological stains Haematoxylin & Eosin and Gomori Trichrome can be used to visualise fibre shape and size, nuclei position, the presence of interstitial cells, blood vessels, connective tissue and adipose tissue. A cross-section of normal muscle should contain fibres that are polygonal in shape and closely situated to each other, with little fibre size variation. Fibre size, which is dependent on age, is uniform in healthy muscle. The number of capillaries present relates to the fibre type and is also age-dependent. Although nuclei are located around the periphery of the muscle cell, it is normal to find internal nuclei in up to three percent of fibres, with a higher occurrence in the muscle cells of physically active people. Peripheral clusters of mitochondria also occur in normal muscle, especially near the capillaries, and may appear as "tramlines", staining red with the Gomori Trichrome stain. There should be minimal connective tissue in normal muscle (171).

Diseased muscle, however, displays diffuse changes in fibre shape, size and distribution. For example, following denervation there is grouping of atrophied fibres. The number of internal nuclei present can vary, but large central nuclei indicate myotubular myopathy, myotonic dystrophy and in some cases, minicore myopathy. Necrosis is identified by pale staining of the fibres and may be associated with phagocytes. Hypercontracted areas stain intensely and represent damaged or necrotic fibres. Regenerating fibres are recognized by their basophilia, but this only represents the early stages. Basophilia may also be apparent in abnormal granular fibres in some conditions. Fibres with structurally abnormal or large clusters of mitochondria appear "ragged red" with the Gomori Trichrome stain (171).

The histochemical stains adenosine triphosphatase (ATPase), succinate dehydrogenase (SDH) and nicotinamide adenine dehydrogenase-transverse reductase (NADH-TR) are used to identify fibre types, reveal structural changes and identify enzyme deficiencies. Type 1 oxidative fibres stain strongly for SDH and NADH-TR owing to their greater mitochondrial content and the reverse is true for glycolytic type 2A and 2B fibres, with type 2B being most extreme. In normal individuals, the proportion of fibre types present varies between muscles and within a muscle. In normal quadriceps, however, the proportion of type 1 fibres is usually less than 55 %. Type 1 fibres are considered to be predominant if more than 55 % of the fibres counted are type 1, and more than 80 % type 2 fibres are required for a type 2 predominance (171). Normal muscle usually contains fewer than 3 % of the transitional type 2X/D fibres.

Diseased muscle, in particular that with neuromuscular disorders, may contain altered fibre type proportions. For example, dystrophic muscle contains more of the regenerating type 2X/D fibres. Type 1 predominance is a common non-specific myopathic feature, but particularly pronounced in the congenital myopathies. Type 2 atrophy is non-specific and can be induced for example in steroid use and muscle disuse. Type 1 atrophy is less common and occurs in myotonic dystrophy and some congenital dystrophies. Grouping of fibres is indicative of a neurogenic disorder, possibly reflecting re-innervation. A fibre deficiency is said to occur when less than 10 % of the fibres constitute a given fibre type. Cores and mini-cores show a zone (usually central) within the fibre with no enzyme activity. Disruption and

disorientation of the myofibrillar network are common structural changes and may give rise to whorled fibres, ring fibres or fibres which are moth-eaten in appearance. Alterations in the distribution of mitochondria are apparent in lobulated fibres in which abnormal aggregations of oxidative enzymes are seen, particularly in triangular peripheral areas of the fibres. This is a non-specific feature. Mitochondrial abnormalities can be detected with SDH and NADH-TR staining (171).

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1.3 THE SYSTEM: THE ENDURANCE RUNNER

Extensive research has been done on human endurance athletes to explain the physiological changes from a sedentary to a highly trained person. This research has also attempted to explain the factors that contribute to performance. The aim of this section, however, is to give a brief overview of the characteristics of the endurance runner. Trained endurance runners may be differentiated from the sedentary population by a number of factors. For example trained endurance runners have increased cardiovascular efficiency as evidenced by lower maximal (3), sub-maximal (23, 206) and resting (23) heart rates, improved parameters of aerobic fitness including maximal oxygen uptake, exercise economy, the lactate / ventilatory threshold and oxygen uptake kinetics (96), and lower body fat levels (5, 193). They also have an increased plasma volume and erythrocyte volume (men only) (185), greater levels of high-density lipoproteins (193), higher bone mineral densities in their lower limbs (7), increased left ventricular volume of their cardiac muscle (122), and improved neuroendocrine function (11, 70, 97, 207). An important consequence of altered neuroendocrine function is an improved regulation of physiological and metabolic processes.

Specifically, the skeletal muscle of endurance runners has an increased capacity for aerobic metabolism (36, 87, 195), a greater proportion of type 1 fibres (36, 36, 39, 63, 195), larger type 1 fibres (36, 39, 63) and abundant subsarcolemmal aggregations of mitochondria located close to the capillaries (39), presumably all adaptations to aid endurance efficiency. The skeletal muscle of rested endurance runners also shows signs of structural pathology including irregular fibre shape, increased connective tissue, more internal nuclei and fibre type grouping. These are suggestive of ongoing fibre injury and may be a functional adaptation necessary to endure the stress of endurance running (178).

From a psychological perspective, distance runners are believed to be well adjusted individuals displaying improved mood states as indicated by good vigour and well-being and less depression, anxiety and fatigue compared to sedentary individuals

(136, 155, 205). Runners forced to reduce their training volume and intensity, however tend to display more negative mood states (208).

Percy Cerutti, the coach of the legendary Australian runner Herb Elliot was quoted as saying:

"If you do not have that almost constant feeling of dissatisfaction with everything, recognizing that no sooner is one pinnacle achieved, one goal realized, [there will be another], success may well elude you" (143).

Some athletes do, as Cerutti suggests, have personalities that are more rigid, goal-oriented and perfectionist in nature and such individuals could be more likely to drive themselves into a state of overtraining or injury (155). In fact, overtraining is a phenomenon that affects up to 65 % of competitive endurance runners (132) and in one study runners with a "Type A" personality sustained significantly more injuries in one year compared to "Type B" personality runners (62). Furthermore, the prevalence of eating disorders amongst competitive female endurance runners (16 %) is higher than the general population (6.6 %) (186) and they tended to have lower self esteem and poorer mental health (91). Therefore as much as exercise may positively affect mood, the personality of athletes and therefore their attitudes towards exercise may have an equally powerful, although not always positive, effect on their well being.

1.4 THE STRESSOR: ENDURANCE EXERCISE

1.4.1 The stress response model

For the purpose of this thesis the endurance runner is viewed as a system. Most systems have a set range of conditions under which they function optimally, and therefore resist disruptions to these conditions to maintain homeostasis. Stress is a state that occurs when a physical, chemical or emotional factor perturbs the equilibrium or homeostasis of a system and a stressor is a stimulus that causes stress. Stress can be positive (eustress) or negative (distress) in nature and when stressors impact a system, there is an ensuing stress response (149). Hans Selye developed a stress response model known as the General Adaptation Syndrome to explain how systems cope with both acute and more chronic stressors (Figure 1.5).

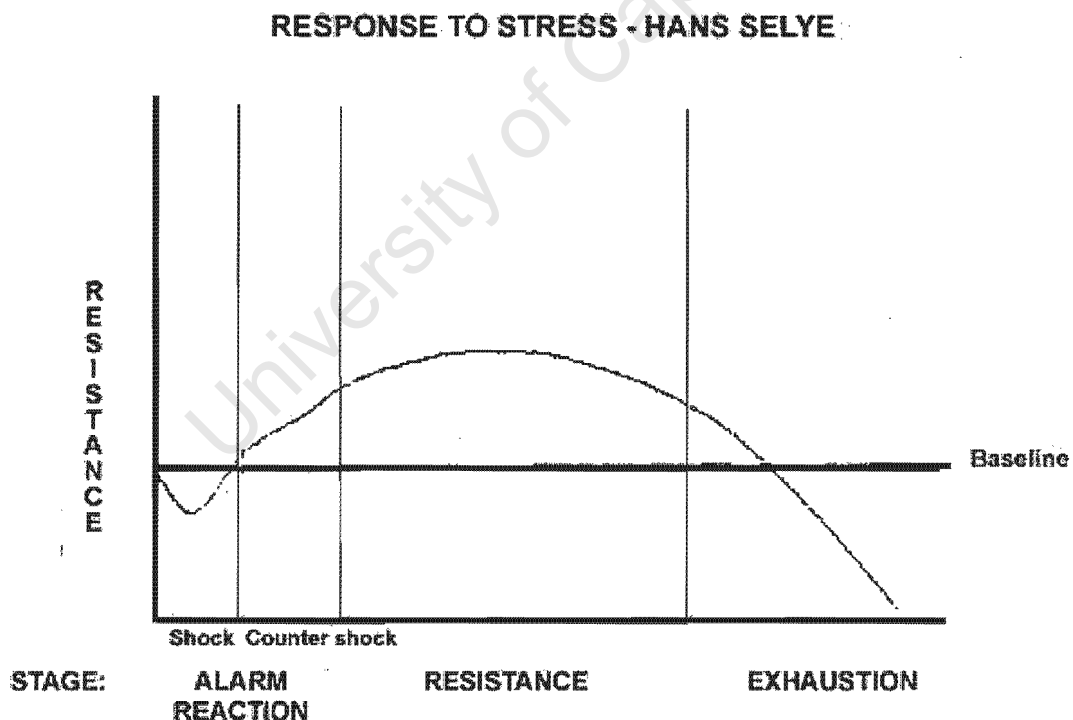


Figure 1.5 Hans Selye's model of response to stress. *The x axis is advancing time and the y axis the degree of resistance exhibited by the system perturbed by a stressor. The response is divided into three phases: alarm reaction, resistance and exhaustion.*

The theory of Selye's stress response model is that all organisms or systems continually exposed to a stressor will pass through three stages in response to the stressor (149). During the initial "alarm reaction" phase, resistance to the stressor is low, presumably as the system's homeostatic state is perturbed. Resistance increases once the initial shock has passed, by which stage homeostasis is most likely being restored. As exposure to the stressor continues, resistance increases to a peak before decreasing during the "resistance" phase. After a long exposure to the stressor resistance decreases to below the baseline levels, leaving the system more vulnerable to the stressor during the phase of "exhaustion".

Selye's stress response model could be applied to runners undergoing a single bout of endurance exercise. Initiation of a training run or race would disturb the homeostatic state of runners at rest (alarm reaction phase), leading to a variety of physiological responses to both restore homeostasis and accommodate the stressor during the activity (resistance phase). To avoid reaching the exhaustion phase, runners would either terminate the training session or race, or adjust exercise levels to resist the stressor better. Similarly, the stress response model can be applied to the span of the running careers of athletes. For example, prior to exposure to endurance running, individuals would be in a state of homeostasis relative to their lifestyles. The initiation of a training programme would send the body of novice runners into the phase of alarm reaction. Their resistance to endurance running would be low as they would not have the necessary adaptations to cope with the stress of running. With each training exposure, however, their resistance would increase, presumably through first acute and then medium term adaptations to the stressor (resistance phase). The same runners in the veteran stages of their careers would likely display chronic adaptations to endurance running, and may be at risk of entering the exhaustion phase. Therefore responses to an acute bout of exercise reflect a system trying to re-establish homeostasis. Adaptations to systematic endurance training are important as they allow the system to increase its resistance to a chronic stressor. This section will focus on the responses and adaptations observed in skeletal muscle, since this is the organ of importance in this thesis.

1.4.2 Acute response to endurance exercise

If Selye's stress response model is applied to a single bout of endurance exercise, then the acute response would occur within seconds to hours after exposure to the stressor. These changes would occur during the alarm reaction phase of the model, presumably in response to the perturbations caused by the stressor, and in a rapid attempt to restore the runner's state of homeostasis. It is beyond the scope of this thesis to present a detailed discussion of these responses. Therefore a brief summary of the changes as reviewed by Flück et al. (63) follows. Neuronal activity increases within seconds to minutes after starting exercise and endocrine changes including an increase in circulating concentrations of growth hormone, renin and epinephrine and a decrease in insulin are also induced within a few minutes. From a metabolic perspective the activities of a number of protein kinases are increased within minutes to hours after starting exercise. The expression of many metabolic proteins (hexokinase II, glucose transporter-4), proteins involved in folding (heat shock protein-70), transcription regulation proteins (C-fos) and some growth factors have also been shown to increase within minutes to hours in response to endurance exercise. Muscle phospholipid synthesis increases within minutes of beginning exercise. Within hours the synthesis of proteins, including fibronectin, increases and there is also an initial decrease in lipid stores, accompanied by an increase in fatty acid oxidation, which continues for the next few weeks (63).

1.4.3 Medium term adaptations to endurance exercise

Adaptation is generally defined as the *"change in structure, function or form that improves an organism's chance of survival within in a given environment"* (212). For the purpose of this thesis the medium term adaptations to endurance exercise would be those changes occurring in the latter part of the alarm response phase or during the resistance phase of Selye's stress response model once the initial disturbance of the stressor has been accommodated. These adaptations would further improve runner's resistance to the stressor and presumably contribute to improvements in performance.

Endurance training results in a number of adaptations that improve the body's capacity for exercise over prolonged periods where oxidative metabolism is important. Common medium term adaptations that occur within weeks to months in response to endurance training might include increased capillary supply to the muscle, increased volume density of mitochondria and capillaries, increased mitochondrial protein concentration, increased activities of the enzymes involved in the mitochondrial electron transport chain and oxidative metabolism, improved lactate transport across the sarcolemma, decreased glycolytic flux and decreased glycolytic enzyme activity, a glycogen sparing effect during sub-maximal exercise, increased lipid stores, increased fat oxidation during sub-maximal exercise, increased maximum oxygen uptake and improved resistance to fatigue at sub-maximal levels (63, 83, 87, 150).

To examine the specific medium term effects of endurance running on skeletal muscle Kuipers et al. (116) studied runners over a 19 – 20 month training period. The subjects used in this study could be considered novice runners since they had never run over 12 km and had never participated in distance running races. They trained for six months in preparation for a 15 km race, then for a further five months before running a 25 km race, and a final seven months before completing a marathon. During the training period, no changes were noted in fibre type or fibre diameter and there were no signs of markers of structural pathology in any of the muscle biopsies taken immediately before the first event, which was the 15 km race. Therefore, the first five months of training during which distances did not exceed 12 km did not result in any pathological changes in the muscle of these runners.

It was only immediately after the 15 km race that the first pathological changes were observed. Markers of structural pathology observed in each biopsy increased during the following five months until the 25 km race, whereafter the degree remained constant. The changes observed in the muscle samples included central nuclei, atrophic fibres, infiltration of macrophages, myoblastic activity, regeneration and central splitting. The degree of structural pathology was not different between male and female runners, or between different fibre types. Ultra-structural changes included irregular z-lines and hypercontracted sarcomeres in the peripheral areas of the muscle fibres. There was no evidence of sarcolemmal damage or mitochondrial

changes. The authors concluded that the training for endurance races was as stressful as racing in terms of the markers of structural pathology found in the skeletal muscle of the runners. Since glycogen stores were not depleted in the affected fibres, the authors also concluded that it was unlikely that glycogen availability was responsible for the ensuing pathological changes. A limitation in this interpretation, however, was that as the biopsy was taken between one and six hours post race, not all of the subjects were fasted, and glycogen synthesis may have occurred in the interim. The main conclusion from this study was that the observed markers of structural pathology in the muscle of endurance runners undergoing both training and racing are minor and transient, and the extent appears to be related to training distance, and not intensity (116).

Another function of medium term adaptations may be to protect the organism or system from future exposures to the same stressor. Experiments in which animals are repeatedly exposed to downhill running, as well as anecdotal evidence from humans, suggests that prior exposure to a task that induces damage to skeletal muscle provides protection against that same stressor on subsequent occasions (16). This phenomenon is known as the "repeated bout effect" (131). The mechanism of this adaptive protection is not well understood but there is evidence that it can be explained by changes in the neuromuscular system, the connective tissue and the muscle cells (131). Despite the lack of understanding of the mechanism of the repeated bout effect, it is known that training minimises morphological changes to skeletal muscle, damage to the sarcolemma, delayed onset muscle soreness, and performance changes following an acute bout of exercise (54). Furthermore, acute bouts of exercise and longer term training in animals and humans increase the activities of anti-oxidant enzymes including superoxide dismutase, catalase and glutathione peroxidase, and the production of heat shock proteins. These responses or adaptations can be interpreted as being protective against the increased production of free radicals often associated with exercise, and should ultimately protect lipids, DNA and protein against oxidative damage (130).

1.4.4 Chronic adaptations to endurance exercise

For the purpose of this thesis, chronic adaptations to endurance exercise are viewed as those necessary to maintain resistance against the ongoing stress of endurance running during the "resistance" phase of Selye's stress response model. Few longitudinal studies exist that document the long-term effects of endurance exercise on skeletal muscle. Therefore studies examining the skeletal muscle of veteran runners are currently the best sources of information regarding the cumulative effects of the continual stress of endurance running on muscle. However, one needs to be cautious in attributing observed changes in the muscle of veteran runners to exposure to running alone, as the possible confounding effects of pre-selection to running and the effects of aging on muscle cannot be ignored.

Hikida et al. studied the gastrocnemius muscle of veteran runners in a trained but rested state prior to a marathon and observed extensive accumulations of subsarcolemmal mitochondria, glycogen and lipid droplets, as well as signs of trauma including disrupted sarcolemmas, erythrocytes and mitochondria in the extra-cellular space, contracture knots, disoriented myofibrils and the presence of leukocytes and macrophages (86). These findings suggested that either most of the runners were not in a fully rested state prior to the marathon, or this degree of disruption is common in the skeletal muscle of trained endurance runners (86) reflecting a chronic adaptation to endurance running. Unfortunately neither the age nor information about the training history of these athletes was given in this study. Sjöström et al. found that the vastus lateralis muscle of rested endurance runners (mean age: 30 ± 4 years) had increased levels of markers of structural pathology including irregular fibre shapes, poorly organised fascicles, increased connective tissue, internal nuclei and grouping of fibre types, but that these abnormalities varied greatly between the subjects. The authors concluded that these observations were probably due to repeated injury and repair and were considered to be pathological for the age of the runners (178).

Therefore, while chronic adaptations presumably maintain runners' resistance to the stress of endurance running, tolerating a chronic stressor may in turn lead to

maladaptations. Indeed, Sharwood et al. showed that veteran distance runners who had accumulated more than 5 000 km in racing had lower measures of neuromuscular efficiency after exercise-induced muscle damage (172). It is tempting to speculate that such maladaptations may contribute to muscle losing its functional characteristics and the ability to adapt to and regenerate in response to exercise training, which may manifest as exercise intolerance.

1.4.5 Intolerance to endurance exercise

For the purpose of this thesis intolerance to endurance exercise is described as an unexpected decline in performance and an inability to tolerate previous levels of training; often accompanied by exercise-associated fatigue and in some cases the development of skeletal muscle symptoms such as fatigue, pain, tenderness or cramping.

In keeping with Selye's stress response model, veteran endurance runners are likely to enter the exhaustion phase of the stress response model at some point during their running career. The following case illustrates such an example: A 64 year old male runner had covered 153 944 km in training and 16 604 km in races over 47 years of running. During this period he completed 212 marathon and ultra-marathon races, and of the 122 marathons he ran he finished 88 in less than three hours. However, from the age of 64 years, his running speed began to decrease at a faster rate than that would be expected for his increasing age (119). He also lost the ability to sustain the training loads to which he was previously accustomed. His decline in performance, or exercise intolerance was most likely related to a reduced resistance to the stress of endurance running, although the causes of this reduced resistance were not established. The remarkable point of this case was that the runner was exposed to and tolerated the stress of endurance running for 47 years before he appeared to enter the exhaustion stage of Selye's stress response model.

Of greater concern are younger athletes reaching this phase of exhaustion and exercise intolerance after far fewer years of endurance running. Derman et al. described a group of athletes presenting with an exercise-associated fatigue and intolerance to exercise that was accompanied by myopathic abnormalities within their skeletal muscle (48). This condition was called "Fatigued Athlete Myopathic Syndrome" (FAMS) (48). More recently the condition was redefined as "Acquired Training Intolerance" (ATI) (75) since it was established that the athletes did not necessarily share a common myopathy or set of myopathies, but rather had a common symptom of an intolerance to exercise. The athletes with ATI have in common a history of a high training volume and a current inability to tolerate former levels of endurance exercise. They experience a sudden and unexplained decline in performance, often associated with a reduced training volume, both of which are more severe than the decrease that would be expected to accompany advancing age. Medical examination reveals that these athletes are free from physiological fatigue conditions, including training-, diet-, travel- or pregnancy-induced fatigue and pathological fatigue conditions, including haematological or metabolic conditions, classic chronic fatigue syndrome, overtraining syndrome or any neuromuscular disorders. In most cases, the athletes with ATI report general skeletal muscle symptoms including excessive delayed onset muscle soreness, muscle stiffness, tenderness and skeletal muscle cramps. Furthermore, these individuals have usually consulted many physicians without getting a satisfactory diagnosis, and do not respond to long periods of rest, or nutritional or psychological support (48, 75).

Biopsies of the vastus lateralis muscle of these athletes showed a greater degree of markers of structural pathology, including fibre size variation, internal nuclei, z-disc streaming and more lipid and glycogen droplets compared to healthy, age-matched control athletes (75). Endurance training and racing is known to induce such changes in the skeletal muscle of apparently healthy runners. In fact, mild levels of markers of structural pathology are thought to be functional adaptations (178), reflective of the continuous state of degeneration and regeneration occurring within the muscle in response to the stimulus of running (116). The greater degree of structural pathology observed in the skeletal muscle of the athletes with ATI may, however, have been an abnormal response. These athletes also had significantly shorter skeletal muscle telomeres compared to asymptomatic athletes. In fact, three of the athletes with ATI were shown to have pathologically shortened telomeres

(33). Collins et al. interpreted this as meaning that the muscle of individuals with ATI had undergone more cycles of damage and repair compared to the age- and mileage-matched healthy control athletes (33). Alternatively, shortened telomeres may reflect excessive oxidative damage to the DNA of the skeletal muscle. This too is plausible as studies have shown that oxidative stress increases as a result of distance running in moderately trained individuals (51).

Therefore evidence from the athletes with ATI suggests that a high exposure to endurance running results in the skeletal muscle becoming more vulnerable to the ongoing stress of endurance exercise by causing abnormalities within the muscle, ultimately resulting in an intolerance to exercise. This is further supported by a case describing an elite male runner who sustained a high training load for a number of years. He experienced an unexplained decline in performance, became intolerant to his previous levels of training and was subsequently shown to have mitochondrial pathology in his lower limb muscles. Interestingly, a biopsy from his triceps muscle was clear of any pathology, indicating that the pathology was localised to the muscles used in running (183). The fact that the pathology was only in the lower limb musculature invites speculation that excessive training may well have led to the mitochondrial pathology, and his subsequent development of exercise intolerance.

1.5 THE CO-STRESSORS: AGING, ACUTE INFECTION, METABOLIC MYOPATHIES, BEHAVIOUR

For the purpose of this thesis endurance running is considered to be the primary stressor on the athlete. However, it is unlikely to be the solitary stressor. Therefore it would be appropriate to explore possible co-stressors that might compound the stress induced by running. Naturally countless co-stressors exist, but this review will focus on a few that are relevant to the athlete, known to impact skeletal muscle and that were observed in the cohorts studied in the experimental chapters of this thesis.

1.5.1 Aging

Chronological aging is characterised by a functional decline in the major biological systems including the cardiovascular (156), metabolic (204), respiratory (78) and neuromuscular (47) systems. These changes manifest as a reduced functional capacity during activities of daily living and exercise. Sarcopenia, or the age-related loss of skeletal muscle mass and function, is thought to be partially responsible for some of the physical and metabolic deteriorations that accompany aging (173). More specifically, the age-related alterations to physical capacity can be at least partially attributed to the observed changes in skeletal muscle strength (125), morphology (128), metabolism (31, 166) and changes in the neuromuscular system (117, 192).

1.5.1.1 The effect of aging on skeletal muscle

By the age of 70 years the cross-sectional area of muscle is reduced by up to 25 – 30 % (130). Although the cross-sectional areas of type 1, oxidative fibres do not appear to change, and the density of hybrid fibres increases, observed changes in the type 2 glycolytic fibres may help explain this phenomenon (63). The volume density and capillary to fibre ratio of muscle fibres decrease with increasing age, and there is also an increase in oxidative damage to the myofibrils and a decrease in the number of motor neurons and motor units (63). Therefore the observed loss of muscle mass with increasing age may be due to a decrease in the total number of

fibres and individual fibre atrophy (130), which in turn may be the result of denervation (76). Muscle strength may be reduced by 30 – 40 % by the age of 70 and this loss of strength continues to fall at a rate of 1 – 2 % per year (130). The decrease in muscle force and power may be attributed to a change in the contractile properties of the motor units in muscle (76), or the age-related atrophy of muscle (130).

A host of skeletal muscle proteins have altered expression with age, thereby affecting muscle function. For example, the expression of some of the proteins involved in glucose storage, fatty acid import and β -oxidation, ATP production and transfer, protein turnover, endocrine response, transcription, cell proliferation, DNA repair and detoxification decrease with increasing age (73, 99). In contrast, the expression of proteins involved in protein folding, growth arrest, cell death, neuronal remodelling, repair of oxidative stress/damage and inflammation during repair increase with advancing age (63, 99).

One of the important effects of aging on skeletal muscle with regards to exercise capacity is a change in its ability to regenerate. Older muscle reportedly has a good capacity for myoblast proliferation and fusion, and subsequent new muscle formation, albeit slightly less efficient compared to younger muscle (138). The reduced efficiency may be attributed to the gradual decrease in the number of satellite cells in older muscle (100, 161) as well as their diminished proliferation rate (161). Alternatively, any impairment in satellite cell activation may contribute to the reduced regenerative capacity observed in older skeletal muscle (34). Other factors intrinsic to the muscle, including changes in the extra-cellular matrix volume and composition, vascularity, expression of growth factors, hormone status (76) and functional characteristics of macrophages (22) could all contribute to the less efficient regeneration of muscle seen in older hosts. All of these factors contribute to changes in the internal milieu of skeletal muscle and may in turn affect the muscles potential for regeneration (76).

1.5.1.2 The effect of aging on endurance performance

While regular endurance training delays the age-associated decline in skeletal muscle strength (188) and the development of disabilities in older individuals (200), the overall decline in race speed during the lifespan is striking (181) and inevitable (119). Age group records reflect the best possible performance by an individual within a certain age category. The U.S. age group records for the standard marathon show that the performance of male athletes declines steadily by approximately 6-9 % each decade between the ages of 30 and 70 years, whereafter the deterioration in performance is greater (98). Another study showed that a group of veteran ultra-endurance runners who had trained at a high volume for a number of years were still able to perform well for their age group (120). This suggests that age related changes in endurance capacity are strongly dependent on the individual.

The effect of age on peak physical performance depends on the physiological demands of the sport as well as the number and interplay of the systems (physiological, biochemical and structural) predominantly involved (181). Therefore changes in systems influencing endurance capacity are important for veteran runners. In general, endurance capacity decreases slowly from the third to the sixth decade, and faster thereafter (67). This age-associated decline can be attributed to a number of factors including changes in training and racing volume (103, 181) increased likelihood of injury (181), changes in physiology including decreased oxygen uptake, heart rate and ventilation rate during maximal exercise (81), declines in both the aerobic and the anaerobic systems, and decreases in strength and co-ordination (181).

In summary, despite the fact that regular exercise slows down some aspects of the age-related deterioration in the body, a decline in endurance performance with increasing age is inevitable.

1.5.2 Acute infection

Moderate exercise improves immune function in individuals who were previously sedentary. This effect is considered to be dose-dependent until a critical point is reached where further increases in training loads may depress the immune system, leaving athletes more susceptible to infection (142). While athletes are not necessarily immuno-deficient, those undertaking exercise that is prolonged, continuous and of a relatively high intensity, are most susceptible to post-exercise immune function depression for up to 24 hours after exercise (71). Therefore endurance training may leave some athletes more vulnerable to infection, which in turn may impact both their skeletal muscle and their endurance performance.

1.5.2.1 The effect of infection on skeletal muscle

Acute infections initiate responses which are both specific and systemic in nature and aimed at the invading micro-organism. Effects of the acute phase response to infection may include cardiac and skeletal muscle wasting, impaired carbohydrate and fat metabolism, as well as disturbances in neuromuscular function (66). Muscle wasting, which is restored following infection, is thought to occur due to a decrease in protein synthesis and an accelerated proteolysis. This catabolic response may be associated with the decreased activities of glycolytic and oxidative pathway enzymes (66). Skeletal muscle biopsies may show areas of myofibrillar degradation and increased vacuolisation and fast twitch muscle fibres are thought to be more susceptible to degradation compared to slow twitch fibres and cardiac muscle. Diffuse myalgia, prominent in certain infections has not been correlated to any structural or ultra-structural alterations, whereas localised myalgia may indicate myositis (focal infiltration of the affected muscles by inflammatory cells) (66). Viral infections from, for example, the Coxsackievirus, influenza, paramyxoviruses, cytomegalovirus and Epstein-Barr virus, have been directly associated with chronic and acute myositis (40).

1.5.2.2. The effect of infection on endurance performance

Exercising while an infection is present is contra-indicated. During either the fever or tissue wasting stage of infection, heart rate during sub-maximal exercise is elevated, suggesting that aerobic capacity is compromised. Muscle strength is also decreased and athletes often report myalgia (66). Of significance is that the effects of an infection may impair performance for an extended period after the symptoms resolve. An elite cyclist underwent physiological testing before, during and after a viral infection (93). One week after the virus his heart rate and RPE were higher during a sub-maximal exercise test and he oxidised less fat compared to before infection. One and ten weeks post-virus his heart rate and blood lactate values were still higher than pre-virus levels during a graded maximal exercise test. Furthermore 50 weeks following the virus he was still unable to complete the maximal test (93).

The extent of the effect that infection has on athletes varies depending on the individual. This may be related to training status as some animal studies have shown that training blunts the effect of an infection. Furthermore, trained humans may have a more efficient immune system than untrained individuals making them better able to cope with infection (66). Therefore the recovery time course from infection varies between individuals and also depends on the type, extent and duration of the infection as well as the effects of bed rest or detraining (66). The recovery of aerobic capacity to pre-infection levels may take between one and three months, whereas strength recovery is faster (one month) but strength endurance is slower (up to four months) (66). Up to two weeks may be required to recover protein loss after an average 36 hour bout of the influenza virus. Recovery from infectious mononucleosis (infection with the Epstein-Barr virus) is even slower since residual effects of the infection may persist for up to one month following supposed clearing up of symptoms. In fact, maximal exercise undertaken during the first month following infectious mononucleosis may lead to a rebound effect resulting in long-lasting fatigue (66). Therefore awareness of an athlete's capability following an infection seems to be a key part of effective recovery. For example, a middle distance runner who was diagnosed as having infectious mononucleosis after both altitude training and over-reaching recovered to pre-infection race form within ten months. Furthermore, he was able to maintain his aerobic fitness during the

recovery phase with the help of a moderated training programme and continuous physiological and medical assessments (4).

A negative consequence of viral infection is post-viral fatigue syndrome. The primary complaints of this syndrome are muscle fatigue and weakness, often exacerbated by physical activity, but controlled tests of muscle power and fatigue usually return normal results (154). Preedy et al. studied female patients in whom symptoms had been present for between one and 17 years (mean time: 4.6 years). The vastus lateralis muscles of the patients were morphometrically normal, as were plasma CK activities, haematological analyses, liver function tests, thyroid function, blood biochemistry results and standard viral screens. However, eighty percent of the patients had a persistent enteroviral infection and a reduced mean muscle RNA composition (154).

Excessive training or competition may contribute to persistent fatigue and recurrent infection but this behaviourally-induced state normally resolves with rest. If the symptoms persist, however, further clinical investigations are recommended (159). In a group of 41 competitive athletes who reported to a clinic with complaints of persistent fatigue and / or recurrent infection, 68 % of the athletes were shown to have an underlying medical condition which was able to explain their symptoms. Although there was no uniformity with respect to the types of conditions experienced, the presence of an unresolved viral illness was the most common finding (159).

In summary, although regular activity is thought to reduce an individual's susceptibility to infection, athletes who engage in prolonged, intense training may be at an increased risk to infection. Furthermore, viral infections limit endurance capacity and affect skeletal muscle metabolism for an extended period of time following the resolution of the symptoms. Recovery from an infection is specifically dependent on the individual.

1.5.3 Metabolic myopathies

A number of case studies in the literature have described classic skeletal muscle disorders in athletes such as carnitine palmitoyltransferase (CPT) deficiency (60), mitochondrial dysfunction (183), myotonia congenita (203) and other abnormal myopathies (50) which cause the dysfunction of skeletal muscle during exercise. The metabolic myopathies are of particular interest in this review since they potentially disrupt skeletal muscle function during exercise and are relevant to a case study presented later on in the thesis. By definition, a metabolic myopathy is a disorder of skeletal muscle due to abnormalities in glycogen, lipid or mitochondrial metabolism, ultimately leading to a failure in energy production (41).

Metabolic myopathies that affect skeletal muscle during exercise include glycogen storage diseases, fatty acid oxidation defects and mitochondrial abnormalities. McArdle's disease (glycogen storage disease) and CPT II deficiency (fatty acid oxidation disorder) are the most common metabolic myopathies (187). The glycogen storage disorders involve both abnormal glycogen breakdown and glucose utilisation, and symptoms usually occur during high intensity exercise. The main features are acute muscle breakdown which often leads to myoglobinuria, muscle cramps and swelling, exercise intolerance. Common complaints include easy fatigability with exertion, and occasional muscle stiffness with exercise (41). Individuals suffering from McArdle's disease lack the enzyme glycogen phosphorylase necessary to break down glycogen, and also have abnormally high stores of glycogen. The result is that these individuals are unable to tolerate anaerobic exercise. Muscle contracture is a feature of this disease and may be the cause of the often-associated myoglobinuria. In other cases of exercise intolerance, individuals who experience muscle contracture, myoglobinuria and also have elevated stores of glycogen might lack the enzyme phosphofruktokinase, a key component in the anaerobic metabolism of glucose (6).

In contrast, disorders of lipid metabolism are usually precipitated by periods of prolonged exercise or fasting. The signs include muscle pain or tightness and myoglobinuria (41). CPT II deficiency is the most common disorder of mitochondrial

β -oxidation and usually presents in late childhood or early adulthood as recurrent episodes of rhabdomyolysis. It is indicated by myoglobinuria and elevated levels of plasma creatine kinase activity after prolonged exercise, fasting, high fat intake, viral infection, fever, emotional stress (199), exposure to cold, general anaesthesia or ingestion of drugs such as diazepam, ibuprofen and valproic acid (12). In general, individuals with CPT II deficiency feel normal between attacks of rhabdomyolysis but report myalgia, muscle tightness (199), stiffness or weakness (12) during the attacks. If the rhabdomyolysis is severe enough, acute renal failure may develop (199). Deficiency of the CPT II enzyme, necessary to transport free fatty acids into the mitochondria for oxidation, prevents β -oxidation of fats in muscle and results in an increase in free fatty acids with subsequent excessive formation of triglycerides. It has been suggested that the resulting lack of energy, particularly once the glycogen stores have been depleted, disrupts the sarcolemma resulting in myoglobinuria (6). Skeletal muscle structure appears normal in 50 % of cases, while muscle lipid storage is found in 20 % of the patients. Other structural abnormalities may include atrophy or necrosis of type 1 fibres. Ultra-structural abnormalities of the mitochondria are unusual (199).

In summary, metabolic myopathies affect an individual's capacity to exercise due to abnormalities in glycogen, lipid or mitochondrial metabolism. Although not common, they are indicated in cases where athletes experience exercise intolerance accompanied by repeated episode of myoglobinuria and muscle fatigue, pain or stiffness.

1.5.4 Behaviour

As mentioned in section 1.3, the personality of competitive athletes often contributes to their drive to succeed, but may also compromise their well-being through their compulsive behaviour or attitude towards exercise. Some athletes over-emphasise the importance of exercise, and are typically characterised by their unwillingness to stop exercising even when it is contra-indicated, such as in times of injury or illness. As opposed to the generally positive effects of exercise on well-being, abuse of

exercise can result in negative psychological changes (158). A pathological attitude towards exercise occurs when individuals continue exercising despite contraindications, and when exercise begins to take precedence over the social aspects of the individual's life (43). Athletes who are pre-occupied with their weight are likely to adopt an obligatory attitude towards their exercise, and obligatory exercisers are more likely to be obsessive compulsive in personality (42). Davis et al. proposed that the combination of excessive pre-occupation with weight and obsessive compulsive tendencies in active and competitive sports women is likely to increase the duration of their exercise sessions as well as their obligatory attitude towards exercise (43). Furthermore, strenuous physical activity might be linked to altered serotonin functioning and an increased obsessive nature, further perpetuating the physical activity and starvation cycle. As obsessiveness increases, so too is the individual's attitude towards exercise likely to become pathological in nature. The interactions between physical activity, starvation and obsessive compulsiveness may be reciprocal in that they influence and encourage each other in a destructive cycle (43).

Many endurance athletes engage in excessive training. While periods of overreaching are considered necessary to tax the athlete's body sufficiently to continue adapting to the stimulus of exercise, reaching a state of overtraining is not desirable. Smith (180) proposes that the repetitive trauma to the musculoskeletal system sustained during periods of high intensity or high volume training with insufficient rest may lead to a state of overtraining. If a local inflammatory response resulting from musculoskeletal stress becomes chronic in nature, a systemic inflammatory response may ensue, elevating the number of circulating cytokines. This in turn may lead to a set of behaviours including reduced appetite or depression to induce recovery (145, 180). Perhaps the most concerning consequence of overtraining to athletes is the subsequent decrease in performance, and the fact that recovery may take up to 12 weeks (158).

In summary, it is clear that while regular exercise may lead to positive mood adjustments in athletes, the attitude of athletes towards exercise, and therefore their behaviour, has a strong effect on their well-being.

1.6 SUMMARY AND CONCLUSIONS OF THE LITERATURE REVIEW

The organ of interest in this thesis, skeletal muscle, is plastic in nature and copes well with the stresses associated with everyday living. Furthermore, it appears to fully recover from minor damage through a refined degeneration and regeneration process. Satellite cells are of particular importance when skeletal muscle has had to withstand excessive cycles of degeneration and regeneration. The proliferative history of satellite cells, in part, determines the remaining regenerative capacity of skeletal muscle. This in turn can be assessed indirectly by measuring the lengths of the telomeres of the satellite cells.

Endurance exercise is a stressor to the system of the endurance runner. Both a single bout of exercise, as well as years of endurance racing and training, challenge the capacity of endurance runners to resist this stressor. An acute bout of endurance exercise disturbs the runner's homeostatic state and induces minor damage to skeletal muscle. However the plastic nature of muscle allows it to recover from exercise-induced muscle damage and to adapt to the stimulus of systematic endurance training. In this manner athletes become more aerobically efficient and better able to tolerate future bouts of endurance exercise. It may be speculated that this transient damage to skeletal muscle contributes to the adaptation necessary to develop resistance to future bouts of endurance exercise.

Longitudinal studies documenting the chronic adaptations of skeletal muscle to endurance running are less well researched and it is difficult to dissociate the confounding influence of aging-induced changes in skeletal muscle from the changes induced by training. However, long term training is likely to lead to chronic damage in skeletal muscle as evidenced by the abnormalities seen in the muscle of trained yet rested runners prior to a marathon. Furthermore some athletes become intolerant to exercise. A high exposure to endurance running is considered a likely trigger for this intolerance, leading to abnormalities within the skeletal muscle including increased levels of markers of structural pathology, shortened telomeres, or mitochondrial myopathy. However, since the human is not an isolated system, endurance running is only one of countless stressors acting on the body. While the

CHAPTER 1

primary focus of this thesis is the effect of chronic exposure to endurance exercise on runners; co-stressors including aging, infection, metabolic myopathies and behaviour, to mention a few, also need to be considered as they all impact both endurance performance and skeletal muscle itself.

University of Cape Town

1.7 OBJECTIVES OF THE THESIS

The primary objective of this thesis is to examine the effects of chronic exposure to endurance running on the athlete, with particular emphasis on skeletal muscle.

Current evidence showing the longitudinal effects on performance of chronic exposure to endurance running is in the form of case reports or anecdotal evidence. Therefore the first study in this thesis aimed to examine the interaction between chronological aging and the cumulative effects of ultra-endurance racing. This was achieved by analysing the longitudinal changes in performance of four age groups of runners who completed a 56 km ultra-marathon race over ten successive years (Chapter 2). The findings from this study gave rise to three questions attempting to understand why, irrespective of age, veteran runners face an inevitable decline in performance, or an intolerance to endurance exercise. Each question was addressed in a separate chapter of the thesis.

- (i) Do several years of endurance running lead to changes in either the physiological or neuromuscular characteristics of veteran runners while running? (Chapter 3)
- (ii) Do veteran and novice runners respond similarly to a bout of exercise-induced muscle damage? (Chapter 4)
- (iii) Does a high exposure to endurance running lead to molecular changes in the skeletal muscle of veteran runners? (Chapter 5)

Finally, in light of the apparent robustness of skeletal in response to a high exposure to endurance running, five case studies describing exercise intolerant runners are presented in Chapter 6. The aim of investigating these cases is to understand the factors or circumstances leading to exercise intolerance in endurance runners.

CHAPTER 2

**THE INTERACTION OF AGING AND TEN YEARS OF RACING ON
ULTRA-ENDURANCE RUNNING PERFORMANCE**

The data presented in this chapter have been published in the following article: Rae, D.E., Bosch, A.N., Collins, M. and Lambert, M.I. The interaction of aging and 10 years of racing on ultra-endurance running performance. *Journal of Aging and Physical Activity*. 13(2): 210 – 222, 2005.

2.1 INTRODUCTION

Chronological aging is characterised by a functional decline in the major biological systems including the cardiovascular (156), metabolic (204), respiratory (78) and neuromuscular systems (47). These changes manifest as a reduced functional capacity during activities of daily living and exercise. More specifically, the age-related alterations to physical capacity can be at least partially attributed to the observed changes in skeletal muscle strength (125), morphology (128), metabolism (32, 166) and the changes in the neuromuscular system (117, 191).

In contrast to the decline in physical capacity associated with chronological aging, regular physical activity induces many favourable adaptations to these biological systems. For example, it is widely accepted that regular moderate intensity exercise improves an individual's risk profile for chronic life-style diseases (194). Furthermore, regular endurance training not only improves exercise capacity (82), but it also delays the age-associated decline in skeletal muscle strength (188) and the development of disabilities in older individuals (200). Importantly, older sedentary people adapt similarly to endurance training compared to their younger counterparts (32, 37), suggesting that regular exercise training delays the functional decline associated with the aging process.

The interactive effect of the two seemingly antagonistic stimuli of chronological aging and endurance training on performance is less well documented. Age group records, which reflect the best possible performance by an individual within a certain age category, clearly show that an age-associated decline in performance is inevitable. The U.S. age group records for the standard marathon show that the performance of male athletes declines steadily by approximately 6 – 9 % each decade between the ages of 30 and 70 years, whereafter the deterioration in performance is faster (98) (Figure 2.1). The decline in physical capacity with age (151) can be attributed to both the aging process and to a decrease in training and racing volume (103). However, these cross-sectional data should be interpreted with caution (127) as they cannot account for individual variability in changes in aging and performance and they do not take into account any cumulative effects of ultra-endurance training and racing.

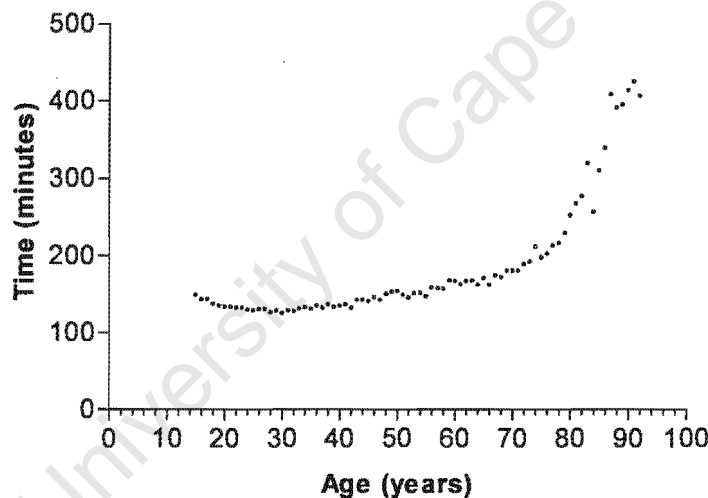


Figure 2.1 USA age group records for the marathon

The analysis of longitudinal data allows changes in performance to be associated with the combined effects of chronological aging and cumulative endurance training. A longitudinal case study showed that the performance of a competitive masters runner changed from a 40 minute 10 km time at the age of 30 years to a time of 61 minutes 59 seconds at the age of 64 years (119). This equates to a 50 % decline in performance over a 34 year period, which is 20 % more than that predicted by the cross-sectional age group records model. This suggests that cross-sectional data on the decline in performance with chronological aging under-predicts these changes.

Alternatively, this decline in performance is not linear. Longitudinal studies of larger groups of masters distance runners show that masters athletes have an improved maximum oxygen uptake, maximum heart rate and minute ventilation, compared to their sedentary peers at any given age (103, 152). However, these studies measured physiological markers of performance as opposed to actual performance. Therefore, the aim of this study was to examine the interaction between chronological aging and the cumulative effects of ultra-endurance racing. To this end, the longitudinal changes in performance of four age groups of runners who ran a 56 km ultra-marathon over ten successive years were analysed.

University of Cape Town

2.2 METHODS

2.2.1 Data source

The data used for this study were obtained from the Two Oceans 56 km Ultra-Marathon database. This electronic database, maintained by the information technology services company, Advanced Software Technologies (www.ast.co.za), is available in the public domain (www.twooceansmarathon.org.za). The database contains information for each person who has competed in the Two Oceans Ultra-Marathon (56 km) since 1970 (n = 49 750). Specifically, these data describe each runner's date of birth, gender, race times and the year in which each race was completed.

2.2.2 Subject characteristics

The data for male and female runners who had completed ten Two Oceans Ultra-Marathons were extracted from the database and used for this study. To ensure that this group was large enough for sufficient statistical power, runners who had also completed 9 or 11 races were included. Specifically, only the data for runners who had completed either 10 or 11 consecutive races, or 10 races over 11 or 12 years, or 9 races over 10 or 11 years were selected from the original database. To select these runners from the rest of the database, data were sorted initially by name, then date of birth and finally by the year of race. Data for runners who did not fall into one of these three categories were excluded from further analysis.

The age of a runner on the day of a race was determined for each race by subtracting the runners year of birth from the year of the race. The runners were then grouped according to the age at which they completed their first race. Those competitors who finished their first race at the ages of 19, 20 or 21 were included in the 20 year old age group (20's). Similarly, the 30 (30's), 40 (40's) and 50 (50's)

year old age groups contained runners who ran their first race at 29, 30 or 31; 39, 40 or 41 and 49, 50 or 51 years old respectively. Data for runners who did not fall into one of these four age groups were also excluded from further analysis.

2.2.3 Race speed analysis

The original database reported race time as hours:minutes:seconds. This was converted to minutes and used to calculate the race speed ($\text{km}\cdot\text{h}^{-1}$) for each race of every runner. The mean race speeds of races one to eleven were determined for each age group and plotted against race number. The mathematical relationship between mean race speed and race number was described best by a third order polynomial function, $y = ax^3 + bx^2 + cx + d$, where y is mean race speed ($\text{km}\cdot\text{h}^{-1}$) and x is race number. The standard deviation and coefficient of variation for each mean race speed of each runner was determined. Finally, changes in race speed from the first to the last race, from the first to the fastest, and from the fastest to the last race were calculated for each age group.

2.2.4 Statistical analysis

An analysis of variance (ANOVA) with repeated measures was used to determine differences in race speed between age group and race number. A one-way ANOVA was used to examine differences in change in race speed between the groups. Tukey's HSD post hoc test was used when the group F value for paired data was significant, while the Fisher LSD post hoc test was used for unpaired data. An independent t-test was used to determine differences between first and last race speeds between groups. A Bonferroni adjustment was made to the α level to reduce the risk of a family-wise error rate. Significance was accepted at $p < 0.050$ or $p < 0.025$ (Bonferroni adjustment). Statistical analysis was performed using Statistica (StatSoft, Inc. 2002. STATISTICA (data analysis software system) version 6.1, Tulsa, Oklahoma, USA).

2.3 RESULTS

2.3.1 Subject characteristics

Data were obtained for 194 males and females who had run 9, 10 or 11 consecutive races in the Two Oceans 56 km Ultra-Marathon over a period of 9 to 12 years. Runners in the 20's group completed their first race at the mean age of 20.5 ± 0.7 years ($n = 20$, of which 19 were males). The mean age of the 30's group at the time of their first race was 30.0 ± 1.0 years ($n = 62$, of which 57 were males). The 40's group completed their first race at the mean age of 39.9 ± 0.9 years ($n = 97$, of which 86 were males) and the 50's group was 49.4 ± 1.0 years old for their first race ($n = 15$, of which 14 were males) (Table 2.1).

Table 2.1 Subject characteristics of the 20's, 30's, 40's and 50's groups.

	20's (n = 20)	30's (n = 62)	40's (n = 97)	50's (n = 15)
Number of males	19	57	86	14
Age at first race (years)	20.5 ± 0.7	30.0 ± 1.0	39.9 ± 0.9	49.4 ± 1.0
Age at last race (years)	30.3 ± 1.2	39.8 ± 1.3	49.4 ± 1.1	58.6 ± 0.7
10 or 11 consecutive races	6	22	48	10
10 races over 11 or 12 years	9	28	25	2
9 races over 10 or 11 years	5	12	24	3

Age data are presented as the mean \pm standard deviation.

2.3.2 Race speed data

All four age groups had similar speeds for their first race. The same was true for the last and fastest races, except that the fastest race speed of the 20's group was significantly faster than that of the 50's group ($p = 0.018$). The fastest race times for the 20's, 30's, 40's and 50's groups occurred after an average of 4.1 ± 2.0 , 4.4 ± 2.1 , 3.7 ± 2.1 and 4.1 ± 2.2 years of racing respectively, and the average time taken to reach peak racing speed was not significantly different between the four groups (Table 2.2).

Table 2.2 Race speed data of the first, fastest and last races for each age group.

	20's (n = 20)	30's (n = 62)	40's (n = 97)	50's (n = 15)	p values
First race speed (km·h⁻¹)	11.1 ± 1.2	11.0 ± 1.3	10.8 ± 1.1	10.3 ± 0.7	¹ 0.999 ⁴ 0.990 ² 0.991 ⁵ 0.767 ³ 0.797 ⁶ 0.950
Fastest race speed (km·h⁻¹)	12.8 ± 1.2	12.2 ± 1.4	11.7 ± 1.1	11.0 ± 1.0	¹ 0.859 ⁴ 0.470 ² 0.095 ⁵ 0.111 ³ 0.018 ⁶ 0.714
Last race speed (km·h⁻¹)	10.8 ± 1.7	10.6 ± 1.6	10.2 ± 1.1	9.7 ± 0.7	¹ 1.000 ⁴ 0.520 ² 0.906 ⁵ 0.356 ³ 0.590 ⁶ 0.949
Time to fastest race (years)	4.1 ± 2.0	4.4 ± 2.1	3.7 ± 2.1	4.1 ± 2.2	0.315

Data are presented as the mean ± standard deviation.

¹ 20's v 30's, ² 20's v 40's, ³ 20's v 50's, ⁴ 30's v 40's, ⁵ 30's v 50's, ⁶ 40's v 50's

The mean speed of the fastest race for all four age groups was significantly faster than that of their first race ($p < 0.001$ for all groups). Similarly, the mean speed of each group's fastest race were also significantly faster than that of their last race ($p < 0.001$ for the 20's, 30's and 40's groups, $p = 0.029$ for the 50's group) (Figure 2.2).

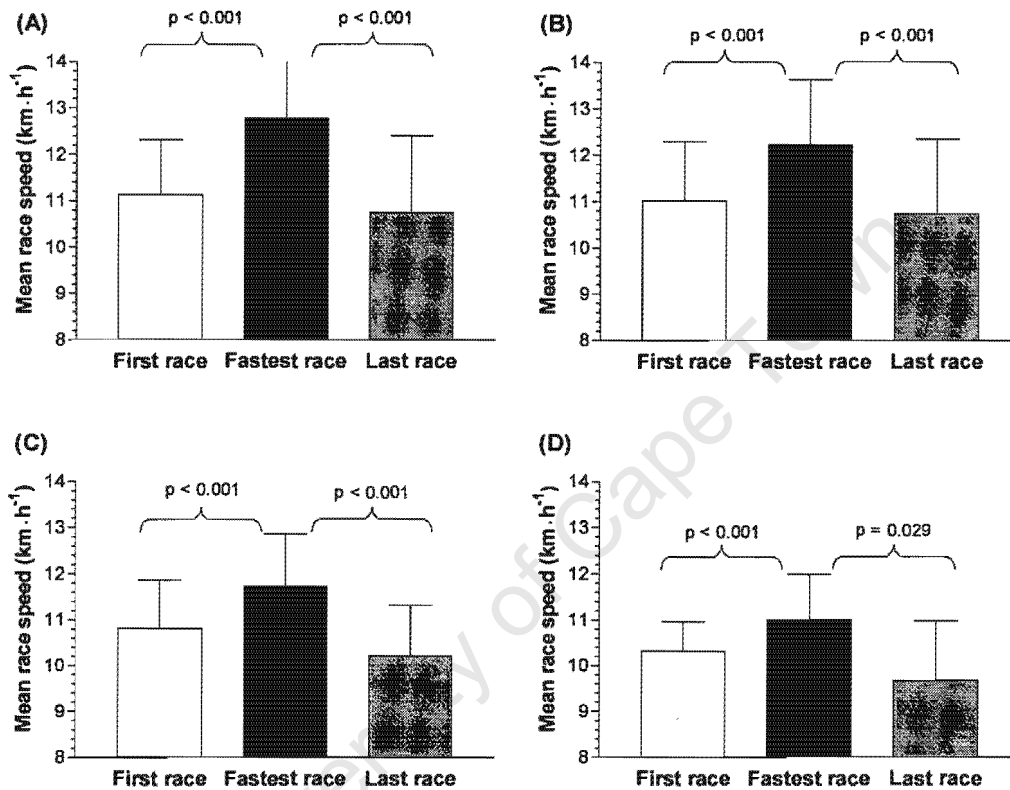


Figure 2.2 The first, fastest and last mean race speeds of the 20's (A), 30's (B), 40's (C) and 50's (D) groups.

Table 2.3 shows the changes in mean race speed from the first to the last race, from the first to the fastest race, and from the fastest to the last race for each of the four age groups. The change in speed between the first and the last race was not different between groups. In contrast, the $15 \pm 10\%$ increase in speed from the first to the fastest race of the 20's group was significantly greater than the $11 \pm 10\%$ ($p = 0.035$), the $9 \pm 7\%$ ($p = 0.001$) and the $7 \pm 6\%$ ($p = 0.001$) increases of the 30's, 40's and 50's groups respectively. The 30's group also showed a greater improvement in speed between the first and the fastest race compared to the 40's

($p = 0.031$) and 50's ($p = 0.031$) groups. This suggests that the 20's and 30's groups had a greater capacity to improve their 56 km race performance compared to the runners who started at the ages of 40 and 50 years old. With respect to the change in mean race speed from the fastest to the last race, the 18 ± 10 % decrease in speed exhibited by the 20's group was significantly greater than the 14 ± 9 % decrease of the 30's group ($p = 0.012$), the 14 ± 6 % decrease of the 40's group ($p = 0.013$) and the 13 ± 7 % decrease of the 50's group ($p = 0.017$). The decreases in speed from the fastest to the last race were not different between the 30's, 40's and 50's groups. Thus the younger runners showed a greater decline in performance once they had reached their peak, compared to the older runners.

Table 2.3 Change in mean race speed from the first to last race, the first to fastest race and the fastest to last race for all four age groups.

	20's (n = 20)	30's (n = 62)	40's (n = 97)	50's (n = 15)	p values
Δ first to last (%)	-3 ± 14	-2 ± 12	-5 ± 8	-6 ± 7	¹ 0.145 ⁴ 0.520 ² 0.001 ⁵ 0.356 ³ 0.003 ⁶ 0.949
Δ first to fastest (%)	15 ± 10	11 ± 10	9 ± 7	7 ± 6	¹ 0.035 ⁴ 0.031 ² 0.001 ⁵ 0.031 ³ 0.001 ⁶ 0.324
Δ fastest to last (%)	-18 ± 10	-14 ± 9	-14 ± 6	-13 ± 7	¹ 0.012 ⁴ 0.833 ² 0.013 ⁵ 0.550 ³ 0.017 ⁶ 0.458

Data are presented as the mean \pm standard deviation.

¹ 20's v 30's, ² 20's v 40's, ³ 20's v 50's, ⁴ 30's v 40's, ⁵ 30's v 50's, ⁶ 40's v 50's

Figure 2.3 depicts the line of best fit for mean race speed and race number for each age group, while figure 2.4 shows the variance around each curve as calculated by the 95 % confidence intervals. Specifically, Figure 2.3 shows the interaction effects of aging and number of races.

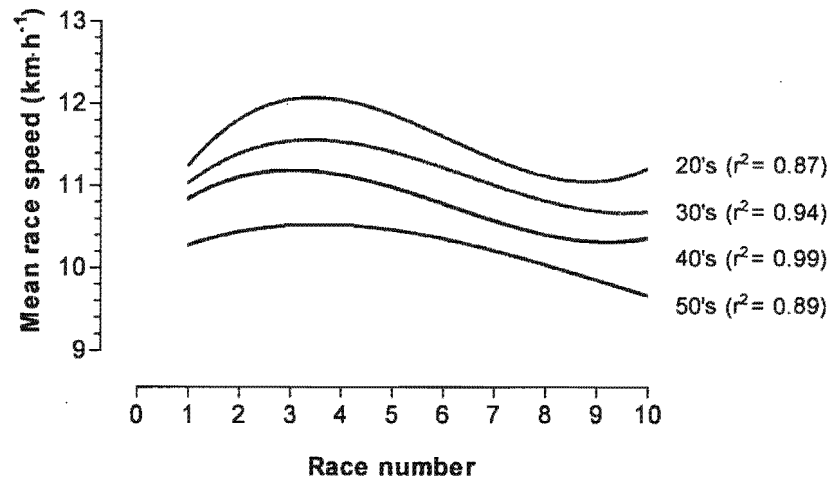


Figure 2.3 Smoothed mean race speed, represented as a 3rd order polynomial, of the ten successive races of the four age groups. For subjects that ran 11 races ($n = 25$), only races 1 – 10 were plotted. The r^2 value in parenthesis after the group label describes the goodness of fit of the third order polynomial used to show the change in mean race speed for each age group. The slight upward turn of some of the traces is likely an artefact of fitting the data to the third order polynomial and does not necessarily indicate that the performance of the runners improved at the end of the ten year race span.

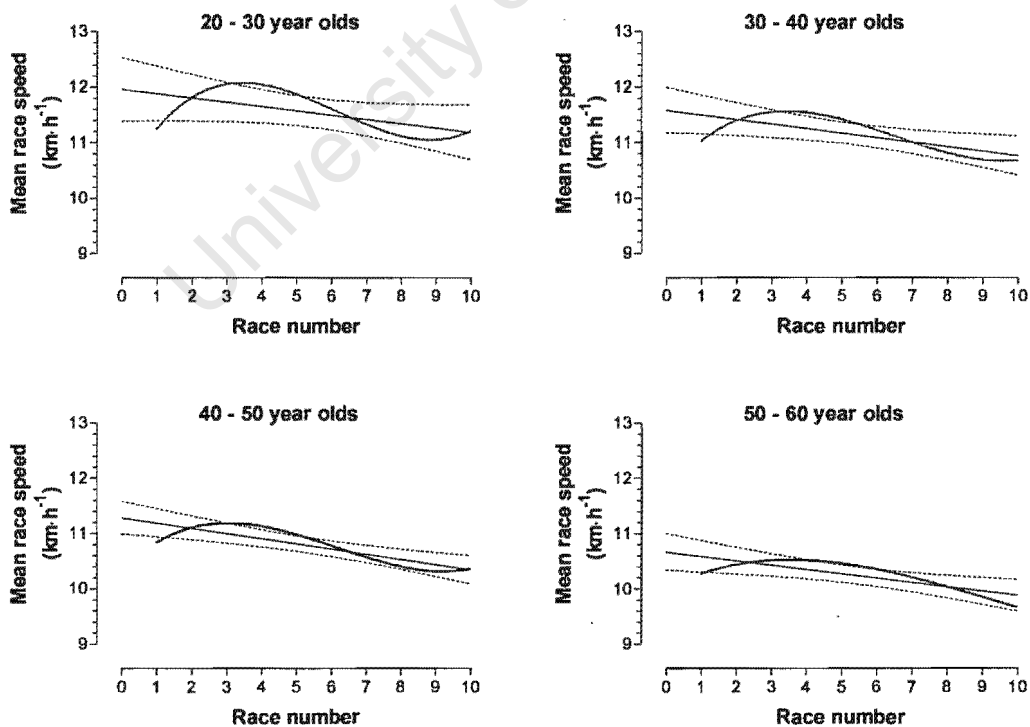


Figure 2.4 The variance around each curve, as determined by the 95 % confidence interval.

Repeated measures analysis of variance (ANOVA) was used to determine the interaction between the four groups and the first, fastest and last races. In the first versus last race analysis, there were significant main effects for group (20's, 30's, 40's and 50's, $p = 0.012$) and race number (first and last race, $p < 0.001$), but there was no interaction between group and race number ($p = 0.321$). This implies that the change in mean race speed from the first to the last race was not different between the four age groups. When the first versus the fastest race data were analysed, there was also a significant main effect for both group ($p = 0.001$) and race number (first and fastest race, $p < 0.001$). In addition, there was an interaction between group and race number ($p = 0.001$), which suggests that the groups differed in the extent to which they improved race speed between the first and fastest races. The fastest race versus the last race was analysed and significant main effects for group ($p < 0.001$) and race number (fastest and last race, $p < 0.001$) were found. There was also an interaction between group and race number ($p = 0.048$) indicating that the decline in mean race speed from the fastest to the last race was different between the four age groups.

Finally, one-way ANOVA was used to determine differences between the last race of one age group and the first race of the subsequent age group to examine the effects of ten years of racing on performance. The mean race speed of the last race of the 20's group ($10.8 \pm 1.7 \text{ km}\cdot\text{h}^{-1}$), for which this group of runners would have been 29 years old, was not significantly different from that of the first race of the 30's group ($11.0 \pm 1.1 \text{ km}\cdot\text{h}^{-1}$) ($p = 0.440$) (Figure 2.5). The last race of the 30's group ($10.8 \pm 1.6 \text{ km}\cdot\text{h}^{-1}$), representing 39 year old runners, was not significantly different compared to the first race of the 40's group ($10.8 \pm 1.1 \text{ km}\cdot\text{h}^{-1}$) ($p = 0.787$). Similarly, the mean speed of last race of the 40's group ($10.2 \pm 1.1 \text{ km}\cdot\text{h}^{-1}$), when the runners would have been 49 years old, was not different from that of the first race of 50's group ($10.3 \pm 0.7 \text{ km}\cdot\text{h}^{-1}$) ($p = 0.713$).

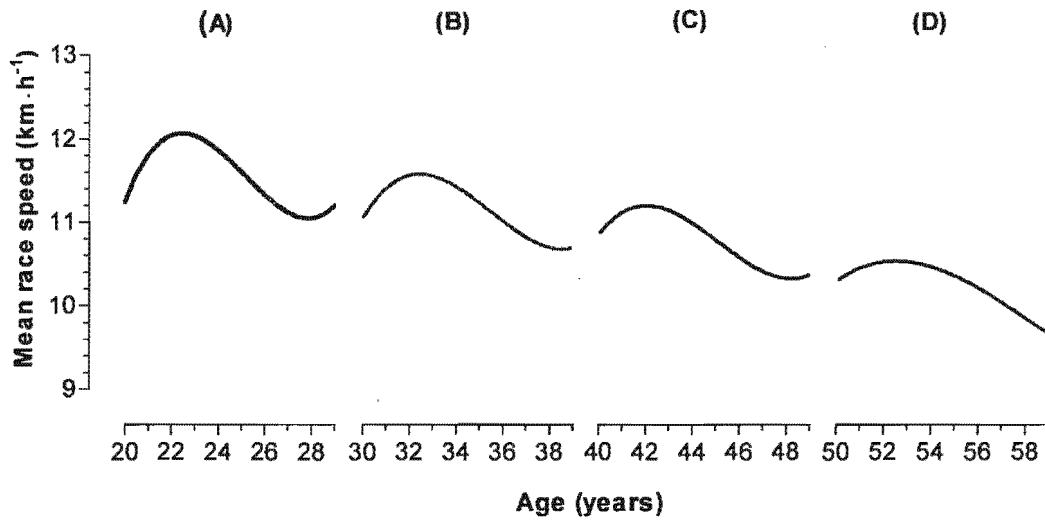


Figure 2.5 The smoothed mean race speed of the ten successive races completed by each of the four age groups plotted along a continuum of age from 20 to 59 years. (A) 20's, (B) 30's, (C) 40's and (D) 50's.

University of Cape Town

2.4 DISCUSSION

The aim of this study was to examine the interaction between chronological aging and the cumulative effects of ultra-endurance racing. Running performance, defined as mean race speed, was determined for each of the 10 races completed by runners in the 20's, 30's, 40's and 50's age groups. The longitudinal data were examined for differences between the groups with respect to change in race speed over the ten year period.

The first important finding relates to the pattern of change in performance of this 56 km race over ten years for each of the four age groups (Figure 2.3). While all four age groups ran their first race at a similar speed and improved their respective performances significantly from the first to the fastest race, the extent of this improvement was greater in the younger runners (20's groups) compared to the older runners (50's group).

These data suggest that runners who completed their first Two Oceans Ultra-Marathon at a younger age had a greater capacity to improve their 56 km ultra-endurance running performance compared to those who first competed in the race later on in life. It is well established that endurance training induces adaptations in various biological systems which result in an improvement in physical capacity in both young and older sedentary individuals (32, 109, 174). Additionally, the extent of adaptation in older people compared to younger people has been shown to be similar (32). However, these adaptations have only been related to changes in physiological measures associated with physical capacity, such as maximum oxygen uptake, maximum heart rate and minute ventilation, and not to actual performance, such as race time for an ultra-endurance event. The data from this study demonstrated that the younger runners (20's and 30's groups) had a greater capacity to improve their absolute and relative 56 km race time compared to older runners (40's and 50's groups). This suggests that chronological age may be one of the factors that affect the extent to which an individual can improve ultra-endurance performance in this particular 56 km race. Presumably younger runners either simply have a greater capacity to adapt to training compared to older runners, or the

younger runners in this study underwent training of a greater volume and / or intensity which in turn lead to greater improvements in race time.

The second significant finding was that each age group took approximately four years to reach peak running speed for this ultra-endurance race (Table 2.2). Assuming that the runners trained regularly and that they were always trying to achieve a personal best time, it seems that regardless of the age at which the runners completed their first race, a period of about four years was required for the manifestation of adaptations associated with peak running performance during this ultra-endurance event. It has been shown that after only eight weeks of regular endurance training significant acute metabolic and respiratory changes in skeletal muscle, presumed to be positive adaptations to the training, have occurred (85). Further adaptations needed to elicit optimal ultra-endurance performance must therefore require a more chronic stimulus. One possible adaptation may be that of learned pacing strategies (118). Alternatively, neuromuscular adaptations such as changes in pre-activation and musculotendinous stiffness may contribute to this delayed adaptation.

Thirdly, this data shows that it was not possible for any of the age groups to compete at their fastest race speed for more than a few years (Figures 2.2 and 2.3). Instead, mean race speed decreased significantly for all four age groups between the fastest and the last race, over a period of approximately six years, to a speed similar to that of the first race. Additionally, the rate at which this decline occurred was different between the four age groups. It is interesting to note that despite the fact that the runners had the necessary adaptations to compete at their optimal capacity in a 56 km race, they were still unable to continue racing at this peak speed. One conclusion might be that chronological aging contributes to this decline in performance. Age-group race records show that there is an inevitable slowing down of race speed (98) with increasing age, which may be attributed to either aging or a decrease in training and racing volume. If chronological aging had been solely responsible for the performance decrement seen in the four age groups in this study, one would have expected the 50's group to have slowed down more than the younger groups. However, the data showed the opposite to be true: the 20's group had a significantly greater decrease in mean race speed between the fastest and

the last race compared to the 30's, 40's and 50's groups. Given that the main difference between the 20's and 50's groups was that the younger group initially improved more and ran faster than the older group, it may be that race speed is an important factor contributing to the seemingly inevitable age-associated decline in 56 km ultra-endurance performance over time when runners under the age of 60 years old are studied. Another possible explanation for the runners in this study being unable to maintain their peak race speed might be that while muscle can adapt to and tolerate a given volume and intensity of training over a number of years, there may be a threshold where after additional training stress leads to maladaptations within the muscle.

The fourth finding was that over the ten year period, the net change in mean race speed between the first and the last race was not different between the four age groups and that the mean race speed of the last race was not significantly slower than the first race for all four age groups. This suggests that the combined effects of ten years of racing and ten years of chronological aging neither improves nor worsens net performance during a 56 km ultra-endurance race in individuals between the ages of 20 and 60 years. Despite a more limited ability to improve 56 km running performance, older runners do not face a greater decline in performance over ten years of racing compared to younger runners. This implies that between the ages of 20 and 60 years, runners are equally susceptible to any performance limiting effects that may be imposed by an accumulation of training and racing distance over a ten year period. One might argue that the older runners would be less likely to train at the same speeds and volumes as the younger runners and would therefore not be subjected to the same cumulative stress. A longitudinal study of older and younger runners following a training programme of the same absolute pace and volume and the same racing schedule over at least ten years may answer this question.

Finally, the effect of ten years of ultra-endurance racing on performance during a 56 km race was examined by comparing the last race of each age group to the first race of the next age group. For example, the mean speed of the last race of the 30's group, who would by then have been 39 years old, was compared to the mean speed of the first race of the 40's group. If competing in the Two Oceans Ultra-

Marathon for ten years has an adverse effect on a runner's performance, one would expect to see a difference in performance between two similarly aged competitors, one of whom had completed ten races, the other having run the 56 km race for the first time. The final finding of this study showed that there was no difference in mean race speed between the last race of one age group and the first race of the subsequent group (Figure 2.5). Therefore, despite ten years of racing, runners between the ages of 20 and 50 years are still able to run this 56 km race at a similar speed to their age matched peers that have only just begun racing. This suggests that ten years of ultra-endurance racing has no adverse effects on performance during a 56 km race. An alternative interpretation is that it appears that ten years of training does not have any advantage over short term training. The difficulty in making this comparison, however, is that the runners are presumably in different stages of adaptation. Those runners completing their first race may not yet have undergone all the training induced adaptations associated with ultra-endurance racing that allow them to reach their optimal performance level and are presumably still going to improve, while those runners competing in their tenth race have passed their optimal performance point and are in the process of decline.

A limitation of this study is that it was assumed that the runners in each age group were relatively inexperienced ultra-marathon athletes when they ran their first race. Although the runners in the study had never run this particular 56 km race before, they may well have completed any number of other marathon or half marathon events. It was not possible, however, to check the validity of this assumption. As the data were analysed as means, it was anticipated that the few subjects deviating from this assumption would not have had a significant impact on the trends and therefore the overall interpretation.

2.5 CONCLUSION

In summary, the data showed that for this 56 km ultra-endurance running race:

- (i) Performance improved and declined at greater rates for runners younger than 40 years of age.
- (ii) Runners younger than 40 years had a greater capacity to improve their performance compared to runners older than 40 years of age.
- (iii) It appears that approximately four years of racing and training are required to reach peak racing speed, regardless of age.
- (iv) It does not seem possible to sustain this peak racing speed for more than a few years.
- (v) The combined effects of ten years of chronological aging and ten years of racing neither improves nor worsens net performance in runners aged between 20 and 60 years of age.

In conclusion, these data suggest that while all four age groups of runners in this study showed similar patterns of change in 56 km race speed over a ten year period, the extent of change in performance was greater in the runners younger than 40 years of age compared to the runners older than 40 years. The veteran runners of a given age, i.e. those who had competed in ultra-endurance races over a span of ten years, ran at a similar speed to novice runners of the same age. The main finding of this study was that despite similar current performance times, the novice runners would almost certainly improve their performance, while the veteran runners were more likely to continue slowing down. This descriptive study raises a number of interesting questions regarding the effects of a high exposure to endurance running and the seemingly inevitable decline in performance facing veteran runners.

- (i) Do several years of endurance running lead to changes in either the physiological or neuromuscular characteristics³ of veteran runners while running? (To be addressed in Chapter 3)
- (ii) Do veteran and novice runners respond similarly to a bout of exercise-induced muscle damage? (To be addressed in Chapter 4)
- (iii) Does a high exposure to running lead to molecular changes in the skeletal muscle of veteran endurance? (To be addressed in Chapter 5)

CHAPTER 3

**A COMPARISON OF VETERAN AND NOVICE ENDURANCE RUNNERS:
Physiological and neuromuscular characteristics during maximal and
sub-maximal running**

3.1 INTRODUCTION

The findings from the previous chapter raise some interesting ideas. Irrespective of age, runners tend to improve during their first few years of ultra-endurance racing, where after they face a slow decline in performance (Figure 3.1). Furthermore, after approximately ten years of training and racing, these "veteran" runners are statistically no better or worse off with regards to performance than when they started. They are, however, in a different phase of adaptation to running training and racing. While the "novice" runners are still likely to adapt to training with the result of improving their performance, the similarly performing "veteran" runners are more likely to become slower, despite the advantage of more years of training and experience.

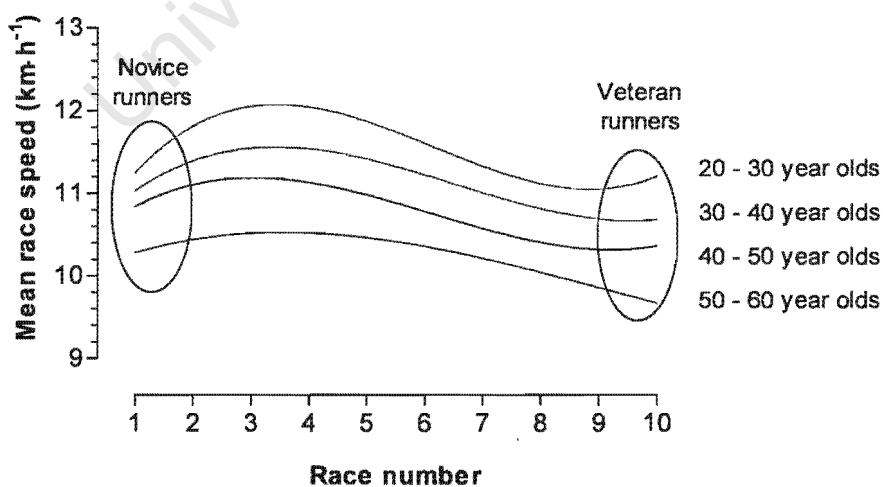


Figure 3.1 Longitudinal performance changes in four age groups of endurance runners who completed ten consecutive Two Oceans 56 km Ultra-Marathon races.

While increasing age is known to reduce physical capacity (148, 173) 30, 40, and even 50 year old runners can hardly be assigned to the elderly population. Therefore something other than, or in addition to, aging must contribute to their reduced capacity to improve performance. Furthermore, since exercise has also been shown to delay physiological changes associated with aging (188, 200), the argument for another precipitating factor becomes stronger. One possibility might be that exposure to running training and racing acts as a chronic stressor to the human body. In fact, Kuipers et al. found that over an 18 month period, changes in muscle morphology were associated with distance covered in training rather than the intensity of the exercise (116). While adaptations to endurance training may be interpreted as being positive, as performance and a general sense of well-being improves, the cumulative stresses associated with repetitive exposure to running may ultimately become a negative stressor. For example, veteran runners who have competed in more than 5 000 km of races have been shown to have a decreased level of neuromuscular efficiency following a bout of exercise designed to induced muscle damage (172).

Therefore the aim of this study was to answer the first question raised in Chapter 2: Do several years of endurance running lead to either physiological or neuromuscular changes in the skeletal muscle of veteran runners while running? It was anticipated that any differences in these characteristics between veteran and novice endurance runners may contribute to understanding the seemingly inevitable decline in performance facing veteran runners.

3.2 METHODS

3.2.1 Subject characteristics

Thirteen healthy distance runners (12 males and one female) were recruited by advertising both publicly and at running clubs in the greater Cape Metropolitan. Seven of these runners, all males, were allocated to the veteran runners group (VET). These runners fell into the "veteran" stage of the 20-30, 30-40 and 40-50 year old groups shown in Figure 3.1 as they had been taking part in endurance races for at least eight years. The remaining six runners (one female) were allocated to the novice runners group (NOV) as they had been running for no more than five years. This group was similar to the "novice" runners of the 20-30, 30-40 and 40-50 year old groups shown in Figure 3.1 as they were in their first few years of endurance running. All subjects participated actively in long distance races. None of the runners suffered from physiological or pathological fatigue conditions, classic chronic fatigue syndrome, overtraining syndrome or any neuromuscular conditions (48). The study was explained in detail to each subject who then completed a consent form (Appendix 1) agreeing to participate in the study. The Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town approved this study (REC reference number: 181/2002).

3.2.2 Experimental design

All runners (VET and NOV) were required to visit the laboratory on two occasions, three days apart. They were asked to refrain from exercising the day before the trial began and not to participate in any races during the week preceding the trial. The runners were, however, allowed to train normally for the duration of the trial.

Visit 1

The study was explained in detail to the runners and, on agreeing to participate, they signed the consent form and completed questionnaires on their personal particulars, medical history, social sport participation (Appendix 1), competitive sport training and racing history as well as a one week diary to ensure they were not currently overtrained (Appendix 2). The body composition of each runner was measured and after they were familiarised with running on a treadmill, they performed a peak treadmill running speed (PTRS) test.

Visit 2

The runners visited the laboratory three days later and were familiarised with the 140 m indoor running track and the pacing lights system that would be used to pace them through a 15 minute sub-maximal run. After the sub-maximal run, they rested for 10 minutes before running a 5 km time trial on the same indoor track at a self-selected pace.

3.2.3 Detailed physiological testing procedures

3.2.3.1 Anthropometry

Body composition measurements included height and weight, seven skinfolds, three girths and the sub-gluteal to above knee height. Height (m) and weight (kg) were used to determine body mass index ($\text{m}\cdot\text{kg}^{-2}$). The biceps, triceps, abdominal, subscapular, supra-iliac, mid thigh and calf skinfolds (mm) formed the sum of seven skinfolds measurement (mm) (165) while percentage body fat was estimated based on the Durnin and Womersley equation (53). Lean body mass (kg) was calculated from mass and percentage body fat. The sub-gluteal, mid thigh and above knee girths as well as the sub-gluteal to above knee height (cm) were used to determine lean thigh volume (cc^2) (102). The same investigator performed all measurements to avoid inter-tester measurement errors.

3.2.3.2 Peak treadmill running speed test

The peak treadmill running speed of each runner was determined using a modified Noakes protocol of the standard VO_2 max test (169) on a motor driven treadmill (Quinton Instruments, Seattle, WA, USA). Female and male participants began the test at speeds of 10 and 12 $\text{km}\cdot\text{h}^{-1}$ respectively, and the speed was increased by 0.5 $\text{km}\cdot\text{h}^{-1}$ every 30 seconds. The test ended when the runner decided that he / she was no longer able to continue at that speed. PTRS was recorded as the fastest speed at which the individual was able to complete the entire 30 second stage.

Heart rate (HR), rating of perceived exertion (RPE), volume of oxygen inspired (VO_2) and ventilation rate (VE) were measured continuously during the test. HR was measured using a Polar Accurex NV heart rate monitor (Polar Electro OY, Kempele, Finland). RPE was determined using Borg's RPE scale (Appendix 3) (13). The Oxycon Alpha analyzer system (Jaeger-Mijnhart, Bunnik, the Netherlands) was used to take respiratory measurements during the test. The system captures breath by breath data and displays this information as an average of adjacent 10 second periods. Before each test, the gas meter was calibrated with a Hans Rudolph 3L syringe (Vacumed, Ventura, CA) and the analyzers were set with room air and a 4 % carbon dioxide : 96 % nitrogen gas mixture.

3.2.3.3 Sub-maximal run

The participants were required to run for 15 minutes at a constant velocity (70 % of their PTRS) on an indoor track. HR, VO_2 and VE were recorded continuously during the run. RPE, electromyography (EMG) and footswitch signals were measured every three minutes on a 20 m straight of the track. EMG and footswitch data were recorded for between eight and ten strides.

Each runner's speed was controlled using a pacing light system (Pacerlite 1) built by Harry Hall, Department of Human Biology, University of Cape Town. HR was recorded using the same Polar heart rate monitor as described in section 3.2.3.2. Participants carried a portable oxygen analyzer (K4 b², Cosmed, Rome, Italy) while running, which measured breath by breath VO_2 and VE. The system consisted of a gas mask, secured to the runners head with a velcro head strap, which was

connected to the analysing unit and battery pack and secured to their trunk using a custom made harness. RPE was determined using the Borg RPE scale (13). The EMG and footswitch signals were recorded using a telemetric EMG system (Telemetry Research System 9000, Noraxon, Arizona, USA).

The EMG electrode sites were at the center of the belly of the vastus lateralis (VL), vastus medialis (VM) and medial gastrocnemius (MG) muscles, and on the tibial tuberosity of the right leg. Hair and dead skin cells were removed from the electrode sites by shaving and sandpapering a patch of skin, approximately 100 cm², around the site. The skin was cleaned with alcohol swabs and two electrodes (Blue Sensor, Medicotest, Denmark) were placed parallel to each other, with approximately 2 cm between the electrode centers, on the muscle belly perpendicular to the direction of the muscle fibres. A single reference electrode was placed on the tibial tuberosity. Strapping tape was placed over the electrodes to minimise their movement and to help prevent sweat from interfering with the signal. The strapped electrodes and leads were secured to the leg using elastoplast tape. The leads plugged into the telemetric transmitter unit, which was strapped to the waist of the subject. The electrode pairs recorded the surface electrical activity (μV) corresponding to the underlying muscle, sampled at a frequency of 2 000 Hz.

A footswitch (Norswitch bilateral telemetric footswitch system, Noraxon, Arizona, USA) was taped to the right foot of each subject. One sensor of the footswitch was taped to the distal phalange of the right hallux, at the point where the toe lifts off the ground and the second sensor was taped similarly to the middle, posterior part of the sole of the foot at the tip of the calcaneus. The footswitch sensors were secured with tape before the runner replaced his / her sock and shoe. The footswitch was also plugged into the EMG transmitter box and recorded heel strike and toe off of the right foot. The electrical signal from the transmitter unit was picked up remotely by the receiver unit, passed through an analogue to digital converter card and stored digitally on a computer. This signal was recorded and synchronised with EMG activity of the three muscles.

3.2.3.4 Time trial

To simulate running under race conditions, the runners completed a 5 km time trial as fast as possible, on the same indoor track described above. As respiratory measurements were not recorded during the time trial, the portable oxygen analyser was removed, but the HR monitor, EMG electrodes and the footswitch remained in place as for the sub-maximal run. HR was recorded continuously during the time trial while time RPE, EMG and footswitch data were recorded for the last eight to ten strides of every kilometer as described in section 3.2.3.3. The runners carried a watch which recorded their run time while the tester gave them the distance as they completed each kilometer and encouraged them verbally.

3.2.4 Data analysis

3.2.4.1 Training and racing data

Each runner completed extensive running training and racing history questionnaires. Using their personal logbooks they retrospectively recorded their annual training and racing information. From this, average training frequency ($\text{days}\cdot\text{wk}^{-1}$) and speed, total training distance, total racing distance and combined racing and training distance were calculated for each runner for three periods - their entire career, their novice years (first two years of running) and the past year. Given that one of the VET runners was an outlier with respect to training, racing and combined training and racing distances, these three variables were log transformed for analysis. Each participant also completed a retrospective diary documenting their training and racing during the eight weeks prior to the first day of the trial. From this their current weekly training distance, frequency and speed were determined.

3.2.4.2 Heart rate

Recorded heart rate was downloaded from the monitor and analysed using the Polar Precision Performance Software (version 2.10.007). Each data point of the continuous raw HR data represented the average value of adjacent five second periods throughout the test. Peak HR during the PTRS test was the highest

recorded value for any five second period. The 3, 6, 9, 12 and 15 minute HR values during the sub-maximal run were the averages of HR data collected for the 30 seconds preceding each time point. The steady state HR value was the average HR from minutes 12 to 15 of the sub-maximal run. The HR values representing the 1st, 2nd, 3rd, 4th and 5th kilometers of the time trial were the averages of the last 30 seconds of each respective kilometer.

3.2.4.3 Respiratory measurements

The respiratory data collected during the PTRS test was used to determine the maximum VO_2 and VE values. These peak values were taken to be the highest values recorded over any ten second period during the test. The portable K4 system was not used during the PTRS test since the reliability of its recording during maximal exercise has not been confirmed. The respiratory data collected during the sub-maximal run was downloaded using the KB b² Data Management Software (version 7.4a, Cosmed, Rome, Italy) and the raw data was subsequently exported and analysed in Microsoft Excel. The last 30 seconds worth of data for each three minute stage was averaged to give minute 3, 6, 9, 12 and 15 values for VO_2 and VE. Steady state values were the average over the last three minutes of the sub-maximal run.

3.2.4.4 Stride characteristics

The exact points of heel strike and toe off for each step were determined manually using the footswitch trace in Noraxon's MyoResearch software (version 2.11.90.81.200, Noraxon, Arizona, USA). For the purpose of this study contact time (CT) is defined as the period from heel strike to toe off of the same foot and flight time (FT) is the period from toe off of the right foot to heel strike of the left foot. A step is defined as the contact time plus the subsequent flight time of the same leg. A stride is defined as the step of the right leg plus the following step of the left leg and stride time (ST) is the time taken to complete a stride. Stride frequency (SF) is the number of strides taken in one second and stride length (SL) is the distance covered in one stride.

The following equations were used to calculate the stride parameters using the heel strike (HS) and toe off (TO) times:

$$\begin{aligned}
 \text{CT (ms)} &= \text{TO time} - \text{HS time} \\
 \text{FT (ms)} &= [(\text{right foot HS time (n+1)} - \text{right foot HS time (n)}) - \text{CT}] / 2 \\
 \text{ST (ms)} &= [\text{HS time (n+1)} - \text{HS time (n)}] \\
 \text{SF (strides}\cdot\text{s}^{-1}) &= 1 / (\text{ST} / 1000) \\
 \text{SL (m)} &= \text{running velocity} / \text{SF}
 \end{aligned}$$

3.2.4.5 Electromyography

The raw digital electromyography (EMG) signal was processed using Noraxon's MyoResearch software (version 2.11.90.81.200, Noraxon, Arizona, USA) and the data analysed in Microsoft Excel. The raw EMG signal was passed through a 50 Hz notch filter to remove any electrical interference from external sources. A 15 – 500 Hz bandpass filter was used to remove movement artefacts (signals below 15 Hz) and non-physiological signals (above 500 Hz). The filtered signal was then smoothed using the root mean square method over 50 ms windows. An integrated EMG (iEMG) value ($\mu\text{V}\cdot\text{s}$) was calculated for each step and then time-normalised to one second ($\mu\text{V}\cdot\text{s}$) $\cdot\text{s}^{-1}$.

The final time-normalised, iEMG values for each time point in the sub-maximal run (3, 6, 9, 12 and 15 minutes) and each kilometer in the time trial (1, 2, 3, 4 and 5 km) were the averages of at least eight strides collected for each of the five time or five distance points respectively. The 6, 9, 12 and 15 minute iEMG values of the sub-maximal run were normalised to the minute 3 iEMG value and expressed as a percentage of this sub-maximal recording. The five time trial iEMG readings were also normalised to the minute 3 sub-maximal iEMG value. iEMG readings were determined for both the pre-activation (PA) phase and ground contact time of the step. The pre-activation phase was the 100 ms period prior to heel strike and ground contact time was the period from heel strike to toe off of the same foot.

3.2.5 Statistical analysis

An independent t-test was used to compare differences between the VET and NOV groups with respect to their descriptive, training and racing characteristics, as well as the physiological, stride and EMG variables measured at steady state during the sub-maximal run. As the VET group was significantly older than the NOV group, an analysis of co-variance for age was performed on all the data. The levels of significance reported in the results section only show the co-variance for age in cases for which the co-variance proved necessary. Since one of the VET runners was an outlier with respect to training distance and total training and racing distance, an independent t-test was performed on the log of these two variables (89). A dependent t-test was used to compare the 5 km time trial performance of each group during the trial to that of their current time and personal best effort. Repeated measures analysis of variance (ANOVA) was used to determine differences between the groups with respect to the physiological, performance, stride and EMG variables measured during the sub-maximal run and the time trial. Correlations were performed using Pearson's Product-Moment with pair-wise deletion of missing data. Statistical significance was accepted at $p < 0.050$.

3.3 RESULTS

3.3.1 Descriptive characteristics

Table 3.1 Descriptive characteristics of the VET and NOV runners.

	VET (n = 7)	NOV (n = 6)	p value
Age (years)	39.7 ± 8.0	28.7 ± 6.9	0.023 *
Height (m)	1.71 ± 0.08	1.73 ± 0.07	0.601
Weight (kg)	68.1 ± 6.6	69.8 ± 11.9	0.749
BMI (kg·m ⁻²)	23.3 ± 1.1	23.1 ± 2.4	0.845
S7 (mm)	67.7 ± 14.0	84.1 ± 18.2	0.094
Body fat (%) ¹	17.3 ± 3.5	19.0 ± 4.8	0.027 *
LBM (kg)	56.4 ± 6.8	56.5 ± 9.8	0.981
LTV (cm ³)	4 026 ± 531	4 103 ± 322	0.768
PTRS (m·s ⁻¹)	5.2 ± 0.4	4.9 ± 0.6	0.215
Peak VO ₂ (ml·kg ⁻¹ ·min ⁻¹)	58.2 ± 5.7	53.8 ± 7.1	0.245
Peak HR (bpm) ¹	185.6 ± 10.7	193.3 ± 11.4	0.687

Data are presented as the mean ± standard deviation.

* Significant difference between the VET and NOV groups.

¹ Co-variance for age was necessary.

LTV: n = 6 for the VET group.

Abbreviations: VET – Veteran runners group, NOV – Novice runners group, BMI – Body mass index, S7 – Sum of 7 skinfolds, LBM – Lean body mass, LTV – Lean thigh volume, PTRS – Peak treadmill running speed, VO₂ – Volume of oxygen inspired, HR – Heart rate, bpm – beats per minute.

The VET runners were approximately 11 years older than the NOV runners ($p = 0.023$) (Table 3.1). To account for this age difference, the descriptive variables were re-analysed using an analysis of co-variance for age. Statistically, this was only necessary for the percentage body fat and peak heart rate variables. Therefore, the p values reported in Table 3.1 represent the levels of significance as determined by an independent t -test for all variables, except percentage body fat and peak heart rate. The VET and NOV groups were matched for anthropometric variables such as height, weight, BMI, sum of seven skinfolds, lean body mass and lean thigh volume, as well as peak treadmill running speed, peak oxygen consumption and peak heart rate (Table 3.1). Percentage body fat, however, was significantly lower in the VET group compared to the NOV group ($p = 0.027$).

3.3.2 Training characteristics

Table 3.2 shows the training characteristics of the VET and NOV runners for three time periods: during their entire running career, during their novice years and during the past year. Both groups began distance running at similar ages, but the VET runners had been running for significantly longer than the NOV runners had ($p = 0.004$), explaining the current age difference between the groups. The VET runners had also accumulated a greater distance in training during their entire career than the NOV runners had ($p = 0.001$). Training frequency, weekly distance and speed of the two groups during their entire running career, their novice years or the past year were not different.

To determine the current training frequency, weekly distance and speed of the groups, the runners were asked to complete retrospective training logs detailing their training during the eight weeks preceding the trial. This data showed that the recent average weekly training distance of the VET group was 45.2 ± 33.2 km, at a frequency of 2.7 ± 0.4 days per week and at a speed of 11.2 ± 1.8 km·h⁻¹. In comparison, the NOV group covered a weekly distance of 25.2 ± 12.6 km, training approximately 2.2 ± 1.5 days per week at a speed of 11.3 ± 2.1 km·h⁻¹. There were

no statistical differences between the current weekly training distance ($p = 0.450$), frequency ($p = 0.231$) or speed ($p = 0.913$) of the two groups.

Table 3.2 Training characteristics of the VET and NOV runners.

	VET (n=6)	NOV (n=5)	p value	
Entire career	Age began running (yrs)	25.0 ± 9.8 (16.0 – 41.0)	24.8 ± 6.6 (17.0 – 35.0)	0.970
	Time running (yrs)	15.1 ± 7.3 (8.0 – 28.0)	2.6 ± 1.7 (1.0 – 5.0)	0.004 *
	Total distance (km)	45 285 ± 56 154 (10 110 – 158 240)	3 275 ± 2 278 (1 040 – 5 810)	0.001 *
	Frequency (days·wk⁻¹)	5 ± 1 (3 – 7)	4 ± 1 (2 – 5)	0.271
	Distance (km·wk⁻¹)	61.2 ± 28.2 (33.4 – 109.8)	38.1 ± 8.6 (25.0 – 47.0)	0.113
	Speed (km·h⁻¹)	11.2 ± 2.2 (9.0 – 13.3)	10.4 ± 0.4 (10.1 – 10.8)	0.392
Novice years	Total distance (km)	3 374 ± 1 935 (1 152 – 5 720)	2 295 ± 1 292 (1 040 – 4 216)	0.317
	Frequency (days·wk⁻¹)	4 ± 1 (4 – 6)	4 ± 1 (2 – 5)	0.555
	Distance (km·wk⁻¹)	46.4 ± 14.7 (32.5 – 70.0)	38.2 ± 11.7 (20.0 – 50.0)	0.338
	Speed (km·h⁻¹)	10.4 ± 1.4 (9.0 – 12.2)	10.1 ± 0.4 (10.1 – 10.8)	0.663
Past year	Frequency (days·wk⁻¹)	5 ± 2 (3 – 7)	4 ± 0 (4 – 5)	0.185
	Distance (km·wk⁻¹)	65.1 ± 28.2 (20.3 – 100.0)	41.2 ± 7.3 (35.0 – 50.0)	0.109
	Speed (km·h⁻¹)	10.4 ± 2.2 (8.3 – 13.0)	10.4 ± 1.1 (10.1 – 12.2)	0.953

Data are presented as the mean ± standard deviation, with the range in parentheses.

* Significant difference between the VET and NOV groups.

Age began running and Time running: $n = 7$ for the VET group.

Abbreviations: VET – Veteran runners group, NOV – Novice runners group.

3.3.3 Racing characteristics**Table 3.3 Racing characteristics of the VET and NOV runners.**

	VET (n=7)	NOV (n=5)	p value	
Entire career	Years in racing	11.9 ± 7.4 (6.0 – 28.0)	2.4 ± 1.7 (1.0 – 5.0)	0.020 *
	Number of races	100 ± 44 (28 – 153)	15 ± 9 (4 – 25)	0.002 *
	Total distance (km)	2 337 ± 1 051 (566 – 3 686)	384 ± 347 (81 – 921)	0.003 *
	PB 5 km time (min)	18.7 ± 3.1 (14.6 – 23.0)	21.0 ± 4.8 (16.1 – 26.2)	0.399
	PB 10 km time (min)	37.3 ± 4.8 (31.0 – 43.1)	44.4 ± 10.1 (33.3 – 56.0)	0.139
	PB 21.1 km time (min)	84.0 ± 9.9 (68.0 – 97.0)	97.2 ± 16.2 (81.3 – 114.7)	0.123
	PB 42.2 km time (hrs)	3.3 ± 0.5 (2.4 – 3.9)	3.8 ± 0.8 (3.2 – 5.0)	0.188
Novice years	Best 5 km time (min)	17.8 ± 2.4 (14.6 – 20.0)	21.4 ± 4.4 (18.9 – 26.2)	0.582
	Best 10 km time (min)	39.0 ± 6.5 (31.0 – 49.7)	46.2 ± 8.2 (36.7 – 56.0)	0.139
	Best 21.1 km time (min)	87.8 ± 14.7 (69.0 – 114.3)	102.1 ± 14.9 (86.1 – 115.0)	0.157
	Best 42.2 km time (hrs)	3.5 ± 0.6 (2.4 – 4.3)	4.0 ± 0.7 (3.3 – 5.0)	0.248
Past year	Best 5 km time (min)	20.0 ± 2.44 (17.3 – 23.0)	21.7 ± 4.1 (17.5 – 26.2)	0.454
	Best 10 km time (min)	39.4 ± 3.6 (35.4 – 44.0)	44.4 ± 8.4 (38.3 – 56.0)	0.139
	Best 21.1 km time (min)	90.6 ± 11.2 (75.0 – 108.0)	97.2 ± 16.2 (81.3 – 114.7)	0.444
	Best 42.2 km time (hrs)	3.5 ± 0.7 (2.6 – 4.8)	3.8 ± 0.8 (3.2 – 5.0)	0.583

Data are presented as the mean ± standard deviation, with the range in parentheses.

CHARACTERISTICS OF VETERAN AND NOVICE RUNNERS

* Significant difference between the VET and NOV groups.

Years in racing: $n = 6$ for the NOV group.

PB 5 km time: $n = 5$ for the VET group and $n = 4$ for the NOV group.

PB 10 and 21.1 km times: $n = 4$ for the NOV group.

Novice years best 5 km time: $n = 4$ for the VET and NOV groups.

Novice years best 10, 21.1 and 42.2 km times: $n = 4$ for the NOV group.

Past year best 5 km time: $n = 5$ for the VET group and $n = 4$ for the NOV group.

Past year best 10 and 42.2 km times: $n = 6$ for the VET group and $n = 4$ for the NOV group.

Past year best 21.1 km time: $n = 4$ for the NOV group.

Abbreviations: VET – Veteran runners group, NOV – Novice runners group, PB = personal best.

When the entire racing career of the runners was examined, the VET group had raced for more years than the NOV group had ($p = 0.020$), competed in more races ($p = 0.002$) and covered a greater distance in racing ($p = 0.003$) (Table 3.3). However, the groups were similarly matched for performance as the VET runner's personal best times for the 5 km, 10 km, 21.1 km and 42.2 km races were not significantly different to those of the NOV group. The same was true for each group's personal best 5 km, 10 km, 21.1 km and 42.2 km race times achieved during their "novice" years, and during the past year. Finally, during their entire career, the VET group had covered a combined training and racing distance of $47\,514 \pm 56\,604$ km, significantly greater than that of the NOV group ($2\,992 \pm 2\,448$ km) ($p = 0.011$).

3.3.4 Sub-maximal run

3.3.4.1 Physiological characteristics

The 15 minute sub-maximal run was a set-paced effort at 70 % of the runners' PTRS. Figure 3.2 shows how the HR (A), VO_2 (B), RPE (C) and VE (D) of the VET and NOV runners changed during the course of the 15 minute sub-maximal run. It was necessary to perform an analysis of co-variance for age for HR. Repeated measures ANOVA found no time-by-group interaction for HR, RPE, VO_2 or VE, indicating that the pattern of change shown for these variables during the sub-maximal effort was similar for both groups.

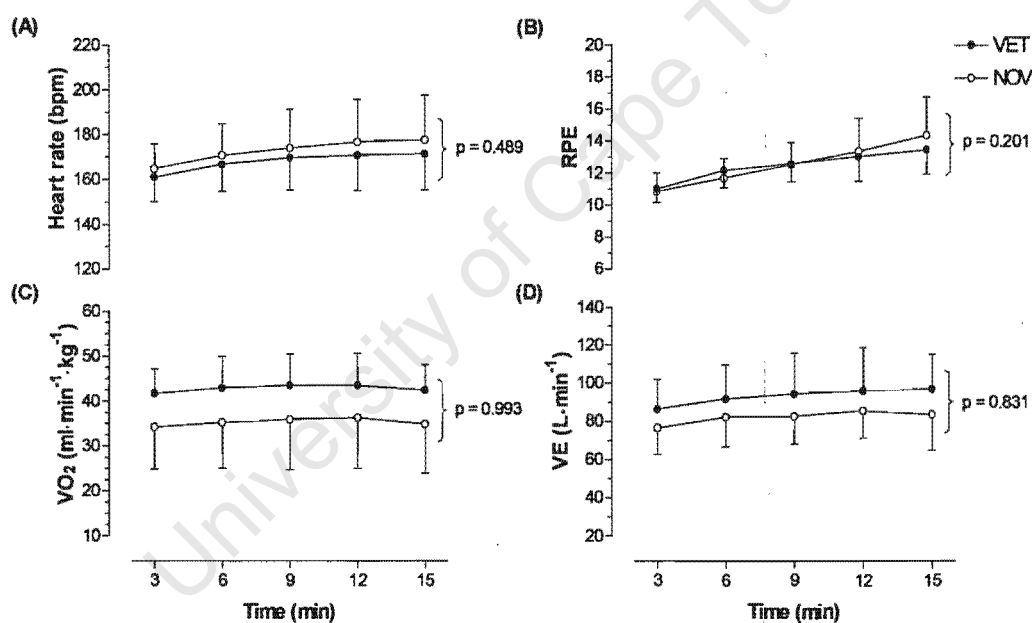


Figure 3.2 Physiological data collected during the 15 minute sub-maximal run. HR (A), RPE (B), VO_2 (C) and VE (D) data were sampled at three minute intervals during the sub-maximal run. The p value on each graph represents the repeated measures ANOVA time-by-group interaction effect.

HR, VO_2 and VE during steady state (minutes 12 – 15) were compared between the groups and the data are presented in Table 3.4. It was necessary to co-vary for age when analysing HR, RPE and VE. There were no differences between the VET and NOV groups with respect to running speed, HR, RPE, or VO_2 at steady state during

the sub-maximal run. The VET runners, however, had a greater VE compared to the NOV runners. When steady state VE was correlated to running speed, a good relationship was found ($r = 0.789$, $p = 0.002$). Given that the VET runners ran at a slightly faster speed compared to the NOV runners, it is likely that the difference seen in steady state VE between the groups was due to running speed differences rather than actual physiological differences between the VET and NOV runners. To determine whether the volume of running accumulated over their entire running careers might affect these physiological variables at steady state during a sub-maximal effort, the log transformed training, racing and combined training and racing distances of both groups were correlated against steady state HR, RPE, VO_2 and VE. No significant relationships were found (data not shown).

Table 3.4 Physiological data of the VET and NOV runners measured at steady state (minutes 12 – 15) during the sub-maximal run.

	VET (n=7)	NOV (n=6)	p value
Speed ($\text{km}\cdot\text{h}^{-1}$)	13.3 ± 1.1	12.2 ± 1.4	0.215
HR (bpm) ¹	166.6 ± 12.8	177.2 ± 19.7	0.347
RPE ¹	13.2 ± 1.5	13.8 ± 2.2	0.644
VO_2 ($\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$)	42.8 ± 6.0	35.3 ± 10.8	0.154
VE ($\text{L}\cdot\text{min}^{-1}$) ¹	96.8 ± 20.4	83.2 ± 17.8	0.033 *

Data are presented as the mean ± standard deviation.

* Significant difference between the VET and NOV groups.

¹ Co-variance for age was necessary.

VO_2 and VE: $n = 5$ for the NOV group.

Abbreviations: VET – Veteran runners group, NOV – Novice runners group, HR – Heart rate, bpm – beats per minute, RPE – Rating of perceived exertion, VO_2 – Volume of oxygen inspired and VE – Ventilation.

3.3.4.2 Stride characteristics

Contact time (CT), flight time (FT), stride length (SL) and stride frequency (SF) were measured every three minutes during the sub-maximal run. Repeated measures ANOVA found a time-by-group interaction for CT ($p = 0.043$) (Figure 3.3). After three minutes, the NOV runners had a longer CT compared to the VET runners, but by minute 9 the CT's were similar and remained so for the rest of the run. There were no time-by-group interactions for FT, SL and SF.

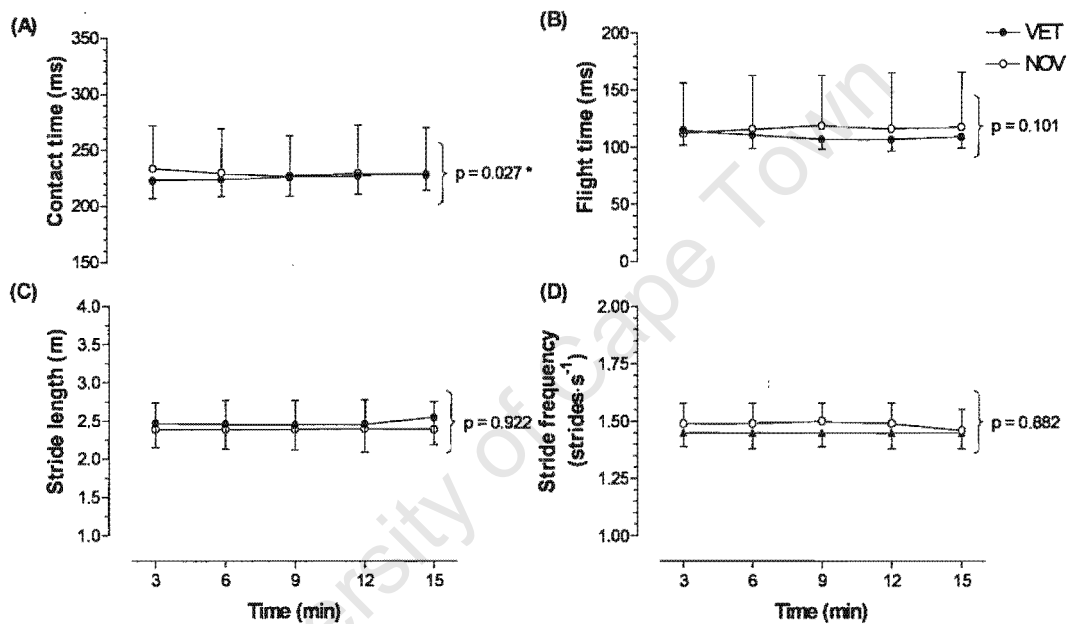


Figure 3.3 Stride characteristics of the VET and NOV runners during the 15 minute sub-maximal run. CT (A), FT (B), SL (C) and SF (D) were calculated every three minutes during the run. The p value on each graph represents the repeated measures ANOVA time-by-group interaction effect. The contact time of the NOV runners was significantly longer than that of the VET runners during the warm-up phase of the run.

The steady state (minutes 12 – 15) values of these stride characteristics were compared between the two groups, and as shown in Table 3.5, the VET runners were similar to the NOV runners. The stride characteristics were also correlated against the log transformed training, racing and combined training and racing distances of the runners, but no relationships were found (data not shown).

Table 3.5 Steady state (minutes 12 – 15) stride characteristics of the VET and NOV runners measured during the sub-maximal run.

	VET (n = 6)	NOV (n = 6)	p value
CT (ms)	224.2 ± 17.0	229.0 ± 42.7	0.792
FT (ms)	111.0 ± 10.8	116.8 ± 48.7	0.764
SL (m)	2.55 ± 0.34	2.40 ± 0.37	0.456
SF (strides·s ⁻¹)	1.48 ± 0.09	1.45 ± 0.07	0.538

Data are presented as the mean ± standard deviation.

Abbreviations: VET – Veteran runners group, NOV – Novice runners group, CT – Contact time, FT – Flight time, SL – Stride length, SF – Stride frequency.

3.3.4.3 Electromyography characteristics

The electrical activity in the vastus lateralis (VL), vastus medialis (VM) and the medial gastrocnemius (MG) muscles was measured during both the pre-activation and ground contact phases at three minute intervals during the sub-maximal run. Figure 3.4 shows a time-by-group interaction for pre-activation phase iEMG of the VL muscle ($p = 0.001$). While the VET group shows a modest decline in pre-activation phase iEMG during the 15 minute run, the NOV group shows an increase. There was a similar trend for the VM muscle. There was no significant interaction for pre-activation phase iEMG of the MG muscle, nor for the ground contact phase iEMG of all three muscles.

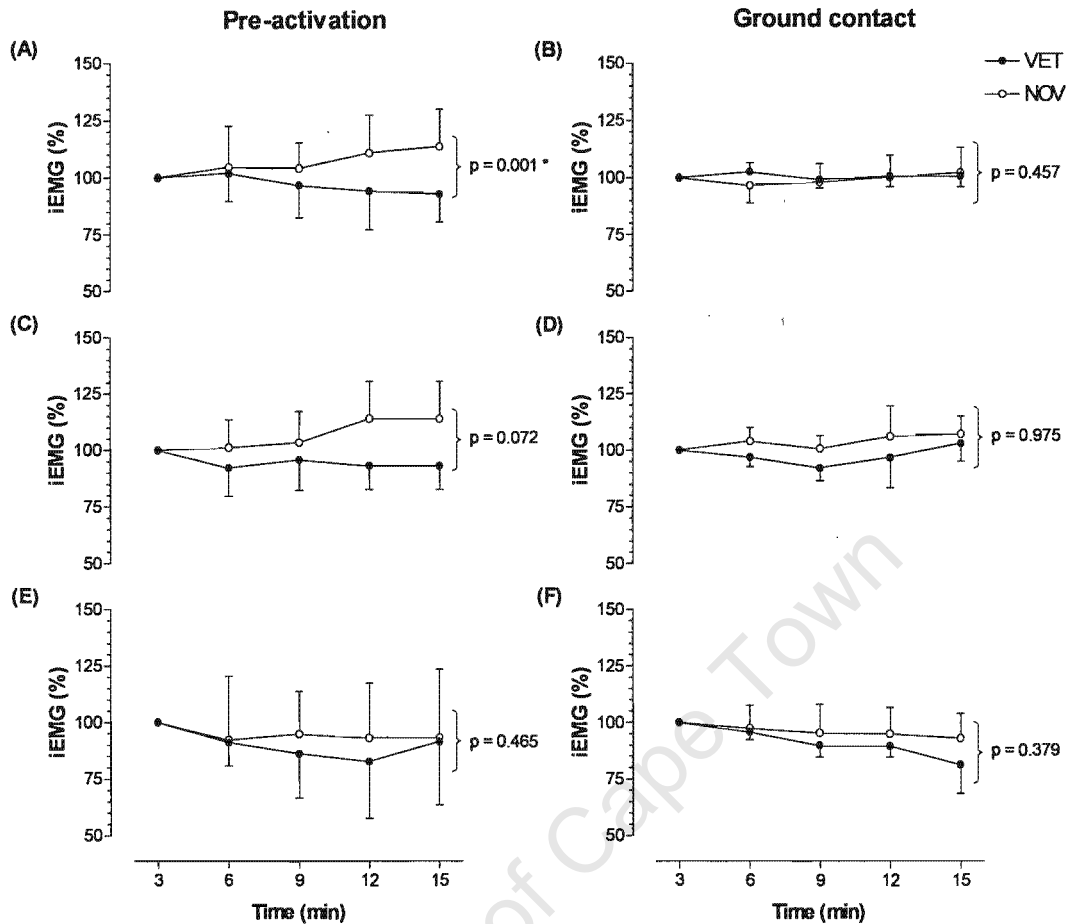


Figure 3.4 EMG characteristics of the VET and NOV runners during the 15 minute sub-maximal run. Pre-activation and ground contact phase iEMG of the vastus lateralis (VL) (A and B), vastus medialis (VM) (C and D) and medial gastrocnemius (MG) (E and F) muscles respectively were calculated from the raw EMG signal recorded every three minutes. Each iEMG value was normalised to the value obtained for the third minute and is expressed as a percentage thereof. The p values on each graph represent the repeated measures ANOVA interaction effect between group and time. There was a significant interaction effect for pre-activation phase iEMG of the VL muscle (panel A), and a similar trend for VM (panel C).

During steady state, the VET runners had lower pre-activation phase iEMG for the VL ($p = 0.030$) and VM ($p = 0.018$) muscles compared to the NOV runners. No differences were found between the VET and NOV runners for pre-activation phase iEMG of the MG muscle, nor for ground contact phase iEMG for all three muscles (Table 3.6). There were no correlations between the EMG variables and the log transformed distance data (data not shown).

Table 3.6 EMG characteristics of the VET and NOV runners measured at steady state (minutes 12 – 15) during the sub-maximal run.

Phase	Muscle	VET (n = 7)	NOV (n = 6)	p value
Pre-activation	VL	92.4 ± 13.7	112.2 ± 15.8	0.030 *
	VM	91.7 ± 9.7	114.0 ± 16.7	0.018 *
	MG	90.8 ± 23.1	93.1 ± 26.7	0.871
Ground contact	VL	100.1 ± 8.7	101.2 ± 4.6	0.788
	VM	100.2 ± 9.1	106.6 ± 7.0	0.278
	MG	86.5 ± 6.1	94.0 ± 11.1	0.183

Data are presented as the mean ± standard deviation.

* Significant difference between the VET and NOV groups.

Pre-activation VM, ground contact VM and MG: n = 6 for the VET group.

Abbreviations: EMG – Electromyography, VET – Veteran runners group, NOV – Novice runners group, VL – Vastus medialis, VM – Vastus lateralis, MG – Medial gastrocnemius.

3.3.5 Time trial

3.3.5.1 Performance and physiological characteristics

The VET and NOV runners completed the 5 km time trial in 20.4 ± 1.6 and 23.3 ± 3.4 minutes respectively. The three minute difference in time between the groups was not statistically significant ($p = 0.065$). Each group's times were not different to either their current 5 km times (VET: $p = 0.396$, NOV: $p = 0.069$) or PB 5 km times (VET: $p = 0.105$, NOV: $p = 0.084$) respectively. Figure 3.5 shows the number of runners completing either the 1st, 2nd, 3rd, 4th or 5th kilometer as their fastest during the time trial. Although there were not enough runners in each group to perform statistics on this data, it can be seen that more VET runners ran fastest in the last kilometer, while more NOV runners recorded their fastest kilometer time in the first kilometer.

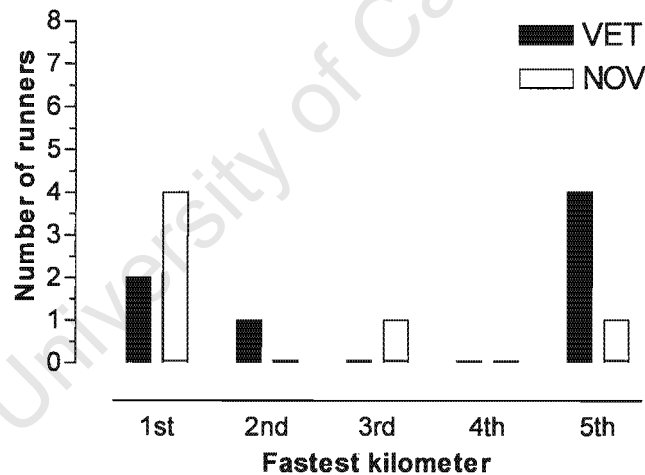


Figure 3.5 Pacing strategies of the VET and NOV runners during the 5 km time trial. The VET and NOV runners displayed different pacing strategies during the time trial. While more VET runners recorded their fastest kilometer split times in fifth kilometer, more NOV runners were fastest in the first kilometer.

Kilometer speed (A), HR (B) and RPE (C) recorded during the time trial is shown in Figure 3.6. It was necessary to perform an analysis of co-variance for age for the heart rate data. There were no significant time-by-group interactions for any of these variables, and the variables were also not significantly correlated to the log transformed distance data (data not shown).

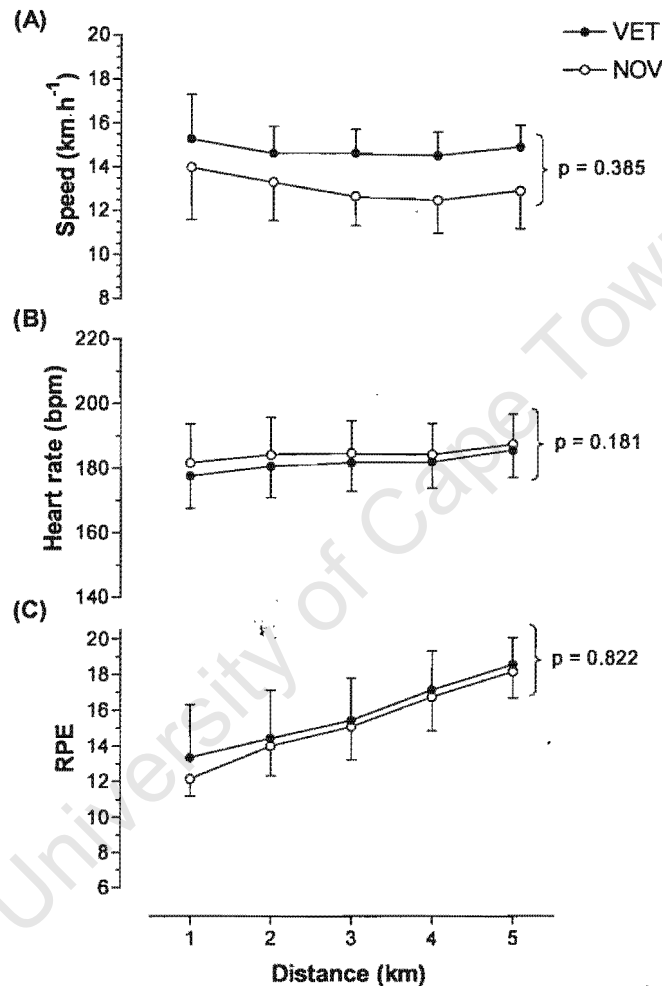


Figure 3.6 Performance and physiological data collected during the 5 km time trial. Speed (A), heart rate (B) and RPE (C) were measured every kilometer. The *p* value on each graph represents the repeated measures ANOVA time-by-group interaction effect.

3.3.5.2 Stride characteristics

Figure 3.7 shows the CT (A), FT (B) and SF (C) variables measured at the end of each kilometer. As each of these parameters changed similarly during the 5 km time trial for the two groups, repeated measures ANOVA found no time-by-group interactions. There were also no relationships found between the stride data and the log transformed distance data (data not shown).

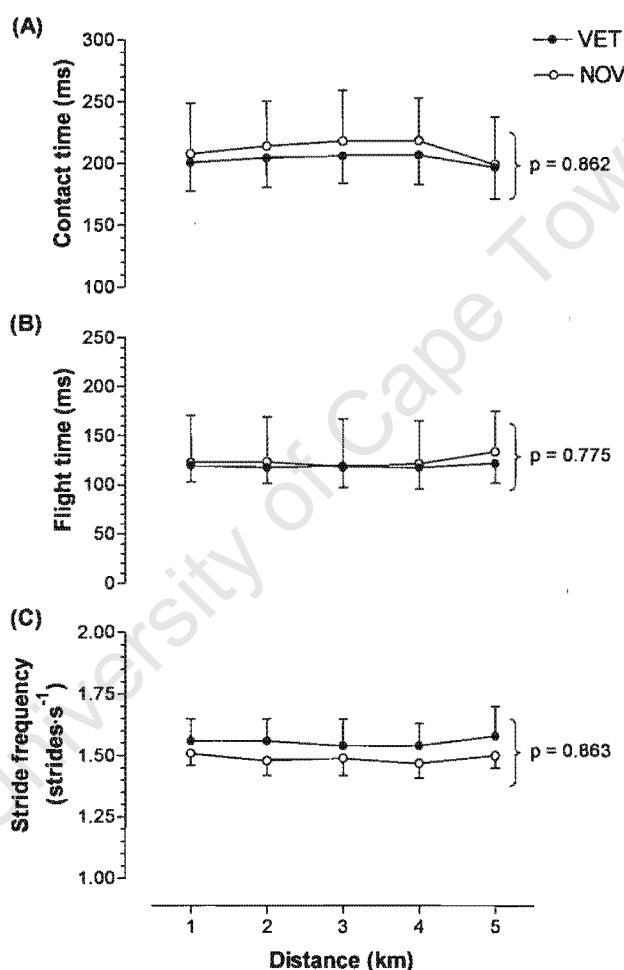


Figure 3.7 Stride characteristics of the VET and NOV runners during the 5 km time trial. CT (A), FT (B) and SF (C) were calculated every kilometer during the time trial. The *p* value on each graph represents the repeated measures ANOVA time-by-group interaction effect.

3.3.5.3 Electromyography characteristics

In contrast to the sub-maximal run, there were no time-by-group interactions for pre-activation or ground contact iEMG measured in the VL, VM and MG muscles during the time trial (Figure 3.8). There were also no significant correlations between the EMG characteristics of the VET and NOV runners during the time trial and the transformed distance data (data not shown).

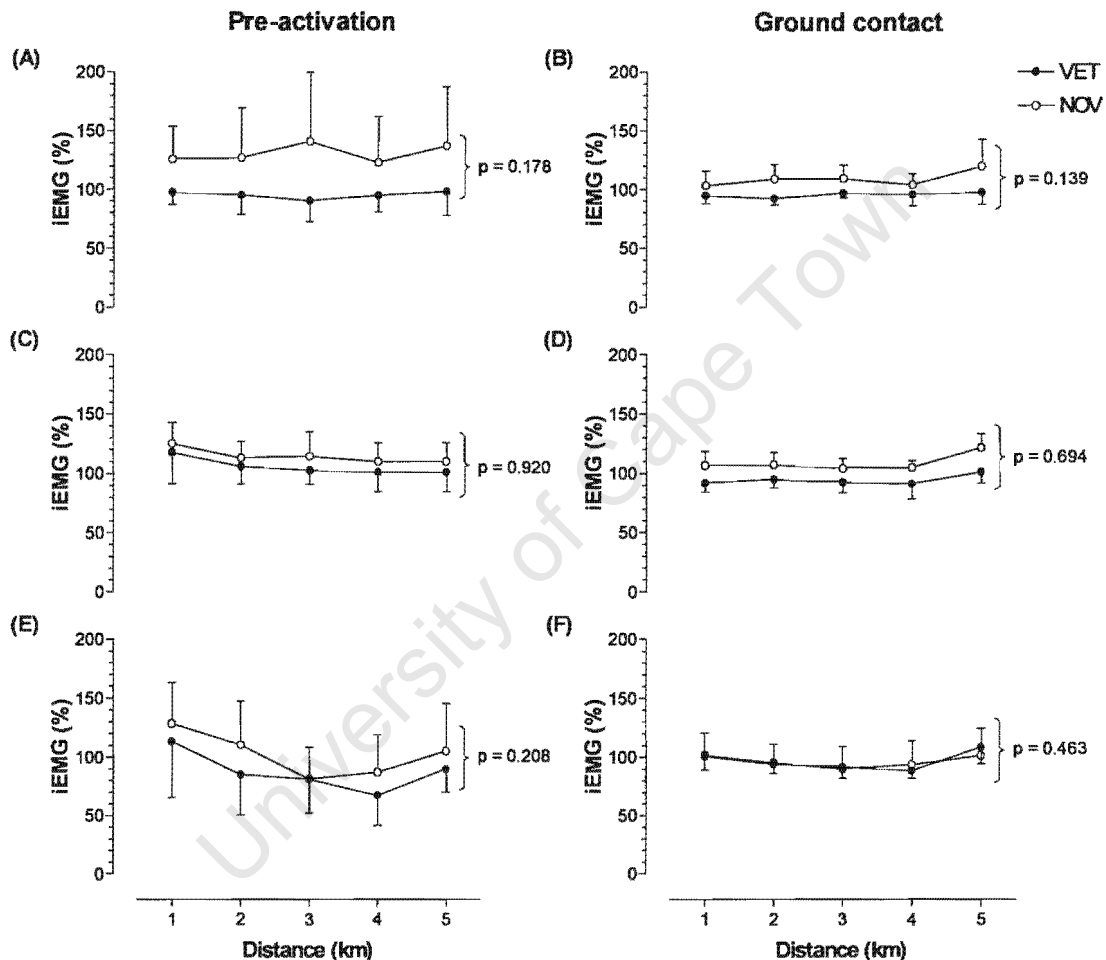


Figure 3.8 EMG characteristics of the VET and NOV runners during the 5 km time trial. Pre-activation and ground contact phase iEMG of the vastus lateralis (VL) (A and B); vastus medialis (VM) (C and D) and medial gastrocnemius (MG) (E and F) muscles respectively were calculated from the raw EMG signal recorded at the end of every kilometer. Each iEMG value was normalised to the value obtained at the third minute of the sub-maximal run and is expressed as a percentage thereof. Each p value is the repeated measures ANOVA interaction effect between group and time.

3.4 DISCUSSION

The aim of this chapter of the thesis was to answer the first question posed in Chapter 2: Do several years of endurance running lead to changes in either the physiological or neuromuscular characteristics of veteran runners while running? Specifically this study compared the physiological and neuromuscular characteristics of veteran and novice runners during sub-maximal and maximal running efforts. The veteran runners used in this study were approximately 11 years older than the novice runners at the time of testing. This was accepted for two reasons. First, it was important that the groups began their running careers at similar ages. Second, by definition, the VET group had run for significantly more years than the NOV group had. As shown in the previous chapter, younger runners have a greater capacity to adapt to years of training and racing compared to older runners (157). By matching the age the participants in this study began running, it was more likely that their potential capacities to adapt to training and racing during their respective running careers were similar. The VET and NOV runners also differed in the estimate of their percentage body fat. The leaner body shape of the VET runners may have been due to their longer exposure to running training and racing.

The training and racing data of the VET and NOV runners shows clearly that the VET runners had endured a greater volume of endurance running compared to the NOV runners. While the health benefits of regular exercise are well documented (108, 114, 170, 184, 194, 211), years of distance running is known, for example, to lead to a degree of persistent low-level structural pathology in the skeletal muscle (86, 201). Presumably this is one of the effects of the ongoing cycles of degeneration and regeneration skeletal muscle endures in response to training. By virtue of their greater volume of endurance running, the VET runners had had a greater exposure to this stressor. However, since the VET and NOV runners had trained at similar intensities (weekly training frequency, distance and speed), the degree of this stress should have been similar for both groups. The VET and NOV runners were matched for current performance times, suggesting they were of a similar calibre. From the design of the study and the matching of the subjects, any differences found between the groups during the sub-maximal run and the 5 km time trial could reasonably be attributed to their different exposures to running.

The main finding of this study was that during the steady state phase of the sub-maximal run the VET runners had less pre-activation phase iEMG in the vastus lateralis and vastus medialis muscles compared to the NOV runners. One interpretation is that endurance runners with a greater exposure to running may experience lower levels of motor unit recruitment in the leg muscles before foot strike during sub-maximal effort running. Pre-activation phase iEMG is thought to be important as it increases the muscle's stiffness in preparation for ground contact and the subsequent propulsion phase (144). Altered muscle stiffness may change the potential for the muscle to absorb impact forces, thereby changing the shock absorption function of the muscles (139). Indeed, Bus has shown that older runners have lower levels of shock absorption as seen by differences in the larger peak forces on impact and larger initial loading rates on ground contact (18). There is, however, no direct evidence to link lower levels of recruitment with decreased shock absorption.

The second finding of this study was that contact time was the only stride characteristic to differ between the two groups during the sub-maximal run. Whereas it remained constant for the VET runners, the NOV runners had a longer ground contact phase at the beginning of the run. After nine minutes, however, the contact times of the two groups were similar and remained so until the end of the run. The large standard deviation seen in the NOV group may be the reason for this initial difference between the groups. Alternatively, if the first six minutes of the run is considered to be the warm-up phase, the NOV runners differed from the VET runners while they were still warming up. One explanation for an increased contact time may indicate a reduced capacity to generate force rapidly and this in turn suggests that perhaps the NOV runners were less efficient from a neuromuscular perspective (139) compared to the VET runners during the warm-up phase of the sub-maximal effort.

The 5 km time trial was used to assess the physiological and neuromuscular characteristics of the VET and NOV runners during a self-paced maximal effort. As neither group's times were significantly different to their current or personal best 5 km times, this test was taken to be a good proxy for maximal performance. The third finding of this study was that a greater exposure to running does not alter the

physiological and neuromuscular characteristics of VET runners during a 5 km time trial. It was interesting to note that the pacing strategies of the two groups appeared to be different. More NOV runners were faster at the beginning of the time trial while more VET runners were able to run fastest at the end. This may relate to the experience of the VET runners. Given their similar performances during the time trial, it was not surprising to see that their heart rate and RPE responses were not different. The VET runners were also indistinguishable from the NOV runners with respect to their neuromuscular characteristics during the time trial.

A minor finding was that at steady state (minutes 12 – 15) during the sub-maximal run, only ventilation rate was different between the groups. However, as ventilation rate was found to be correlated to running speed, and as the VET runners were slightly faster than the NOV runners, this difference may have been a product of running speed differences between the groups. The tendency for oxygen consumption to be different between the groups may be explained by the fact that the two NOV runners with unusually low oxygen consumption values (22.1 and 26.0 ml·min⁻¹·kg⁻¹) may be considered outliers. As there was no evidence to show that the VET runners were different to the NOV runners with respect to the physiological variables measured in this study, it can be concluded that a greater exposure to running neither impairs nor improves the heart rate, rating of perceived exertion, oxygen consumption or ventilation responses of endurance runners to a sub-maximal effort.

3.5 CONCLUSION

In light of the fact that the veteran runners had indeed had a greater exposure to endurance running compared to the novice runners, the main findings of this study were:

- (i) The veteran runners had less EMG activity in the vastus lateralis and vastus medialis muscles during the pre-activation phase of the stride during steady state of the sub-maximal run.
- (ii) The novice runners had a longer ground contact component of their stride during the warm-up phase of the sub-maximal run.
- (iii) The veteran and novice runners were indistinct with respect to the physiological and neuromuscular variables measured in this study during the time trial.

In conclusion, the difference in pre-activation phase EMG between the veteran and novice runners at a sub-maximal pace suggests that the quadriceps muscles of the veteran runners may have a decreased capacity for shock absorption. However, since there were no differences between the groups during the time trial, it is still not clear why veteran runners are almost certainly beyond achieving personal best performance times, yet novice runners are still likely to be able to improve their endurance performance. Therefore, in response to the question that this study tried to answer, it seems that years of endurance running may well lead to neuromuscular changes within the skeletal muscle of veteran runners. However, these changes do not appear to manifest during a time trial type run, but only during the steady state phase of a sub-maximal effort.

CHAPTER 4

A COMPARISON OF VETERAN AND NOVICE ENDURANCE RUNNERS:**Response to and recovery from exercise-induced muscle damage****4.1 INTRODUCTION**

One of the key differences between veteran and novice runners is that the veterans have had a greater exposure to the stress of endurance running. By definition, a stressor is a stimulus that causes stress and endurance exercise is a known stressor to the runner. In particular, endurance training and racing causes minor levels of damage to the skeletal muscle (86, 141, 201). Due to the plastic nature of this organ (63), however, exercise-induced muscle damage is transient (116), reversible (201) and thought to contribute to the adaptation to running (178). In fact, repetitive bouts of exercise-induced muscle damage have been shown to have a protective effect on subsequent bouts of exercise (126, 131). The effect of years of exposure to even these minor levels of exercise-induced muscle damage, however, has not been thoroughly explored.

As a result of chronic exposure to endurance running, the skeletal muscle of veteran athletes would have endured and recovered from many more bouts of exercise-induced muscle damage compared to that of novice runners. Since a group of veteran runners who had accumulated more than 5 000 km in races displayed decreased levels of neuromuscular efficiency following a bout of exercise-induced muscle damage (172), it is tempting to speculate that their ability to respond to and recover from exercise-induced muscle damage is impaired. An impaired capacity to adapt to these repetitive bouts of exercise-induced muscle damage may explain the decline in performance ultimately observed in veteran runners, defined as consistently slower times than their previously best performances.

Therefore the aim of this study was to answer the second question posed in Chapter 2: Do veteran and novice runners respond similarly to a bout of exercise-induced muscle damage? Specifically, three questions were addressed in this study:

- (i) Do veteran and novice runners sustain a similar extent of muscle damage after being exposed to the same relative bout of exercise?
- (ii) Do veteran and novice runners recover similarly from exercise-induced muscle damage with respect to blood markers of muscle damage, muscle pain and muscle power?
- (iii) Does the bout of exercise-induced muscle damage cause differences in the physiological and neuromuscular characteristics of veteran and novice runners one week later?

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4.2 METHODS

4.2.1 Subject characteristics

The same runners were used to form the veteran (VET, $n = 7$) and novice (NOV, $n = 6$) groups as described in Chapter 3 (section 3.2.1). The study was explained in detail to each runner who then completed a consent form (Appendix 1) agreeing to participate in the study. The Research Ethics Committee, Faculty of Health Sciences, of the University of Cape Town approved this study (REC reference number: 181/2002).

4.2.2 Experimental design

All runners (VET and NOV) were required to visit the laboratory on ten occasions over a two week period. They were asked to refrain from exercising the day before the trial began and were also not to have raced in the week preceding the trial, but were allowed to train normally during the trial. A schematic of the experimental design of this study is shown in Figure 4.1.

MUSCLE DAMAGE IN VETERAN AND NOVICE RUNNERS

Day 1	Visit 1	➡	<ul style="list-style-type: none"> • Informed consent • Questionnaires
Day 2			<ul style="list-style-type: none"> • Body composition assessment • Squat and squat jump familiarisation • Squat 5RM
Day 3			<ul style="list-style-type: none"> • Treadmill familiarisation • PTRS test
Day 4	Visit 2	➡	<ul style="list-style-type: none"> • Muscle pain measurements • Squat jumps
Day 5			<ul style="list-style-type: none"> • Indoor track familiarisation • 15 minute sub-maximal run • Muscle pain measurements
Day 6			<ul style="list-style-type: none"> • 5 km time trial • Muscle pain measurements
Day 7	Visit 3	➡	<ul style="list-style-type: none"> • PRE blood sample • Muscle pain measurements • Downhill run
Day 8	Visit 4	}	<ul style="list-style-type: none"> • POST blood sample • Muscle pain measurements
Day 9	Visit 5		<ul style="list-style-type: none"> • Blood sample • Muscle pain measurements • Squat jumps
Day 10	Visit 6		
Day 11	Visit 7	}	<ul style="list-style-type: none"> • Muscle pain measurements • Squat jumps
Day 12	Visit 8		<ul style="list-style-type: none"> • Indoor track familiarisation • 15 minute sub-maximal run • Muscle pain measurements • 5 km time trial
Day 13	Visit 9		
Day 14	Visit 10	➡	<ul style="list-style-type: none"> • Muscle pain measurements

Figure 4.1 Schematic representation of the testing protocol used in this study. Each subject visited the laboratory for testing on ten occasions during a two week period. Baseline information was collected during visits one and two and the subjects were familiarised with techniques and equipment to be used during the trial. Visit three constituted the intervention phase of the study, namely the downhill run. Visits four to nine took place 24 hours apart over six

CHAPTER 4

consecutive days and monitored changes in serum creatine kinase activity, muscle pain and muscle power in response to the intervention. The final visit took place one week after the downhill run and reassessed the variables measured during visit two.

Visit 1: Baseline measurements

The study was explained in detail to the runners and, on agreeing to participate, they signed the consent form and completed questionnaires on their personal particulars, medical history and social sport participation (Appendix 1), competitive sport training and racing history as well as a one week diary to ensure they were not currently over-trained (Appendix 2). The body composition of each participant was measured and they were familiarised with both the squat and the squat jump techniques. Each runner then performed a squat five repetition maximum test, which was used to estimate their squat one-repetition maximum weight. After a 30 minute rest the subjects were familiarised with running on a treadmill. Finally they performed a peak treadmill running speed test.

Visit 2: Pre-intervention tests

The runners visited the laboratory three days later and completed five squat jumps, which were used to measure lower limb muscle power. They were then familiarised with running on the 140 m indoor running track and adjusting their pace in accordance with the pacing lights system that would be used to pace them through a 15 minute sub-maximal run. After the sub-maximal run, the participants rested for ten minutes before running a 5 km time trial on the same indoor track at a self-selected pace. Subjective and objective measurements of muscle pain were recorded before the squat jumps, after the sub-maximal run and after the time trial.

Visit 3: Intervention

When the runners returned to the laboratory three days later, a resting blood sample (Pre) was drawn and subjective and objective lower limb muscle pain were measured. Muscle damage of the lower limbs was induced by running downhill on a treadmill declined to -10° for 40 minutes at a speed of 70 % of their peak treadmill

running speed (168). Immediately after the test, a second blood sample (Post) was drawn and muscle pain levels were re-assessed.

Visits 4 – 9: Recovery tests

The participants visited the laboratory at 24 hour intervals for six consecutive days after the intervention visit. During these six days the 24-, 48-, 72-, 96-, 120- and 144-hour blood samples were taken, muscle pain was assessed and the subjects performed five squat jumps.

Visit 10: Post intervention tests

The final visit took place one week after the downhill run. The 168-hour blood sample and pain measurements were taken and the runners repeated the five squat jumps, the 15 minute sub-maximal run and the 5 km time trial as for visit 2.

4.2.3 Detailed physiological testing procedures

The procedures used to assess body composition, peak treadmill running speed, the 15 minute sub-maximal run and the 5 km time trial were identical to those described in Chapter 3 (section 3.2.3). The additional procedures for the squat five-repetition maximum test, the muscle power test, the 40 minute downhill run and the assessment of muscle pain are described below.

4.2.3.1 Squat five-repetition maximum

To calculate the weight the runners would use for the squat jumps later in the trial, it was first necessary to determine the weight they could squat in a single maximal effort. Given that the VET and NOV runners were mostly unfamiliar with weight training, their squat one-repetition maximum values were estimated from the weight that they were able to squat in a five-repetition maximum test. The protocol for this test was modified from that described by Baechle and Earle (2). Subjects first warmed up by cycling for ten minutes on a cycle ergometer and stretching. They

were familiarised with the squat technique in which they began by standing with their feet hip width apart and toes pointing 5 degrees outwards. They squatted down until their thighs were parallel to the floor and their knees were at 90 °, but not over their toes, and then returned to standing. All participants performed ten warm up squats with no weight followed by five more warm up squats with a 20 kg bar (men) or a 10 kg bar (women) before resting and stretched for a further five minutes. The tester estimated the initial barbell weight and asked the participants to perform as many squats as possible. If they reached ten repetitions they were stopped and rested for five minutes while the weight of the barbell was increased. Once again the runners performed as many squats as possible, which was usually between three and five, and this number was used to estimate the maximum value they would be able to squat in one repetition.

4.2.3.2 Muscle power test

Muscle power of the lower limbs was assessed using a squat jump. Participants warmed up for five minutes on a bicycle ergometer, stretched and then performed ten squats without weights and five squats with light weights as described in section 4.2.3.1. After a five minute rest, they completed five successive squat jumps carrying a barbell weighted to 30 % of the estimated weight they could squat in one repetition (refer to section 4.2.3.1). The subjects squatted down to a position in which their thighs were parallel to the floor and their knees bent to an angle of approximately 90 °. They held this position for one second and on the verbal cue of the tester jumped up as high and as fast as possible. Subsequent jumps were performed immediately. Muscle power was determined using a device called a FitroDyne (Fitronic, Bratislava, Slovakia), which attaches to the barbell and measures upward velocity ($\text{m}\cdot\text{s}^{-1}$) of the squat jump. Muscle power (W) was calculated from the product of the velocity and force (combined mass of subject and barbell x gravity) of the jump (94). The highest and lowest of the five recorded values were excluded and the final muscle power value was calculated as the average of the remaining three values. This measurement was assumed to be a proxy for lower limb muscle power. A previous study showed the test to be repeatable with 95 % limits of agreements of 17 ± 96 W (94).

4.2.3.3 Downhill run

The downhill run was the intervention component of the trial and was designed to induce damage in the skeletal muscle of the runner's lower limbs, similar to that experienced as a result of a training run. The test was performed on the same treadmill as described in the previous chapter. Briefly, the runners warmed up for five minutes at a self-selected pace on the treadmill, and then ran for 40 minutes at 70 % of their peak treadmill running speed with the treadmill declined to -10° . Heart rate was recorded continuously and rating of perceived exertion was noted every five minutes.

4.2.3.4 Muscle pain assessment

Muscle pain of the lower limbs was assessed both subjectively and objectively. For the subjective assessment, the runners rated the pain in their lower limb muscles using a ten point scale, where 0 represented no pain and 10 extreme pain (Appendix 4) (13), for four categories: general pain, pressure pain, stretch pain and pain during the involvement of the leg in activities of daily living. The sum of these four values constituted the subjective muscle pain score. Objective muscle soreness was measured using a round ended pressure probe, which was constructed by the Biomedical Engineering Department of the University of Cape Town. The probe, calibrated so that a depression of 1 cm is equivalent to a force of 4 Newtons, was pressed into the muscle to the point at which it elicited soreness. The degree of soreness was recorded as follows: 4 cm of probe depression corresponded to a score of 0; 3 cm to a score of 1; 2 cm a score of 2; 1 cm a score of 3 and 0 cm a score of 4 (169). Nine readings were taken using a grid to identify the sites. The center of the grid was positioned to coincide with the site for the mid thigh skinfold measurement (i.e. at the mid point of the anterior surface of the thigh). Each objective muscle pain score was the sum of these nine readings.

4.2.4 Blood sampling and analysis

A 5 ml sample of blood was drawn from the ante-cubital vein and collected in lithium heparin vacutainer tubes at rest before (Pre) and immediately after (Post) the

downhill run, and then at 24 hour intervals for one week. The samples were immediately centrifuged at 3 000 x g for 10 minutes at 4 °C, after which the serum was removed and stored in microfuge tubes at -20 °C until further analysis. The serum creatine kinase (CK) activity of each sample was determined using a commercial enzymatic assay kit (CK-NAC-activated, Boehringer Mannheim Automated Analysis for BM / Hitachi Systems 704, Meylan, France) and a spectrophotometer (Beckman, DU-62, Beckman Instruments, Fullerton, California, USA). The level of CK activity in the serum was used as an indication of skeletal muscle membrane disruption.

4.2.5 Data analysis

The performance, physiological, stride and electromyography data were analysed as described in the Chapter 3 (section 3.2.4).

4.2.6 Statistical analysis

Repeated measures analysis of variance (ANOVA) was used to compare differences between the groups with respect to changes in serum creatine kinase activity, muscle power and steady state HR and RPE during the sub-maximal run in response to the intervention. Repeated measures ANOVA was also used to compare changes in each group's Pre (before the downhill run) and Post (one week after the downhill run) performance, physiological, stride and EMG variables measured during the sub-maximal run and the time trial. Friedman's two-way ANOVA was used to measure differences in subjective pain recorded for each individual. As the VET and NOV groups were not matched for age, all analyses were performed using an analysis of co-variance (ANCOVA) that accounted for age. The ANCOVA statistics were only reported for those variables for which co-variance for age proved necessary. Statistical significance was accepted at $p < 0.050$.

4.3 RESULTS

4.3.1 Subject characteristics

The descriptive, training and racing characteristics of the VET and NOV runners were presented in Chapter 3 (sections 3.3.1, 3.3.2 and 3.3.3).

4.3.2 Intervention efficacy

The intervention phase of the study was designed to induce damage in the lower limb skeletal muscle of the VET and NOV runners, similar to what would be experienced following a training run. To this end the participants completed a 40 minute run on a treadmill declined to -10° at a speed of 70 % of their peak treadmill running speed.

4.3.2.1 Serum creatine kinase activity

Figure 4.2 (A) shows the change in serum creatine kinase (CK) activity in both the VET and NOV runners in response to the downhill run. The resting CK activity was adjusted to 100 % so that subsequent values were normalised relative to each individual's starting point. Serum CK activity increased for both the VET and NOV runners by approximately 75 %, reaching a peak 24 hours after the intervention. The large standard deviations of both groups at 24 and 48 hours indicate the variability of the inter-individual responses to the downhill run. The CK activity of both groups returned to resting values within 72 hours. As indicated by the p-value on Figure 4.2 (A), there was no time-by-group interaction effect ($p = 0.921$). In fact, the pattern of change in CK activity in response to and during the week after the intervention was remarkably similar for the VET and NOV runners.

4.3.2.2 Muscle pain

Both subjective and objective muscle pain were measured. However, only subjective pain is reported as the very large standard deviations in objective pain from 24 to 72 hours after the downhill run made the data difficult to interpret. Figure 4.2 (B) shows the change in subjective muscle pain as reported by the VET and NOV runners in response to and during the week following the downhill run. The values plotted on the graph are the absolute pain scores. Muscle pain increased three-fold for both groups, but unlike CK activity, remained elevated until 48 hours post intervention before returning to resting values approximately 96 hours later. The pain responses of both the VET and NOV groups followed similar courses for the week after the downhill run explaining the lack of a time-by-group interaction effect ($p = 0.699$).

4.3.2.3 Muscle power

The change in lower limb muscle power following the downhill run is shown in Figure 4.2 (C). As for serum CK activity the data are expressed as values relative to resting muscle power. In contrast to the responses of CK activity and muscle pain to the downhill run, the muscle power of both the VET and NOV groups increases steadily throughout the trial. Furthermore the two groups' muscle power increased in a similar fashion, as indicated by the repeated measures ANOVA time-by-group interaction p-value ($p = 0.538$).

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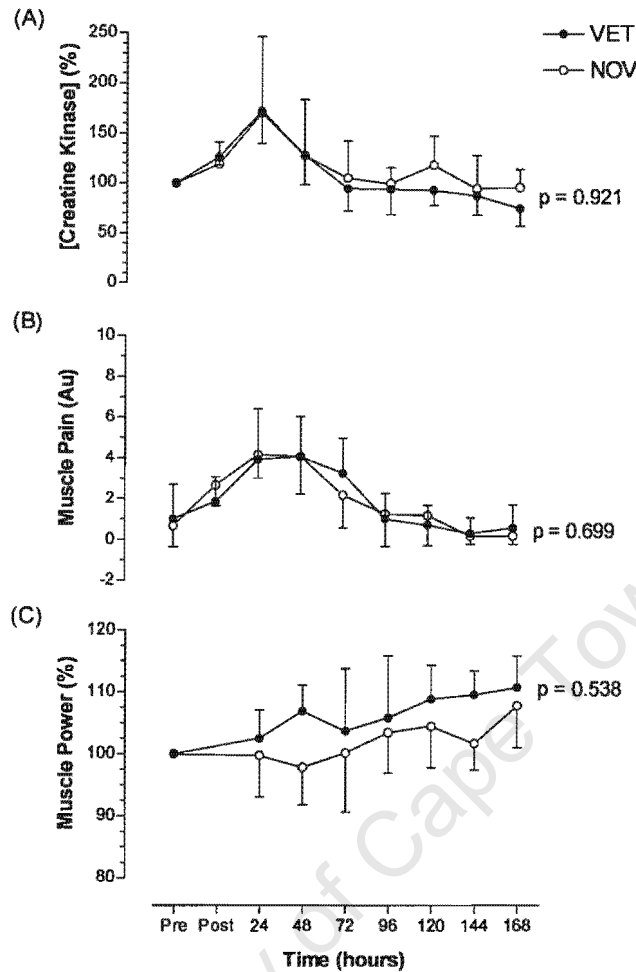


Figure 4.2 Creatine kinase activity, subjective muscle pain and muscle power responses to the downhill run for the VET (n = 7*) and NOV (n = 6) runners. Serum creatine kinase activity (A), subjective muscle pain (B) and muscle power (C) were measured, before, immediately after and at 24 hour intervals for one week after the VET and NOV runners completed the downhill run. The creatine kinase activity and muscle power data are expressed relative to their respective resting values. The p values on the graphs are the repeated measures ANOVA time-by-group interaction effects. * n = 6 for muscle power data. Au – Arbitrary units

4.3.3 Intervention effect on sub-maximal running characteristics

The physiological, stride and electromyography (EMG) characteristics of the VET and NOV runners were recorded every three minutes during the 15-minute sub-maximal run both before (Pre) and one week after (Post) the downhill run. To assess the effects of the intervention on the VET and NOV runners' sub-maximal running characteristics, the Pre data of each group were compared to its Post data using repeated measures ANOVA. In addition, the steady state (minutes 12 – 15) data of the two groups were also compared.

4.3.3.1 Physiological characteristics

Figure 4.3 shows the heart rate (HR) (A), rating of perceived exertion (RPE) (B), oxygen uptake (VO_2) (C) and ventilation rate (VE) (D) responses of the VET runners during the sub-maximal effort before (Pre) and one week after (Post) the downhill run. There were no time-by-intervention interactions for any of the measured variables, indicating that the physiological characteristics of the VET runners during a sub-maximal effort were not altered by the downhill run. The corresponding responses of the NOV group are also shown in Figure 4.3 (panels E – H). The p values on the graph show that one week after the downhill run HR (E), RPE (F), VO_2 (G) and VE (H) of the NOV runners during the sub-maximal effort were similar compared to before the intervention.

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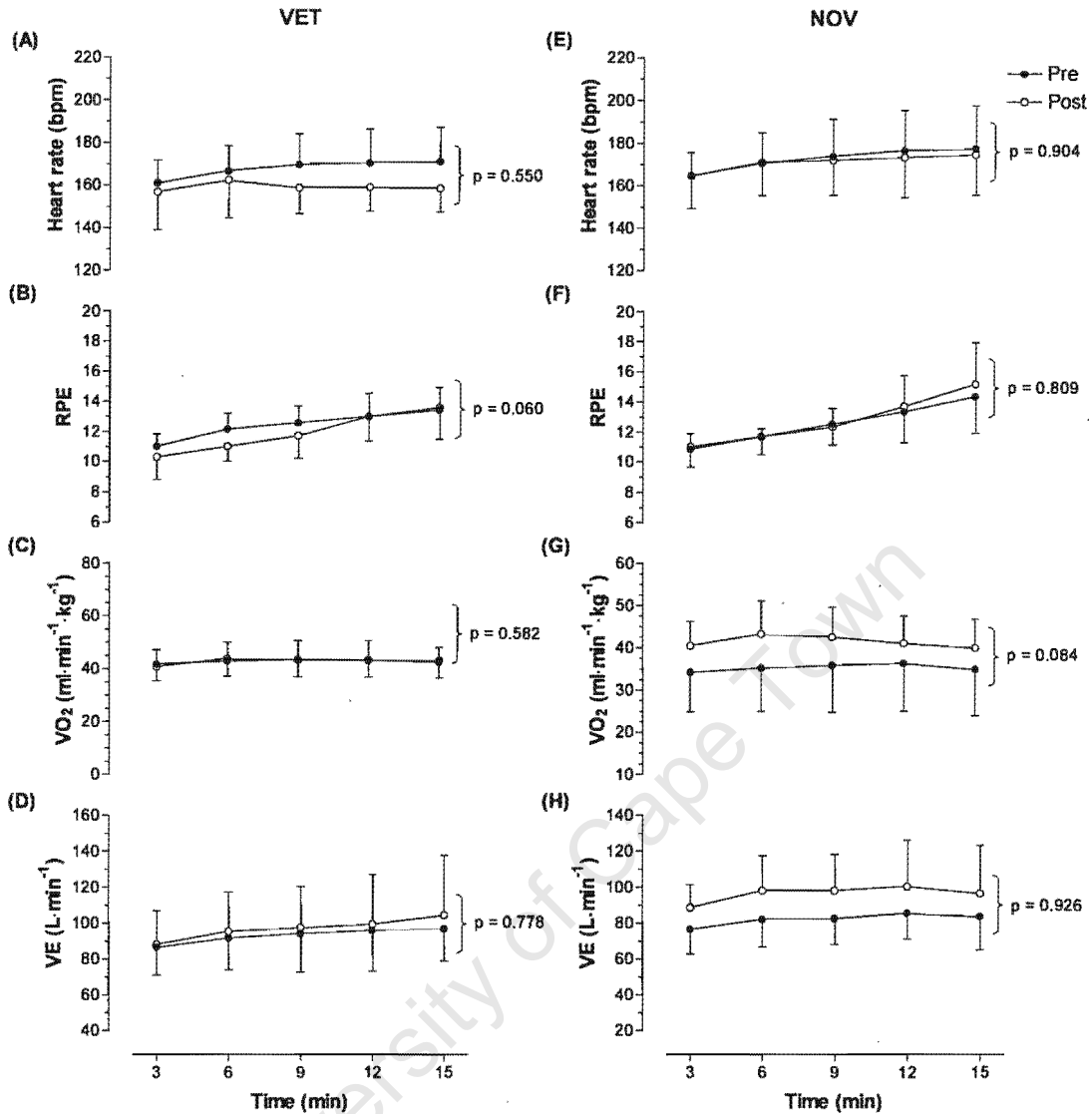


Figure 4.3 Physiological responses of the VET ($n = 7^*$) and NOV ($n = 6^{**}$) runners during a sub-maximal effort before (Pre) and one week after (Post) the downhill run. HR, RPE, VO_2 and VE of the VET (panels A – D) and NOV (panels E – H) runners. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data. * $n = 5$ for HR, VO_2 and VE data. ** $n = 4$ for VO_2 and VE data.

As can be seen in Figure 4.4, steady state (minutes 12 – 15) HR (A), RPE (B), VO_2 (C) and VE (D) of the VET and NOV runners were also similar during the sub-maximal effort before and after the downhill run.

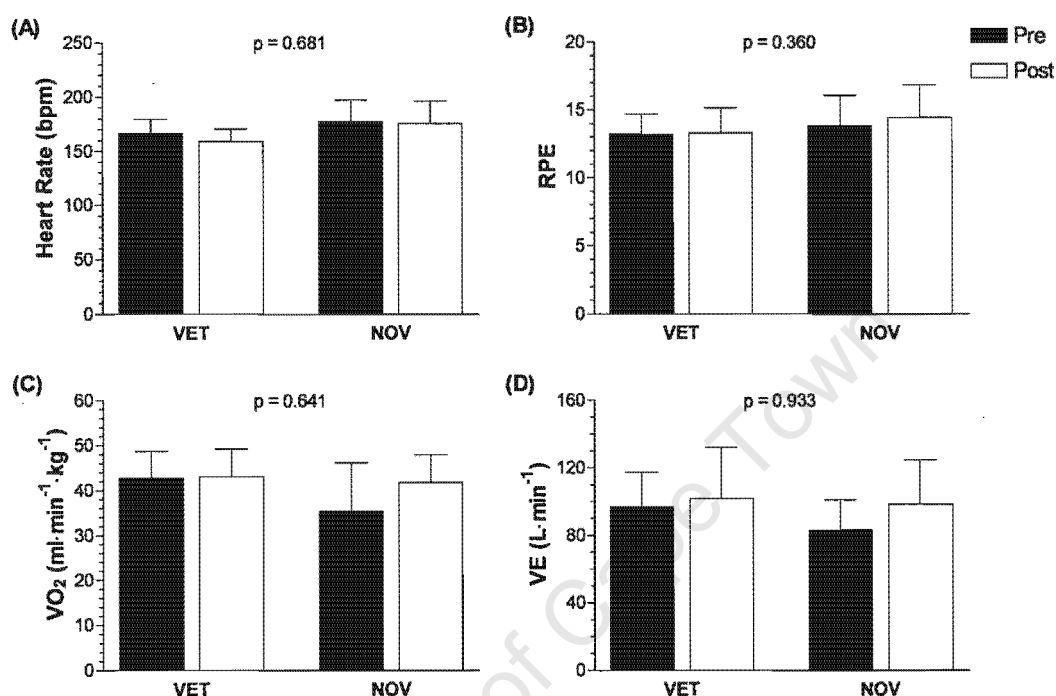


Figure 4.4 Steady state physiological characteristics of the VET ($n = 7^*$) and NOV ($n = 6^{**}$) runners during a sub-maximal effort before (Pre) and one week after (Post) the downhill run. Steady state HR (A), RPE (B), VO_2 (C) and VE (D). The p value on each graph is the repeated measures ANOVA time-by-group interaction effect. The solid bars represent the Pre data, while the clear bars represent the Post data. * $n = 5$ for HR, VO_2 and VE data. ** $n = 4$ for VO_2 and VE data.

4.3.3.2 Stride characteristics

Figure 4.5 shows contact time (CT) (A), flight time (FT) (B), stride length (SL) (C) and stride frequency (SF) (D) of the VET runners during the sub-maximal effort before (Pre) and one week after (Post) the downhill run. As indicated by the p values on each graph, there were no significant differences in the way these stride characteristics of the VET runners changed during the sub-maximal run in response to the exercise-induced muscle damage. The same was true for the NOV runners (Figure 4.5 panels E – G).

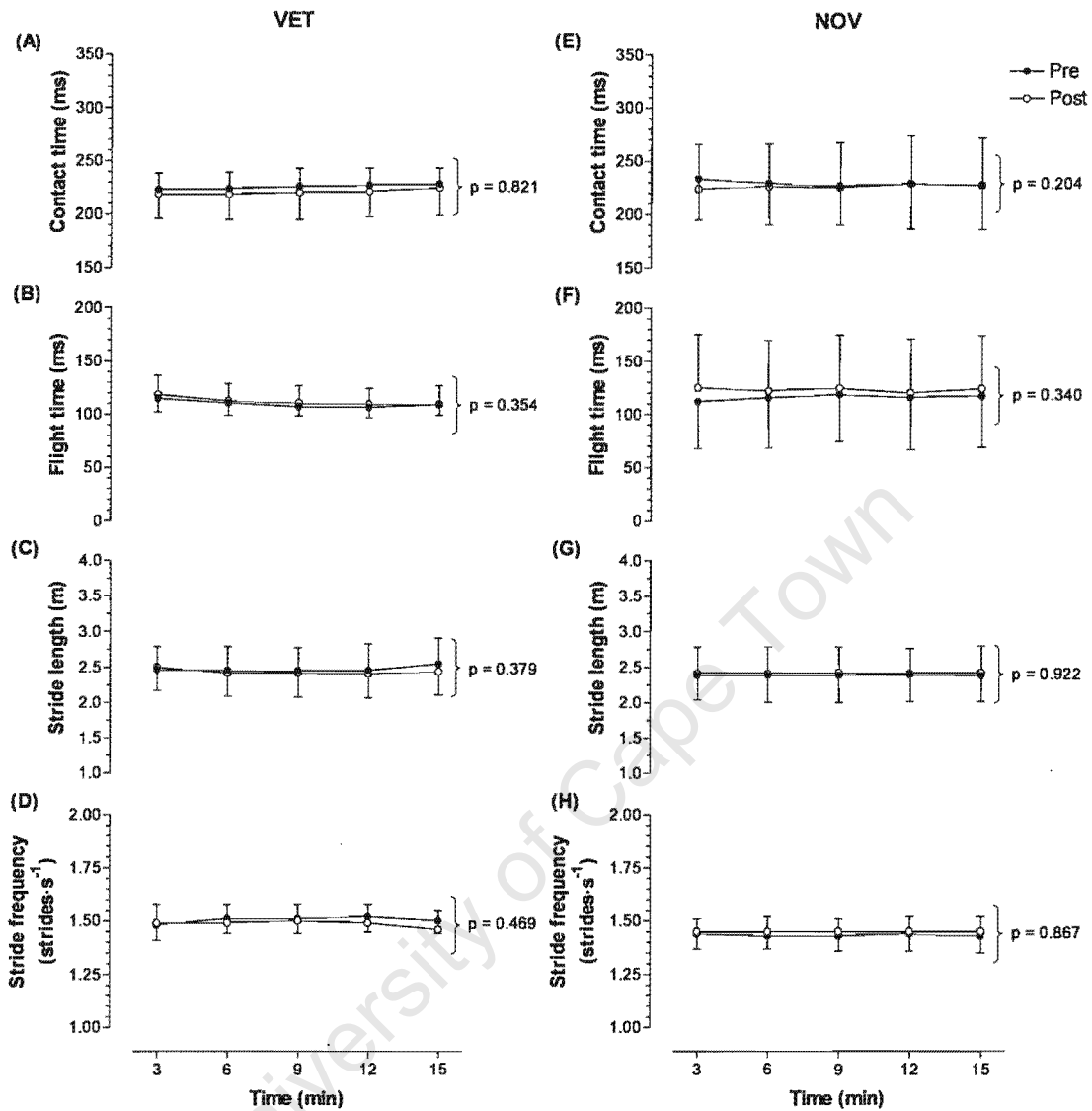


Figure 4.5 Stride characteristics of the VET (n = 7) and NOV (n = 6) runners during a sub-maximal effort before (Pre) and one week after (Post) the downhill run. CT, FT, SL and SF of the VET (panels A – D) and NOV (panels E – H) runners. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data.

As for the physiological variables, no differences were found in each group's steady state (minutes 12 – 15) stride characteristics before and after the downhill run (Figure 4.6).

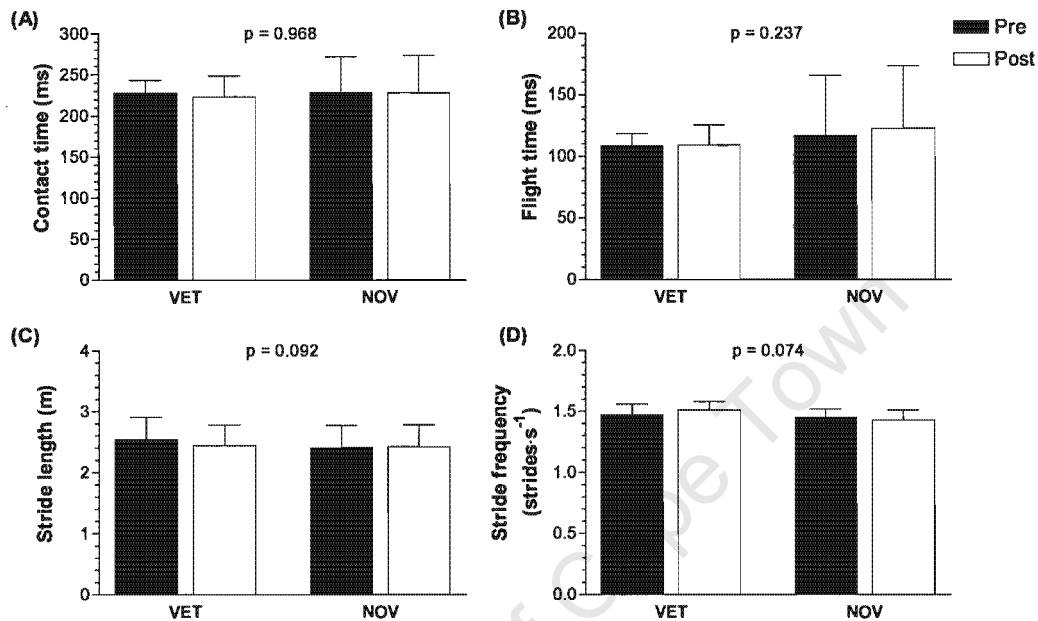


Figure 4.6 Steady state stride characteristics of the VET (n = 7) and NOV (n = 6) runners during a sub-maximal effort before (Pre) and one week after (Post) the downhill run. Steady state CT (A), FT (B), SL (C) and SF (D). The p value on each graph is the repeated measures ANOVA time-by-group interaction effect. The solid bars represent the Pre data, while the clear bars represent the Post data.

4.3.3.3 Electromyography characteristics

Normalised iEMG amplitude during both the pre-activation and ground contact phases were measured in the vastus lateralis (VL), vastus medialis (VM) and medial gastrocnemius (MG) muscles. The patterns of change in the VL pre-activation (A) and ground contact (B), VM pre-activation (C) and ground contact (D), and MG pre-activation (E) and ground contact (F) phase iEMG of the VET runners during the sub-maximal run were similar before (Pre) and one week after (Post) the downhill run (Figure 4.7).

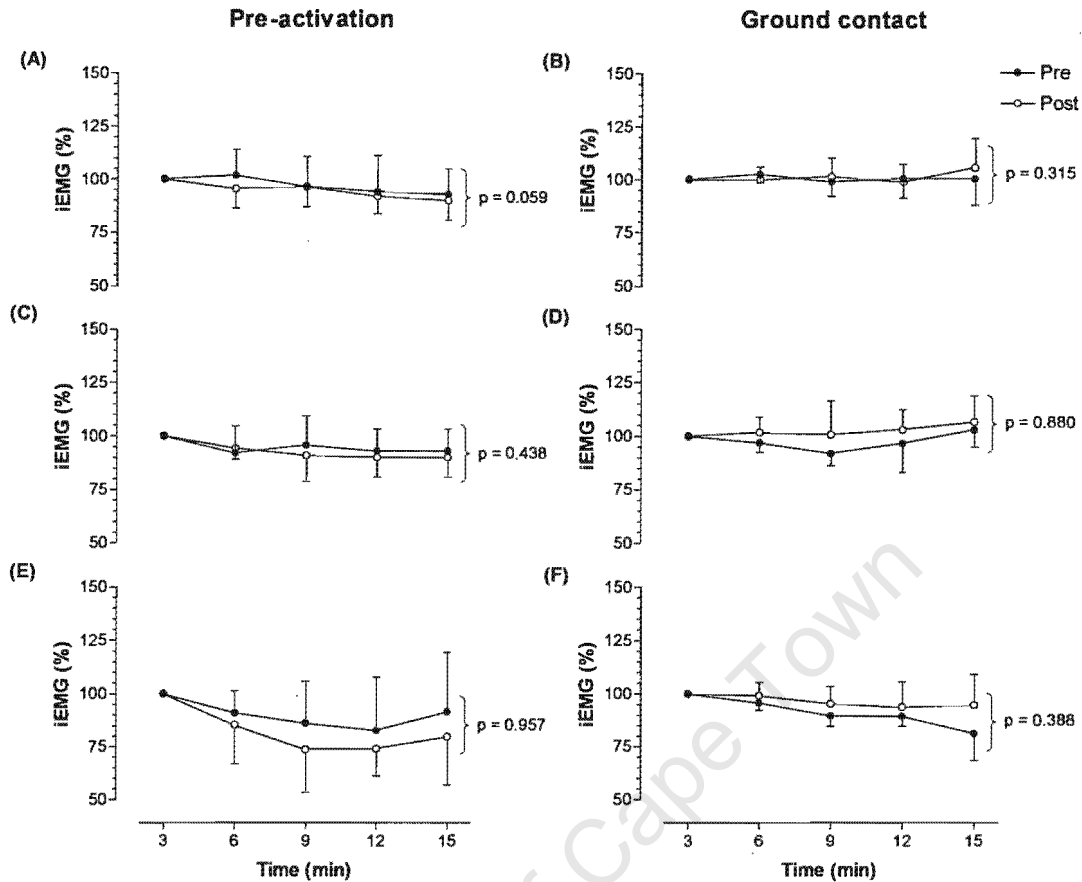


Figure 4.7 EMG characteristics of the VET (n = 7*) runners during a sub-maximal effort before (Pre) and one week after (Post) the downhill run. VL pre-activation (A), VL ground contact (B), VM pre-activation (C), VM ground contact (D), MG pre-activation (E) and MG ground contact (F) phase iEMG of the VET runners. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data. * n = 6 for VL contact, VM pre-activation and contact, and MG contact iEMG data.

With the exception of the pre-activation phase iEMG of the VM muscle, these same EMG characteristics measured in the NOV runners were also not different before (Pre) and one week after (Post) the downhill run (Figure 4.8). As can be seen in panel (C), pre-activation iEMG of the VM muscle increased during the sub-maximal effort before the downhill run, whereas it decreased after the intervention. This indicates a significant effect of the intervention on the way that pre-activation phase

iEMG of the VM muscle in NOV runners changed during the sub-maximal run ($p = 0.028$).

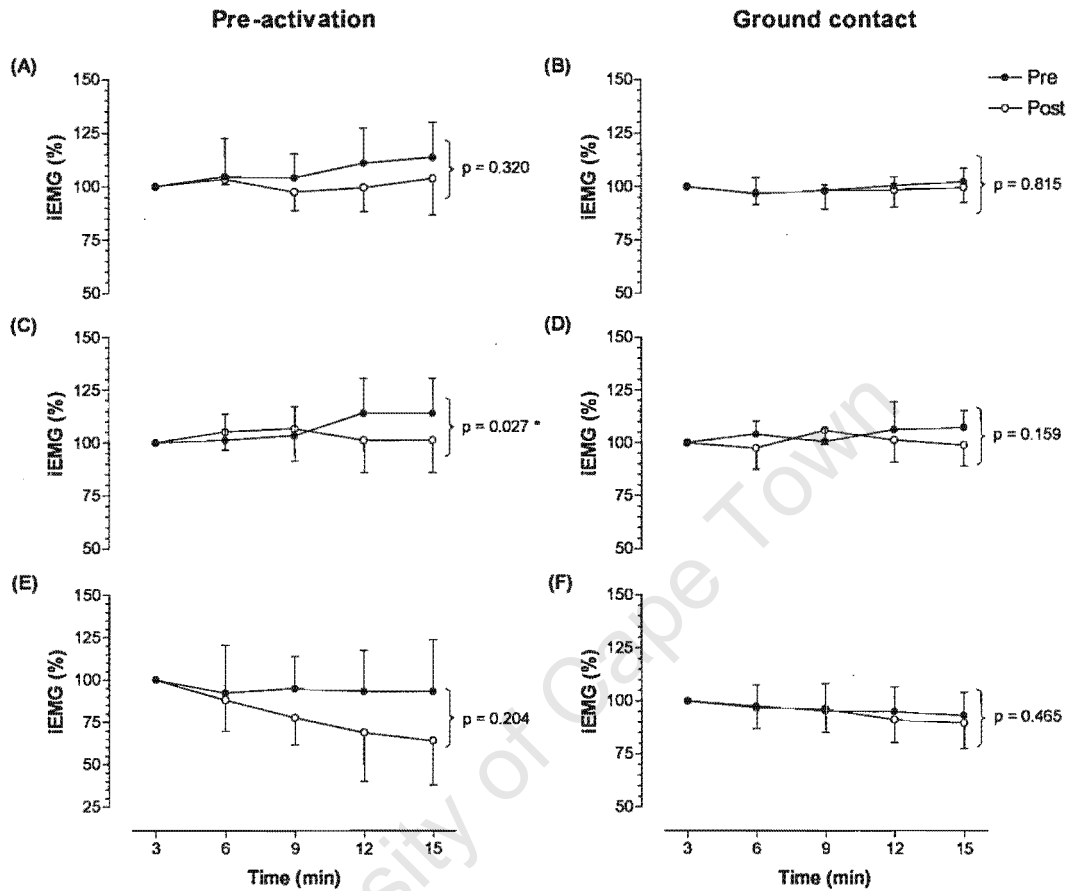


Figure 4.8 EMG characteristics of the NOV ($n = 6$) runners during a sub-maximal effort before (Pre) and one week after (Post) the downhill run. VL pre-activation (A), VL ground contact (B), VM pre-activation (C), VM ground contact (D), MG pre-activation (E), MG ground contact (F) phase iEMG. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data.

The same EMG characteristics measured at steady state were compared before (Pre) and one week after (Post) exercise-induced muscle damage. However, repeated measures ANOVA found no significant differences in the steady state (minutes 12 – 15) EMG characteristics between the VET and NOV groups (Figure 4.9).

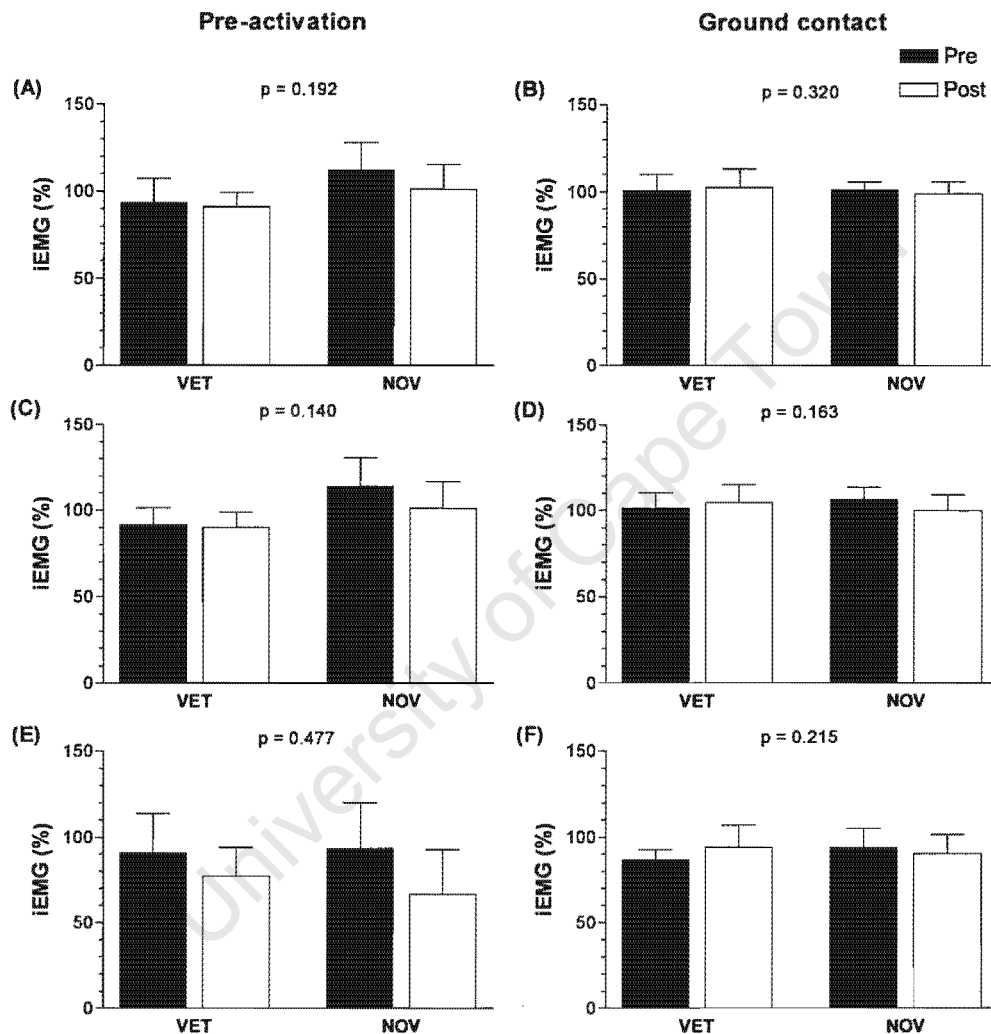


Figure 4.9 EMG characteristics of the VET (n = 7*) and NOV (n = 6) runners during steady state of a sub-maximal effort before (Pre) and one week after (Post) the downhill run. Steady state VL pre-activation (A), VL ground contact (B), VM pre-activation (C), VM ground contact (D), MG pre-activation (E) and MG ground contact (F) phase iEMG. The p value on each graph is the repeated measures ANOVA time-by-group interaction effect. The solid bars represent the Pre data, while the clear bars represent the Post data. * n = 6 VL contact, VM pre-activation and contact, and MG contact iEMG data.

4.3.4 Intervention effect on maximal running characteristics

The performance, physiological, stride and EMG characteristics of the VET and NOV runners were also recorded every kilometer during the 5 km time trial before (Pre) and one week after (Post) the downhill run. As for the sub-maximal run analysis, the Pre data of each group was compared to the Post data using repeated measures ANOVA.

4.3.4.1 Physiological characteristics

The kilometer speed (A), HR (B) and RPE (C) of the VET runners measured during the time trial before (Pre) and one week after (Post) the intervention are shown in Figure 4.10 (panels A – C). There were no significant time-by-intervention interactions for these variables, suggesting that the downhill run did not affect the speed, HR or RPE of the VET runners during a maximal effort. The same was true for the NOV group (Figure 4.10, panels D – F).

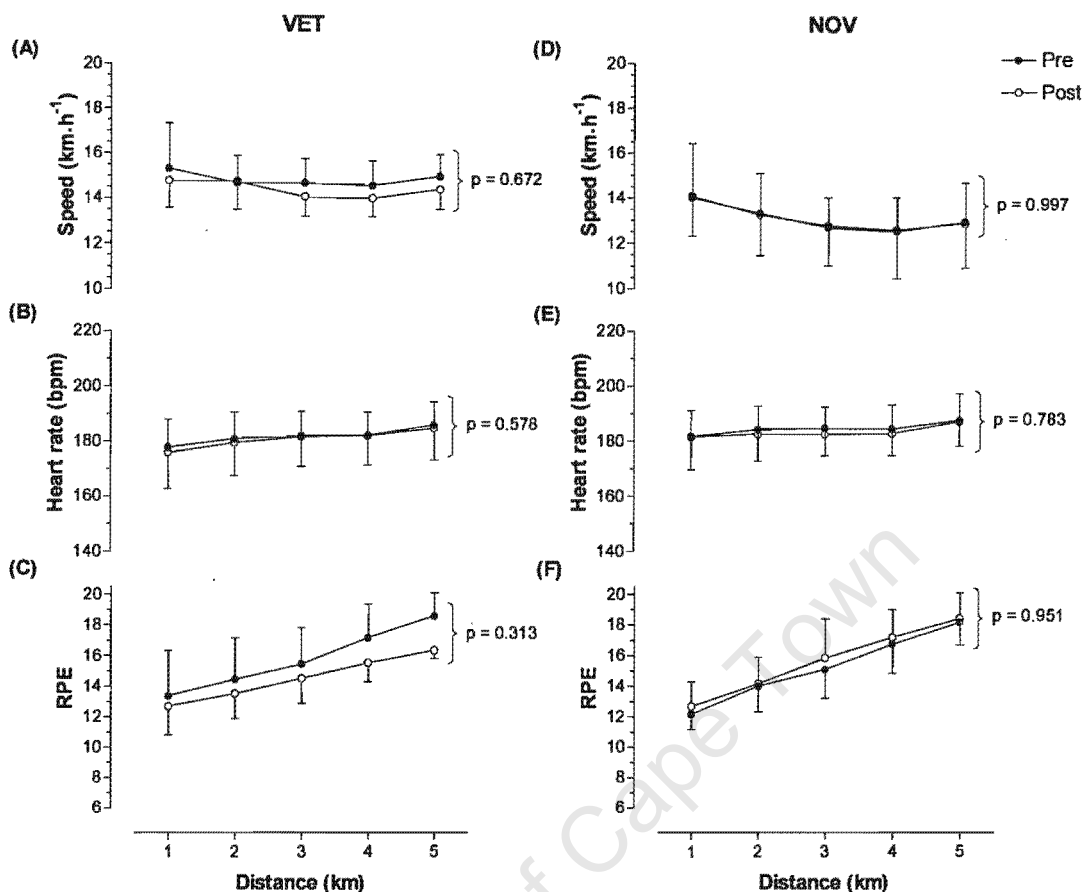


Figure 4.10 Performance and physiological responses of the VET ($n = 6$) and NOV ($n = 7$) runners during a 5 km time trial before (Pre) and one week after (Post) the downhill run. The speed, HR and RPE of the VET (panels A – C) and the NOV (panels D – F) runners. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data.

4.3.4.2 Stride characteristics

As can be seen from the p values on Figure 4.11 (panels A – C), there were no time-by-intervention interactions for CT (A), FT (B) or SF (C) when the Pre and Post time trial efforts of the VET runners were compared. Similarly, the stride characteristics of the NOV runners during the maximal effort were also not different before and one week after exercise-induced muscle damage (Figure 4.11 panels D – F).

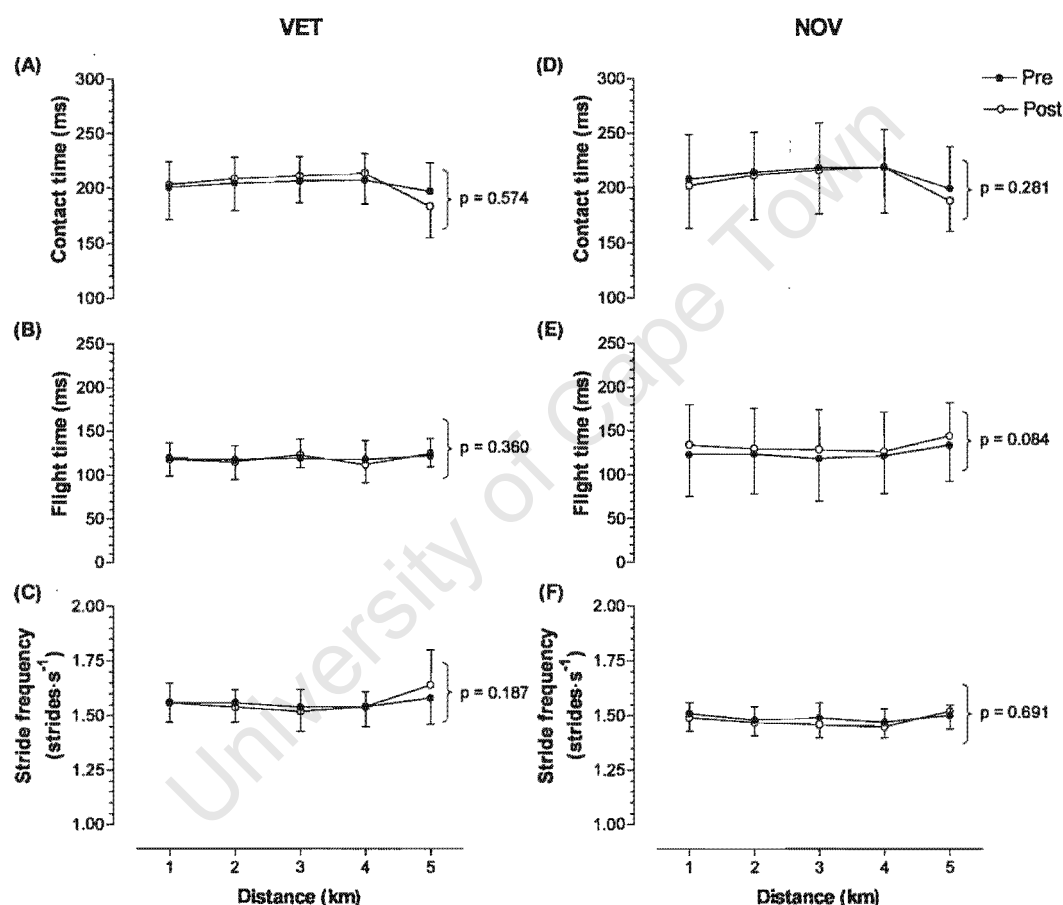


Figure 4.11 Stride characteristics of the VET ($n = 5$) and NOV ($n=6$) runners during a 5 km time trial before (Pre) and one week after (Post) the downhill run. CT, FT and SF of the VET (panels A – C) and NOV (panels D – F) runners. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data.

4.3.4.3 Electromyography characteristics

Pre-activation and ground contact phase iEMG of the VL, VM and MG muscles during each group's time trial were measured before (Pre) and one week after (Post) the downhill run. Figure 4.12 shows that the VET runners displayed no significant changes in any of these EMG characteristics during a maximal effort in response to the downhill run. The large standard deviations seen in the pre-activation phase iEMG of the MG muscle shows that there was large inter-individual variation for this measurement, making the data difficult to interpret.

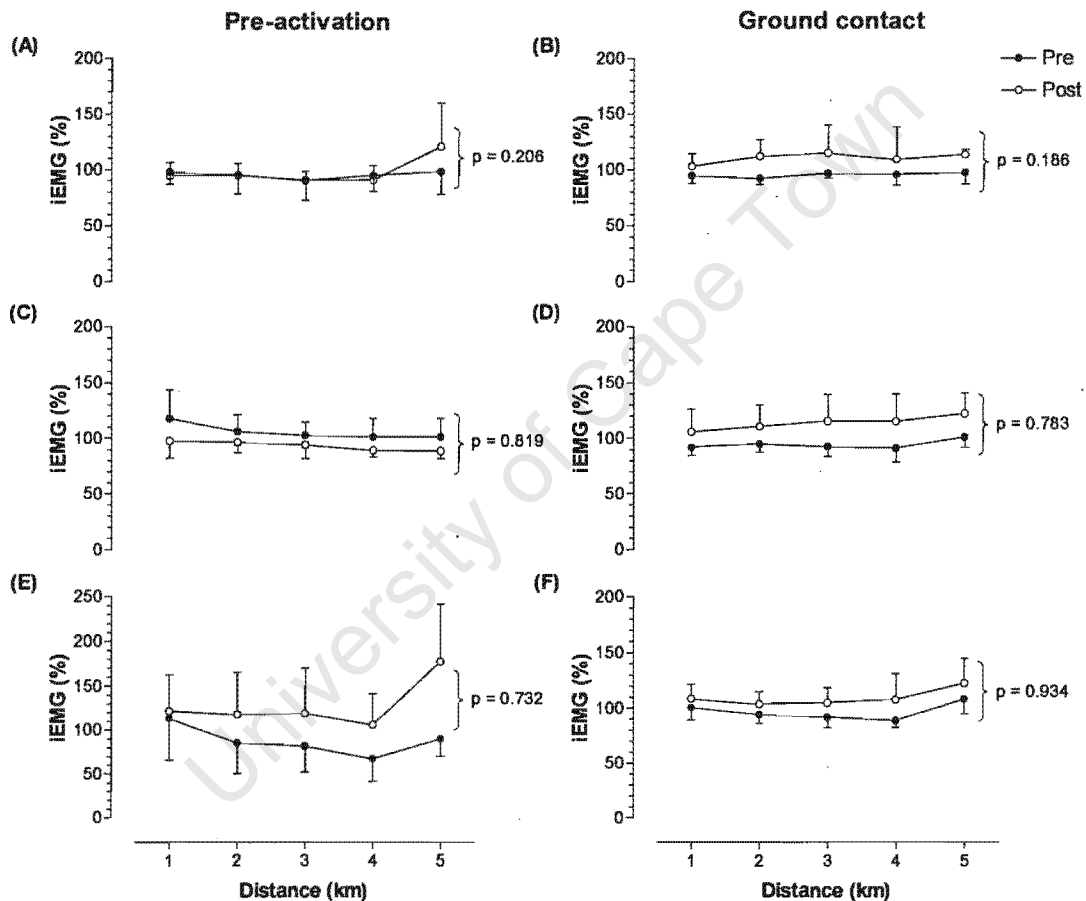


Figure 4.12 EMG characteristics of the VET ($n = 5$) runners during a 5 km time trial before (Pre) and one week after (Post) the downhill run. VL pre-activation (A), VL ground contact (B), VM pre-activation (C), VM ground contact (D), MG pre-activation (E) and MG ground contact (F) phase iEMG of the VET runners. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data.

Figure 4.13 shows that the EMG characteristics of the NOV runners also did not change in response to the intervention.

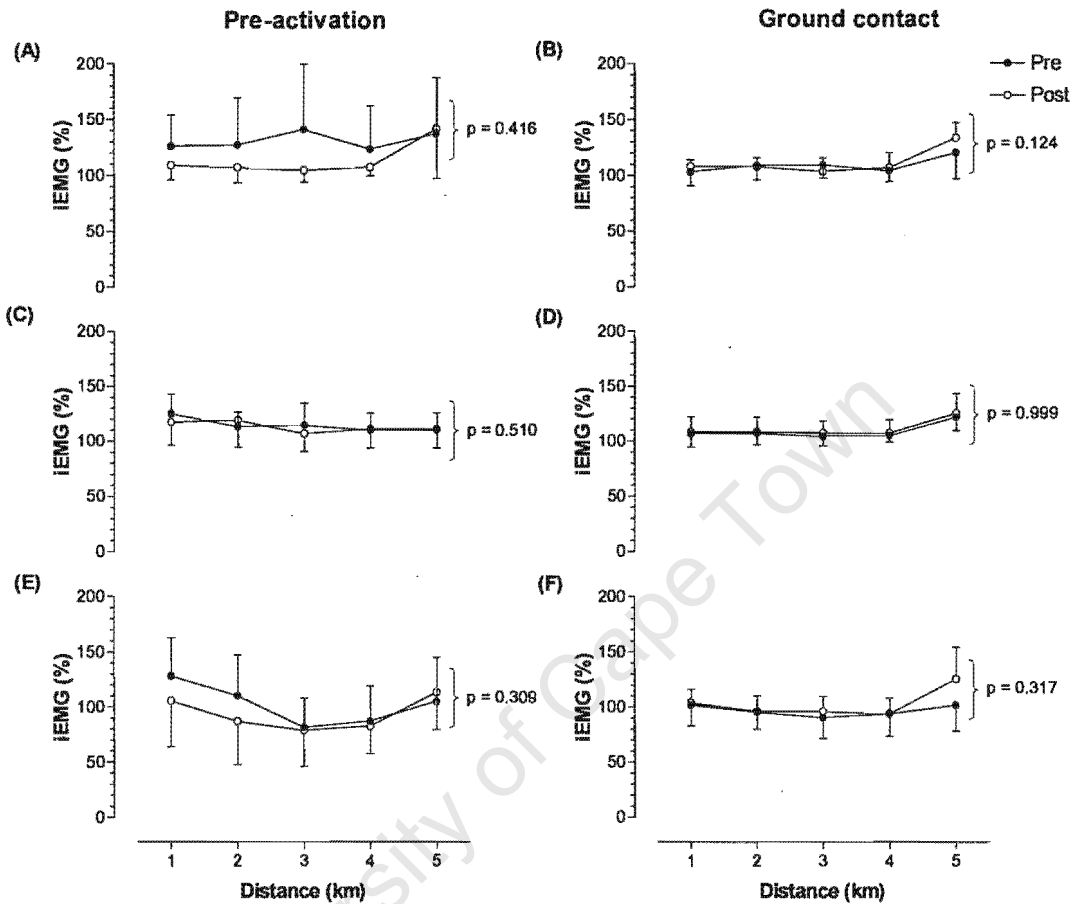


Figure 4.13 EMG characteristics of the NOV ($n = 6^*$) runners during a 5 km time trial before (Pre) and one week after (Post) the downhill run. VL pre-activation (A), VL ground contact (B), VM pre-activation (C), VM ground contact (D), MG pre-activation (E) and MG ground contact (F) phase iEMG of the NOV runners. The p value on each graph is the repeated measures ANOVA time-by-intervention interaction effect. The closed circles represent the Pre data, while the open circles represent the Post data. * $n = 5$ for MG pre-activation iEMG data.

4.4 DISCUSSION

The aim of this chapter of the thesis was to address the second question posed in Chapter 2: Do veteran and novice runners respond similarly to a bout of exercise-induced muscle damage? The results show that the protocol used to induce muscle damage was effective as both the VET and NOV runners incurred clinical signs of damage to their muscles following the downhill run, as seen by the increases in serum creatine kinase activity and subjective muscle pain scores. The severity of this damage is in agreement with previous studies from this laboratory which used a similar protocol (52, 168).

The main finding of this study was that there was no difference in the magnitude of exercise-induced muscle damage between the VET and NOV runners. Furthermore, the time taken by the VET and NOV runners to recover clinically from the downhill run as defined by serum creatine kinase activity (72 hours) and subjective muscle pain (96 hours) was similar for both groups. Therefore, it can be concluded that a greater exposure to distance running does not make veteran runners any more susceptible to exercise-induced muscle damage than novice runners. Alternatively, a lower exposure to running does not make novice runners more prone to the same damage.

The tool used to assess muscle power in the lower limbs of the runners in this study, namely the squat jump, was not effective. All runners steadily improved their muscle power score during the trial. This is contrary to a study by Chambers et al. (24) which showed that muscle power in a group of ultra-endurance runners decreased for 18 days following a 90 km road race. Several other laboratory studies have shown that muscle function is impaired for up to three weeks after exercise-induced muscle damage (19, 20, 28, 57). One explanation for the findings in this study may be that as the non-resistance trained runners became more familiar with the squat jump their technique improved, masking any possible change in muscle power caused by the exercise-induced muscle damage. Therefore, in future such studies with runners, either a one week familiarisation period should be used, or another method to assess muscle power that is more suitable to runners needs to be found.

One week after the downhill-run, the VET and NOV runners repeated the sub-maximal run and the time trial. The results from this study show that the downhill run did not affect the physiological, stride or EMG characteristics during a sub-maximal running effort of either the VET or NOV groups. There was one exception to this for the NOV runners. While the pre-activation phase iEMG in the vastus medialis muscle increased during the last six minutes of the sub-maximal run before the intervention, it failed to do so one week after the downhill run. A similar, but non-significant trend was seen for the vastus lateralis muscle. It is tempting to speculate that the exercise-induced muscle damage lead to a change in the pre-activation phase iEMG in the vastus medialis muscle of the NOV runners at steady state during a sub-maximal effort. However, these data need to be interpreted with caution as the standard deviations are large. Furthermore, there were also no differences between the VET and NOV runners in the way these variables measured at steady state changed from before to after the intervention.

The same physiological, stride and EMG characteristics of the VET and NOV runners during a 5 km time trial were compared before and after the downhill run. The data from this study shows that none of these variables changed in response to the exercise-induced muscle damage. There are two possible interpretations of these data. Firstly, the magnitude of the exercise-induced muscle damage in this study was not sufficient to induce changes in the physiological or stride characteristics of the VET and NOV runners during sub-maximal or maximal running trials. Secondly, one week may be sufficient time for both the VET and NOV to recover fully from the exercise-induced muscle damage induced in this study. Unfortunately, the design of the study precludes us from deciding which interpretation is more accurate.

A limitation to this study is the relatively small sample size used. Therefore the data were interpreted with caution. In hindsight, the design of the study may also have been a limiting factor to the investigation. Changes in the performance, physiological and neuromuscular characteristics of the runners were assessed one week after the bout of exercise designed to induce muscle damage. Most endurance runners train between three and seven times per week and are therefore presumably able to recover quickly from exercise-induced muscle damage produced through training.

Therefore one week may have been too long a recovery period to observe any residual changes associated with exercise-induced muscle damage in healthy individuals.

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4.5 CONCLUSION

In summary, the main findings of this study were:

- (i) Using this study design, the downhill run caused a similar extent of exercise-induced muscle damage in both the VET and NOV runners, as shown by their levels of serum creatine kinase activity after the downhill run.
- (ii) The post-damage results showed that the recovery time courses for both groups were similar with respect to serum creatine kinase activity and muscle pain.
- (iii) One week after the insult, the running performance and the physiological and neuromuscular characteristics measured in the sub-maximal and maximal running tests were not different to the values measured before the downhill protocol in both the VET and NOV runners.

In conclusion, this study answered the question it set out to address. Veteran and novice runners do respond similarly to a bout of exercise-induced muscle damage. Therefore it is unlikely that a vulnerability to, or incomplete recovery from exercise-induced muscle damage explains the decline in performance observed in veteran runners who have had a large exposure to running over several years.

CHAPTER 5

**A COMPARISON OF THE VASTUS LATERALIS MUSCLE OF
ENDURANCE RUNNERS AND SEDENTARY INDIVIDUALS:****Markers of structural pathology and determination of the regenerative
capacity****5.1 INTRODUCTION**

When one examines the effect of exercise on skeletal muscle, it is apparent that acute bouts of either resistance (147), sprint (106) or endurance (86) exercise result in muscle damage. Training for distance running, in particular, has been shown to elicit transient pathological changes in human leg skeletal muscle such as central nuclei, internally split fibres, irregular Z-lines and basophilic fibres (116), indicative of both muscle fibre disruption and repair. Similar changes have been noted following a foot race, such as a marathon (201). Thus single bouts of endurance exercise result in acute damage to skeletal muscle, but this damage is usually minor and repairable (115, 116).

As discussed in Chapter 1 (section 1.2.2.1), the skeletal muscle of a person that trains often presumably sustains regular exercise-associated damage and, as a result, would be subjected to repeated cycles of damage and regeneration. Repair to this damage can still be in effect one week after the bout of exercise (86, 116, 201), and as many athletes train most days of the week, it is reasonable to believe that their muscle is in a constant state of repair. Since skeletal muscle is a post-mitotic tissue, specialized muscle pre-cursor cells called satellite cells, are responsible for its repair following damage (161). The role of satellite cells in the repair of skeletal muscle following damage, such as sustained during exercise, is discussed in detail in Chapter 1 (section 1.2.3.2).

Briefly, during regeneration, quiescent satellite cells are activated to enter the cell cycle and proliferate to increase the number of myogenic cells available for repair. Activated satellite cells along a myofibre migrate to the site of injury. Following the proliferation phase some of the satellite cells become terminally differentiated and fuse either to damaged myofibres for repair, or to each other to form new myofibres. Other satellite cells, however, return to the quiescent state and are restored under the basal lamina (129), presumably to replenish the pool of satellite cells to be used for subsequent rounds of regeneration (25). Satellite cells do not, however, have an infinite capacity to proliferate. This is illustrated in Duchenne's muscular dystrophy patients where skeletal muscle undergoes excessive degeneration and regeneration cycles compared to healthy age-matched individuals. The satellite cells are constantly activated to initiate repair and actually reach senescence prematurely. Ultimately this limits the ability of the muscle of a Duchenne's patient to regenerate as the proliferative capacity of the satellite cells are greatly reduced (44, 161).

Since the skeletal muscles of endurance runners are subjected to repeated cycles of degeneration and regeneration, years of endurance training may similarly limit the ability of muscle to repair, possibly due to the decreased proliferative capacity of the satellite cells. In addition, well trained runners have been shown to have increased levels of oxidative damage to the DNA of peripheral leukocytes following a standard marathon (196). As oxidative stress decreases the proliferative capacity of the satellite cells of myoblasts *in vitro* (160), it may also interfere with skeletal muscle DNA, and indirectly, the capacity of satellite cells to proliferate in response to damage.

The potential for skeletal muscle to regenerate depends, in part, on the number of available satellite cells, their remaining proliferative capacity, as well as environmental and oxidative stressors (138, 162). In general, the number of satellite cells present in muscle decreases with age (162), suggesting that the continual, low level turnover within muscle with advancing age places some demand on the satellite cell pool. Excessive regeneration over an extended period of time, however, would presumably place a far greater demand on satellite cells to aid repair, thereby reducing the size of the satellite cell pool more noticeably than with natural aging. The remaining replicative capacity of satellite cells can be determined indirectly by

measuring their telomere lengths. Since a detailed discussion of mean and minimum telomere lengths in the skeletal muscle of healthy and diseased populations is presented in Chapter 1 (section 1.2.3.3), a brief summary follows. Telomeres are simple repetitive DNA sequences $(TTAGGG)_n$ located at the tips of eukaryotic chromosomes. Each time a satellite cell divides, its DNA loses a small portion of this telomeric sequence (45). Once a critical length is reached, the cell becomes senescent so that it can no longer play a role in regeneration. Minimum telomere length measured in the skeletal muscle of healthy aging humans decreases by approximately 13 base pairs (bp) per year, suggesting a continual, low level of turnover in myonuclei. Therefore, since minimum telomere length corresponds to the nuclei which have undergone the most divisions, it can be used as a measure of the regenerative history of satellite cells (44). In contrast, mean telomere length of skeletal muscle has been shown to be remarkably similar in young, adult and elderly humans, indicating that normal uninjured muscle is very stable from early development through to old age (45).

The skeletal muscle of healthy sedentary individuals would presumably have undergone a normal degree of regeneration as the consequence of everyday living and aging. Therefore, the aim of this study was to compare the skeletal muscle of apparently healthy veteran endurance runners to that of sedentary controls to assess the effects of a large exposure to running on muscle with respect to markers of structural abnormalities and telomere length. In so doing, this study aims to answer the third question raised in Chapter 2: Does a high exposure to running lead to molecular changes in the skeletal muscle of veteran runners?

5.2 METHODS

5.2.1 Subject characteristics

Nineteen apparently healthy runners (13 males and 6 females) were recruited by advertising both publicly and at running clubs in the greater Cape Metropolitan. These subjects formed the athlete group (ATH). They had all run at least 40 km per week in training for a minimum of seven years and actively participated in long distance races during this time. Nineteen untrained individuals (11 males and 8 females) were recruited through public advertising and formed the sedentary group (SED) for this study. The SED individuals reported that they had never done more than two days of social exercise per week, and had never participated in competitive sport beyond school.

5.2.2 Experimental design

The ATH and SED subjects were required to visit the laboratory on a single occasion. All participants were asked not to exercise on the day of, as well as the day before, their visit. The ATH group was also asked not to race or do training runs of longer than 20 km in the week preceding the trial. On arrival at the laboratory, the study was explained in detail to each participant. Once the subjects agreed to participate, they signed a consent form (Appendix 5) and completed questionnaires on their personal particulars, medical history and social sport participation (Appendix 1). The ATH group also completed competitive sport training and racing history questionnaires, as well as a one week diary to ensure that they were not currently overtrained (Appendix 2). The body composition of each subject was measured by the same researcher to avoid inter-individual errors. Finally, a physician took a muscle biopsy from the right vastus lateralis muscle of each subject. The Research Ethics Committee, Faculty of Health Sciences, of the University of Cape Town approved this study (REC reference number: 181/2002).

5.2.3 Data collection procedures

The procedure used to assess body composition was identical to that described in Chapter 3 (section 3.2.3.1). The additional procedure for the muscle biopsy is described below.

5.2.3.1 Muscle biopsy

The muscle biopsy site (mid portion of the belly of the vastus lateralis muscle) was shaved to remove hair and cleaned with an anti-septic (Betadine). Approximately 3.6 mg of local anaesthetic (Xylotox E80-A, containing 20 mg lignocaine hydrochloride and 12.5 µl adrenaline per 1 ml) (Adcock Ingram Limited, Bryanston, South Africa) was administered to the skin and underlying fat and fascia. Once the anaesthetic had taken effect, a small incision was made through the skin, subcutaneous fat, and fascia into the underlying muscle. A 5 mm Bergström needle was used to penetrate the muscle and remove a sample of approximately 100 mg (8, 58). One portion of the muscle tissue was mounted in OCT embedding medium (Tissue-Tek; Miles Laboratories Inc, Naperville Illinois, USA), frozen in isopentane chilled by liquid nitrogen and stored at -80 °C until subsequent fibre-typing and histological analysis. A second portion of tissue were placed in a cryotube, snap-frozen in liquid nitrogen and stored at -80 °C for later determination of telomere length.

5.2.4 Muscle sample analysis

5.2.4.1 Fibre type determination

Fibre type proportions were determined for each subject using the myofibrillar myosin adenosine triphosphatase (ATPase) stain. Serial cross-sections of each muscle biopsy were cut at -20 °C using a cryostat microtome (Leica Universal, HM 500), mounted on glass slides coated with 3-aminopropyltriethoxysilan, and air dried for at least ten minutes at room temperature. Three slides were prepared for each subject; two with muscle cross-sections 20 µm in thickness for the pH 4.3 and

4.6 ATPase stains and one with sections 10 μm thick for the pH 9.4 ATPase stain. The pH 4.3, 4.6 and 9.4 sections were incubated for 15 minutes at room temperature in their respective buffers (pH 4.3 – 2:1 vol:vol 0.2 N acetic acid : 0.2 M sodium acetate; pH 4.6 – 1:2 vol:vol 0.2 N acetic acid : 0.2 M sodium acetate and pH 9.4 - 20 mM sodium barbitone, 36 mM calcium chloride). All sections were then incubated for five minutes in the pH 9.4 buffer. Next, the pH 4.3, 4.6 and 9.4 sections were incubated for 32, 17 and 7 minutes respectively at 37 °C in pre-warmed ATP solution (20 mM sodium barbitone, 18 mM calcium chloride, 5.4 mM ATP disodium salt). All sections were washed with 90 mM calcium chloride before being placed in 2 % cobalt chloride for either six minutes (pH 4.3 and 4.6 sections) or three minutes (pH 9.4 sections). The slides were then washed in 10 mM sodium barbitone, rinsed in distilled water and developed in either 7 % (pH 4.3 and 4.6 sections) or 1 % (pH 9.4 sections) ammonium sulphide. After removing excess ammonium sulphide by washing the slides in running tap water for ten minutes, the sections were dehydrated for one minute each in 70 %, 90 % and absolute ethanol, cleared in xylol, mounted in entellen mounting medium and covered with coverslips. The laboratory method for this staining procedure is attached in Appendix 6.

Approximately 200 fibres per subject were classified as either as type 1, 2A, 2B or 2X/D based on their myosin ATPase activity (15). For sections stained at pH 4.3, the type 1 fibres appeared dark brown, the type 2A and 2B fibres appeared very light in colour and the type 2X/D fibres were the moderately brown fibres. At pH 4.6, the fibre types 1, 2B and 2X/D all appeared dark brown, while the type 2A fibre was very light in colour. And at pH 9.4, type 1 fibres appeared moderately brown in colour and fibre types 2A, 2B and 2X/D were dark brown. Each of the four fibre types was expressed as a percentage of the total number of fibres counted for that subject. Digital images of the sections were taken using a Kodak DC290 Zoom Digital Camera (Eastman Kodak Company, Rochester, NY, 14650, made in Japan) at a zoom of 77 mm, exposure of 1/30 seconds and at a magnification of 10X. The Kodak MDS290 (Microscopy documentation system, Eastman Kodak Company, 2002) and Adobe Photoshop software (version 5.5) (© 1989-1999, Adobe Systems Inc Microsoft Corporation) were used to visualize the sections.

5.2.4.2 Histological analysis

Cross-sections of each muscle biopsy were exposed to various histological stains to determine the level of structural abnormalities in each sample. Consecutive sections were cut and mounted on glass slides, as described in section 5.2.4.1, and stained with haematoxylin and eosin, nicotinamide adenine dehydrogenase-tetrazolium reductase and succinate dehydrogenase. The methods for these staining procedures are attached in Appendix 6. Specifically:

Haematoxylin and eosin stain:

Muscle cross-sections 20 μm in thickness were stained in Mayers haematoxylin solution (6.2 mM haematoxylin, 1 mM sodium iodate, 0.1 M aluminium potassium sulphate, 0.3 M chloral hydrate and 5.2 mM citric acid) for ten minutes, rinsed in tap water to remove excess haematoxylin, differentiated in 1 % acid alcohol (1:99 vol:vol concentrated hydrochloric acid : 70 % ethanol) and blued in running tap water for eight minutes. The sections were then stained in eosin solution (4.8 mM eosin, 2 mM phloxin B) for ten minutes, washed briefly in tap water to remove excess eosin and dehydrated for one minute each in 70 %, 90 % and absolute ethanol. Xylol was used to clear the sections, which were then mounted in entellen mounting medium and covered with a coverslip. The myonuclei stained blue while the cytoplasm and connective tissue of the muscle section stained pink.

Nicotinamide adenine dehydrogenase-tetrazolium reductase stain:

Muscle was sectioned 7 μm thick for this stain. The sections were incubated for 45 minutes at 37 °C in a 0.2 M Tris-Cl buffer (pH 7.4) containing 1.2 mM Nitro Blue Tetrazolium (NBT) and 1 M nicotinamide adenine dehydrogenase (NADH). Each slide was then rinsed in distilled water before being mounted in glycerine jelly and covered with a coverslip. Any NADH present in the section stained blue and the darker the blue hue, the greater the oxidative potential of the cell.

Succinate dehydrogenase stain:

Muscle sections 15 μm in thickness were incubated for 45 minutes at 37 °C in a solution containing a 1:9 (vol:vol) ratio of solution A (0.6 M sodium succinate, 1 M

hydrochloric acid, pH 7) and solution B (1.4 M NBT, 60 mM Tris buffer). The slides were drained and placed in Bakers formal calcium solution (0.1 M calcium acetate, 4 % formaldehyde) for 15 minutes at room temperature, then washed in distilled water, mounted in glycerine jelly and covered with a coverslip. The mitochondria of the muscle samples stained purple.

A pathologist, blinded to the identity and grouping of the subjects, examined each section for signs of structural pathology as indicated by the following markers: internal nuclei, fibre size variation, necrosis and degeneration, inflammation, regeneration and the accumulation of subsarcolemmal mitochondria. A score of 0 was assigned if the marker was either not present in the section or present at acceptable levels. A score of 1 was given when the marker was present at mild levels and a score of 2 represented severe levels of the marker. The scoring sheet used by the pathologist is attached in Appendix 7. To ensure that the scoring of the pathologist was repeatable and accurate, the same pathologist analysed each sample on three separate occasions. Each sample's score for a particular marker was that which was assigned on at least two of the three occasions, so that kappa statistics determined that there was good agreement between the diagnoses of each subject (1). The total score for a subject was the sum of his / her individual marker scores. Digital images of each section were taken as described in section 5.2.4.1.

5.2.4.3 DNA extraction from skeletal muscle

Genomic DNA was extracted from the snap-frozen muscle samples according to the method described by Decary et al. (44). Briefly, approximately 10 mg of frozen muscle was ground to a powder in liquid nitrogen and digested overnight in a shaking water bath (55 °C) in 650 µl of proteinase K digestion buffer (10 mM Tris-Cl, pH 8; 100 mM EDTA, pH 8; 100 mM sodium chloride and 1 % Triton X-100) containing 20 U·ml⁻¹ proteinase K. To extract the nucleic acids, one volume (650 µl) of phenol / chloroform / isoamyl alcohol (25:24:1 vol:vol:vol) was added to each sample, which was then inverted for five minutes and centrifuged at 1200 x g for five minutes. The supernatant was transferred to a new microfuge tube and this step was repeated. One volume of chloroform / isoamyl alcohol (24:1 vol:vol) was added to each sample, which was then inverted for five minutes, centrifuged at 1 200 x g for five minutes and the supernatant was transferred to a new sterile microfuge tube.

The DNA was precipitated by adding one volume of 7.5 M ammonium acetate and 100 % ethanol (1:4 vol:vol), and after pelleting the DNA by centrifugation at 13 000 x g for 5 minutes at room temperature. The supernatant was discarded. The DNA pellet was washed in 0.5 ml of 70 % ethanol and dried under a light for approximately one hour. Finally, the DNA was resuspended in 100 µl of TE buffer (10 mM Tris-Cl, pH 8; 1 mM EDTA, pH 8) by incubation at room temperature overnight and stored at 4 °C until further analysis. The laboratory method for this process is attached in Appendix 8.

5.2.4.4 Skeletal muscle telomere length analysis

The extracted DNA was used to determine minimum, mean and maximum terminal restriction fragment (TRF) lengths by Southern blot analysis using a ^{32}P -(TTAGGG)₃ probe to estimate telomere length. The DNA was digested with the restriction enzyme *Hinf1* to generate the terminal restriction fragments containing the TTAGGG tandem-repeat sequence and a sub-telomeric fragment of the non-TTAGGG DNA according to the methods described by Renault et al. (160) and Decary et al. (45). Briefly, 10 µl of DNA was added to a microfuge tube containing 10 µl of the digestion cocktail (2 µl Buffer H, 6 µl dH₂O and 2 µl *Hinf1*) which was allowed to incubate for four hours at 37 °C. The digested DNA was then resolved on a 0.7 % agarose gel. The gel was dried under vacuum at 60 °C for approximately one hour, denatured in 0.5 M sodium hydroxide / 1.5 M sodium chloride for ten minutes and neutralized in 0.5 M Tris (pH 8) / 1.5 M sodium chloride for ten minutes. Finally, the gel was incubated in 5x standard saline citrate (SSC) with the ^{32}P -end-labelled (CCCTAA)₃ probe at 37 °C for 8 – 12 hours and then washed in three lots of 3x standard SSC at 48 °C for 20 minutes each to detect the terminal restriction fragments.

The dried, treated gels containing the TRFs hybridized to the ^{32}P -end-labelled (CCCTAA)₃ probe were exposed to x-ray film (BioMax, Kodak, EIS, Massey, France) with a BioMax transscreen (BioMax, Kodak, Massey, France). Minimum, mean and maximum TRF lengths were determined as described by Harley et al. (79) and Vaziri et al. (198). The signal responses were analysed by a computer-assisted system using NIH Image 1.62 (densitometric data of one-dimensional gels) and ProFit (densitometric profiles analysis) software. The minimum, mean and

maximum TRF length was determined three times for each sample, on three independent gels. The mean TRF length (L) was calculated by integrating the signal intensity above background over the entire TRF distribution as a function of TRF length using the formula: $L = \sum(OD_i \cdot L_i) / \sum(OD_i)$, where OD_i and L_i are the signal intensity and TRF length, respectively, at position i on the gel image. To determine the minimum value of TRF length in a homogenous way for all samples, the densitometric profile of the TRF length was integrated over the distance of migration. The minimum TRF length corresponds to 95 % of this integration. The laboratory methods used to determine TRF length are included in Appendix 9.

5.2.5 Statistical analysis

An independent t-test was used to compare the descriptive characteristics, medical history, fibre type composition and telomere lengths of the ATH and SED groups, as well as the difference in telomere length of the smokers and non-smokers in the SED group. Analyses of co-variance for age and perceived life stress were also applied to the telomere length analysis. The current running training and racing data of the ATH group were compared to that of their entire running career using a dependent t-test. The Mann-Whitney U test for non-parametric data was used to compare the total scores of the two groups and the difference in the ratio of type 2B fibres of the groups. The ratio of males to females in each group, as well as the incidence of each marker of pathology was analysed using a Pearson Chi Square test. A Kappa statistic (κ) was calculated to determine the level of agreement between the pathologist's successive scores (1). A moderate agreement ($\kappa = 0.59$) was obtained between the first and second scores in the internal nuclei category, a fair agreement ($\kappa = 0.38$) existed between the second and third scores of the fibre size variation category, there was good agreement ($\kappa = 0.78$) between the second and third scores for the necrosis and degeneration category, while the level of agreement between the second and third scores for the aggregations of subsarcolemmal mitochondria category was very good ($\kappa = 0.83$). Correlations were performed using a Pearson Product-Moment test for parametric and a Spearman Rank Order test for non-parametric data, with pair-wise deletion of missing data. Partial correlations were performed controlling for age, perceived life stress and

smoking where applicable. The 95 % confidence intervals (CI) around each correlation coefficient were calculated using the spreadsheet "Calculating likely (confidence) limits and likelihoods for true values" in: *A new view of statistics*, downloaded from www.sportsci.org/resource/stats/index.html (89). Statistical significance was accepted at $p < 0.050$.

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5.3 RESULTS

5.3.1 Subject characteristics

The ATH and SED groups were matched for gender, age, height, weight, BMI, percent body fat, lean body mass and lean thigh volume (Table 5.1). The sum of seven skinfolds measurement of the ATH group was, however, significantly lower than that of the SED group ($p = 0.011$).

Table 5.1 Descriptive characteristics of the ATH and SED subjects.

	ATH (n = 19)	SED (n = 19)	p value
Number of males	13 (68 %)	11 (58 %)	0.501
Age (yrs)	42.5 ± 6.7	38.7 ± 9.5	0.157
Height (m)	1.70 ± 0.10	1.70 ± 0.08	0.937
Weight (kg)	66.8 ± 13.6	69.2 ± 13.9	0.608
BMI (kg·m⁻²)	23.1 ± 3.4	24.0 ± 4.4	0.495
S7 (mm)	74.6 ± 27.1	106.2 ± 43.0	0.011 *
Body fat (%)	19.9 ± 5.5	23.7 ± 7.1	0.082
LBM (kg)	53.4 ± 10.0	53.1 ± 11.0	0.923
LTV (cc)	4 541 ± 1 496	3 919 ± 940	0.144
Perceived life stress	4.3 ± 1.8	5.1 ± 1.8	0.146

Data are presented as the mean ± standard deviation, except for the number of males (n) with the frequency (%) in parenthesis.

** Significant difference between the ATH and SED groups.*

S7, Body fat, LBM and LTV: n = 18 for the ATH group. LTV: n = 18 for the SED group.

Abbreviations: ATH – Athlete group, SED – Sedentary group, BMI – Body mass index, S7 – Sum of seven skinfolds, LBM – Lean body mass, LTV – Lean thigh volume.

SKELETAL MUSCLE OF RUNNERS AND SEDENTARY INDIVIDUALS

All subjects were screened for medical conditions and no cases of coronary heart disease, cancer, stroke, diabetes, metabolic disease, renal disease, or neuromuscular disorders were reported. Table 5.2 shows the current medical conditions of the ATH and SED groups. The ATH group had suffered from more running related injuries compared to the SED group ($p < 0.001$) and more members of the SED group currently smoked compared to the ATH subjects ($p < 0.001$).

Table 5.2 Medical conditions reported by the ATH and SED subjects.

	Condition	ATH (n = 19)	SED (n = 19)	p value
Cardiorespiratory	Hypertension ¹	2	0	0.146
	Hypercholesterolaemia	3	0	0.071
	Palpitations	1	0	0.311
	Asthma	2	2	-
	Allergies ²	4	7	0.283
	Other respiratory complaints ³	1	3	0.290
Neurological	Epilepsy ⁴	1	0	0.311
Musculoskeletal	Osteoarthritis	1	0	0.311
	Lower back pain	5	5	-
	Gout	1	0	0.311
	Exercise associated muscle cramps	3	0	0.071
	Past running injury ⁵	11	0	<0.001 *
Psychological	Depression ⁶	2	1	0.547
Other	Anorexia	1	0	0.311
	Gynaecological ⁷	3	3	-
	Smoking habit	0	10	<0.001 *

Data are expressed as frequencies.

¹ *One of the two hypertensive subjects was on medication to treat the condition.*

² *Allergies included grass, pollen, shellfish and animal products.*

³ Other respiratory complaints included repeated infections of the upper respiratory tract, sinusitis and post nasal drip.

⁴ One subject suffered from epilepsy for which he had 3 three surgical interventions (14, 6 and 4 years ago) and for which he is currently on medication.

⁵ Past running injuries included six cases of ITB, two achilles tendon injuries, an ankle sprain, anterior knee pain, plantar fasciitis, a foot injury, past ACL reconstruction, a torn meniscus, a hamstring tear and lower back pain. None of the injuries were current and most were treated with rest, physio, ice and anti-inflammatories. Two of the cases of ITB required cortisone injections and the ACL reconstruction was performed surgically.

⁶ One of the subjects was currently being medicated for depression.

⁷ Gynaecological complaints included irregular menstruation, ammenorrhoea or menorrhagia.

Abbreviations: ATH – Athlete group, SED – Sedentary group, ITB – Ilio-tibial band, ACL – Anterior cruciate ligament.

5.3.2 Running training and racing characteristics of the ATH group

The ATH group's running training information is presented in Table 5.3. On average, the runners in the ATH group had been running for sixteen years, during which time they covered approximately 43 370 km in training and 6 195 km in races. All the runners still trained regularly and participated in races. However, the training records of the ATH group from the eight weeks before the trial indicated that their current training frequency and distance were lower than during their entire career ($p = 0.008$ and $p = 0.038$ respectively).

Table 5.3 Training characteristics of the ATH group (n = 18).

	Mean \pm SD	Range
Age began running (years)	27.1 \pm 7.9	15.0 – 40.0
Time running (years)	15.8 \pm 5.8	7.5 – 26.0
Entire career		
Total training distance (km)	43 370 \pm 21 433	15 278 – 89 550
Training frequency (days\cdotwk$^{-1}$)	5 \pm 1	3 – 6
Training distance (km\cdotwk$^{-1}$)	65.8 \pm 18.7	29.5 – 92.2
Max training distance (km\cdotwk$^{-1}$)	121.4 \pm 40.9	52.5 – 190.0
Training speed (km\cdoth$^{-1}$)	11.2 \pm 1.2	9.8 – 13.9
Current		
Training frequency (days\cdotwk$^{-1}$)	4 \pm 1	2 – 6
Training distance (km\cdotwk$^{-1}$)	51.3 \pm 23.1	19.9 – 109.6
Training speed (km\cdoth$^{-1}$)	11.5 \pm 1.4	9.0 – 14.4

Entire career training frequency, distance and speed data are the group's average over all their years of running, while the current data reflect the training they had done eight weeks prior to the trial.

Complete training data was only available for 18 of the 19 ATH runners, current training data: n = 15.

Abbreviations: ATH – Athlete group, SD – Standard deviation.

The racing data of the ATH group is presented in Table 5.4. Their current race times for the 5, 10, 21.1 and 42.2 km distances were significantly slower than their personal best times for these same races ($p = 0.011$, $p < 0.001$, $p < 0.001$ and $p = 0.005$ respectively).

Table 5.4 Racing characteristics of the ATH group (n = 18).

	Mean \pm SD	Range
Time racing (years)	14.6 \pm 5.8	7.5 – 26.0
Number of races	356 \pm 344	34 – 1234
Entire career		
Total racing distance (km)	6 195 \pm 5 401	967 – 18 420
PB 5 km time (min)	18.4 \pm 2.3	15.0 – 23.1
PB 10 km time (min)	38.6 \pm 5.2	32.0 – 49.7
PB 21.1 km time (min)	86.3 \pm 11.4	72.0 – 114.5
PB 42.2 km time (hrs)	3.2 \pm 0.5	2.6 – 4.2
Past year		
Best 5 km time (min)	21.4 \pm 2.2	17.1 – 24.0
Best 10 km time (min)	43.0 \pm 6.4	32.0 – 57.0
Best 21.1 km time (min)	96.4 \pm 11.3	72.0 – 120.0
Best 42.2 km time (hrs)	3.7 \pm 0.6	2.6 – 4.7

Entire career number of races, total racing distance, PB 10 km, PB 21.1 km, PB 42.2 km time: n = 17. Entire career PB 5 km time: n = 16. Past year best 5 km time: n = 10. Past year best 10 km time: n = 14. Past year best 21.1 km time: n = 15. Past year best 42.2 km time: n = 12.

Abbreviations: ATH – Athlete group, SD – Standard Deviation.

In contrast to the ATH group, the SED group led a sedentary life as defined by the fact that they have not done more than two sessions of exercise per week, and had not participated in any competitive sports

5.3.3 Fibre type analysis

A portion of each vastus lateralis muscle biopsy sample was used to determine fibre type composition. This information is presented in Table 5.5. The SED group had a higher proportion of type 2A fibres ($p = 0.006$). The groups were similarly matched for proportions of types 1, 2B and 2X/D fibres, although there were trends for the ATH group to have higher proportions of type 1 and 2B fibres.

Table 5.5 Fibre type distribution in the vastus lateralis muscle of the ATH and SED groups.

	ATH (n=15)	SED (n=17)	p value
Type 1 (%)	58.0 ± 13.3	48.2 ± 15.6	0.069
Type 2A (%)	36.7 ± 10.1	50.2 ± 15.0	0.006 *
Type 2B (%)	3.5 ± 7.1	0.4 ± 1.2	0.202 ¹
Type 2X/D (%)	1.7 ± 1.6	0.9 ± 1.6	0.164

Data are presented as the mean ± standard deviation.

** Significant difference between the ATH and SED groups.*

¹ A Mann-Whitney U test for non-parametric data was used since Levene's test for homogeneity indicated a significant variance in the standard deviation values.

Abbreviations: ATH – Athlete group, SED – Sedentary group.

5.3.4 Histological analysis

Serial cross-sections of the vastus lateralis muscle biopsy were exposed to various histological stains to determine the level of markers of structural pathology including internal nuclei, fibre size variation, necrosis and degeneration, inflammation, regeneration and the excessive accumulation of subsarcolemmal mitochondria. As there were no signs of either inflammation or regeneration in the sections examined, only the incidences of the four markers identified are presented. Figure 5.1 shows examples of the markers of structural pathology as visualised using the histological staining methods described in section 5.2.4.2.

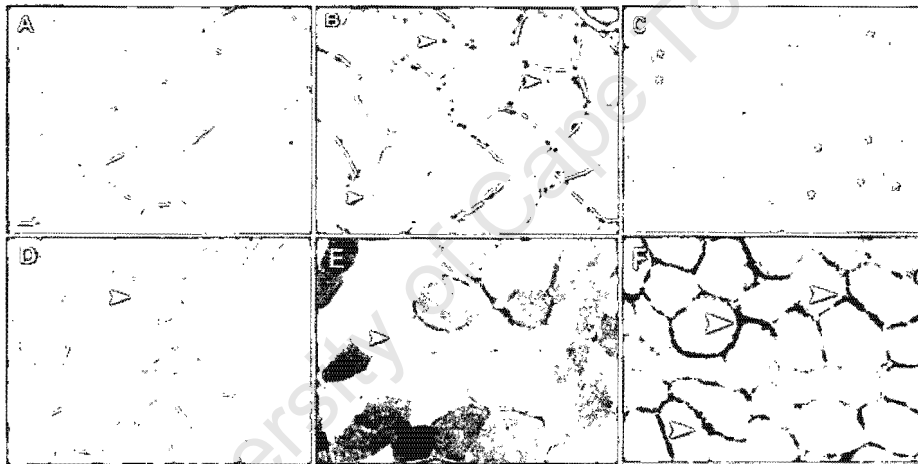


Figure 5.1 Visualisation of the markers of structural pathology in the skeletal muscle of the ATH and CON groups. (A) Control sample (Haematoxylin & Eosin (HE) stain, 10x); (B) Internal nuclei, indicated by arrowheads (HE stain, 10x); (C) Fibre size variation, indicated by asterisks (HE stain, 10x); (D) Necrotic cells, indicated by an arrowhead (HE stain, 10x); (E) Control sample with arrowhead indicating minimal aggregations of subsarcolemmal mitochondria (Nicotinamide adenine dehydrogenase – tetrazolium reductase (NADH-TR) stain, 10x); (F) Increased aggregations of subsarcolemmal mitochondria, indicated by the arrowheads (NADH-TR stain, 10x).

The ATH group had significantly more internal nuclei and greater subsarcolemmal aggregations of mitochondria compared to the SED group ($p = 0.016$ and $p < 0.001$ respectively). There were no significant differences between the groups with respect to the incidence of fibre size variation or signs of necrosis and degeneration (Figure 5.2).

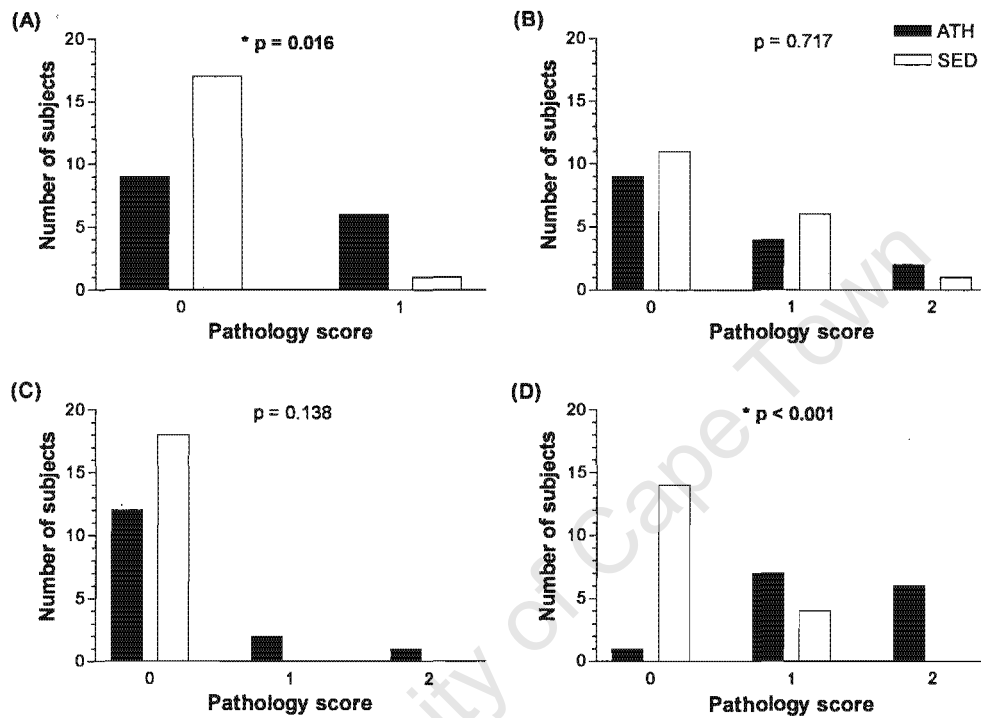


Figure 5.2 Incidence of markers of structural pathology in the vastus lateralis muscle samples of the ATH and SED groups. (A) Internal nuclei, (B) fibre size variation, (C) necrosis and degeneration, (D) subsarcolemmal aggregations of mitochondria.

The total pathology score of the ATH group (2.5 ± 1.6 units) was significantly greater than that of the SED group (0.7 ± 0.9 units) ($p = 0.001$). There was no correlation between the total pathology score and age ($r = 0.15$, $p = 0.411$, $n = 33$) when the data for both groups were pooled. There was also no significant correlation between total training distance and the total pathology score of the ATH group ($r = 0.16$, $n = 14$). Finally, there was no relationship between the total pathology score of the ATH group and their training distance ($r = 0.10$, $p = 0.778$, $n = 11$), frequency ($r = -0.17$, $p = 0.617$, $n = 11$) or speed ($r = -0.44$, $p = 0.178$, $n = 11$) during the eight weeks prior to the biopsy.

5.3.5 Telomere length analysis

The minimum, mean and maximum terminal restriction fragment (TRF) lengths measured in the ATH group were not statistically different to those of the SED group (Table 5.6).

Table 5.6 TRF lengths measured in the vastus lateralis muscle of the ATH and SED groups.

	ATH (n=18)	SED (n=19)	p value
Minimum TRF length (kbp)	6.9 ± 0.9 (5.3 – 8.0)	7.0 ± 0.8 (5.9 – 9.0)	0.805
Mean TRF length (kbp)	10.0 ± 1.0 (7.5 – 11.1)	10.0 ± 1.0 (8.4 – 12.1)	0.643
Maximum TRF length (kbp)	15.1 ± 1.8 (10.8 – 18.0)	14.1 ± 1.6 (11.1 – 16.7)	0.089

Data are presented as the mean ± standard deviation with the range in parenthesis.

Maximum TRF length: n = 16 for ATH and 18 for SED.

Abbreviations: TRF – Terminal restriction fragment, ATH – Athlete group, SED – Sedentary group and kbp – Kilobase pair.

Possible confounding variables to telomere length include age (79), life stress (55), mood disorders (175) and smoking (137). Although the two groups were statistically matched for age and perceived life stress (Table 5.1), an analysis of co-variance for these two variables was performed on the data to ensure they did not affect the results. As can be seen in Table 5.7, TRF lengths of the two groups were still not different when age and perceived life stress were accounted for. The analysis was repeated excluding these three individuals (2 ATH, 1 SED) with depression. This also had no effect on TRF length differences between the groups (data not shown).

Table 5.7 TRF length analysis accounting for possible confounding variables such as age, perceived life stress and depression.

	ATH (n=18)	SED (n=19)	p(A)	p(S)	p(D)
Minimum TRF length (kbp)	6.9 ± 0.9 (5.3 – 8.0)	7.0 ± 0.8 (5.9 – 9.0)	0.753	0.817	0.810
Mean TRF length (kbp)	10.0 ± 1.0 (7.5 – 11.1)	10.0 ± 1.0 (8.3 – 12.1)	0.558	0.516	0.773
Maximum TRF length (kbp)	15.1 ± 1.8 (10.8 – 18.0)	14.1 ± 1.6 (11.1 – 16.7)	0.138	0.057	0.150

Data are presented as the mean ± standard deviation with the range in parenthesis.

The p(A) column represents the analysis of co-variance results when age was taken into account.

The p(S) column co-varied for perceived life stress.

The p(D) column contains the p-values obtained when the analysis was repeated excluding the three subjects with depression.

Abbreviations: TRF – Terminal restriction fragment, ATH – Athlete group, SED – Sedentary group, and kbp – Kilobase pair.

Approximately half of the SED group were smokers, whereas none of the ATH group smoked. To assess the effect of smoking on telomere length in this study, the data of the SED group were re-analysed and are presented in Table 5.8. The data show that there was no difference in minimum, mean or maximum TRF length between the SED smokers and non-smokers. This analysis was not altered when the age of the SED group was taken into account (Table 5.8).

Table 5.8 TRF lengths measured in the vastus lateralis muscle of the smokers and non-smokers in the SED group.

	Smokers (n=10)	Non-smokers (n=9)	p value	p (coV)
Minimum TRF length (kbp)	7.2 ± 0.9 (6.2 – 9.0)	6.8 ± 0.5 (5.9 – 7.5)	0.327	0.264
Mean TRF length (kbp)	10.0 ± 1.2 (8.6 – 12.1)	9.9 ± 0.8 (8.3 – 10.8)	0.887	0.781
Maximum TRF length (kbp)	13.9 ± 1.9 (11.7 – 16.7)	14.2 ± 1.4 (11.1 – 15.8)	0.688	0.442

Data are presented as the mean ± standard deviation with the range in parenthesis.

Maximum TRF length: n = 9 for Smokers group.

Abbreviations: TRF: Terminal restriction fragment, coV – Co-variance for age, and kbp – Kilobase pair.

The data from the ATH and SED groups were pooled and no correlations were found between minimum TRF length and age ($r = -0.11$, $p = 0.507$, $n = 37$), perceived life stress ($r = 0.17$, $p = 0.323$, $n = 37$) or total pathology score ($r = 0.02$, $p = 0.894$, $n = 32$) (Figure 5.3). The oldest SED subject (63 years) appeared to be an outlier (Figure 5.3). The analysis was therefore repeated excluding him, but the results remained the same (data not shown). The analysis was also repeated excluding the three subjects with depression, but the result remained the same when minimum TRF length was correlated against age ($r = -0.14$, $p = 0.428$, $n = 34$), perceived life stress ($r = -0.18$, $p = 0.317$, $n = 34$) or total pathology score ($r = 0.07$, $p = 0.705$, $n = 29$). Finally, the correlation was repeated controlling for smoking, but the results were still not different (Age: $r = -0.04$, $p = 0.835$, $n = 37$; Perceived life stress: $r = 0.18$, $p = 0.502$, $n = 37$; Total pathology score: $r = 0.09$, $p = 0.650$, $n = 34$). The same was true for the mean and maximum TRF length (data not shown).

SKELETAL MUSCLE OF RUNNERS AND SEDENTARY INDIVIDUALS



Figure 5.3 Relationship between minimum telomere length and age (A), perceived life stress (B) and total pathology score (C) of the ATH (closed circle) and SED (open circle) groups.

When the data of the ATH group was examined alone, significant correlations were found between minimum telomere length and the number of years the athletes had been running for ($r = -0.63$, 95 % CI: $-0.85 - -0.21$, $p = 0.007$, $n = 17$) as well as the total number of hours they had spent training ($r = -0.51$, 95 % CI: $-0.80 - 0.04$, $p = 0.034$, $n = 17$) (Figure 5.4 A and B). Although not significant, there was a trend for minimum telomere length to be related to the log of the runner's total training distance ($r = -0.46$, $p = 0.062$, $n = 17$) (Figure 5.4 C). No such relationships were found for either mean or maximum TRF length (data not shown).

The data for minimum telomere length of the ATH group was re-analysed controlling for general life stress, but the results remained the same (Years running: $r = -0.63$, 95 % CI: $-0.85 - -0.21$, $p = 0.009$, $n = 17$; Hours training: $r = -0.50$, 95 % CI: $-0.79 - -0.03$, $p = 0.048$, $n = 17$; Total training distance: $r = -0.46$, 95 % CI: $-0.77 - -0.03$, $p = 0.075$, $n = 17$). When the two athletes with depression were excluded from the analysis, minimum telomere length was still found to be correlated to years in running ($r = -0.63$, 95 % CI: $-0.86 - -0.17$, $p = 0.013$, $n = 15$), but the hours spent training relationship was reduced to a trend ($r = -0.48$, $p = 0.071$, $n = 15$), and no relationship was demonstrated with the log of training distance ($r = -0.42$, $p = 0.121$, $n = 15$).

SKELETAL MUSCLE OF RUNNERS AND SEDENTARY INDIVIDUALS

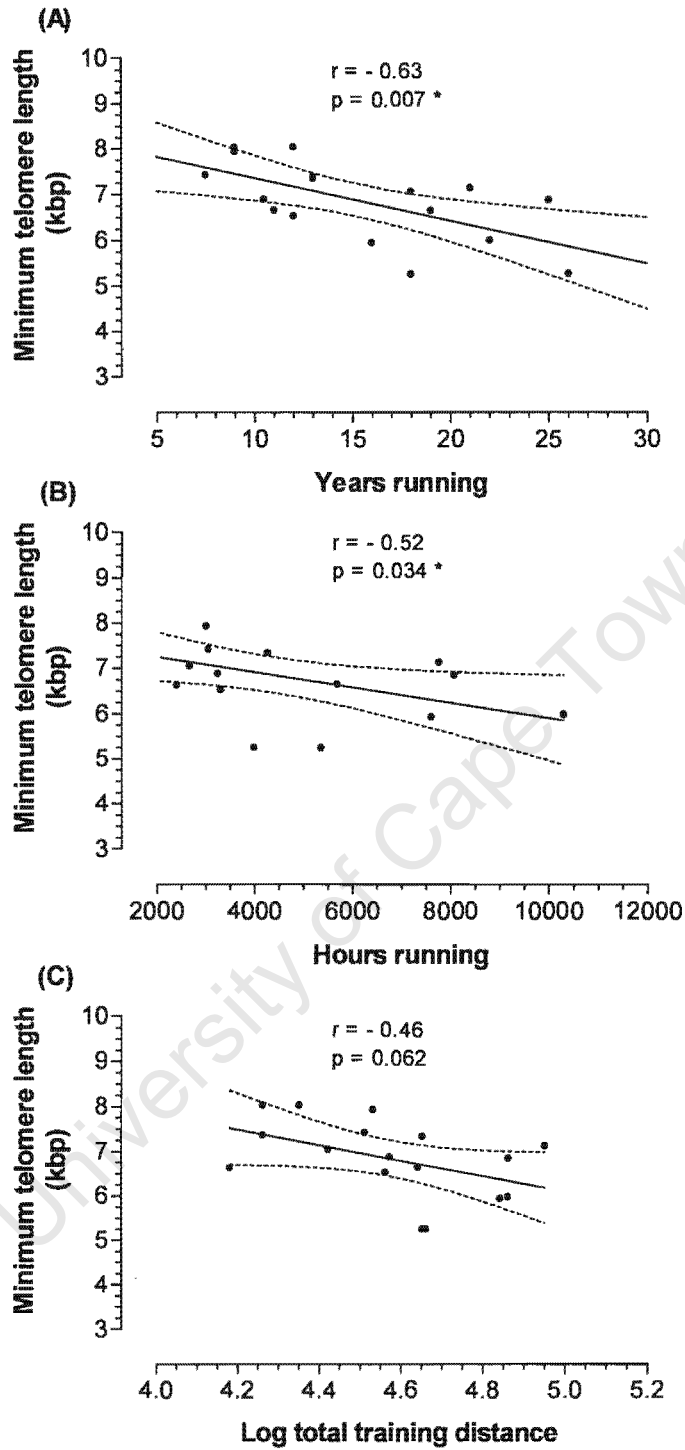


Figure 5.4 Relationship between minimum telomere length and years in running (A), hours spent training (B) and log of the total training distance (C) of the ATH group. The solid line represents the line of best fit, while the dotted lines are the 95 % confidence intervals. * indicates a significant correlation.

The relationship between minimum telomere length and the change in performance (personal best to current time) of the ATH group over the 21.1 and 42.2 km distances was also examined. Figure 5.5 shows that although these correlations were not statistically significant, they were trends for athletes with shorter minimum lengths to show greater declines in performance over both the 21.1 km ($r = 0.52$, $p = 0.056$, $n = 17$) and the 42.2 km ($r = 0.54$, $p = 0.071$, $n = 12$) race distances.

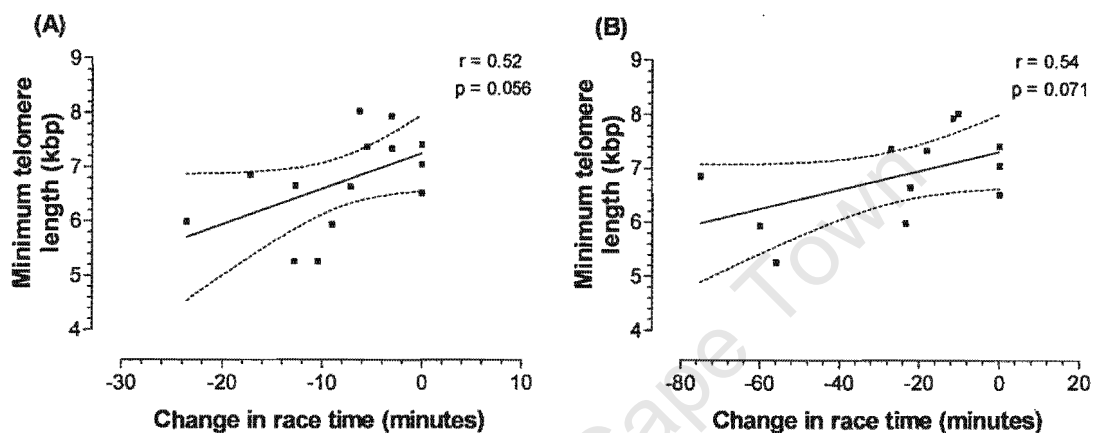


Figure 5.5 Relationship between minimum telomere length and change in performance from personal best to current race time for the 21.1 km (A) and the 42.2 km (B) distances of the ATH group. The solid line represents the line of best fit, while the dotted lines are the 95 % confidence intervals.

When the same relationships were tested controlling for general life stress, the results were similar for the 21.1 km distance ($r = 0.51$, $p = 0.078$, $n = 17$) and the 42.2 km distance ($r = 0.52$, $p = 0.099$, $n = 12$). Finally, when the two athletes with depression were removed from the analysis, the trend for minimum telomere length to be related to change in 21.1 km performance was unaltered ($r = -0.56$, $p = 0.056$, $n = 12$), while the change in 42.2 km performance became significant ($r = -0.68$, 95 % CI: 0.09 – 0.92, $p = 0.032$, $n = 10$). However, the sample sizes for these two analyses are small and the results need to be interpreted with caution.

5.4 DISCUSSION

The aim of this study was to answer the third question posed in Chapter 2: Does a high exposure to running lead to molecular changes within the skeletal muscle of veteran endurance runners? The main finding was that the minimum, mean and maximum telomere lengths measured in the ATH group were not different to those of the SED group (Table 5.6). The results were unchanged when possible confounding variables such as age, perceived life stress, depression (Table 5.7) and smoking (Table 5.8) were accounted for.

It was anticipated that mean telomere length would be similar between groups. Within post-mitotic skeletal muscle tissue, there is a large variation in telomere length since myonuclei are added at varying times during the lifespan (refer to Chapter 1, section 1.2.3.3). Mean telomere length therefore, represents this heterogeneity. Since very little myonuclei turnover occurs in the skeletal muscle of healthy individuals between young and elderly adulthood, however, mean telomere length has been found to be relatively stable throughout the lifespan of humans (45). It is only in extreme cases of disease, such as Duchenne's muscular dystrophy, that mean telomere length is excessively shortened (44). That mean telomere length was similar between the ATH and SED groups, and to published values (44), suggests that the level of myonuclei turnover in both groups to date was of a normal degree.

The fact that the minimum telomere lengths of the groups were similar, however, was contrary to the hypothesis of this study. It was theorised that since the skeletal muscle of the runners had had a large exposure to endurance exercise, and presumably more exercise-induced muscle damage, their satellite cells may have been activated to proliferate and repair the damage more frequently. Over many years, this in turn may have lead to their skeletal muscle having a compromised ability to regenerate either by virtue of a smaller satellite cell pool, or by a reduced proliferation capacity of the satellite cells. Since minimum telomere length in skeletal muscle is an indirect measure of the remaining replicative potential of the satellite cell, one would expect to find shorter minimum telomere lengths in the skeletal

muscle of the runners if excessive repair and regeneration cycles had indeed compromised the replicative potential of the satellite cells. This theory was prompted by the fact that a previous study comparing athletes with acquired training intolerance to healthy athletes found the fatigued athletes to have significantly shorter telomeres in their vastus lateralis muscle. The authors suggested that the muscle of the fatigued athletes may have undergone more degeneration and regeneration cycles compared to the healthy athletes (33). The fact that the ATH and SED subjects in this study had similar minimum telomere lengths suggest that the telomeres of healthy athletes are at no more risk of facing excessive shortening compared to sedentary individuals. Therefore, it seems that some factor other than exposure to endurance running may have triggered excessive telomere shortening in the fatigued athletes (33). This will be addressed in Chapter 6 of this thesis.

A number of reasons may explain the similar minimum telomere lengths of the ATH and SED groups in this study. One might be that the groups were not fundamentally different. With respect to exercise, however, this is unlikely. While the ATH group covered approximately 50 000 km in distance running over a period of 15 years, the SED group did not run at all, let alone participate in social exercise.

A second reason might be that the repeated bouts of exercise-induced muscle damage associated with the endurance running of the ATH group were not of a high enough grade to activate satellite cells excessively, as is seen in cases of Duchenne's muscular dystrophy (44). Cramer et al. showed that after a single bout of unaccustomed, high intensity exercise, the number of satellite cells was increased, indicating activation, but that this did not automatically lead to terminal differentiation (38). They suggest that either a subsequent bout of exercise or myofibre lesions are necessary to stimulate terminal differentiation of the activated satellite cells. Therefore, although the ATH group did have a greater exposure to endurance running, it may not have been sufficiently stressful to cause excessive proliferation of the satellite cells, thereby reducing their potential to replicate in the future. The role of satellite cells in response to exercise is discussed in detail in Chapter 1 (section 1.2.3.2).

A third possibility may be that any telomere shortening in skeletal muscle satellite cells that does occur as a result of long term endurance exercise may be reversed before a critical length is reached. The enzyme telomerase, a ribonucleoprotein, extends telomere length by adding TTAGGG repeats to the 3' end of the DNA strand (10). Furthermore, it has been shown to target cells with the shortest telomere lengths, presumably to restore their length to prevent cell dysfunction or death (84). However, it is usually only the mitotically active cells in a given tissue that express telomerase (74). Although telomerase has been shown to increase telomere length in skeletal muscle cells *in vitro* (49, 210), it does not seem likely that telomerase is activated, or even present, in healthy skeletal muscle *in vivo* since it appears only to be active in either germline or immortal (tumour) cells, and not healthy mortal cells and (105). Therefore it seems unlikely that telomerase plays a role in extending telomere length in healthy skeletal muscle.

A fourth explanation may be that the SED group was exposed to a stressor other than exercise that had a comparable effect on minimum telomere length to a high exposure to endurance exercise. Significantly, half of the SED group members are current smokers, while none of the ATH group smoked. Smoking has been shown to be a stressor to the body which results in shortened telomeres of leukocytes (137, 197). Whether this also affects the telomeres of the skeletal muscle is not clear. When the telomere lengths of the smokers and non-smokers in the SED group were compared, however, no differences were found (Table 5.8), suggesting that smoking alone may not have been a significant stressor to compromise the telomere lengths of the SED group.

The second finding of this study was that the minimum telomere length of the ATH group was correlated to years in distance running and hours spent training. There was also a tendency for minimum telomere length to be correlated to training distance (Figure 5.4). Time spent training and distance covered in training can both be seen as measures of exposure to endurance running. This finding fits with the main hypothesis of the study; the greater the exposure to endurance running, the shorter the minimum telomere lengths would be. Indirectly, this also suggests that veteran runners may have shorter minimum telomere lengths compared to novice runners since the major difference between them would be volume of racing and

training. It would be interesting to further explore whether this may explain the performance decline facing veteran runners. Particularly since there was a tendency in this study for minimum telomere length to be correlated to the decline in race speed of the ATH group over both the 21.1 and 42.2 km distances. This result complements the finding by Kuipers et al. that the frequency of structural changes observed in the muscle of endurance trained individuals increases with training distance, and not necessarily training intensity (116).

There is evidence that individuals exposed to chronic, high levels of life stress have shorter telomere lengths and lower telomerase activity in their peripheral blood mononuclear cells, as well as greater levels of oxidative stress compared to those individuals who have experienced more acute or lower levels of life stress. The authors suggest that this is likely evidence for the anecdotal "premature aging" observed in individuals with higher levels of psychological stress (55). Furthermore, individuals with mood disorders such as depression have been shown to have shorter leukocyte telomere lengths compared to those with no psychiatric illnesses (175), adding to the pool of evidence linking psychological stress to shorter telomeres. The fact that perceived life stress had no influence on the relationships between minimum telomere length and the exposure to running variables suggested that psychological stress was not a contributing co-stressor in this study. When the two depressed athletes were excluded from the analysis, however, only years in distance running remained significantly correlated to minimum telomere length. Since removing athletes with depression from the analysis marginalised the relationship between minimum telomere length and the exposure to factors associated with running, depression may be considered a co-stressor to exposure to endurance running. That is, athletes with a high exposure to endurance running combined with depression may be more susceptible to adverse effects within their skeletal muscle. These results should, however, be interpreted with caution since the sample size for this analysis was reduced.

Histological stains were used to visualise some of the common markers of structural pathology in the skeletal muscle of the ATH and SED subjects. The third finding of this study was that the ATH group had more internal nuclei and greater aggregations of subsarcolemmal mitochondria, and therefore a higher total score

compared to the SED group. Greater numbers of internal nuclei and aggregations of subsarcolemmal mitochondria were also noted in the muscle of the athletes with acquired training intolerance studied previously (75). The results from this study confirm the fact that the skeletal muscle of runners is distinct from that of sedentary individuals, presumably as a result of exposure to the stressor, endurance exercise. These observations are, however, common in the muscle of apparently healthy endurance athletes (116). Internal nuclei are considered to be evidence of cell regeneration (201), while a greater content of mitochondria within skeletal muscle is thought to be a positive aerobic adaptation to endurance training (90, 92). It seems unlikely that the muscle of the ATH group had been compromised by the presence of more internal nuclei and greater subsarcolemmal aggregations of mitochondria since their muscles showed no signs of dysfunction. Therefore, increased levels of these markers of structural pathology observed in the muscle of endurance athletes does not seem to be associated with underlying muscle "pathology".

A number of minor findings of this study are worth brief mention. First, minimum telomere length was not related to age when the data for the ATH and SED groups were pooled together (Figure 5.3). Telomeres shorten significantly during development while satellite cells constantly contribute to muscle growth. During adulthood, however, satellite cells are activated less often and as a consequence telomere length of adult skeletal muscle remains stable (45). Decary et al. have shown that with aging, there is a small decrease (approximately 13 base pairs per year) in the minimum telomere length of the vastus lateralis muscle sampled from individuals aged 9 months to 86 years old (44). In contrast, Renault et al. found no difference in either mean or minimum telomere length of the biceps brachii and masseter muscles of twelve individuals ranging in age from 20 to 83 years old (162). While the results from this study are in agreement with Renault et al. (162), the fact that a different muscle (vastus lateralis) was examined and that the subject population in this study only spanned from 26 to 63 years in age, limits the comparison between the studies. Alternatively, the relatively narrow age span of the subjects from this study was not large enough to observe any changes in telomere length with age.

The second minor finding was that the SED group had a greater proportion of the type 2A fibres compared to the ATH group. There was also a trend for the ATH group to have more type 1 fibres. Since it is widely accepted that endurance trained athletes have higher proportions of type 1 fibres and sedentary individuals tend to have more of the type 2 fibres (36, 189, 213) this finding is in line with the literature. The reasons for these trends, however, are still under debate. Much of the confusion is as a result of the large inter-individual variation in fibre type composition. Simoneau et al. report that 15 % of the variation observed in fibre type composition can be explained by the muscle sampling and technical variance, 40 % by environmental factors and the remaining 45 % by genetics (176). While traditional thinking is that fibre composition is genetically determined (111) and training effects on composition were negligible, training has since been shown to alter fibre type distribution (177) and the genetic component appears to be less significant than originally thought (163). Given that the primary difference between the ATH and SED groups was their exposure to endurance training it is tempting to suggest that the difference in fibre type proportions observed between the groups was due to training. Alternatively, the athletes may innately have had a greater proportion of type 1 fibres and were therefore more inclined to participate in endurance activities since their physiological make up supported it.

A third minor finding of this study was that the ATH group had a lower sum of seven skinfolds measurement compared to the SED group. This difference can be explained by the fact that the ATH group was well trained compared to the SED group and that endurance training, in particular, is known to alter body composition by decreasing body fat (77).

Finally, this study does have some limitations. The first is that the SED group contains smokers, and since smoking is known to affect telomere length in leukocytes (137), its potential effect on skeletal muscle telomeres cannot be ignored. Second, three of the subjects, two ATH and one SED, had depression, which may be a confounding factor since telomere lengths of individuals with mood disorders have been shown to be shorter compared to asymptomatic controls (175). Third, since some of the samples were unable to be analysed for fibre type

distribution and levels of structural pathology due to technical difficulties, the sample sizes for these factors were reduced.

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5.5 CONCLUSION

In summary, the three main findings of this study were:

- (i) The minimum, mean and maximum telomere lengths measured in the vastus lateralis muscle of the ATH group were similar to those of the SED group.
- (ii) The minimum telomere length of the ATH group was correlated to their years in distance running and hours spent training.
- (iii) The muscle of the ATH group had more internal nuclei and greater aggregations of subsarcolemmal mitochondria compared to the SED group.

In conclusion, the findings from this study suggest that while exposure to endurance running may increase the demand for repair and regeneration of muscle, the remaining regenerative capacity of the muscle of veteran runners is similar to that of sedentary individuals. This suggests that the capacity of muscle to repair and regenerate in response to exercise-induced injury is large. However, based on the relationship between minimum telomere length and exposure to endurance running, it is tempting to speculate that with sufficient exposure to endurance running the telomeres of veteran runners may ultimately reach a critical minimum length, thereby impairing the regenerative capacity of muscle. Finally, this study showed that the muscle of endurance trained individuals was distinct from that of a sedentary population in terms of markers of structural pathology such as internal nuclei and aggregations of subsarcolemmal mitochondria. Furthermore, since the levels of these markers measured in the muscle of the runners in this study were not associated with muscle dysfunction, they were not deemed to be pathological in nature. In answer to the question that this study addressed, there was no molecular evidence in the vastus lateralis muscle of the veteran runners to suggest that a high exposure to endurance exercise might adversely affect skeletal muscle. Therefore the muscle of apparently healthy individuals aged between 20 and 60 years appears

to be a robust organ with ample capacity to repair and regenerate in response to the stress of years of endurance running.

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CHAPTER 6

EXERCISE INTOLERANCE:**A case series of fatigued endurance runners****6.1 INTRODUCTION**

This thesis has explored the effects that a high exposure of running has on endurance runners, with particular emphasis on their skeletal muscle. Based on Selye's model of stress response (149) (discussed in Chapter 1, section 1.4.1), chronic exposure to a stressor ultimately results in the system or organism entering a phase of exhaustion where its resistance to the stressor begins to fall. The seemingly inevitable decline in performance experienced by veteran endurance runners, irrespective of age, demonstrated in Chapter 2 is likely a result of their entering this exhaustion phase. In an attempt to understand why veteran runners reach this point, it was shown in Chapter 3 that they may have altered neuromuscular characteristics when running at a sub-maximal effort compared to novice runners. It was also shown that years of endurance training does seem to mildly impact the remaining replicative capacity of the skeletal muscle of veteran runners (Chapter 5). While these two findings may in part help explain the seemingly inevitable decline faced by veteran runners, they are not conclusive. This suggests that, as indicated in Chapter 5, skeletal muscle is a robust organ that shows a remarkable tolerance to a high exposure to endurance running. Therefore, either some other property of skeletal muscle may be involved with the decline in performance observed in veteran runners, or another organ or system is associated with exercise intolerance. It is beyond the scope of this thesis, however, to explore these other organs or systems further.

Perhaps another approach to examining the effect of a large exposure of endurance exercise on runners is to investigate cases where runners have developed an

intolerance to exercise. Derman et al. (48) described a group of athletes presenting with an exercise-associated fatigue and intolerance to exercise that was accompanied by abnormalities within their skeletal muscle. Historically, this condition has been called Fatigued Athlete Myopathic Syndrome (FAMS) (48), and more recently Acquired Training Intolerance (ATI) (75). An explanation for the change in name was discussed in Chapter 1 (section 1.4.2). The athletes with ATI had in common a history of a high training volume and a current inability to tolerate former levels of endurance exercise. They experienced a sudden and unexplained decline in performance, often associated with a reduced training volume, both of which were more severe than the decrease that would be expected to accompany natural aging. Medical examination revealed that these athletes were free from physiological fatigue conditions, such as training-, diet-, travel- or pregnancy-induced fatigue and pathological fatigue conditions, such as haematological or metabolic conditions, classic chronic fatigue syndrome, overtraining syndrome or any neuromuscular disorders. In most cases, the athletes with ATI reported general skeletal muscle symptoms such as excessive delayed onset muscle soreness, muscle stiffness, tenderness and skeletal muscle cramps. Furthermore, these individuals had usually consulted many physicians, unsuccessfully, and had not responded to long periods of rest or nutritional support (48, 75).

Biopsies of the vastus lateralis muscle of athletes with ATI showed a greater degree of structural abnormalities, such as fibre size variation, internal nuclei, z-disc streaming and more lipid and glycogen droplets compared to healthy, age-matched control athletes (75). Endurance training and racing is known to induce such changes in the skeletal muscle of apparently healthy runners. These mild levels of structural abnormalities are thought to be functional adaptations (178), reflective of the continuous state of degeneration and regeneration occurring within the muscle in response to the stimulus of running (116). The greater degree of structural abnormality observed in the skeletal muscle of the athletes with ATI, however, could be considered to be pathological in nature.

In addition to the structural abnormalities, the athletes with ATI also had significantly shorter skeletal muscle telomeres compared to asymptomatic athletes. In fact, three of these athletes were shown to have pathologically shortened telomeres (33).

Collins et al. interpreted this as meaning that the muscle of the athletes with ATI had undergone more cycles of damage and repair compared to the age- and mileage-matched healthy Control group (33). In the previous chapter, it was hypothesised that a large exposure to endurance running and hence more cycles of damage and repair, would lead to shortened telomeres in the skeletal muscle of veteran endurance runners compared to sedentary individuals. However, this proved to be untrue. Although there was a relationship between telomere length and exposure to running in the veteran runners, the telomere lengths of the asymptomatic runners were not shorter compared to those of the sedentary individuals. These results suggested that the remaining replicative capacity of the skeletal muscle of the veteran runners did not appear to have been compromised by a high exposure to endurance running. Therefore some factor other than a large exposure to endurance exercise might have been responsible for the accelerated telomere shortening observed in the muscle of the athletes with ATI (44). For example, since it has been shown that moderately trained individuals sustain greater levels of oxidative stress as a result of distance running (51), shortened telomeres may reflect excessive oxidative damage to the DNA of the skeletal muscle. While the cause of the higher levels of abnormalities and shorter skeletal muscle telomeres of the athletes with ATI is unknown, it is tempting to speculate that they are related to their intolerance to endurance exercise.

Evidence from Chapter 5, as well as the documented findings relating to the athletes with ATI, suggests that while runners appear to tolerate a chronic exposure to endurance running with few ill effects, exceptions do exist where the onset of exercise intolerance may be associated with abnormalities within the runners' skeletal muscle. Five cases of endurance runners suffering from intolerance to endurance exercise as evidenced by their exercise-associated fatigue and various skeletal muscle symptoms are discussed in this chapter. The aim of presenting these case studies is to better understand the factors or circumstances leading to exercise intolerance in endurance runners. It is anticipated that the cases may provide clues to possible triggers to either the dysfunction of exercising skeletal muscle or the development of exercise intolerance. This may help the early recognition and prevention of such cases in the future.

6.2 METHODS

6.2.1 Patient recruitment

During the past ten years, a number of endurance athletes have consulted physicians at the Sports Medicine Clinic at the Sports Science Institute of South Africa with complaints of persistent fatigue accompanied by a reduced tolerance to exercise training and a decline in performance. From a clinical perspective, these patients had suffered from exercise-associated fatigue for more than one year and reported skeletal muscle symptoms including excessive delayed onset muscle soreness, stiffness, muscle tenderness and skeletal muscle cramps (48). A group of these patients were studied at this laboratory between four and eight years ago and became known as "athletes with acquired training intolerance" (ATI) (75, 182) due to their shared sudden intolerance to endurance exercise. Five such patients are presented in this chapter as case studies. To protect the identity of the patients they are referred to by a fictitious set of initials.

An Ironman triathlete (Case 1, AA) and an endurance runner (Case 2, AB) presented at the clinic with similar symptoms to the athletes with ATI and agreed to be examined systematically with their data contributing to case studies. A unique case of two identical twins also presented itself. One twin (Case 3, AC) was an endurance runner until he developed ATI which forced him to stop running; in contrast his twin brother (Case 3, SC) had led a relatively sedentary life. Both agreed to undergo testing to compare the effects of high volume endurance training and exercise-associated fatigue on genetically matched skeletal muscle. A runner (Case 4, AD) from the original group of patients with ATI studied at this laboratory (182) subsequently suffered an adverse event while running an ultra-marathon and revisited our laboratories in search of an explanation. A second runner (Case 5, AE), also from the original group of athletes with ATI (182), appeared to make a complete recovery from her exercise intolerance and agreed to be examined to better understand any changes that might have contributed to her recovery.

6.2.2 Clinical evaluation

Each athlete was examined by a sports physician using the clinical diagnostic approach to the athlete with chronic fatigue as described by Derman et al. (48). This diagnosis ruled out the likelihood that the patients suffered from any physiological or training-induced fatigue conditions, overtraining syndrome, chronic fatigue syndrome, fibromyalgia, any known neuromuscular disorder, cardiac or other chronic disease or any known depressive disorder for which they needed medication; and confirmed that they were not using any medication known to induce fatigue or alter muscle function.

6.2.3 Special investigations

6.2.3.1 All cases

Following their consultation with the sports physician, each patient was required to visit the laboratory on a single occasion. All patients were asked to refrain from exercising on the day of, as well as the day before their visit. They were also not to race or do training runs of longer than 20 km in the week preceding their visit. On arrival at the laboratory, the study was explained in detail to each athlete. Once the subjects agreed to participate, they signed a consent form (Appendix 5) and completed questionnaires detailing their personal particulars, medical history, social sport participation (Appendix 1), and competitive sport training and racing history (Appendix 2). They were also asked to keep a one week diary to ensure that they were not currently overtrained (Appendix 2). The body composition of each patient was measured before a physician took a muscle biopsy from their right vastus lateralis muscle. Since these patients were being documented as a case series, additional tests were deemed appropriate for two of the cases based on the unique features of their individual presentation.

6.2.3.2 Case 2

Three days after the standard clinical and experimental assessment described in sections 6.2.2 and 6.2.3, patient AB returned to the laboratory for an assessment of his physiological and neuromuscular characteristics while running (as per the methods described in Chapter 3, sections 3.2.2 and 3.2.3), as well as his response to exercise-induced muscle damage (as described in Chapter 4, sections 4.2.2 and 4.2.3). Briefly, the patient performed a peak treadmill running speed test three days after the standard experimental assessment. Four days later he returned to complete a 15 minute sub-maximal run at 70 % of his peak treadmill running speed, followed by a 5 km time trial. On his fourth visit, three days later, he performed a 40 minute downhill run on a treadmill declined to -10° , designed to induce muscle damage. Blood samples were drawn at rest before, immediately after, and at 24 hour intervals for one week following the downhill run to measure serum creatine kinase concentration. Subjective levels of pain were also assessed at the same time intervals. One week after the downhill run he repeated the 15 minute sub-maximal run and the time trial.

6.2.3.3 Case 4

Following the standard experimental assessment, patient AD returned to the laboratory the next day and performed a test to examine his clinical response to and recovery from exercise-induced muscle damage (Chapter 4, sections 4.2.2 and 4.2.3). Briefly, muscle damage of the lower limbs was induced by running downhill on a treadmill (-10°) for 40 minutes. The speed of the treadmill was set to the same speed as his previous visit to our laboratories ($11.5 \text{ km}\cdot\text{h}^{-1}$). Rectal temperature was measured every two minutes during the downhill run, as well as every minute during the six minute flat recovery run. Blood samples were drawn at rest before, immediately after, and at 24 hour intervals for one week following the downhill run on the treadmill to measure serum creatine kinase activity. Subjective levels of pain were also assessed at the same time intervals. Blood glucose measurements and a dipstick urine analysis for haemoglobin were performed before and immediately after the run.

The Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town approved this study (REC reference number: 181/2002).

6.2.4 Data collection procedures

The procedure used to assess body composition was identical to that described in Chapter 3 (section 3.2.3.1). A muscle biopsy was taken from the midbelly of the right vastus lateralis muscle as described in Chapter 5 (section 5.2.3.2). One portion of the muscle tissue was prepared, stored and used for fibre-typing and histological analysis as described in Chapter 5 (sections 5.2.4.1 and 5.2.4.2). A second portion of tissue was prepared, stored and used for the determination of telomere length as described in sections 5.2.4.3 and 5.2.4.4 of Chapter 5.

6.3 CASE REPORTS

6.3.1 Case 1

6.3.1.1 Case history

AA began training for distance running events at the age of 19 years and during the subsequent 17 years sustained a high training load (Table 6.1) while competing in both ultra-endurance running and triathlon events. At his peak he trained seven days per week covering 240 km at an average speed of $14.1 \text{ km}\cdot\text{h}^{-1}$. He amassed approximately 79 000 km during his running career. While training for the 2001 South African Ironman Triathlon (age 36 years) AA became unusually fatigued. He recalled feeling as though he had a virus at the time but believed that his symptoms of fatigue, muscle soreness, muscle cramps during exercise, shortness of breath and an elevated heart rate were related to his high training load. However, he was unable to recover as usual from the race and the exercise-associated fatigue that he experienced prevented him from managing even short distances in training (Table 6.1). Initial blood tests could not explain his prolonged exhaustion.

A few months later he completed a standard marathon, but in his slowest time to date (2h58min), 25 minutes slower than his personal best time. After eight months he still felt unable to recover from even a 10 km training run and described feeling as though he had a persistent cold. He consulted a homeopath who broadly diagnosed him as having a "viral illness and chronic fatigue". Results from further blood tests conducted showed that he had a slightly raised mean cell haemoglobin, elevated total serum cholesterol and low density lipoprotein (LDL) levels and that there was evidence of a previous Hepatitis A infection. An acupuncturist deemed him to be "overtrained" and treated him accordingly. He reduced his training intensity for three months and attempted a standard marathon but was unable to complete the race. Figure 6.1 shows the decrease in his race times for the Two Oceans 56 km Ultra-Marathon and the 90 km Comrades Marathon, two annual races that take place in Cape Town and KwaZulu Natal respectively in South Africa, following the onset of his intolerance to endurance exercise.

Table 6.1 Running training and racing characteristics of AA prior to (Pre) and for the duration of (During) his exercise intolerance, as well as his current characteristics.

	Period		
	Pre	During	Current ¹
Time (years)	17	1	-
Total training distance (km)	76 500	2 500	-
Total racing distance (km)	n.a.	n.a.	-
Combined training and racing distance (km)	n.a.	n.a.	-
Annual training volume (km·yr ⁻¹)	4 500	2 500	2 500
Training distance (km·wk ⁻¹)	90	50	50
Training frequency (days·wk ⁻¹)	7	6	6
Training speed (km·h ⁻¹)	14.1	n.a.	13.3
42.2 km time (h:min)	2h33min	n.a.	2h58min

¹ Current training data are those recorded during the eight weeks prior to this assessment and current race times are of his most recent races.

Abbreviations: n.a. – not available

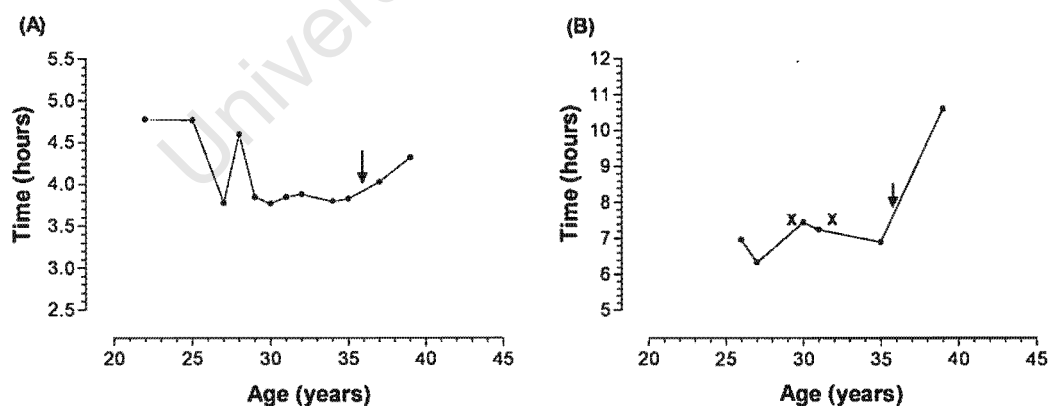


Figure 6.1 AA's annual performance changes for the Two Oceans 56 km Ultra-Marathon (A) and the 90 km Comrades Marathon (B) during the course of his athletic career. The arrows indicate the year of the onset of his exercise intolerance. The crosses indicate races that he began but did not complete.

One year after the onset of his intolerance to endurance exercise, AA consulted a sports physician at the Sports Medicine Clinic at the Sports Science Institute of South Africa and agreed to participate in this study. A medical history questionnaire revealed that he had no family history of chronic or cardiovascular diseases, and besides Perthes disease of the hips, he was free of any chronic illnesses or neuromuscular disorders and did not use any chronic medication. Although he had never been formally diagnosed with overtraining syndrome, he admitted that he did regularly overtrain. Likewise, he had never been diagnosed with depression, but recalled periods of feeling depressed in the past.

6.3.1.2 Clinical evaluation and present symptomatology

AA weighed 74.1 kg, was 1.79 m tall, had a BMI of 23.1 and was 37 years old at the time of assessment. He had normal blood pressure (120/70 mmHg) and a resting heart rate of 45 beats per minute. He reported a high level of perceived life stress (10/10, measured on a subjective 10 point scale). AA's primary symptoms were ongoing exercise-associated fatigue, excessive delayed onset muscle soreness (DOMS), stiffness, tenderness and exercise-associated muscle cramps which all worsened in hot, humid environments. He reported that his fatigue only affected his capacity to exercise and did not influence his social or work abilities. There was no indication that AA suffered from chronic fatigue syndrome at the time of examination and no other obvious abnormalities were detected.

6.3.1.3 Special investigations

A stress ECG and a lung function test found no abnormalities. Blood test results showed that he had mildly elevated levels of resting serum creatine kinase activity ($201 \text{ U}\cdot\text{L}^{-1}$, normal range: $15 - 195 \text{ U}\cdot\text{L}^{-1}$ at 37°C) and $[\text{CO}_2]$ ($30.2 \text{ mmol}\cdot\text{L}^{-1}$, normal range: $22.0 - 30.0 \text{ mmol}\cdot\text{L}^{-1}$). There was also evidence for a past activation of the Epstein Barr virus.

Skeletal muscle analysis

Unfortunately no data were available from the histological analysis of his vastus lateralis muscle. Analysis of his skeletal muscle telomeres revealed that his

minimum, mean and maximum terminal restriction fragment (TRF) lengths were 4.9, 8.2 and 12.1 kilobase pairs (kbp) respectively. These values were all shorter than those obtained from groups of apparently healthy athletes (min: 6.9 ± 0.9 kbp, mean: 10.0 ± 1.0 kbp, max: 15.1 ± 1.8 kbp) and sedentary individuals (min: 7.0 ± 0.8 kbp, mean: 10.0 ± 1.0 kbp, max: 14.1 ± 1.6 kbp). In fact the minimum TRF lengths of his muscle were noticeably shorter than those of both the athletic and sedentary control groups (Figure 6.2).

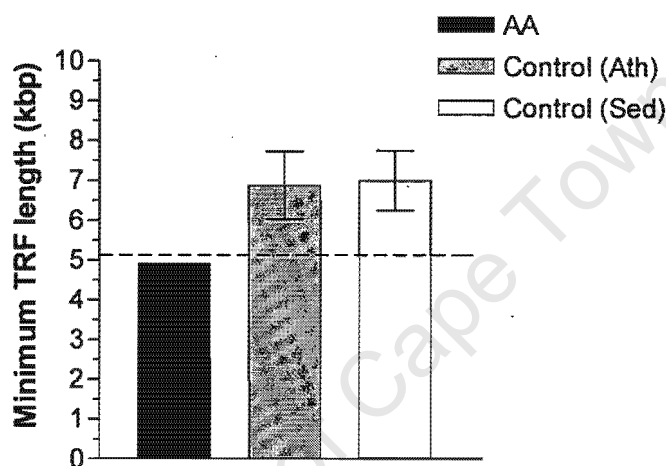


Figure 6.2 Minimum TRF lengths of AA and the Control groups (Athletic and Sedentary populations). The horizontal line represents two standard deviations of the athletic control group mean.

6.3.1.4 Discussion

One year after the onset of AA's exercise intolerance a biopsy of his vastus lateralis muscle revealed that his minimum telomere lengths were more than two standard deviations shorter than both asymptomatic athletes and sedentary controls. Since telomere length is considered to be a marker of the replicative history of muscle (44), the muscle of AA may have undergone more cycles of degeneration and regeneration compared to the control groups. Alternatively, the DNA in his lower limb skeletal muscle may have been subjected to greater levels of oxidative damage (160). Irrespective of the mechanism, this finding suggests that the remaining replicative capacity of the satellite cells in AA's skeletal muscle was lower than that

of healthy control athletes and sedentary individuals. Therefore it is plausible that AA's shortened telomeres were associated with his high exposure to endurance exercise, and in turn his current intolerance to exercise.

By all accounts AA presented as a typical athlete with ATI, where a large exposure to the stress of endurance running appears to have compromised his skeletal muscle. However, his case history suggests that two other co-stressors (viral infection and behaviour) may also have contributed to his exercise intolerance. AA described a definite onset of his intolerance to endurance exercise while training for the 2001 Ironman triathlon. He became progressively more exhausted and recalled feeling as though he had a persistent viral infection. However, he ignored these symptoms, did not rest during this period and completed the triathlon. Acute infections lead to an acute phase response triggered by cytokines, and correlated to fever, malaise and anorexia (66). Friman et al. suggested that the purpose of this systemic response is to mobilize nutrients for the increased needs of the activated immune system, as well as for energy production and tissue repair, and that effects included wasting of striated muscle, degradation of performance-related metabolic enzymes and sub-optimal central circulatory function (66). Thus a virus can potentially impair both the aerobic and anaerobic performance capacity of athletes, the effects of which may be present for as long as one year following infection (66, 164). Jakeman described an elite athlete that contracted a viral illness, subsequently experienced a decline in performance, and whose altered steady state metabolism during exercise and chronic tachycardia took in excess of 50 weeks to resolve (93). Evidence from this case indicated that both the skeletal and cardiac muscles of the athlete were affected by the virus.

Since AA did not consult a doctor or have blood tests conducted at the time, it is not possible to definitively state that he suffered from a viral infection. However, subsequent blood tests showed that he had experienced past infections of both the Epstein-Barr and Hepatitis A viruses. Given the symptoms and circumstances surrounding the onset of AA's intolerance to endurance exercise, it is not unreasonable to believe that a virus acted as a co-stressor in triggering his intolerance to exercise. Since the skeletal muscle of athletes is particularly vulnerable for an extended period of time following viral infection (66, 164), it is

tempting to speculate that AA's continued efforts at high volume and high intensity racing and training did not allow him sufficient rest to recover fully from the possible infection.

The second co-stressor that needs to be considered in this case is that of behaviour. It is not unusual for athletes to have a personality that is more rigid, goal-oriented and perfectionist in nature, but such individuals could be more likely to drive themselves into a state of overtraining or injury (155). AA illustrates how the behaviour of a goal-oriented athlete might contribute to the occurrence of exercise intolerance. As he was determined to accommodate the necessary training to compete in the Ironman triathlon, he ignored his symptoms, continued with his demanding training schedule and completed the race. Furthermore, despite still being intolerant to high volume training, AA persists with preparing for and competing in endurance races. Placing an abnormal amount of importance on exercise and being unwilling to stop exercising, even when it is contra-indicated such as in times of injury or illness is suggestive of a pathological attitude towards exercise (43). It seems likely that the combined stressors of AA's chronic exposure to endurance exercise, a viral infection and his attitude towards his sports brought about his intolerance to exercise.

6.3.2 Case 2

6.3.2.1 Case history

AB took up distance running when he was 18 years old and ran for approximately 16 years before experiencing any problems. While he covered a modest 42 000 km in training during his asymptomatic years, he completed in excess of 100 marathons. At his peak he trained seven days per week, covering an average of 70 km each week at a speed of approximately 15 km·h⁻¹. Throughout his running career he accumulated 48 934 km (Table 6.2).

Table 6.2 Running training and racing characteristics of AB prior to (Pre) and for the duration of (During) his exercise intolerance, as well as his current characteristics.

	Period		
	Pre	During	Current ¹
Time (years)	16	1	-
Total training distance (km)	42 120	1 560	-
Total racing distance (km)	4 494	250	-
Combined training and racing distance (km)	46 614	1 810	-
Annual volume (km·yr ⁻¹)	2 913	1 810	1 560
Training distance (km·wk ⁻¹)	50.6	30.0	30.0
Training frequency (days·wk ⁻¹)	6.3	5.0	5.0
Training speed (km·h ⁻¹)	13.0	10.0	10.0
42.2 km time (h:min)	2h59min	5h20min	-
21.1 km time (h:min)	1h15min	1h35min	-

¹ Current training data are those recorded during the eight weeks prior to this assessment and current race times are of his most recent races.

AB believed that his intolerance to endurance exercise began on completion of a standard marathon during his sixteenth year of endurance racing at which time he was 34 years old. During the race he suffered from stomach cramps at around the 28 km mark, and described feeling a complete lack of energy and painful muscles after 36 km. He completed the marathon in a time of 5h20min, 2h20min slower than his personal best time. Four months later he attempted his seventh 90 km Comrades Marathon, but was unable to finish the race. Since then he has suffered from exercise-associated fatigue, sore leg muscles and cramping while running. He has difficulty completing longer races and experiences painful quadriceps and hamstring muscles after about 15 km of running. He was unable to recall any likely triggers for the onset of these symptoms and despite undergoing a range of tests and examinations, no explanation has been found. Figure 6.3 shows the change in AB's race times for both the Two Oceans 56 km Ultra-Marathon and the 90 km Comrades Marathon. While his performance in these races had been on a continual decline since his first race, of significance was that he had been unable to complete a number of attempts at each race since the onset of his intolerance to endurance exercise. Either his reduced training level had not allowed him to sufficiently prepare for ultra-endurance races, or the race distances themselves were too demanding given his symptoms.

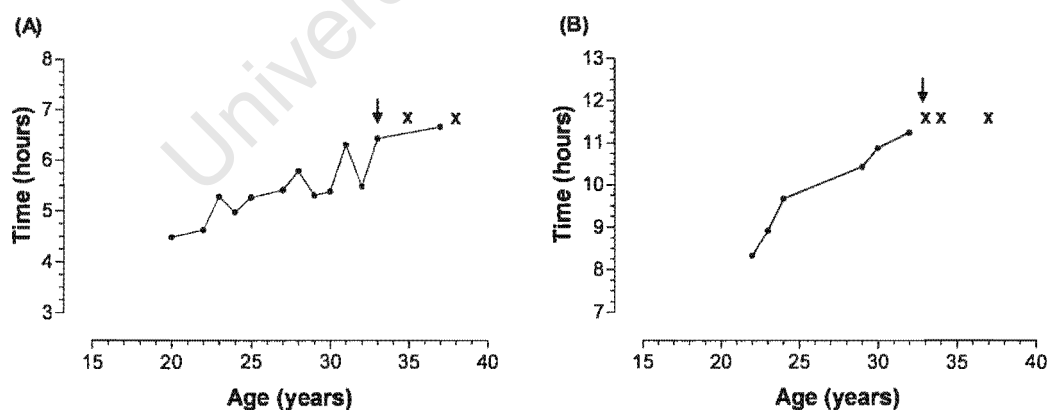


Figure 6.3 AB's annual performance changes for the Two Oceans 56 km Ultra-Marathon (A) and the 90 km Comrades Marathon (B) during the course of his athletic career. The arrows indicate the year of the onset of his exercise intolerance. The crosses indicate races that he began but did not complete.

AB's exercise intolerance persisted for a year and continued to limit both his training (Table 6.2) and racing (Figure 6.3) capacities, prompting him to consult a physician at the Sports Medicine Clinic. He agreed to undergo testing in an attempt to establish the cause of his symptoms. His medical history questionnaire revealed that he had never been diagnosed with chronic fatigue syndrome, glandular fever, neuromuscular disorders, depression or overtraining. He had a family history of stroke, but no other chronic illnesses or diseases. He reported using medication (type not reported) to treat lower back pain which had been present for two months, and occasionally suffered from exercise-related gastro-intestinal complaints. While he reported periods of feeling depressed, he had never been diagnosed with depression.

6.3.2.2 Clinical evaluation and present symptomatology

AB weighed 97.6 kg, was 1.91 m tall, had a BMI of 26.8 and was 35 years old at the time of assessment. His perceived level of life stress was low (2/10). His primary symptoms included exercise-associated fatigue, and painful, cramping skeletal muscles. As for AA, AB's fatigue only affected his capacity to exercise. There was no indication that he suffered from chronic fatigue syndrome at the time of examination. No further abnormalities were detected at the time of assessment.

6.3.2.3 Special investigations

Skeletal muscle analysis

A biopsy sample of the vastus lateralis muscle of AB was taken to assess levels of structural abnormalities and telomere length. Muscle fibre type analysis showed him to have approximately 57 % type 1 and 43 % type 2A fibres, similar to the expected ratio for the quadriceps muscle in healthy individuals (~ 55 % type 1) (171). There was no evidence of internal nuclei, fibre size variation, necrosis, degeneration, inflammation or degeneration within his muscle. There was, however, a mild degree of subsarcolemmal mitochondria aggregation, giving him a total pathology score of 1.0 unit. Healthy, symptom free athletes had a total pathology score of 2.5 ± 1.6 units, while sedentary controls had a score of 0.7 ± 0.9 units. Therefore, histological examination of his skeletal muscle provided no information to help explain his symptoms of fatigue and muscle pain.

His minimum, mean and maximum TRF lengths were measured as 7.1, 9.7 and 13.8 kbp respectively. These were similar to those of both the apparently healthy Athletic (min: 6.9 ± 0.9 kbp, mean: 10.0 ± 1.0 kbp, max: 15.1 ± 1.8 kbp) and Sedentary (min: 7.0 ± 0.8 kbp, mean: 10.0 ± 1.0 kbp, max: 14.1 ± 1.6 kbp) control groups. In particular, his minimum TRF length was not different to that of the Athletic and Sedentary control groups (Figure 6.4). In this regard, AB was unlike the "typical" athletes with ATI. While he displayed similar signs and symptoms, his skeletal muscles showed no evidence of structural abnormalities or shorter telomere lengths compared to either asymptomatic athletes or sedentary individuals. Therefore AB underwent further testing in an attempt to elucidate the cause of his exercise intolerance.

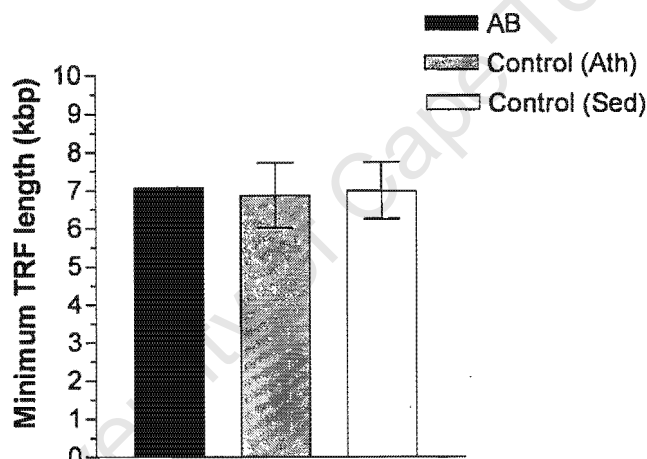


Figure 6.4 Minimum TRF lengths of AB and the Control groups (Athletic and Sedentary populations).

Physiological and neuromuscular analysis

As mentioned in section 6.2.4.2, AB underwent the same tests described in Chapters 3 and 4 to assess his physiological and neuromuscular characteristics while running, as well as his response to and recovery from exercise-induced muscle damage. He was compared to the 13 veteran and novice runners studied in Chapters 3 and 4 who acted as a Control group. AB was of the same age and had similar sum of seven skinfolds, percentage body fat, peak treadmill running speed and peak heart rate measurements compared to the Control runners. He was, however, taller, heavier and had a higher BMI (Table 6.3).

Table 6.3 Descriptive characteristics of AB and the Control group.

	AB	Control (n = 13)	
		Mean \pm SD	Range
Age (years)	34	34.5 \pm 9.3	21.0 – 47.0
Height (m)	1.91 ***	1.72 \pm 0.07	1.58 – 1.82
Weight (kg)	97.6 ***	68.9 \pm 9.0	50.5 – 85.3
BMI (kg·m⁻²)	26.8 *	23.2 \pm 1.7	19.7 – 26.9
S7 (mm)	82.6	75.3 \pm 17.6	48.6 – 113.6
Body fat (%)	19.0	18.1 \pm 4.1	13.0 – 23.4
PTRS (m·s⁻¹)	16.5	18.2 \pm 1.8	15.5 – 20.5
Peak HR (bpm)	183.0	189.2 \pm 11.3	171.0 – 212.0

Data of the Control group are presented as the mean \pm the standard deviation.

* Indicates a value outside of one standard deviation of the Control group mean.

** Indicates a value outside of the Control group range.

Abbreviations: SD – Standard deviation, BMI – Body mass index, S7 – Sum of 7 skinfolds, PTRS – Peak treadmill running speed, VO₂ – Volume of oxygen inspired, HR – Heart rate, bpm – Beats per minute.

Since the Control group included veteran and novice runners, their exposure to endurance running was varied. Although AB began running at a younger age, had run for more years, covered a greater distance, and trained more frequently and at a higher volume compared to the average Control runner, his exposure was still within the range of the Control group (Table 6.4). Therefore a comparison between AB and the Control group's running characteristics was deemed appropriate.

Table 6.4 Training and racing characteristics of AB and the Control group.

	AB	Control (n = 13)	
		Mean \pm SD	Range
Age began running (yrs)	18	24.9 \pm 8.3	16 – 41
Time running (yrs)	17	9.9 \pm 8.5	1 – 28
Total distance (km)	49 984	27 580 \pm 46 143	1 121 – 161 211
Frequency (days \cdot wk $^{-1}$)	6.3	4.2 \pm 1.2	2.0 – 6.7
Distance (km \cdot wk $^{-1}$)	61.8	50.7 \pm 23.9	25.0 – 109.8
Speed (km \cdot h $^{-1}$)	9.8	10.8 \pm 1.5	9.0 – 13.4
PB 21.1 km time (h:min)	1h15m *	1h29min \pm 0h08min	1h08min – 1h45min
PB 42.2 km time (h:min)	2h59min	3h28min \pm 0h40min	2h22min – 4h57min

Data of the Control group are presented as the mean \pm the standard deviation.

** Indicates a value outside of one standard deviation of the Control group mean.*

Age began running and Time running: n = 12.

Abbreviations: SD – Standard deviation, PB – Personal best.

AB completed a 15 minute sub-maximal run to assess his physiological and neuromuscular characteristics while running at a sub-maximal pace. The data collected for AB during steady state (minutes 12 – 15) of the sub-maximal run were compared to those of the Control runners (Table 6.5). AB's speed, HR, RPE, VO_2 and VE during steady state were not different to those of the Control group. Of interest, however, was the unusual relationship between his steady state speed and VE. In general, VE is linearly related to running speed until a work rate corresponding to approximately 50 – 70 % of maximal oxygen uptake, where after ventilation rate begins to rise exponentially (153). Since running economy is the relative oxygen consumption (35) or ventilation rate (153) for a standardized running speed, AB's high VE relative to the Control group, despite his slower absolute running speed suggested that he had poor running economy.

Table 6.5 Physiological characteristics of AB and the Control group measured at steady state (minutes 12 – 15) during the sub-maximal run.

	AB	Control (n= 13)	
		Mean \pm SD	Range
Speed ($\text{km}\cdot\text{h}^{-1}$)	11.6	12.7 \pm 1.3	10.9 – 14.4
HR (bpm)	168.0	168.0 \pm 18.2	149.0 – 206.0
RPE	14	14 \pm 2	11 – 18
VO_2 ($\text{ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$)	41.7	42.5 \pm 5.9	34.0 – 50.0
VE ($\text{L}\cdot\text{min}^{-1}$)	126.0	101.6 \pm 24.8	75.5 – 142.2

Data of the Control group are presented as the mean \pm the standard deviation.

Note: HR for Controls: n = 11, VO_2 and VE for Controls: n = 9.

Abbreviations: SD – Standard deviation, HR – Heart rate, bpm – Beats per minute, RPE – Rating of perceived exertion, VO_2 – Volume of oxygen inspired and VE – Ventilation rate.

AB had a similar flight time and stride length compared to the Control group during steady state of the sub-maximal run, but a longer contact time and a lower stride frequency (Table 6.6). Stride frequency is related to running speed (speed = stride frequency x stride length) and is also equal to $0.5 \times (\text{contact time} + \text{flight time})$. Since this was a set-paced run (70 % of peak treadmill running speed), AB appeared to use a lower stride frequency to maintain the required relative speed. This would have been achieved by increasing stride length, or increasing contact and flight time. Since his stride length was similar to that of the Control group, his longer ground contact time was likely responsible for his slower stride frequency.

Table 6.6 Stride characteristics of AB and the Control group measured at steady state (minutes 12 – 15) during the sub-maximal run.

	AB	Controls (n = 13)	
		Mean \pm SD	Range
CT (ms)	289.6 *	225.6 \pm 34.0	174.6 – 302.2
FT (ms)	106.2	115.4 \pm 35.2	57.4 – 186.0
SL (m)	2.64	2.43 \pm 0.33	2.03 – 3.01
SF (strides \cdot s ⁻¹)	1.26 * **	1.47 \pm 0.08	1.27 – 1.57

Data of the Control group are presented as the mean \pm the standard deviation.

* Indicates a value outside of one standard deviation of the Control group mean.

** Indicates a value outside of the Control group range.

Abbreviations: SD – Standard deviation, CT – Contact time, FT – Flight time, SL – Stride length, SF – Stride frequency.

EMG activity was measured in three muscles (vastus lateralis (VL), vastus medialis (VM) and medial gastrocnemius (MG)) during two different phases of the running stance. The pre-activation phase is the 100 ms prior to the foot making contact with the ground and thought to be the phase during which the muscles are preparing for the impact of ground contact and the subsequent propulsion phase. The ground contact phase occurs while the foot is in contact with the ground. Integrated EMG (iEMG) during steady state was expressed relative to the iEMG value obtained during the first three minutes of the run. AB's pre-activation phase iEMG in both the VL and VM muscles were lower than those of the Control group, suggesting less preparation of these muscles prior to the ground contact. During the ground contact phase, the VM muscle of AB showed higher levels of iEMG compared to the Control group (Table 6.7).

Table 6.7 EMG characteristics of AB and the Control group measured at steady state (minutes 12 – 15) during the sub-maximal run.

Phase	Muscle	AB	Controls (n = 13)	
			Mean \pm SD	Range
Pre-activation	VL	84.6	95.9 \pm 12.2	82.6 – 124.3
	VM	78.1 *	95.2 \pm 13.2	76.6 – 120.3
	MG	87.3	72.1 \pm 21.4	41.9 – 102.8
Ground contact	VL	105.8	100.7 \pm 8.9	86.6 – 120.7
	VM	131.1 * **	102.6 \pm 9.3	87.4 – 123.8
	MG	87.6	92.4 \pm 11.7	74.7 – 116.7

Data of the Control group are presented as the mean \pm the standard deviation.

* Indicates a value outside of one standard deviation of the Control group mean.

** Indicates a value outside of the Control group range.

VL ground contact for the Control group: n = 11.

Abbreviations: EMG – Electromyography, SD – Standard deviation, VL – Vastus medialis, VM – Vastus lateralis, MG – Medial gastrocnemius.

Fifteen minutes after the sub-maximal run AB completed a 5 km time trial during which physiological, stride and neuromuscular characteristics were measured. His time for the run was 25min54s, slower than all but one member of the Control group (mean: 22min10s \pm 2min49s), and his average speed was 11.6 km·h⁻¹ (Control group: 13.7 \pm 1.5 km·h⁻¹). Given that his personal best times for both the 21.1 and 42.2 km distances were faster than those of the Control group, his slower 5 km time at present confirmed the effect his exercise intolerance has had on his race ability.

Figure 6.5 shows the kilometer split times and heart rate of AB and the Control runners as measured at the end of each kilometer of the time trial. AB's pacing strategy during the run was similar to that of the Control group; they all slowed down progressively from the first to fourth kilometer. At the end of the time trial, however, the Control group was able to increase speed while AB ran his slowest time in kilometer five. His heart rate during the time trial was consistently lower, but followed a similar pattern of change to that of the Control group.

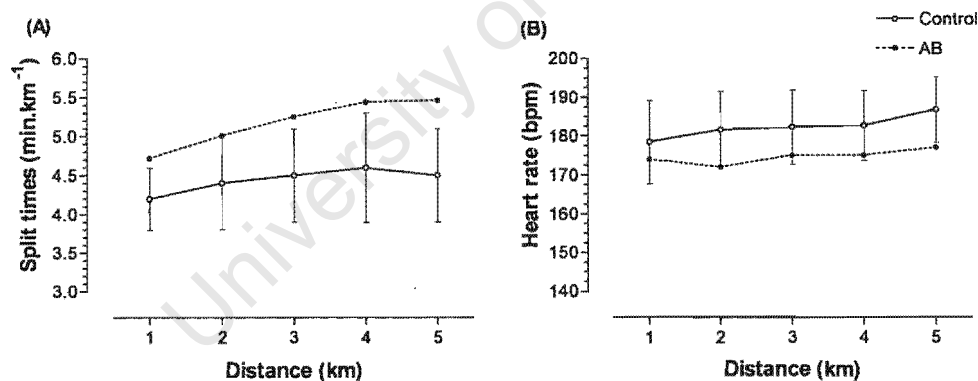


Figure 6.5 Comparison of the kilometer split times (A) and heart rate (B) of AB and the Control group during a 5 km time trial. The mean and standard deviation of the Control group are shown by the solid line with open circles, while AB's data is represented by the dotted line with closed circles.

Contact time, flight time and stride frequency of AB during the time trial were compared to those of the Control runners (Figure 6.6). The longer contact time, shorter flight time and lower stride frequency of AB were consistent with his slower running speed.

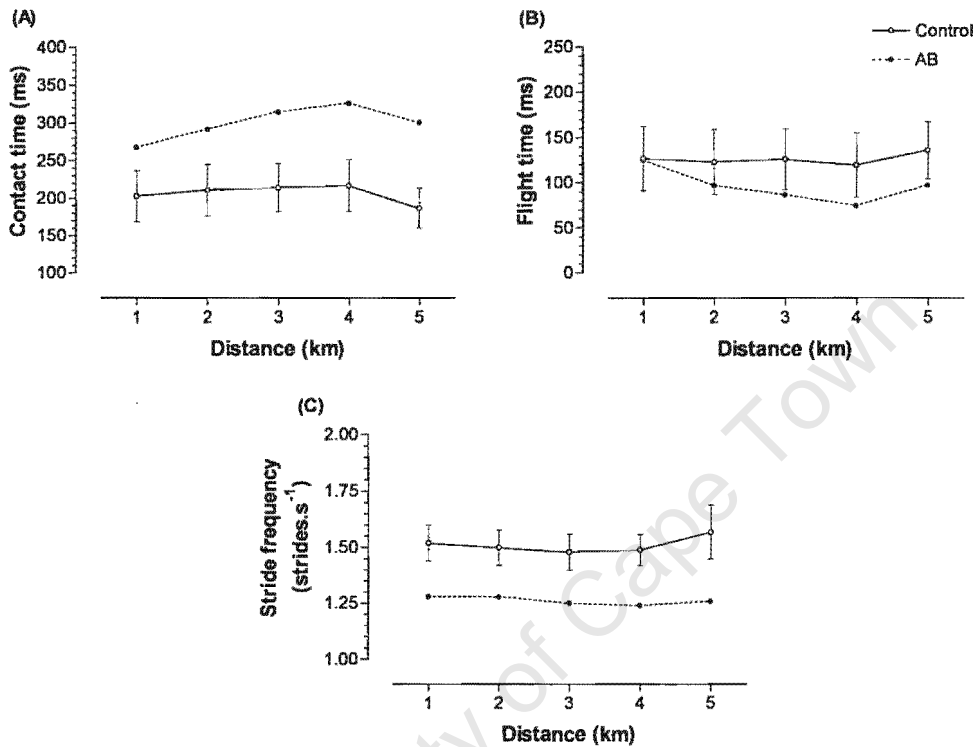


Figure 6.6 Comparison of the stride characteristics of AB and the Control group during the 5 km time trial. (A) Contact time. (B) Flight time. (C) Stride frequency. The mean and standard deviation of the Control group are shown by the solid line with open circles, while AB's data is represented by the dotted line with closed circles.

EMG activity during the time trial was measured at the end of each kilometer and expressed relative to the value recorded after three minutes of the sub-maximal run. Pre-activation phase iEMG of the VL, VM and MG muscles of AB were lower than those of the Control group. While the iEMG levels of VL and MG muscles were similar to those of the Control group during the ground contact phase, VM iEMG was noticeably higher at the end of the time trial (Figure 6.7).

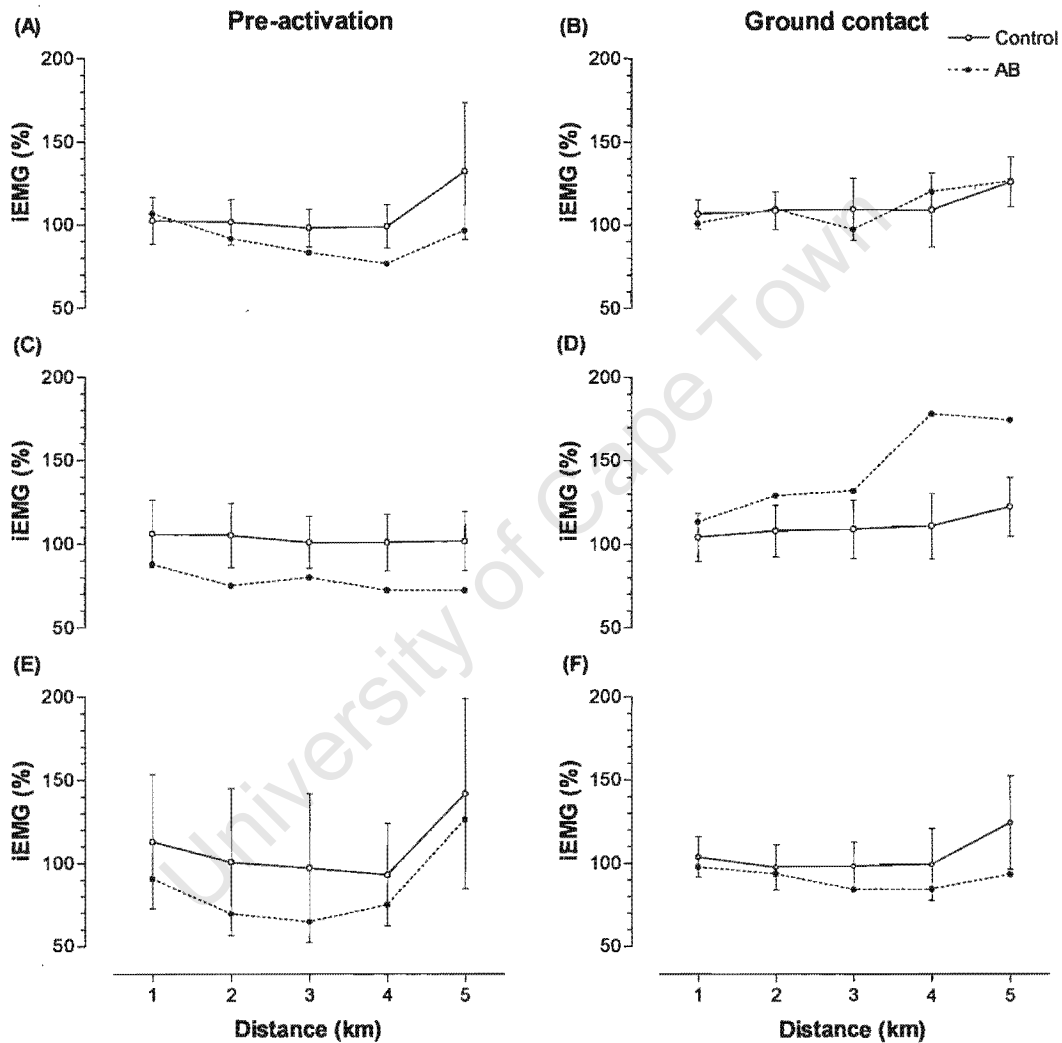


Figure 6.7 Comparison of the EMG characteristics of AB and the Control group during the 5 km time trial. (A) VL pre-activation and (B) ground contact phase iEMG. (C) VM pre-activation and (D) ground contact phase iEMG. (E) MG pre-activation and (F) ground contact phase iEMG. The mean and standard deviation of the Control group are shown by the solid line with open circles, while AB's data is represented by the dotted line with closed circles.

Three days later AB completed a 40 minute run at a speed of 70 % of his peak treadmill running speed on a treadmill declined to -10° to induce muscle damage. Figure 6.8 shows his response to the downhill run compared to that of the Control group. His peak level and change in creatine kinase activity during the week following the run was not different to that of the Control group. This indicated that the extent of muscle membrane disruption he incurred and the time required to repair this damage was not different to healthy Control runners. His subjective pain response, however, was unusual. He reported no pain during the first 48 hours, when signs of damage and corresponding muscle pain usually peak. Instead he reported two peaks in pain 72 and 120 hours after the run. Either he experienced a delayed sensation of muscle pain in response to the exercise-induced muscle damage, or he misunderstood or misinterpreted his level of pain.

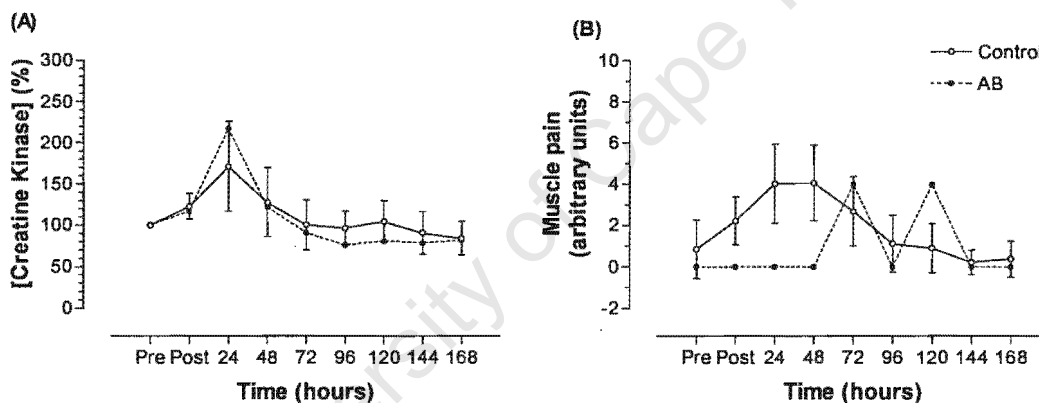


Figure 6.8 Creatine kinase activity (A) and muscle pain (B) response of AB and the Control group to exercise-induced muscle damage. *The mean and standard deviation of the Control group are shown by the solid line with open circles, while AB's data is represented by the dotted line with closed circles. Creatine kinase activity was normalised to resting levels (Pre).*

6.3.2.4 Discussion

AB did not present as a typical athlete with ATI. Despite his similar symptoms of exercise associated fatigue and impaired performance, his exposure to running ($2\,913\text{ km}\cdot\text{yr}^{-1}$) was modest, there was no evidence of structural abnormalities in his skeletal muscle biopsy, nor were his telomere lengths different to those of healthy controls. AB did however, display neuromuscular anomalies while running. Firstly, while running at steady state during a set-paced sub-maximal effort, AB's vastus lateralis and vastus medialis muscles showed lower levels of pre-activation phase EMG activity compared to the Control group. EMG activity during the pre-activation phase of the stride is thought to be important as it increases the muscle's stiffness in preparation for ground contact and the subsequent propulsion phase (144). Altered muscle stiffness may change the potential for the muscle to absorb impact forces, thereby changing the shock absorption function of the muscles (139). Therefore the quadriceps muscles of AB may have had impaired shock absorption properties.

Secondly, AB's vastus medialis muscle showed higher levels of EMG activity during the ground contact phase of his stride while running at a sub-maximal pace. A similar trend was observed during the 5 km time trial. This over-activity was most apparent at the end of the time trial, presumably when AB was most fatigued. Higher levels of EMG activity in the vastus lateralis and vastus medialis muscles during the ground contact phase may indicate a neuromuscular inefficiency since their EMG activity during this phase remains constant between speeds of 7.2 and $16.2\text{ km}\cdot\text{h}^{-1}$ (68). Furthermore, the longer ground contact and flight times of AB's stride during both the sub-maximal run and the time trial compared to the control runners indicate a reduced capacity to generate force rapidly, also suggestive of a reduced neuromuscular efficiency (139).

Finally, one of the symptoms reported by AB was that of exercise-associated muscle cramps, specifically in his quadriceps, hamstring and abdominal muscles. While the EMG activities of his hamstring and abdominal muscles were not measured, it is tempting to speculate that the over-activity observed in his vastus medialis muscle might be related to the cramps he experienced during longer duration exercise. Given his symptoms of exercise-associated fatigue and skeletal muscle cramping

after long distances, as well as his neuromuscular abnormalities while running, the possibility of an underlying myopathy needs to be further investigated. There is a group of muscle disorders in which patients present with impaired activation of muscle contraction or relaxation. Impaired activation or relaxation of the contractile apparatus may be due to an intrinsic muscle dysfunction such as of the sarcolemma, sarcoplasmic reticulum, or excitation-contraction coupling. Alternatively it may be due to a primary abnormality in the central nervous system, motor nerves or neuromuscular junction. However these disorders are usually associated with atrophy secondary to disuse or denervation (123). While AB may not have such a severe disorder, it would be prudent to further examine his neuromuscular system for an underlying myopathy.

University of Cape Town

6.3.3. Case 3**6.3.3.1. Case history**

Case 3 describes two identical twins that are now 63 years old. The twins participated in cricket, athletics and boxing while at school, but both stopped formal exercise thereafter. The sedentary twin (SC) led a sedentary lifestyle after leaving school, but began walking once a week three years ago. In contrast, the athletic twin (AC) took up distance running at the age of 34 years. During the subsequent 11 years he completed nearly 60 marathons, and trained seven days per week, covering about 80 km each week at approximately 12 km·h⁻¹. At his peak, AC ran 120 km per week in training and completed his fastest marathon in a time of 2h56min at the age of 40 years. During his 11 years of symptom-free running he accumulated more than 59 000 km (Table 6.8) and completed over 500 races.

Table 6.8 Running training and racing characteristics of AC prior to (Pre) and for the duration of (During) his exercise intolerance.

	Period	
	Pre	During
Time (years)	11	5
Total training distance (km)	51 220	3 900
Total racing distance (km)	8 522	-
Combined training and racing distance (km)	59 742	-
Annual volume (km·yr ⁻¹)	5 431	780
Training distance (km·wk ⁻¹)	78.6	20.8
Training frequency (days·wk ⁻¹)	6.6	3.3
Training speed (km·h ⁻¹)	11.8	10.2
42.2 km time (h:min)	2h56min	-
21.1 km time (h:min)	1h23min	-

During his twelfth year of distance running AC (age 46) became ill with a viral infection the week before he was to run his tenth Winelands Marathon, an annual 42.2 km race in South Africa. As it was to be an important run – he would become one of only ten runners to compete in all ten of these events – he decided to participate in the race despite having been ill. He felt unusually lethargic during the race and recalls having a mildly sore chest. He was determined, however, to finish this hilly course in a silver medal time and so ignored his symptoms and ran the race at a high intensity. He completed the race in 3h13min, but reported being pale and exhausted at the end. Two days later he resumed training but his legs were heavy and fatigued, forcing him to rest for a few more days. He recalled having a sensation of whole body fatigue, but in particular his legs felt lethargic. He attempted a 15 km run a few weeks after the marathon, but his legs were so tired that he had to walk the second half of the course. During the next five years he went through repeated cycles of rest followed by attempts to run. For the most part he could not complete his training runs and resorted to walking. He described his recovery from each training run as feeling similar to recovering from a marathon. The symptoms of fatigue and lethargy persisted for almost six years and although they did not affect his occupational or social activities they had a severe effect on his exercise capacity. During this period he barely completed 20 km per week in training (Table 6.8) since he needed so much time to recover from each session, and his race times got progressively slower (Figure 6.9).

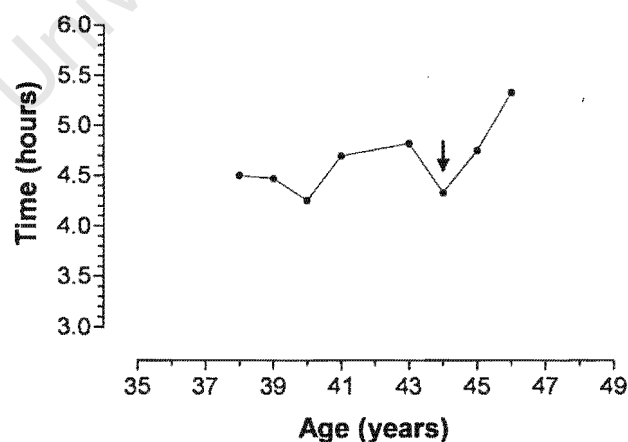


Figure 6.9 AC's annual change in performance for the Two Oceans 56 km Ultra-Marathon during the course of his athletic career. *The arrow indicates the year of the onset of his exercise intolerance.*

Since he was unable to train at his previous volume and mindful of future repercussions, he decided to stop running all together. During the past thirteen years, AC has continued to exercise six days per week including three weight training and two cardiovascular sessions in a gym, and one outdoor walk. At present, he purposefully trains at low intensity to avoid any relapse. Despite his continued fitness training, a recent assessment of his lower limb strength showed that he was below average for his age. A one repetition maximum leg press test predicted his maximum to be 85 kg, lower than his age and gender predicted maximum of 106 kg.

The medical histories of the twins revealed that neither had been diagnosed with chronic fatigue syndrome, Epstein-Barr viral infection, any neuromuscular disorders, overtraining or depression and neither suffered from any chronic or cardiac diseases. AC had, however, contracted the hepatitis virus before he began distance running in his early thirties, and SC had suffered from bilharzia in his teens. The only chronic illness within their family was cancer, but the type was not specified. Neither twin used any chronic medication at the time of the assessment, and had not done so in the past. AC smoked cigarettes for approximately 13 years, but stopped when he began running, while SC had smoked cigarettes for the past 40 years.

6.3.3.2 Clinical evaluation and present symptomatology

AC weighed 69.0 kg, was 1.79 m tall and had a BMI of 21.5. He no longer experienced exercise-associated fatigue or any of the skeletal muscle symptoms that were present 13 years ago during his phase of exercise intolerance. SC (85.4 kg, 1.84 m, BMI of 25.2) did not currently and had never experienced excessive, unexplained fatigue or skeletal muscle symptoms. The twins were 63 years old at the time of assessment.

6.3.3.3 Special investigations

Skeletal muscle analysis

Analysis of the vastus lateralis muscle biopsies taken from the twins showed that the fibre type proportion of AC was 83 % type 1 and 17 % type 2A, while SC had

approximately 65 % type 1, 34 % type 2A and 1 % type 2X/D. AC had a degree of fibre size variation and significant aggregations of subsarcolemmal mitochondria, giving him a total pathology score of 3.0 units. In comparison, SC had only minor aggregations of subsarcolemmal mitochondria, and a total pathology score of 1.0 unit. The total pathology score of AC was similar to that of apparently healthy control runners (2.5 ± 1.6 units) and SC's total pathology score was also similar to that of sedentary controls (0.7 ± 0.9 units). The minimum, mean and maximum TRF lengths of AC were 6.6, 10.0 and 15.2 kbp respectively, while the minimum and mean TRF lengths of the SC were 7.9 and 10.9 kbp respectively. Figure 6.10 shows the minimum TRF lengths of the twins in comparison to apparently healthy control runners and sedentary individuals. While neither was significantly different to those of the controls, a difference between the twins was noticeable.

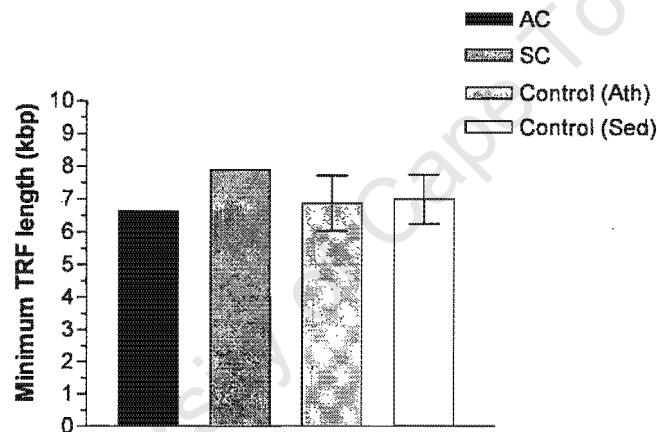


Figure 6.10 Minimum TRF lengths of AC, SC and the Control groups (Athletic and Sedentary populations).

6.3.3.4 Discussion

Like AA, AC's case history indicated that he had a typical profile of an athlete with ATI, despite the lack of muscle biopsy evidence to assess structural abnormalities or telomere length at the peak of his running career. He too identified the onset of his exercise intolerance and associated symptoms as training for and racing a marathon while recovering from a bout of flu. Therefore, as for AA, co-stressors such as viral infection and behaviour may have exacerbated the stress of a large exposure to

endurance running, precipitating his exercise intolerance. Since AC had not participated in endurance running for the past 13 years and no longer experienced the symptoms of exercise-associated fatigue, muscle pain and “heavy legs”, this case also provided evidence that the negative effects of a stressor can be reversed and the associated symptoms relieved if the stressor is removed.

Assessment of AC’s skeletal muscle biopsy taken 13 years after the removal of the stressor provided an opportunity to observe whether his period of exercise intolerance had resulted in any long-term structural damage to his muscle. At the age of 63 years his vastus lateralis muscle displayed moderate levels of structural abnormalities (fibre size variation and subsarcolemmal mitochondria). The extent of these abnormalities was similar to the levels seen in healthy endurance runners but greater than the sedentary population and, as discussed in Chapter 1 (section 1.4.3), likely evidence of adaptation to endurance training. However, since adaptation to exercise training reverses with detraining (63), and AC stopped endurance running 13 years ago, these findings are difficult to explain. Since he still exercises in the gym most days of the week, it would be interesting to know if this training could evoke the variation in fibre size and aggregations of subsarcolemmal mitochondria ordinarily observed in the muscle of endurance runners. Alternatively, the observed structural abnormalities were remnants of his past running career. The telomere lengths measured in AC’s muscle were similar to those of the athletic and sedentary control populations suggesting that despite his large exposure to running earlier on in his life, the remaining replicative capacity of his muscle had not been compromised.

Comparison of AC to his sedentary identical twin brother (SC) provided a unique opportunity to observe any long-term effects a high exposure to endurance running might have on the phenotype of genetically matched skeletal muscle. SC also acted as an age control when examining AC’s muscle for residual effects of his running career thereby removing the possible confounder of age. AC and SC are monozygotic twins. Therefore, they have the same chromosomal DNA sequence, other than a few DNA replication errors, and share all their nuclear DNA, thereby accounting for their seemingly identical phenotypes. It is possible, however, for monozygotic twins to have minor differences in their phenotypes. Traditionally,

environmental factors have been assumed to be responsible for differences in phenotype. Current thinking, however, is that in addition to environmental factors, epigenetic factors need to be considered as molecular controllers of phenotype. Epigenetics refers to DNA and chromatin modifications and epigenomic profiles are established during cell differentiation and embryonic morphogenesis (209). In the case of these twins, for example, epigenetics may explain the 5 cm height difference between them.

Comparison of the skeletal muscle of the twins showed that AC had noticeably more type 1 fibres (83 %) compared to his sedentary twin (65 %). Given that this difference was nearly 20 % it is unlikely that it could be attributed to either measurement variance, (approximately 6 %) (176), or even their epigenomic profiles. While the expression of the slow type myosin heavy chain (MHC) 1 gene is shown to be higher in elite marathon runners and trained ultra-endurance athletes and lower in sprinters and weight lifters, the extent of expression due to genetic predisposition versus training is an area still under debate (63). There are some studies, however, that have shown that even short periods of endurance training lead to an increase in MHC 1 isoform expression and a down-regulation in the expression of MHC 2X mRNA (63). Therefore, it is reasonable to attribute the observed difference between the twins to one of their primary behavioural differences: a large volume of endurance training versus a sedentary lifestyle.

The muscle of AC also had a greater degree of structural abnormality (fibre size variation and marked aggregations of subsarcolemmal mitochondria) compared to SC. Since the level of abnormality observed within SC's muscle was similar to the sedentary control group, it seems likely that the twins' muscle did not contain any innate abnormalities. The difference between the twins was likely reflective of the current differences in their levels of activity. However, should AC's current fitness training not be responsible for the fibre size variation and significant subsarcolemmal aggregations of mitochondria observed in his muscle, they may be considered as residual effects from his previous chronic exposure to endurance running.

The minimum skeletal muscle telomere length of AC was approximately 1.3 kbp shorter than that of SC. It is possible that this difference falls within the normal range of variation when telomere lengths are measured; however, no such published values were found. Alternatively, the difference may already have been apparent at birth, or it became apparent during their formative years. Mean telomere length changes very little during adulthood (45) while minimum telomere length decreases by approximately 13 bp per year with aging in healthy individuals, but by as much as 187 bp per year in response to a large insult on the muscle's regeneration capacity, such as observed in patients with Duchenne's muscular dystrophy (44). Therefore, if the twins reached adulthood with similar telomere lengths, the current observed difference may have been due to their differences in behaviour. This suggests that AC's high exposure to endurance training did indeed have an effect on the proliferative capacity of his satellite cells, but that this effect was not to the detriment of his muscle, since his telomere lengths were similar to healthy sedentary controls. A final observation is that SC had smoked cigarettes for 40 years, a habit which has been shown to shorten leukocyte telomeres (137, 197) presumably in response to the stress of this behaviour. Therefore, SC's telomeres may also have sustained additional shortening related to smoking, making the difference between the twins' telomere lengths even more significant.

6.3.4 Case 4**6.3.4.1 Case history**

AD began distance running at the age of 43 years and covered 36 799 km during his 15 year running career. He ran for nine years without experiencing any problems and his training and racing characteristics for this nine year period (Pre) are presented in Table 6.9.

Table 6.9 Running training and racing characteristics of AD prior to (Pre) and for the duration of (During) his exercise intolerance, as well as his current characteristics.

	Period		
	Pre	During	Current ¹
Time (years)	9	6	-
Total training distance (km)	17 576	9 674	-
Total racing distance (km)	6 098	3 451	-
Total running distance (km)	23 674	12 465	-
Annul volume (km·yr ⁻¹)	2 630	2 078	1 870
Training distance (km·wk ⁻¹)	57.8	48.6	55.7
Training frequency (days·wk ⁻¹)	5.0	5.0	3.1
Training speed (km·h ⁻¹)	12.8	10.8	9.7
PB 42.2 km time (h:min)	3h38min	4h48min	4h27min
PB 21.1 km time (h:min)	1h33min	2h13min	2h30min

¹ Current training data are those recorded during the eight weeks prior to this assessment and current race times are of his most recent races.

During his ninth year of running (age 51 years) AD completed two races feeling more tired than usual, but attributed this to a trip overseas, a shortage of training time and a recent bad cold. For the next six years (age 51 – 56 years) his running was plagued by symptoms such as excessive sweating and skeletal muscle fatigue and pain. He nevertheless managed to maintain a similar volume of training, albeit it at a slower speed (Table 6.9), and continued attempting ultra-distance races. He described feeling nauseous during races, was often unable to complete them, and would need up to two weeks to recover from each race. His race times, particularly for the 90 km Comrades Marathon, were notably slower in the years following the onset of his symptoms (Figure 6.11).

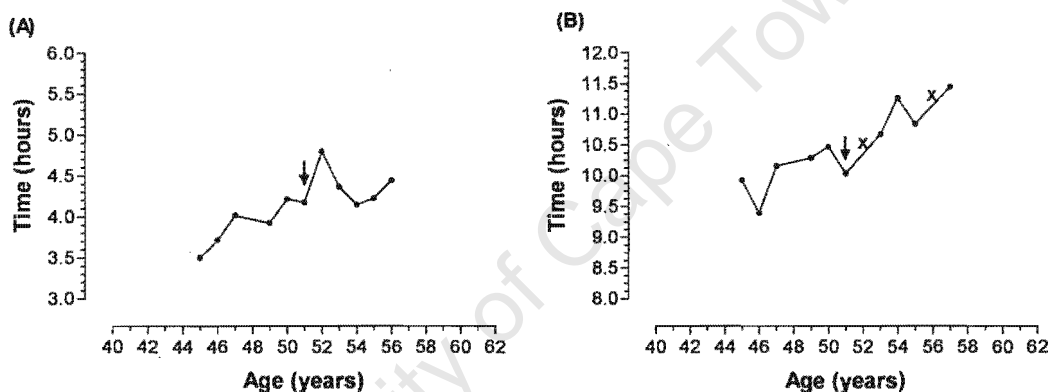


Figure 6.11 AD's annual change in performance for the 42.2 km distance (A) and the 90 km Comrades Marathon (B). The 42.2km time plotted for each age represents AD's best race over that distance for that year. The Comrades Marathon times are those achieved each year he completed the race. The arrows indicate the year of the onset of his exercise intolerance. The crosses indicate races he began but was unable to finish.

His general practitioner conducted blood tests but found no abnormalities, and while a sports physician diagnosed him as having mild exercise-induced asthma, a cardiologist, a pulmonologist and a neurologist were unable to find specific problems. AD also consulted the sports physicians at the Sports Science Institute of South Africa (age 52 years), and was diagnosed as having ATI. He agreed to participate in a study examining such athletes (182). Briefly, his physiological test results from that study were not extraordinary and routine blood tests were negative

except for evidence of a past activation of the Epstein Barr virus. He was also screened for HIV, Coxsackie virus, Bilharzia and Brucellosis, as these conditions present with symptoms of fatigue and sweating, but the results were negative. A biopsy of his vastus lateralis muscle revealed that he had some degree of fibre size variation and aggregations of sub-sarcolemmal mitochondria (75), but both were consistent with levels observed in the skeletal muscle of healthy endurance-trained individuals (75, 116). His minimum telomere length was measured as 4.9 kbp, similar to symptom-free endurance runners (5.3 ± 0.7 kbp) (33). The conclusion of this assessment was that the low level of structural abnormalities observed in his muscle may have been associated with his current inability to tolerate the volumes of training and racing to which he was accustomed (182). His intolerance to endurance exercise persisted for a further four years, prompting him to undergo another full medical examination. Again, his health was found to be normal in all respects, with the exception of borderline hypertension (150/90 mmHg supine and 170/90 mmHg erect) and a mild non-specific neutropenia and lymphopenia.

Five years after the onset of his intolerance to endurance exercise, AD attempted his tenth 90 km Comrades Marathon. Six weeks prior to the race he was placed on lisinopril, (an angiotensin-converting-enzyme inhibitor) to treat his hypertension. To reduce his pain during the race he also took six tablets containing 450 mg of paracetamol and 35 g of orphenadrine citrate over a 12 hour period, three times more than he had used previously. These tables are reported to have analgesic, anti-pyretic and skeletal muscle relaxant properties. After 30 km his legs began cramping and he experienced severe quadriceps pain. He collapsed approximately 1 km from the finish of the race with whole body muscular fatigue such that he could not raise his upper body. He was taken to the medical tent immediately and given fluids intravenously. He was markedly oliguric and the urine that he did eventually pass was dark brown, possibly indicative of myoglobinuria. He returned home but continued to experience nausea, vomiting, weakness, shaking and severe muscle pain, predominantly in his legs, but also in other muscles.

Two days after his collapse AD was hospitalized where he presented as anxious, distressed and clinically mildly dehydrated. His blood pressure was 130/60 mm Hg and examination of his chest and heart were normal. Serum creatine kinase activity

was severely elevated at $101\,682\text{ U}\cdot\text{L}^{-1}$ (37°C), almost 400 times above the normal upper limit ($260\text{ U}\cdot\text{L}^{-1}$ at 37°C), indicating that he had incurred more than 25 times the amount of leakage of intra-muscular creatine kinase into the blood than usually sustained by healthy runners for the same event ($\sim 4\,000\text{ U}\cdot\text{L}^{-1}$ at 37°C , unpublished data from this laboratory). Serum sodium and calcium concentrations, red cell count and haematocrit levels were below the normal reference range values, whereas potassium, urea, creatinine, myoglobin and white cell count were elevated (Table 6.10). The enzymes alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and aldolase were measured at 465, 1 943, 2 581 and $247\text{ U}\cdot\text{L}^{-1}$ respectively, i.e. the enzymes were 10, 48, 11 and 32 times more elevated compared to normal. These clinical results were consistent with rhabdomyolysis and acute renal failure (26, 27, 135). Fluids were initiated with furosemide in an attempt to force diuresis. Haemodialysis was begun since, despite urine output improving, creatinine continued to rise and he developed worsening uraemic symptoms.

AD remained in intensive care for five days while being treated for acute renal failure. He suffered from muscle myalgia and atrophy, weight fluctuations of 10 kg, and developed gout, which persisted for seven months. On discharge from hospital his serum urea and creatinine levels, and white blood cell count were still elevated, and his haemoglobin, red cell count and haematocrit levels were low (Table 6.10). Since he was also hypertensive on discharge, a calcium channel blocker (type unknown) was initiated, but stopped when he became hypotensive. He was dialysed twice as an outpatient and made good recovery with normalization of his renal function one month later. At this time, haemoglobin, red cell count and haematocrit were still below the lower limit of the reference range. Five weeks after the race he was assessed by a sports physician who measured borderline hypertension (140/82 mm Hg) and found evidence of hyperuricaemia and non-specific tissue damage (increased serum ferritin). Despite weeks of rest, AD had difficulty in both regaining his muscle mass and recommencing training due to excessive fatigue while running.

Table 6.10 Haematological data of AD from when he was admitted to hospital two days after the 2002 90 km Comrades Marathon to one month after the race.

	Time after race (days)							
	2	3	4	5	6	7	14	26
Sodium (135 – 150 mmol·L ⁻¹)	130*	129*	131*	135	138	135	141	139
Potassium (3.5 – 5.0 mmol·L ⁻¹)	5.2*	4.1	4.4	4.2	3.8	3.5	4.7	4.3
Chloride (96 – 108 mmol·L ⁻¹)	97	100	105	100	102	95*	104	104
Bicarbonate (23 – 27 mmol·L ⁻¹)	24	20*	18*	24	26	27	26	27
Urea (3.4 – 7.4 mmol·L ⁻¹)	24.4*	26.4*	28.4*	18.1*	14.4*	11.2*	10.8*	7.3
Creatinine (80 – 115 μmol·L ⁻¹)	332*	408*	536*	473*	433*	377*	140*	83
Calcium (2.15 – 2.50 mmol·L ⁻¹)	2.10*	2.06*			2.58*		2.45	
Phosphate (0.87 – 1.45 mmol·L ⁻¹)	1.40	2.09*			1.70*		1.13	
Myoglobin ¹ (19 – 98 μg·L ⁻¹)	8220*				1300*			
Haemoglobin (14.0 – 18.0 g·dL ⁻¹)	14.1	12.7*	12.0*	11.4*	11.0*	10.6*		13.5
Red cell count (4.6 – 6.0 × 10 ¹² ·L ⁻¹)	4.5*	4.0*	3.8*	3.6*	3.5*	3.4*		4.4*
Haematocrit (42 – 52 %)	42*	38*	35*	34*	34*	31*		40*
White cell count (4.0 – 10.0 × 10 ⁹ ·L ⁻¹)	10.9*	8.4	6.7	5.5	5.5	6.8		7.0

The normal range and units of each variable are given in parentheses

** indicates a value outside of the normal range*

¹ *The value for myoglobin was that measured in the serum*

Six months after the 2002 90 km Comrades Marathon AD visited our laboratory for the second time. He had two reasons for visiting: Firstly, he still sought answers to both his ongoing intolerance of endurance running and the cause of his collapse during the 2002 90 km Comrades Marathon. Secondly, he was determined to complete his tenth 90 km Comrades Marathon and wanted to have his decision to compete medically sanctioned. A medical history questionnaire indicated that he had borderline hypertension, high cholesterol, gout, and occasionally suffered from exercise-induced asthma. He was free from other chronic diseases and was not currently overtrained. At the time of his assessment he was taking allopurinol for his symptoms of gout. He described an adverse reaction to diclofenac including a sensation of tingling in his body and occasional collapse. AD reported that he did not suffer from depression.

6.3.4.2 Clinical evaluation and present symptomatology

AD weighed 84.5 kg, was 1.84 m tall, had a BMI of 25 and was 57 years old at the time of his assessment. He reported a moderately high level of perceived life stress (7/10). His primary symptoms, excessive sweating and fatigue when running, had been present for six years and substantially affected his ability to train and race but no other aspects of his life. Secondary symptoms included excessive delayed onset muscle soreness, stiffness and weakness, and more recently, exercise-associated muscle cramping. There was no evidence that AD suffered from chronic fatigue syndrome and no other obvious abnormalities were detected at the time of this assessment.

6.3.4.3 Special investigations

Blood tests returned normal results, but confirmed a past activation of the Epstein Barr virus.

Skeletal muscle analysis

A biopsy of his vastus lateralis muscle was taken according to the methods described in section 6.2.5. Unfortunately, due to technical reasons, it was not possible to use histological staining to examine the markers of pathology in the

sample. Determination of the TRF lengths of his skeletal muscle telomeres showed that the minimum, mean and maximum lengths were 7.1, 9.6 and 14.2 kbp respectively. Specifically, the minimum TRF length of AD was similar to that of asymptomatic control runners (6.9 ± 0.1 kbp) and apparently healthy sedentary individuals (7.0 ± 0.8 kbp) (Figure 6.12 A). The same analysis performed at this laboratory four years ago (1999), two years after the onset of AD's intolerance to endurance exercise, also showed that his minimum TRF length was not different to that measured in control athletes at that time (Figure 6.12 B). Although the absolute TRF lengths measured in 1999 and for this current study (2003) could not be compared due to limitations in the technique, it was useful to observe that in both instances AD's minimum TRF length was similar to the respective Control groups.

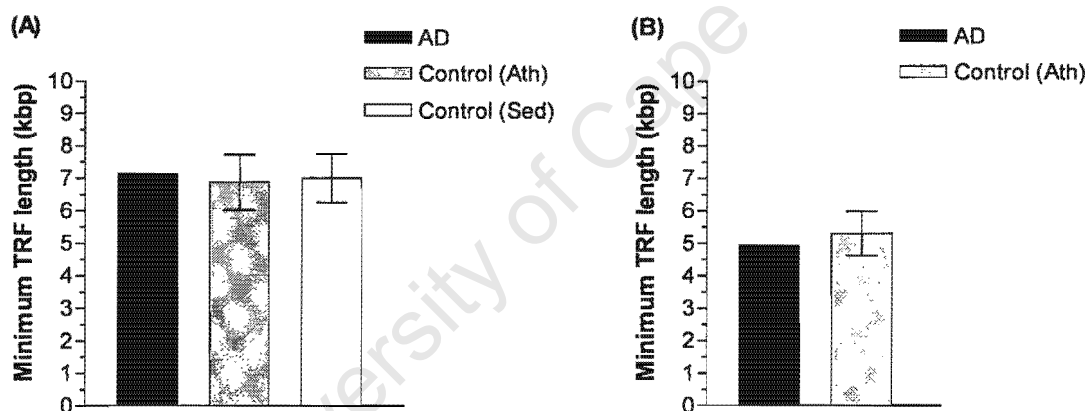


Figure 6.12 Minimum TRF lengths of AD and the Control groups (Athletic and Sedentary populations) measured at present (A) and soon after the onset of his intolerance to endurance exercise (B).

Physiological testing

In an attempt to offer AD advice regarding the effect of his intolerance to endurance exercise on his performance, his response to exercise-induced muscle damage was assessed according to the methods described in section 6.2.3.1. The main finding was that after a bout of exercise designed to induced muscle damage, he sustained a normal degree of muscle damage as indicated by his serum creatine kinase levels, and his recovery during the following week was also deemed to be normal (Figure 6.13 A). Similarly, he did not experience any further skeletal muscle pain as measured by a subjective pain rating scale (Appendix 4) compared to other athletes (Figure 6.13 B). AD's rectal temperature measured during the downhill run reached a peak of 38.7 °C, suggesting that his core temperature was effectively regulated. Together these data show that AD responded normally to a single bout of exercise designed to induce muscle damage and provided no evidence to help explain his exercise-associated fatigue or excessive sweating.

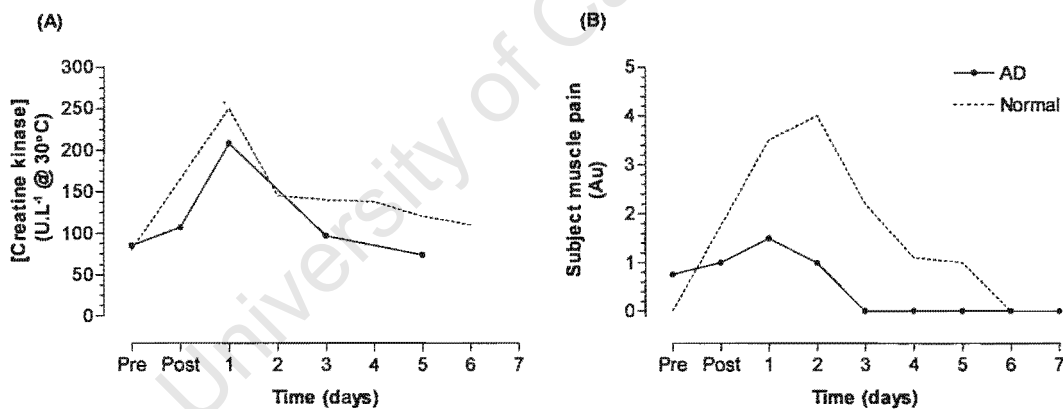


Figure 6.13 AD's change in serum creatine kinase activity (A) and subjective muscle pain (B) following a 40 minute downhill run designed to elicit muscle damage. The response of AD is depicted by the solid line with closed circles. The dotted line (Normal) represents the average response of a number of healthy runners studied previously at this laboratory (168). Au – Arbitrary units.

Although these special investigations provided no evidence as to the cause of AD's exercise intolerance or his case of rhabdomyolysis and acute renal failure, he was advised not to attempt his tenth 90 km Comrades Marathon. Despite this advice, he completed his tenth race in his slowest time to date, 11h20min but with no major ill effects, apart from evidence of muscle damage as indicated by significantly raised serum creatine kinase activity and myoglobin levels (Table 6.11 and Figure 6.14).

Table 6.11 Blood biochemistry data of AD after the 2003 90 km Comrades Marathon.

	Time after race (days)		
	1	3	7
Sodium (135 – 150 mmol·L ⁻¹)	138	140	139
Potassium (3.5 – 5.0 mmol·L ⁻¹)	3.6	4.4	4.1
Chloride (96 – 108 mmol·L ⁻¹)	105	102	102
Bicarbonate (23 – 27 mmol·L ⁻¹)	21 *	27	28 *
Urea (3.4 – 7.4 mmol·L ⁻¹)	6.4	5.4	7.4
Creatinine (80 – 115 μmol·L ⁻¹)	88	74 *	78 *
Calcium (2.15 – 2.50 mmol·L ⁻¹)	2.43	-	-
Phosphate (0.87 – 1.45 mmol·L ⁻¹)	1.06	-	-
Creatine kinase (0 – 190 U·L ⁻¹ at 37 °C)	12 233 *	2 442 *	305 *
Myoglobin (16 – 76 ng·mL ⁻¹)	261 *	-	-

The normal range and units of each variable are given in parentheses.

** Indicates values outside of the normal ranges.*

His maximum creatine kinase activity of 12 233 U·L⁻¹ (at 37 °C), was recorded the day after the race. Although this was eight times lower than his maximum value reached the year before (101 602 U·L⁻¹ at 37 °C), it was still three times greater than that of healthy athletes in the same race, suggesting that his skeletal muscle cell membranes was still more susceptible to exercise-induced muscle damage compared to asymptomatic runners. Despite sustaining a larger degree of muscle

damage, the permeability of his skeletal muscle membranes resolved following a similar time course as apparently healthy runners (Figure 6.14).

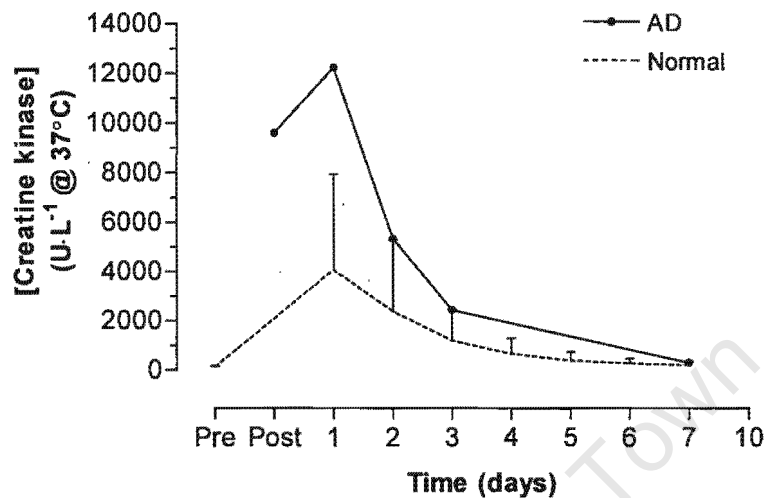


Figure 6.14 Creatine kinase activity of AD following the 2003 90 km Comrades Marathon. The solid line with the closed circles represents the response of AD, while the dotted line is the normal response of healthy runners in the same race with the error bars representing one standard deviation from the mean (unpublished data from this laboratory).

6.3.4.4 Discussion

There were two primary issues for AD. The first was the cause of his initial intolerance to endurance exercise and the second the cause of his collapse and development of rhabdomyolysis and subsequent acute renal failure (ARF) during a 90 km ultra-marathon. AD's exposure to endurance running prior to the onset of his intolerance to endurance exercise (17 576 km over 8 years, $\sim 42 \text{ km}\cdot\text{wk}^{-1}$) was modest. Since the previous chapters in this thesis have shown that skeletal muscle has a large capacity to tolerate endurance running, it is unlikely that his exposure to running alone was responsible for his exercise intolerance. This was validated, in part, by the fact that the minimum telomere lengths measured in his skeletal muscle both at present and six years ago were no different to either apparently healthy control athletes or sedentary individuals. A biopsy of his vastus lateralis muscle measured two years after the onset of his exercise intolerance found structural

abnormalities such as internal nuclei and aggregations of subsarcolemmal mitochondria, the levels of which were consistent with the effects of endurance training (178) and similar to those of the apparently healthy control athletes. Furthermore, when AD's response to, and recovery from, a single bout of exercise-induced muscle damage was assessed, it was found to be normal. Thus neither a high exposure to endurance running, structural abnormalities, short telomeres or a decreased integrity of his skeletal muscle cell membranes were likely related to his symptoms of fatigue, muscle pain or excessive sweating.

A study of 41 athletes found that in 68 % of the cases an underlying medical condition was responsible for their persistent fatigue (159). AD consulted a range of medical experts who found no clinical condition that could explain his fatigue except perhaps for the presence of mild exercise-induced asthma (EIA). Although chronic asthma is one of the respiratory illnesses that may explain exercise-associated fatigue (159), no direct evidence was found in the literature for limb skeletal muscle abnormalities or dysfunction in asthmatics. Therefore, although AD's mild exercise-induced asthma may have been a potential cause of his exercise-associated fatigue, it was not deemed to be a likely explanation for his exercise intolerance since it could not account for his other symptoms of skeletal muscle pain and excessive sweating.

AD did however associate the onset of his intolerance to endurance exercise with both travel and illness, therefore the possibility that a viral infection contributed to the onset of his symptoms was considered. Although he was not formally diagnosed with a specific virus at the time, later blood tests showed that AD had experienced a past activation of the Epstein-Barr virus. Unlike AA and AC who were undergoing high training loads followed by stressful races while either suffering from or recovering from an illness, AD had neither high current training loads, nor a history of a large exposure to endurance running. Therefore either high intensity training does not make runner more susceptible to infection, or low intensity training makes runners just as susceptible to infection, or a viral infection was not the primary trigger for the onset of AD's subsequent symptoms. Given the history of AD, however, a viral infection must be considered as a possible co-stressor contributing to the onset of his intolerance to exercise.

AD's collapse and development of rhabdomyolysis and acute renal failure (ARF) after the 90 km Comrades Marathon is intriguing since an ultra-endurance race does not cause irreversible or irreparable damage to skeletal muscle, renal function or electrolyte balance under normal circumstances (69). Furthermore, AD had completed nine 90 km Comrades Marathons previously, including three during his period of exercise intolerance, and had reportedly never experienced a similar episode. One possible trigger might have been the environmental conditions on the day. There have been a number of cases described in the literature where athletes have developed exertional rhabdomyolysis under hot environmental conditions (14, 110, 121, 146). The average ambient temperature during the race in which he collapsed was 20 °C (range: 10.5 – 25.5 °C), and the average relative humidity was 49.3 % (range: 25.3 – 91.0 %). Therefore it seems unlikely that excessively hot or humid conditions during the race alone could have triggered the case of rhabdomyolysis. Alternatively, the combined ingestion of lisinopril and the paracetamol / orphenadrine citrate tablets might have been related to the rhabdomyolysis and subsequent development of ARF. There is, however, no evidence in the literature indicating that these two drugs might combine to cause either rhabdomyolysis or ARF. Of interest is that the paracetamol / orphenadrine citrate tablets are reported to have analgesic, anti-pyretic and skeletal muscle relaxant properties and are typically used to combat skeletal muscle pain during ultra-endurance races. These tablets may have masked early warning signs of problems within AD's muscle, thereby allowing him to continue running while causing severe damage to his muscle.

Analysis of his serum creatine kinase (CK) activities after both the 90 km Comrades Marathon during which he collapsed (2002), and his unremarkable run the following year (2003) showed that his skeletal muscle cell membranes suffered greater damage compared to asymptomatic athletes on both occasions. In contrast, his response to a single, 40 minute bout of exercise designed to induce muscle damage as measured in our laboratory was normal. This suggests that he had a greater susceptibility to rhabdomyolysis or excessive muscle damage following ultra-endurance distances. While moderate to severe levels of muscle damage are common following ultra-endurance races (86, 179), extreme rhabdomyolysis under

mild environmental conditions might be triggered by a myopathy such as a glycogen storage disease, muscular dystrophy or an inflammatory myopathy (61). In cases where endurance athletes present with fatigue, muscle pain and exercise intolerance, rhabdomyolysis, myoglobinuria, or a marked increase in CK activity after exercise, and where common causes are not diagnosed, metabolic myopathies, including mitochondrial myopathies should be considered (50, 190).

A similar case to AD was described by Tarnopolsky et al. (187). An active 47 year old male began to experience muscle pain, particularly in his quadriceps following exercise. He reported having a flu-like episode in his 20's but he continued exercising over the years until he began to suffer from myalgia, and was hospitalised with acute renal failure. He subsequently recovered and can now complete moderate (6 or 7 km) running or swimming sessions. Laboratory results showed that his muscle creatine kinase activity levels fluctuated from normal to about $800 \text{ mmol}\cdot\text{L}^{-1}$ without excessive exercise (normal: $< 230 \text{ mmol}\cdot\text{L}^{-1}$). Forearm ischemic tests were normal, as was electron microscopic analysis of his muscle biopsy. He was finally diagnosed as having a severe carnitine palmitoyltransferase (CPT) II deficiency (187). This is the most common disorder of mitochondrial β -oxidation and usually presents in late childhood or early adulthood as recurrent episodes of myoglobinuria after prolonged exercise, fasting, high fat intake, viral infection, fever or even emotional stress. If the rhabdomyolysis is severe enough, ARF may follow. In general, individuals with CPT II deficiency feel normal between attacks of rhabdomyolysis but report myalgia and muscle tightness during the attacks (199). Recurrent rhabdomyolysis has been shown to be associated with this metabolic myopathy (113). Common symptoms of this deficiency are myalgias and pigmenturia that occur during periods of fasting or prolonged exercise and with superimposed colds or flu (187).

Primitive evidence for an argument of a metabolic abnormality, and perhaps CPT II deficiency in the case of AD, would include his complaint of excessive sweating and muscle fatigue during distance running, his development of rhabdomyolysis and subsequent ARF, and his severely elevated levels of circulating CK activity after the 90 km Comrades marathon the year following his collapse. Therefore, it was recommended that AD be tested for metabolic myopathies.

Finally, as for AA and AC, the behaviour of AD needs to be considered as a possible co-stressor. In the first instance, AD only decreased his training load by approximately 17 % following the onset of his intolerance to endurance exercise, and continued to attempt ultra-endurance races. Should his skeletal muscle have been compromised by a viral infection, for example, his determination to resume training and racing at previous levels most likely did not afford his muscle the opportunity to fully recover. Secondly, AD was determined to complete his 10th 90 km Comrades Marathon before ending his distance running career. Despite his exercise-associated fatigue, sore muscles, excessive sweating and reduced training volume he prepared for and attempted the race, ingesting a high volume of the paracetamol / orphenadrine citrate tablets. This behaviour showed a dogged determination and perhaps desperation to race at all costs. Both instances illustrate an individual determined to undertake endurance running despite a number of signs that the activity might be damaging.

6.3.5 Case 5**6.3.5.1 Case history**

AE began competitive distance running when she was 20 years old and during her 24 year running career covered approximately 130 070 km. For the first 11 years she maintained an average annual training distance of over 7 500 km, running every day of the week. Her training and racing data for this period (Pre) are presented in Table 6.12.

Table 6.12 Running training and racing characteristics prior to (Pre), for the duration of (During) and after (Post) the patient's exercise intolerance, as well as her current characteristics.

	Period			
	Pre	During	Post	Current ¹
Time (years)	11	9	4.5	-
Total training distance (km)	83 200	25 440	18 660	-
Total racing distance (km)	2 202	65	503	-
Total running distance (km)	85 402	25 505	19 163	-
Annual volume (km·yr ⁻¹)	7 763	2 833	3 578	4 500
Training distance (km·wk ⁻¹)	145.5	57.8	75	82.8
Training frequency (days·wk ⁻¹)	7.0	6.6	7.0	5.8
Training speed (km·h ⁻¹)	11.2	9.2	9.1	10.8
42.2 km time (h:min)	2h49min	-	3h40min	3h40min
21.1 km time (h:min)	1h23min	1h33min	1h43min	1h47min

¹ Current training data are those recorded during the eight weeks prior to this assessment and current race times are of her most recent races.

By the age of 30 years, AE had accumulated in excess of 83 000 km in endurance training and racing. During her eleventh year of high volume endurance running she completed her tenth successive 90 km Comrades Marathon, an annual race which she had won twice before (ages 21 and 22 years). Prior to the race she ran between 140 and 160 km per week in training with no rest days. She recalled experiencing excessively heavy legs and weight loss related to her training levels at that time. She also suffered from painful skeletal muscles after races, which would sometimes last for up to two weeks depending on the distance she had run. Significantly, she felt unable to recover from her tenth 90 km Comrades Marathon and attributed her ensuing fatigue to too much training over the years. She was forced to make a noticeable decrease in her training volume and raced very little in the subsequent nine year period during which she suffered from exercise-associated fatigue (Table 6.12). During this period she was diagnosed as having chronic fatigue and attributed this, along with two pregnancies and a significant life stress event, to the extended duration of her exercise-associated fatigue. The diagnosis of chronic fatigue may, however, have been inappropriate as she described her fatigue as only affecting her exercise capacity and no other areas of her life. Another important feature of this case was that AE suffered from an eating disorder.

Five years after the onset of her intolerance to endurance exercise (age 36 years) AE's fatigue was at its worst and she volunteered to participate in a study at this laboratory investigating athletes with acquired training intolerance (ATI). Despite her exercise-associated fatigue and reduced training volume, her maximum oxygen uptake was estimated at $59.7 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ (182) indicating a good level of cardio-respiratory fitness. Histological staining of a biopsy of her vastus lateralis muscle showed that she had an extraordinary predominance of type 1 fibres (91 %). The skeletal muscle biopsy also showed that she had significantly more fibre size variation, internal nuclei and aggregations of subsarcolemmal mitochondria compared to healthy control athletes (75). Furthermore, her minimum, mean and maximum TRF lengths (1.2, 3.8 and 10.0 kbp respectively) were not only significantly shorter than those of healthy mileage-matched control athletes, but were classified as being pathologically short (33). Therefore, based on these investigations and despite her reduced training and racing volumes, AE's skeletal muscle still demonstrated significant disturbances. Furthermore, the extremely

shortened telomeres indicated that either the proliferative capacity of her satellite cells had been significantly reduced, presumably through excessive cycles of degeneration and regeneration, or the DNA had been exposed to significant levels of oxidative stress. These were both plausible options given her large exposure to endurance training and racing. It is tempting to speculate that the exercise-associated fatigue experienced by AE was related to the state of her skeletal muscle.

Nine years after the onset of her intolerance to endurance exercise she was finally able to increase her training volume again and by the age of 43 years was running approximately 100 km per week and described her training condition as excellent. She also resumed ultra-marathon racing, but initially required long periods to recover from the races. Despite being slower in training (Table 6.12) and racing (Figure 6.15) compared to before, she no longer suffered from exercise-associated fatigue and could not be classified as an athlete with ATI.

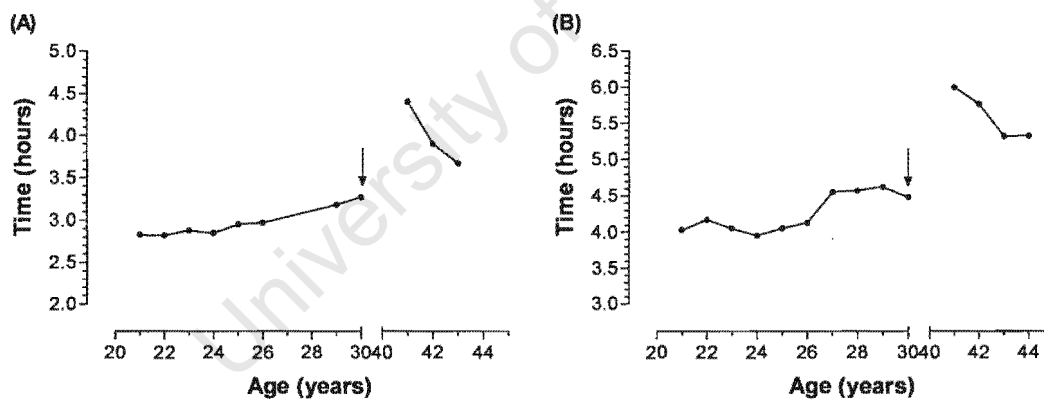


Figure 6.15 AE's annual performance changes for the 42.2 km distance (A) and the Two Oceans 56 km Ultra-Marathon (B) during the course of her running career. Each 42.2 km race time represents her fastest standard marathon time that year. She rarely competed in races while she was fatigued (age 32 to 40), but she did continue training at a reduced volume. The arrows indicate the year of the onset of her intolerance to exercise.

Figure 6.15 shows the change in performance of AE for the standard marathon and the Two Oceans 56 km Ultra-Marathon during her career. Since she stopped

competing when she began to experience the fatigue associated with running, the effect that it had on her race ability was unclear. The large decline in performance seen when she resumed racing at the age of 41 years may have been attributed to her advancing age, decreased training volume, residual effects of fatigue, or any combination of these three factors. That she was able to improve each subsequent year over both distances suggests that her performance was more likely related to her training volume which increased from 55 to 90 km per week during her return to competition.

Given her remarkable progress following nine years of exercise intolerance, AE agreed to return to our laboratories for follow up testing. Her medical history revealed a family history of coronary heart disease, high blood pressure, cancer, asthma and depression. She had previously been diagnosed with anorexia, depression and chronic fatigue and had used exercise, restrained eating and laxatives to control her weight. She reported occasionally experiencing fainting spells and dizziness upon standing, both of which she attributed to her low blood pressure.

6.3.5.2 Clinical evaluation and present symptomatology

AE weighed 51.1 kg, was 1.68 m tall, had a BMI of 18.1 and was 43 years old at the time of assessment. The anthropometric values were similar to those recorded during her previous visit to the laboratory. Her perceived level of life stress was high (8/10). AE reported that she no longer suffered from exercise-associated fatigue and that she did not have any musculoskeletal symptoms, such as muscle pain, stiffness, tenderness or cramps, related to running.

6.3.5.3 Special investigations

Results from routine blood tests were normal except for evidence of a past activation of the Epstein Barr virus.

Skeletal muscle analysis

Histological staining of cross-sections of the biopsy of her vastus lateralis muscle showed that her fibre type predomination was still type 1 (83 %), and that she had a significantly larger degree of internal nuclei, fibre size variation, evidence of necrosis and degeneration as well as large aggregations of subsarcolemmal mitochondria compared to apparently healthy runners. Her total pathology score was 7.0, noticeably higher than the mean value obtained for apparently healthy control runners (2.5 ± 1.6 units) and sedentary individuals (0.7 ± 0.9 units). This suggests that her skeletal muscle was still in a state of ongoing disruption, seemingly beyond the expected level in the muscles of a healthy endurance trained runner. This current pathology score was also similar to the score of 9.0 units she obtained when her skeletal muscle was examined during her period of exercise intolerance. Therefore despite her symptoms associated with ATI having resolved, her skeletal muscle still appeared to be in a similar state of excessive disruption and repair. Figure 6.16 illustrates the increased levels markers of structural pathology in the muscle of AE compared to an apparently healthy control runner. The control runner was matched to AE for age at which they began running (AE: 20 and Control: 21 years), years spent running (AE: 24 and Control: 22 years), career average weekly training distance (AE: 100 and Control: 90 km), current weekly training distance (AE: 83 and Control: 100 km) and PB 42.2 km time (AE: 2h49min and Control: 2h52min).

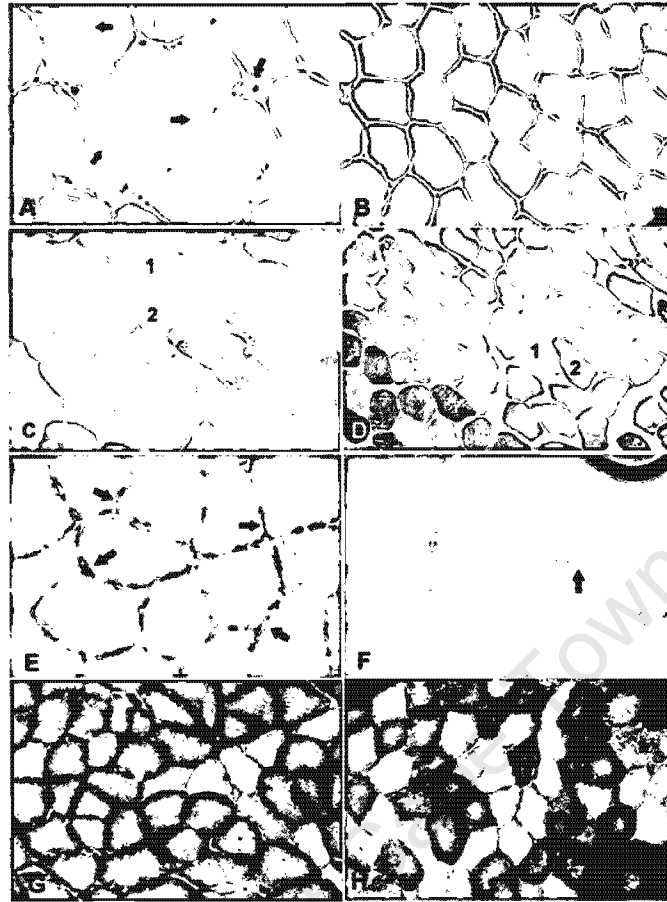


Figure 6.16 Cross-sections of the vastus lateralis muscle from AE (panels A, C, E and G) and a healthy control runner (panels B, D, F and H). Panels A and B show muscle cross-sections stained with Gomori Trichrome (10x). The arrows in panel A show that the muscle of AE had evidence of internal nuclei, abundant connective tissue, fibre atrophy and fibre splitting. In contrast, the muscle of the control runner in panel B shows normal polygonal shaped cells and regular fibre size. Panels C and D show muscle stained with ATPase at pH 9.4 (10x). Panel C highlights the predominance of type 1 fibres in the muscle of AE. In contrast, the muscle of the control runner has a more normal fibre type distribution (panel D). The numeral 1 indicates a type 1 fibre (lighter in colour) and the numeral 2 a type 2 fibre (darker in colour). The SDH stain was used on the sections shown on panels E and F (20x). The arrows in panel E show the accentuated, lobular accumulations of subsarcolemmal mitochondria in the muscle of AE, while panel F depicts the more normal accumulations in the control runner. The NADH-TR stain (10x) was used on the muscle cross-sections shown in panels G and H. The dense dark outlines of the muscle fibres of AE in panel G further highlight the unusually high levels of subsarcolemmal mitochondria compared to the control runner (panel H).

The minimum and mean skeletal muscle TRF lengths of AE were measured as 6.9 and 10.6 kbp respectively, similar to those of both apparently healthy control athletes (min: 6.9 ± 0.9 kbp, mean: 10.0 ± 1.0 kbp) and sedentary individuals (min: 7.0 ± 0.8 kbp, mean: 10.0 ± 1.0 kbp) (Figure 6.17 A). These results were particularly striking since when AE had visited this laboratory eight years earlier (1998) at the peak of her fatigue, her minimum TRF length was pathologically short compared to age-matched control athletes at that time (Figure 6.17 B). While the absolute values of her minimum TRF length measurements from 1998 and 2006 could not be compared, it is clear that there had been a change in their length relative to asymptomatic control athletes.

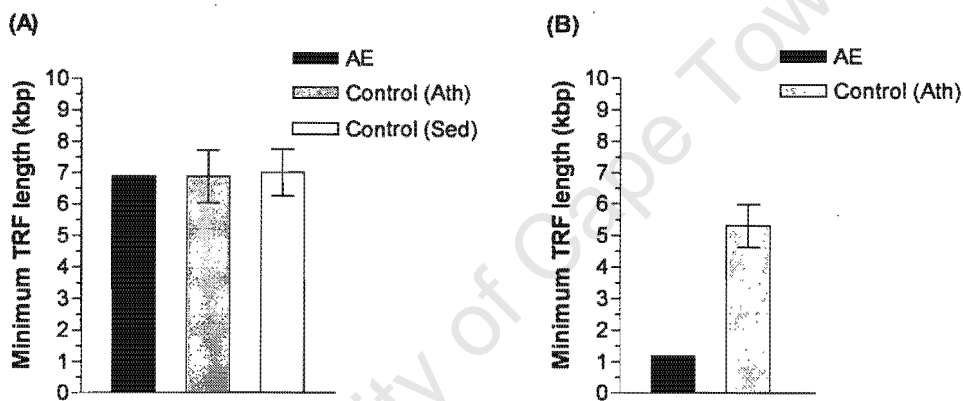


Figure 6.17 Minimum TRF lengths of AE and the Control groups (Athletic and Sedentary populations) measured at present (2003) (A) and at the peak of her exercise intolerance period (1998) (B).

By all accounts, AE had made a remarkable recovery from her extended bout of exercise-associated fatigue as she was once again able to tolerate a high training load and compete in ultra-endurance races. Evidence from her skeletal muscle, however, was conflicting. While telomere length had apparently resolved, her skeletal muscle still showed marked signs of structural abnormalities. Unfortunately, seven months after her visit to our laboratories she passed away suddenly at the age of 44 years. Following a detailed autopsy investigation, the pathologists were unable to determine the cause of her death.

6.3.5.4 Discussion

The clinical picture of AE during her phase of exercise intolerance was consistent with that of an athlete with ATI. Her large exposure to endurance running may well have been associated with the unusually high levels of structural abnormalities and significantly short minimum telomere lengths measured while she was exercise intolerant. Of interest was that the initial analysis of her muscle performed when she first visited this laboratory, was six years after the onset of her intolerance to endurance exercise. During those six years she had dramatically reduced her training volume (62 %) and all but stopped racing. If her past exposure to endurance running was solely responsible for the state of her muscle, either a very long period of time was necessary to reverse the effects, or her current training volume was still high enough to maintain the state of ongoing damage and repair, or some other factor was responsible.

Her case history suggests that in addition to the stress of endurance training, her behaviour was an important co-stressor, likely contributing to her extended period of fatigue. Firstly, given her high training volume of approximately 100 km per week and the fact that she took no rest days suggests that AE was almost certainly perpetually in a state of over-reaching or even overtraining. Over-reaching is a short period where athletes increase their intensity or volume of training, resulting in minor performance decrements while they adapt to the increased stimulus of training. With rest, or a reduction in training load, they recover and often then observe improvements in performance. Should athletes be in a state where training stimulus continually outweighs rest or recovery periods, they are likely to reach a state of overtraining (180). Furthermore, over-reaching and overtraining leave athletes more vulnerable to infection and its consequences (71, 180).

Secondly, AE suffered from an eating disorder which had been present since her early teen years. At various stages in her life she had suffered from anorexia, bulimia and related depression. Individuals suffering from anorexia experience a loss in skeletal muscle mass due to protein degradation, which is usually accompanied by muscle dysfunction (loss in strength and fatigue), proximal limb weakness (64), and there may also be EMG evidence of neuropathy (56). Muscle biopsies from anorexic patients typically display segmental loss of myofibrils,

abundant glycogen granules, type 2 fibre atrophy, (133), higher proportions of type 1 fibres with a smaller cross-sectional size, no type 2B fibres, an increased presence of the normally rare type 2X/D fibres, a 50 % reduction in glycolytic enzymes and a 15 % reduction in the activity of the mitochondrial enzymes citrate synthase and hydroxyacyl-CoA dehydrogenase compared to healthy sedentary controls (56). Significantly, anorexia produces a disturbance in skeletal muscle metabolism (134). Since it is clear that of the eating disorders, anorexia in particular has a direct impact on skeletal muscle, it is remarkable that AE was such a successful endurance athlete in her early career. It also seems likely, however that the combined stresses on her skeletal muscle of a high exposure to endurance running together with an eating disorder contributed to her intolerance to endurance exercise. Together her tendency to overtrain along with her eating disorder fit the picture of a female athlete with strong obsessive tendencies towards their exercise (42, 43).

The current assessment of AE's skeletal muscle indicated that in addition to being free of the symptoms associated with ATI, her minimum telomere lengths were no longer pathologically short. In fact, they were no different from healthy control runners or sedentary individuals. This information was difficult to interpret as it suggested that relative to control athletes, the telomeres of AE had in effect lengthened. Current literature suggests that telomere length in skeletal muscle, a post-mitotic tissue, is remarkably stable since the tissue undergoes very little growth during adulthood (45). Furthermore, the enzyme telomerase, which is responsible for extending telomere length, ought not to be active in skeletal muscle since its activity has only been demonstrated in germline or immortal cells (10). In fact telomerase has only been found to be active in extending telomere length in skeletal muscle *in vitro* (49, 210). Therefore it is difficult to explain the large difference in her minimum telomere lengths observed during her period of intolerance to exercise and after the apparent resolution of her symptoms.

Despite the apparent normalisation of her telomere lengths, there was still a large degree of structural abnormality present in her skeletal muscle. While it could be argued that these abnormalities were related to her resumed high volume of training, they were still significantly greater than those observed in asymptomatic endurance runners. Of further concern was that there were signs of degeneration in

the biopsy sample of AE that were not observed in the biopsies of the apparently healthy control runners, or the other four cases presented in this chapter. One explanation might be that the skeletal muscle of AE was innately more susceptible to exercise-induced muscle damage, or had a less efficient mechanism for repair. Alternatively, the observed high levels of structural abnormalities in the muscle of AE were more reflective of her eating disorder than her exposure to endurance running. Therefore in the case of AE, a combination of stressors was likely related to her prolonged exercise intolerance: a chronic exposure to endurance running and a group of obsessive behavioural tendencies including overtraining and an eating disorder. Her sudden death however, remains inexplicable.

University of Cape Town

6.4 SUMMARY

Table 6.13 summarises the key points of each case. Common to all five cases was a sudden and seemingly inexplicable onset of exercise-associated fatigue that lasted for an extended period of time. This fatigue forced all the runners to reduce their training volume, limited their performance capacity and was accompanied by general skeletal muscle symptoms such as fatigue, soreness and exercise-associated cramps. The athletes could not be diagnosed as having chronic fatigue syndrome, however, as although their fatigue affected their capacity to exercise, it did not affect the social, occupational or educational aspects of their lives (88). Furthermore medical examination found no other probable causes for their fatigue. Bruckner et al. describe fatigue as being pathological in cases where rest and a decreased training load are unable to resolve it, and athletes simultaneously experience a decrease in performance (17). Using this definition, it was appropriate to label the five cases described in this Chapter as “pathologically fatigued”. Despite their common symptom of exercise intolerance it was not possible to place all five cases into one group. Each athlete differed from the next with respect to exposure to endurance running, calibre of athlete, likely triggers for their intolerance to endurance exercise, extent of change in training load and performance capacities, age of onset of the intolerance, detected skeletal muscle abnormalities, formal diagnoses, duration of symptoms and outcome in terms of symptoms (Table 6.13).

Table 6.13 Summary of the five cases presented in this series of exercise-intolerant runners.

	AA	AB	AC	AD	AE
Years running ¹	17	16	11	8	11
Distance (km) ¹	76 500	46 614	51 220	17 576	85 280
Exposure (km·yr⁻¹) ¹	4 500	2 913	4 656	2 197	7 753
PB 42.2 km	2h33m	2h59m	2h56m	3h38m	2h49m
Age at onset	36	33	44	51	30
Perceived trigger	High training load, illness	Unknown	Illness	Travel, illness	High training load
Symptoms	Fatigue ² , muscle pain, EAMC sweating	Fatigue ² , muscle pain, EAMC	Fatigue ² , muscle pain, heavy legs	Fatigue ² , muscle pain, sweating	Fatigue ² , muscle pain, heavy legs
Duration (yrs)	1	1	5	5	9
Current symptoms	Same	Same	None	None	n/a
Δ training load	↓ 55 %	↓ 40 %	↓ 74 %	↓ 17 %	↓ 62 %
Δ performance	↓ 16 %	↓ 77 %	↓ 12 %	↓ 32 %	↓ 30 %
Skeletal muscle abnormalities	Short telomeres	NM anomalies	None	None	Structural abnormality
Clinical diagnoses ³	CF	None	None	EIA	Eating disorder, CF
Outcome	Persistent fatigue	Persistent fatigue	Stopped running	ER, ARF, still fatigued	Deceased

¹ Exposure to running prior to the onset of their intolerance to exercise

² Exercise-associated fatigue

³ In the period during which the intolerance was present

Abbreviations: EAMC – Exercise-associated muscle cramps, NM – Neuromuscular, CF – Chronic fatigue, EIA – Exercise-induced asthma, ER – Exertional rhabdomyolysis,

The development of exercise intolerance in the original group of athletes with ATI studied at this laboratory was attributed to an association between their past high volumes of endurance running and increased levels of structural pathology (75) and shortened telomeres (33) in their skeletal muscle. Of the five cases of exercise intolerant athletes presented in this study, two of whom were from the original study, only one (AE) displayed significant levels of structural abnormalities. However, this athlete had also had an eating disorder for several years which may have explained the disturbances observed in her muscle. Furthermore, only the two cases with the highest exposures to endurance running (AA and AE) had significantly shorter skeletal muscle telomeres during their respective periods of exercise intolerance compared to asymptomatic control athletes and sedentary individuals. Therefore, the observations from this study suggest that unless the manifestation of these abnormalities occurs long after the symptoms of exercise intolerance have begun, neither is fundamental to the development of exercise intolerance and are therefore not necessarily strong diagnostic tools for evaluating such cases. The observations from this study also support the findings in Chapter 5 that a large exposure to endurance running is unlikely to compromise skeletal muscle from a morphological or structural perspective. Furthermore, while telomere length may be related to exposure to endurance running, the remaining regenerative capacity of muscle in both asymptomatic and exercise-intolerant runners does not appear to have been compromised.

Despite the fact that structural abnormalities and shortened telomeres could not be attributed to all of these cases, the unusual findings in the skeletal muscle of AA and AE cannot be ignored. Perhaps measuring telomere lengths is not a sufficiently sensitive tool to gauge the extent to which muscle's remaining capacity to repair injury has been challenged. AA and AE had the highest accumulated training volumes and the shortest telomeres during their respective periods of exercise intolerance. It may be speculated that if too many of their satellite cells were in danger of reaching premature senescence, the exercise-associated fatigue experienced by these runners might have been a mechanism to reduce the stress of endurance running, thereby protecting the muscle.

The most important observations of these case studies were the presence of significant co-stressors in four of the five cases and the uniqueness of each case. The histories of the cases indicated that three of the runners may have continued training and racing while a viral infection was present; four of the cases may have had tendencies of an obsessive attitude towards exercise, one of the cases appeared to have underlying neuromuscular abnormalities, another an underlying metabolic myopathy, and one had an eating disorder. Each one of these co-stressors likely compounded the primary stressor, endurance training. Alternatively, the stress of endurance training compounded the underlying stress of the "co-factor".

Therefore, it can be speculated that while the stress of endurance training alone may not be capable of sending athletes into a state of exercise intolerance, the addition of a co-stressor may lower the resistance of runners sufficiently that they enter the state of exhaustion on Selye's model of stress response. Furthermore, patients AC and AE both demonstrated that removal of the primary stressor (endurance running) by modification of the secondary stressor (behaviour) reversed the symptoms associated with exercise intolerance such as exercise-associated fatigue and various skeletal muscle symptoms. In summary, these cases indicate that a range of co-stressors in combination with the stress of endurance running may explain why athletes develop intolerance to endurance exercise. Furthermore, understanding the unique nature of each case might help clinicians in the effective treatment of exercise intolerance in athletes.

6.5 CONCLUSION

Traditionally, acquired training intolerance in athletes was explained by myopathic changes within their skeletal muscle as a result of their high exposure to endurance running. Contrary to this concept and the original hypothesis of this study, the previous chapters in this thesis have shown that a large exposure to endurance running does not seem to threaten the state of skeletal muscle sufficiently to explain the development of exercise intolerance experienced by some athletes. Therefore, the aim of presenting these five cases of fatigued runners was to elucidate other potential stimuli for the onset of exercise intolerance in veteran athletes. In particular, this chapter aimed to better understand the factors or circumstances leading to exercise intolerance. The following observations were made from examining these case studies:

- (i) Co-stressors such as viral infection, behaviour and an underlying myopathy were determined as major contributing factors to the onset of exercise intolerance in these cases.
- (ii) The triggers for, and the manifestation of, exercise intolerance for each case was unique.
- (iii) Structural pathology and shortened telomeres in skeletal muscle are not necessarily associated with the development of exercise intolerance.

In conclusion, cases of exercise intolerance in athletes are unique. The most important lesson from these cases was that a high exposure to endurance running alone does not necessarily cause athletes of varying ages to become intolerant to exercise. Instead, the presence of a significant co-stressor is a stronger predictor for the likely development of exercise intolerance.

CHAPTER 7

SUMMARY AND CONCLUSIONS

7.1 OVERVIEW

The objective of this thesis was to examine the effects of chronic exposure to endurance running on the athlete, with particular emphasis on skeletal muscle.

7.1.1 Chapter 2

There is not much longitudinal data on the change in endurance performance of runners. Therefore the aim of this study was to examine the interaction between chronological aging and the cumulative effects of endurance racing. To this end, the longitudinal changes in performance of four age groups of runners who completed a 56 km ultra-marathon over ten successive years were analysed.

In summary, the data showed that for this 56 km ultra-endurance running race:

- (i) Performance improved and declined at greater rates for runners younger than 40 years of age.
- (ii) Runners younger than 40 years had a greater capacity to improve their performance compared to runners older than 40 years of age.
- (iii) It appears that approximately four years of racing and training are required to reach peak racing speed, regardless of age.
- (iv) It does not seem possible to sustain this peak racing speed for more than a few years.

- (v) The combined effects of ten years of chronological aging and ten years of racing neither improves nor worsens net performance in runners aged between 20 and 60 years of age.

In conclusion, these data suggest that while all four age groups of runners showed similar patterns of change in race speed over a ten year period, the extent of change in performance was greater in the runners younger than 40 years compared to the runners older than 40 years. Furthermore, while the runners who had competed in ultra-endurance races over a span of ten years (i.e. veteran runners) ran at a similar speed to novice runners of the same age, the novice runners would almost certainly still improve their performance, while the veteran runners were more likely to continue slowing down.

The impending improvement in performance of the novice runners was easier to understand as they are still likely to develop medium and long term adaptations to endurance running, as well as gain experience in training and racing which together ought to allow them to run better races. The decline in performance of the veteran runners was more difficult to explain. While it would be tempting to attribute it to aging, this is not a reasonable conclusion as the 30, 40 and 50 year old veteran runners from this study can hardly be classified as aged. Therefore, race age, or exposure to endurance running, as opposed to chronological age seems to be a more important factor. One possibility is that advancing race age may induce chronic changes in the runner that ultimately limit performance capacity. Since endurance running is known to stress skeletal muscle, three questions were addressed in this thesis in an attempt to establish whether changes in the skeletal muscle of veteran runners could explain their seemingly inevitable decline in performance.

7.1.2 Chapter 3

The aim of this study was to answer the following question: Do several years of endurance running lead to changes in either the physiological or neuromuscular characteristics of veteran runners while running? The main findings of this study were:

- (iv) The veteran runners had less EMG activity in the vastus lateralis and vastus medialis muscles during the pre-activation phase of the stride during steady state of the sub-maximal run.
- (v) The novice runners had a longer ground contact component of their stride during the warm-up phase of the sub-maximal run.
- (vi) The veteran and novice runners were indistinct with respect to the physiological and neuromuscular variables measured in this study during the time trial.

In conclusion, the difference in pre-activation phase EMG between the veteran and novice runners at a sub-maximal pace suggests that the quadriceps muscles of the veteran runners may have a decreased capacity for shock absorption. However, since there were no differences between the groups during the time trial, it is still not clear why veteran runners are almost certainly beyond achieving personal best performance times, yet novice runners are still likely to be able to improve their endurance performance. Therefore, in response to the question that this study tried to answer, it seems that several years of endurance running may well lead to neuromuscular changes within the skeletal muscle of veteran runners. However, these changes do not appear to manifest during a time trial type run, but only during the steady state phase of a sub-maximal effort.

7.1.3 Chapter 4

The aim of this study was to answer the following question: Do veteran and novice runners respond similarly to a bout of exercise-induced muscle damage? The main findings of this study were:

- (i) The downhill run caused a similar extent of exercise-induced muscle damage in both the veteran and novice runners, as shown by their levels of serum creatine kinase activity after the downhill run.
- (ii) The post-damage results showed that the recovery time courses for both groups were similar with respect to serum creatine kinase activity and muscle pain.
- (iii) One week after the insult, the running performance and the physiological and neuromuscular characteristics measured in the sub-maximal and maximal running tests were not different to the values measured before the downhill protocol in both the veteran and novice runners.

In conclusion, the veteran and novice runners in this study did respond similarly to a single bout of exercise designed to induce muscle damage. Therefore, it seems unlikely that a vulnerability to, or incomplete recovery from, exercise-induced muscle damage could explain the decline in performance observed in veteran runners who have had a high exposure to running over the years.

7.1.4 Chapter 5

The aim of this study was to answer the following question: Does a high exposure to endurance running lead to molecular changes in the skeletal muscle of veteran runners? Specifically, this study compared markers of structural pathology in and the regenerative capacity of the vastus lateralis muscle of apparently healthy

veteran endurance runners to those of sedentary controls. The main findings of this study were:

- (iv) The minimum, mean and maximum telomere lengths measured in the vastus lateralis muscle of the ATH group were similar to those of the SED group.
- (v) The minimum telomere length of the ATH group was correlated to their years in distance running and hours spent training.
- (vi) The muscle of the ATH group had more internal nuclei and greater aggregations of subsarcolemmal mitochondria compared to the SED group.

In conclusion, the findings from this study show that while exposure to endurance running may increase the demand for repair and regeneration of muscle, the remaining regenerative capacity of the muscle of veteran runners is similar to that of sedentary individuals. This suggests that the capacity of muscle to repair and regenerate in response to injury is large. However, based on the relationship between minimum telomere length and exposure to endurance running, it is tempting to speculate that with sufficient exposure to endurance running the telomeres of veteran runners may ultimately reach a critical minimum length, thereby impairing the regenerative capacity of muscle. This study also showed that the muscle of endurance trained individuals is distinct from that of a sedentary population in terms of markers of structural pathology such as internal nuclei and aggregations of subsarcolemmal mitochondria. Furthermore, since the levels of these markers measured in the muscle of the runners in this study were not associated with muscle dysfunction, they were not deemed to be pathological in nature.

In answer to the question that this study addressed, despite a relationship between exposure to endurance running and the regenerative capacity of skeletal muscle, there was no molecular evidence in the vastus lateralis muscle of the veteran runners to suggest that a high exposure to endurance exercise might adversely affect their muscle. Therefore the muscle of apparently healthy individuals aged

between 20 and 60 years appears to be a robust organ with ample capacity to repair and regenerate in response to the stress of years of endurance running.

7.1.5 Chapter 6

The evidence from chapters 3, 4 and 5 of this thesis was not able to explain conclusively the seemingly inevitable decline in performance facing veteran endurance runners irrespective of age. In particular, skeletal muscle appears to be a robust organ that comfortably tolerates the stress of a high exposure to endurance running. Therefore a different research design was adopted for this study. Five endurance runners suffering from intolerance to endurance exercise as evidenced by their exercise-associated fatigue, various skeletal muscle symptoms and an unexplained sudden decline in performance were studied. The following observations were made:

- (iv) Co-stressors such as viral infection, behaviour and an underlying myopathy were determined as major contributing factors to the onset of exercise intolerance in these cases.
- (v) The triggers for, and the manifestation of, exercise intolerance for each case were unique.
- (vi) Structural pathology and shortened telomeres in skeletal muscle are not necessarily associated with the development of exercise intolerance.

In conclusion, cases of exercise intolerance in athletes are unique. The most important lesson from these cases was that a high exposure to endurance running alone does not necessarily cause athletes of varying ages to become intolerant to exercise. Instead, the presence of a significant co-stressor is a stronger predictor for the likely development of exercise intolerance.

7.2 INTERPRETATION

The objective of this thesis was to examine the effects of chronic exposure to endurance running on the athlete, with particular emphasis on skeletal muscle. Chronic exposure to endurance running is a stressor to the athlete. An analogy can be drawn between a runner's response to this stressor during his / her athletic career and the response of a system or an organism to a stressor as described by Hans Selye in his model of stress response (Figure 7.1 A). Briefly, a novice runner takes up endurance running and for the first few weeks of training experiences the alarm reaction phase since the body has not yet adapted to the stress of this form of exercise. However, as training continues during the ensuing months the novice runner enters the resistance phase as the body adapts to running, increasing resistance to the stressor. This presumably coincides with the beginning of the runner's improvement in performance. The length of time a runner remains in the resistance phase is variable; however it is in this stage where one would expect to find most veteran runners. It may be argued that the longevity of the resistance phase is dependent on achieving the appropriate balance between training stress and recovery and regeneration. Should a runner's career be cut short, for example due to injury, the stress of endurance running would be removed and the runner would not enter the stage of exhaustion. Should the veteran runner continue, however, the model suggests that at some point resistance to the stressor would begin to decrease. This may manifest as a reduced capacity to adapt to the stimulus of training, or exercise intolerance, and would coincide with the exhaustion stage of Selye's stress response model.

This concept is supported by the longitudinal study of changes in endurance performance presented in Chapter 2 of this thesis. Figure 7.1 illustrates the analogy between the pattern of change in ultra-endurance performance of runners over a ten year span and the stress response pattern proposed by Selye. As novice runners build up resistance to the stressor, so too does their performance improve. Similarly, following a large exposure to endurance running, resistance decreases in veteran runners, coinciding with their seemingly inevitable decline in performance. Of particular interest to this thesis were the skeletal muscle factors contributing to the

decrease in resistance and corresponding decline in performance experienced by veteran runners.

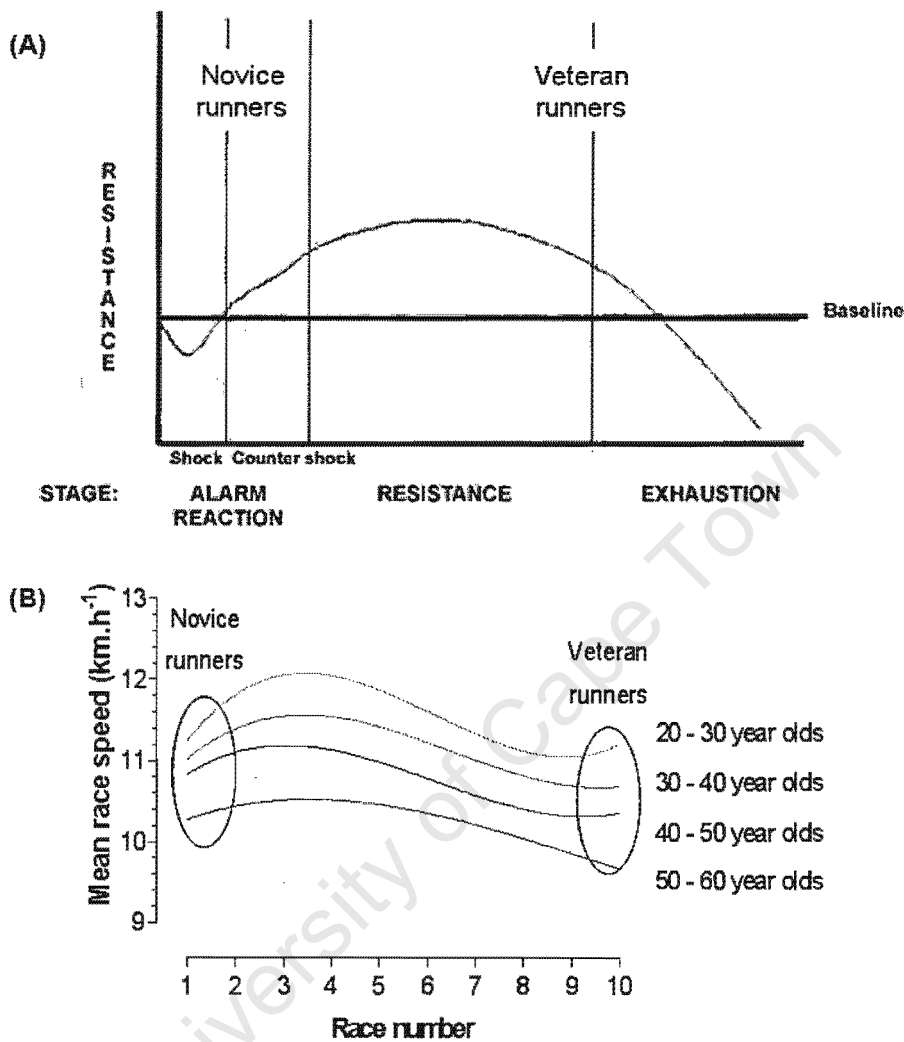


Figure 7.1 (A) Hans Selye's model of stress response and (B) the longitudinal change in ultra-endurance performance of runners in four different age groups over ten years. An analogy can be drawn between the response of an organism to a stressor and the chronic exposure of runners to the stress of endurance running. Novice runners would be in the alarm reaction stage of the model as they build resistance to the stressor through adaptation. Veteran runners ultimately experience a decrease in resistance to the stressor, as evidenced by their seemingly inevitable decline in performance. The slight upward turn of the performance traces in Figure 7.1 (B) may be an artefact of the fitting of the data to a third order polynomial. They are not necessarily indicative of improved race times of the veteran runners towards the end of the ten year span.

Therefore, it appears that the performance capacity of athletes is diminished with chronic exposure to endurance running. One explanation for the decline in performance experienced by veteran runners would be that of chronological aging (Figure 7.2). The aging-related effects on the systems and organs of the body, such as skeletal muscle, would presumably reduce the resistance of runners to the chronic stress of endurance running.

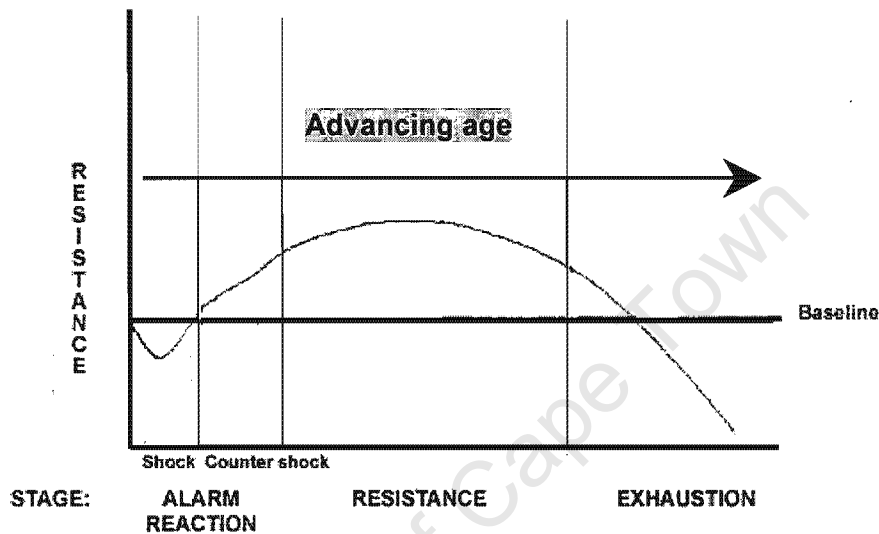


Figure 7.2 Chronological aging of endurance runners may account for their entry into the exhaustion phase of Selye's stress response model.

The findings from Chapter 2 of this thesis, however, show that the endurance performance of veteran runners decreases irrespective of age (Figure 7.1 B). Although advancing age certainly contributes to the decrease in resistance of runners to endurance exercise, as evidence by the fact that the older age groups showed smaller overall improvements in race times; chronological aging cannot be the primary cause. Some other factor common to the veteran runners studied in Chapter 2 must explain the seemingly inevitable transition to the stage of exhaustion. The obvious commonality would be that of a high exposure to endurance running (Figure 7.3), and indeed this would fit with the traditional thinking that one of the primary triggers for exercise intolerance is a prior large volume of running training and racing.

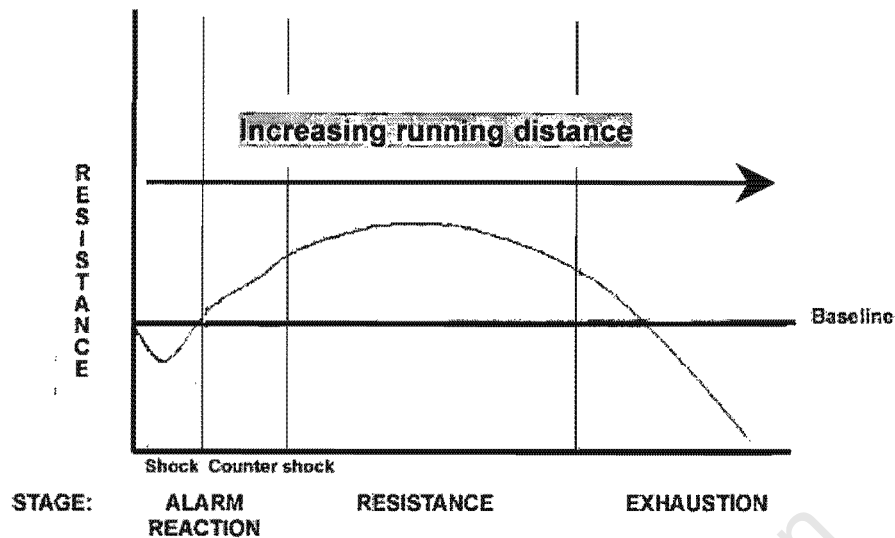


Figure 7.3 A high volume of accumulated distance in training and racing may account for endurance runners reaching the exhaustion phase of Selye's stress response model.

To lend credence to this model, a large exposure to endurance running would need to produce an effect on human endurance athletes that decreases their resistance to the stressor. For example, advancing age reduces the functional capacity of various systems of the body, thereby reducing the ability of masters athletes to tolerate the stress of endurance running. Therefore the following possible mechanisms by which a large exposure to running might decrease the resistance of the veteran runner to endurance exercise were investigated:

- (i) Changes in the physiological or neuromuscular systems of veteran runners
- (ii) An impaired ability of veteran runners to respond to and recover from exercise-induced muscle damage
- (iii) Molecular changes in the skeletal muscle of veteran runners

The findings from the studies presented in Chapters 3 and 5 of this thesis suggest two possible mechanisms to explain how a high volume of endurance running may

ultimately lead to exercise intolerance (Figure 7.4). Firstly, the vastus lateralis and vastus medialis muscles of the veteran runners displayed decreased EMG activity during the pre-activation phase of the stride, suggestive of a reduced capacity for shock absorption, when running at a sub-maximal effort (Chapter 3). Secondly, a relationship was found between total time spent training and minimum telomere length in the muscle of veteran runners. Therefore it could be hypothesised that continued exposure to the stress of endurance running may result in telomeres reaching a critical minimum length in the future, thereby reducing the remaining capacity of muscle to repair and regenerate in response to injury (Chapter 5). Further associative studies would be necessary, however, to demonstrate conclusively a relationship between either of these proposed mechanisms and the development of exercise intolerance.

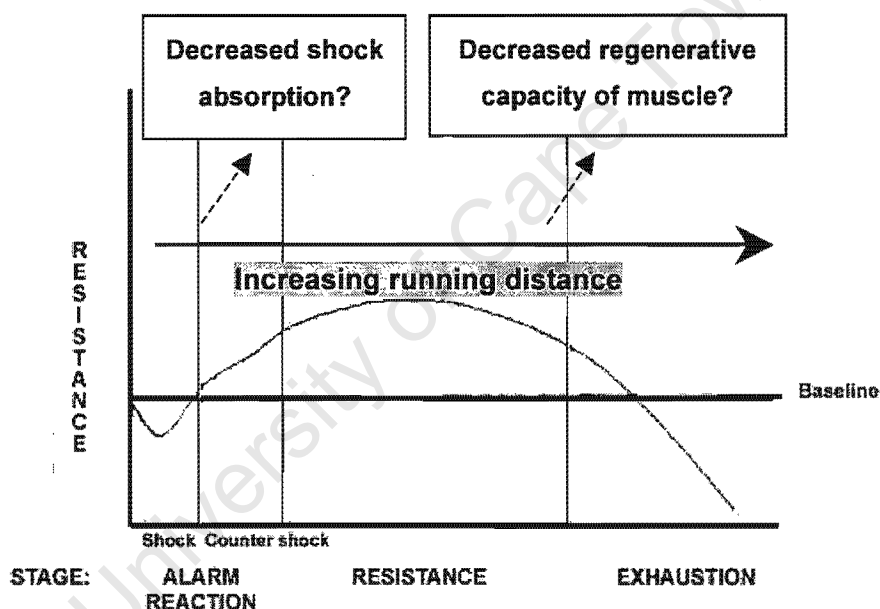


Figure 7.4 Possible mechanisms by which a large exposure to endurance running may lead to a decrease in the veteran athlete's resistance to exercise.

This thesis demonstrated that from a molecular perspective the vastus lateralis muscle of apparently healthy veteran runners (42.5 ± 6.7 years old), which had been subjected to 15 years of endurance running (65 km per week), was in no way compromised compared to the skeletal muscle of sedentary individuals. This suggests that in a healthy population, a chronic exposure to endurance running is unlikely to stress this robust organ sufficiently to explain the development of

exercise intolerance. Perhaps other systems or organs are more vulnerable to a high exposure to endurance running than skeletal muscle. Alternatively, some factor other than a large exposure to endurance running, and independent of aging, is responsible for veteran runners reaching the exhaustion stage. Furthermore, by virtue of the fact that the veteran runners studied in this thesis were still performing reasonably well with no signs of maladaptation within their vastus lateralis muscle, they may have represented a unique population of runners more resistant to the chronic stress of endurance exercise.

Therefore, Chapter 6 of this thesis investigated exercise intolerant veteran runners in an attempt to better understand the factors or circumstances by which runners might enter the exhaustion phase of Selye's stress response model. The unique nature of each case demonstrated that neither advancing age nor a large exposure to endurance running was a good predictor of the development of intolerance to exercise. Instead a number of co-stressors were likely responsible for either compounding the primary stressor (endurance running) or diminishing the runners resistance to stress (Figure 7.5).

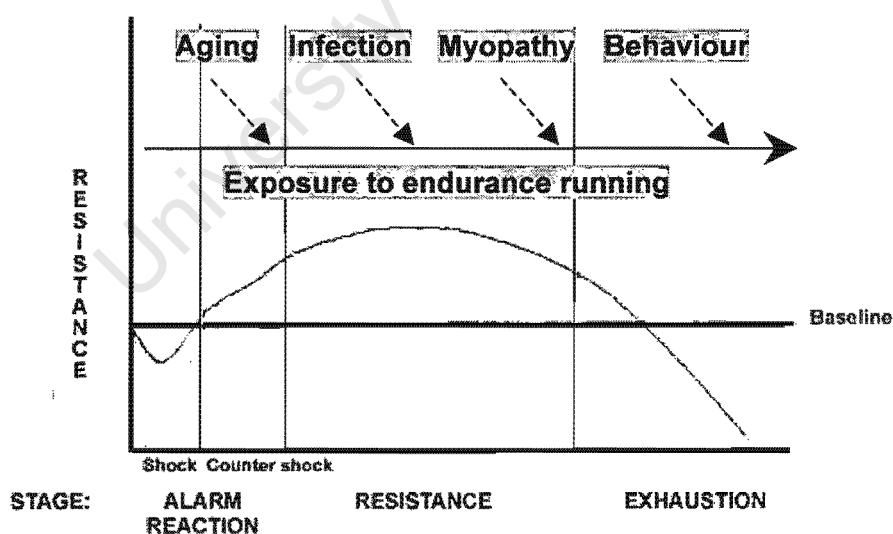


Figure 7.5 Co-stressors including aging, infection, an underlying myopathy or behaviour may compound the primary stressor, exposure to endurance running, resulting in an athlete becoming intolerant to exercise.

In conclusion, the data from this thesis showed that apparently healthy endurance runners can, under normal circumstances, tolerate a large exposure to endurance training and racing with no ill effects. That is, they comfortably remain in the resistance phase of Selye's model of stress response. Furthermore, the skeletal muscle is a robust organ with a large capacity to tolerate a chronic exposure to endurance running. While the stress of aging might explain the decline in performance experienced by masters runners, it does not explain the similar decline observed in younger runners. Similarly, since athletes with varying degrees of exposure to running may present with exercise intolerance, a large exposure to this stressor cannot be the singular cause of the reduced performance capacity of veteran athletes. Instead, the presence of co-stressors in combination with the stress of endurance running appears to be a far better predictor of the likelihood of runners developing exercise intolerance. This modifies previous thinking, which attributed maladaptations within the system of the endurance runner to a high exposure to endurance exercise.

7.3 LIMITATIONS

A limitation to the study presented in Chapter 2 was that it was assumed that the runners in each age group were relatively inexperienced ultra-marathon athletes when they ran their first 56 km race. Although the runners in the study had never run this particular 56 km race before, they may well have completed any number of other marathon or half marathon events. It was not possible, however, to check the validity of this assumption. As the data were analysed as means, it was anticipated that the few subjects deviating from this assumption would not have had a significant impact on the trends and therefore the overall interpretation.

A limitation to the studies presented in Chapters 3 and 4 was the relatively small sample size used. Therefore the data were interpreted with caution. In hindsight, the design of the study presented in Chapter 4 may also have been a limiting factor to the investigation. Changes in the performance, physiological and neuromuscular characteristics of the runners were assessed one week after the bout of exercise designed to induce muscle damage. Most endurance runners train between three and seven times per week and are therefore presumably able to recover quickly from exercise-induced muscle damage produced through training. Therefore one week may have been too long a recovery period to observe any residual changes associated with exercise-induced muscle damage in healthy individuals.

The first limitation to the study presented in Chapter 5 was that the SED group contained smokers. Since smoking is known to affect telomere length in leukocytes, its potential effect on skeletal muscle telomeres cannot be ignored. The second limitation was that three of the subjects (two athletes and one sedentary individual) reported having depression. This may be a confounding factor since telomere lengths of individuals with mood disorders have been shown to be shorter compared to asymptomatic controls. Both of these limitations were addressed statistically by using an analysis of co-variance. Thirdly, since it was not possible to analyse all of the biopsy samples for fibre type distribution and levels of structural pathology due to technical difficulties, the sample sizes for these factors were reduced.

7.4 FUTURE RESEARCH

Future research needs to explore the two proposed mechanisms by which a large exposure to endurance running may contribute to the decline in performance facing veteran runners. Firstly, the neuromuscular characteristics of otherwise healthy veteran runners who are clearly in the decline phase of their running careers could be compared to those of runners who are still performing close to their peak capacity. Secondly, the relationship between skeletal muscle minimum telomere length and exposure to running needs to be investigated further. For example, the effect of shorter telomeres on the remaining replicative capacity of the satellite cells in muscle could be examined.

A study designed to investigate the effect of oxidative damage as a result of endurance exercise on both telomere length and the proliferative capacity of satellite cells would also be useful. Furthermore, a comparison between the muscles directly involved in running (for example, vastus lateralis) versus those not (for example, deltoid) would provide insight as to the specificity of the impact of a chronic exposure to endurance running on the musculature of a runner. Finally, other systems or organs potentially involved in the decline in performance facing veteran runners could be investigated.

7.5 CLINICAL IMPLICATIONS

While many apparently healthy runners appear to tolerate a large exposure to endurance running with few ill effects, athletes presenting with an unexplained intolerance to endurance exercise are cause for concern. The findings from this thesis have clinical implications for any number of people coming into contact with exercise intolerant athletes including physicians, exercise physiologists, physiotherapists, dieticians, psychologists, exercise trainers, coaches and not least of all, the athletes themselves. Exercise intolerance is an important clinical symptom and should not be trivialised. Although broad in nature, it may well indicate a serious underlying condition. Recognising that co-stressors such as acute infection, behaviour or an underlying myopathy may compound the stress of chronic endurance training and racing is important. Therefore a detailed case history surrounding the onset of exercise intolerance as well as the athlete's habits relating to exercise is also recommended to identify possible co-stressors. In cases where behaviour is a likely co-stressor, the athlete needs to be counselled as to the importance of balancing rest and recovery with the stimulus of training. In cases where exercise intolerance is persistent, does not reverse with adequate rest and symptoms are worsened with exercise, the existence of an underlying myopathy should be further investigated. In conclusion, since cases presenting with exercise intolerance appear to be heterogeneous in nature, a multi-disciplinary approach is appropriate for the successful treatment of these athletes.

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University of Cape Town

APPENDIX 1



DEPARTMENT OF HUMAN BIOLOGY
FACULTY OF HEALTH SCIENCES

UNIVERSITY OF CAPE TOWN



UCT / MRC RESEARCH UNIT FOR EXERCISE
SCIENCE AND SPORTS MEDICINE



ACQUIRED TRAINING INTOLERANCE RESEARCH STUDY

<i>CONTENTS</i>	<i>ACTION</i>
Consent Form	ALL subjects to read and sign
Personal Particulars	ALL subjects to complete
Medical History	ALL subjects to complete
Body Composition Assessment	ALL subjects to be assessed
Social Sport/Activity Participation	ALL subjects to complete
Competitive Sport Training	ALL subjects to complete

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INFORMED CONSENT

ALL subjects to be read and sign
Delete and sign phrases not applicable

I, the undersigned, have been fully informed about the UCT/MRC Research Unit for Exercise Science and Sports Medicine within the Department of Human Biology, University of Cape Town study on the Acquired Training Intolerance (ATI). I agree to complete a questionnaire disclosing my personal details and information relating to my medical, racing and training history and psychological state. I understand that all the information that is collected during the study will be treated confidentially, will only be used for scientific research purposes and that my name and personal particulars will not be released under any circumstances.

I understand that the following measurements / tests **may** be conducted on me during this study, as described in the preceding General Information section:

All subjects:

- Body composition assessment - Height, weight, skinfold, girth and diameter measurements.
- VO₂ max test (treadmill) - An incremental running test in which females begin at 10 km/hr and males begin at 12 km/hr. The speed of the treadmill will be increased by 0.5 km/hr every 30 seconds. The test will end when the subject decides he / she is unable to complete a 30 second stage.
- Muscle power test – 5 squats to be performed as fast as possible.
- Submaximal run test - A 15 minute run at 70 % of peak treadmill running speed on an indoor track.
- 5km run time trial - A 5 km run to be completed as fast as possible on an indoor track.
- Downhill run – A 40 minute downhill run on a treadmill at a slope of -10°. Running speed will be at 70% of peak treadmill running speed.
- Blood sample - Approximately 25 ml of blood will be drawn from the ante-cubital (forearm) vein on 8 occasions.
- 4-minute saliva sample will be collect on 8 occasions.

I have been fully informed about the risks inherent in participation in this trial. I understand that downhill run to be performed in this study is intended to induce muscle damage and that my legs may be sore and tender for about one week after the run. I understand that the drawing of blood from the antecubital (forearm) vein is invasive and has certain risks.

I understand that my blood samples will only be used for the purposes explained to me, namely to measure markers of exercise-induced muscle damage and markers of inflammatory responses. I also understand that some of the blood samples and the saliva samples will be sent to our collaborators (Prof Lucille Smith) at the Department of Sports and Physical Rehabilitation Sciences within the Faculty of Health Sciences at Technikon Pretoria to the analysis of markers of inflammatory responses.

I agree to participate in the study and I have been informed that I will be free to withdraw from the study at any time if I so wish. I also understand that I will be free to request that my samples are destroyed before the completion of the study. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive, where applicable, an estimate of my percentage body fat, VO_2 max and fibre type upon completion of the study if I should desire it.

Subject's name: _____

Signature: _____

Investigator's name: _____

Signature: _____

Witness's name: _____

Signature: _____

Date: _____

PERSONAL PARTICULARS**ALL** subjects to complete

Surname: _____

First name(s): _____

Postal address: _____

Code: _____

E-mail address: _____

Day time phone number: () _____

Cell phone number: _____

Date of birth (dd/mm/yy): _____

Gender (M or F): _____

Height (m): _____ Weight (kg): _____

Major sport: _____

Smoker (please circle): No, never Yes, ex Yes, current

If yes, for how many years did you smoke? _____

If yes, how many cigarettes did you smoke per day? _____

If you have stopped smoking, when did you stop? _____

MEDICAL HISTORY

ALL subjects to complete

1. Cardiac and / or chronic disease

Have you ever been diagnosed with any of the following diseases? (Please tick)

<input type="checkbox"/> Coronary heart disease	<input type="checkbox"/> High blood pressure	<input type="checkbox"/> Cancer
<input type="checkbox"/> Psychiatric abnormalities	<input type="checkbox"/> Epilepsy	<input type="checkbox"/> Asthma
<input type="checkbox"/> Renal disease	<input type="checkbox"/> Tuberculosis	<input type="checkbox"/> Diabetes
<input type="checkbox"/> High cholesterol	<input type="checkbox"/> Stroke	

Other diseases: _____

Comment: _____

Briefly describe the **intervention** used to treat this disease.

Disease	Intervention	Date
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Please describe the **specialist** you saw for this disease.

Disease	Specialist	Date
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

2. Family History

Does anybody in your family suffer from any of the following conditions?

(Please use the following abbreviations: F = father M = mother

GF = grandfather GM = grandmother)

<input type="checkbox"/> Coronary heart disease	<input type="checkbox"/> High blood pressure	<input type="checkbox"/> Cancer
<input type="checkbox"/> Psychiatric abnormalities	<input type="checkbox"/> Epilepsy	<input type="checkbox"/> Asthma
<input type="checkbox"/> Renal disease	<input type="checkbox"/> Tuberculosis	<input type="checkbox"/> Diabetes
<input type="checkbox"/> High cholesterol	<input type="checkbox"/> Stroke	

3. Cardiovascular history

Have you ever experienced the following? (Please tick)

<input type="checkbox"/> Chest pain at rest	<input type="checkbox"/> Chest pain during exercise
<input type="checkbox"/> Dizziness during exercise	<input type="checkbox"/> Fainting spells
<input type="checkbox"/> Excessive shortness of breath	<input type="checkbox"/> Palpitations
<input type="checkbox"/> Previous heart complaints	<input type="checkbox"/> History of Rheumatic fever
<input type="checkbox"/> High blood pressure	<input type="checkbox"/> High blood cholesterol

Other cardiovascular complaints: _____

Comment: _____

4. Respiratory history

Have you ever experienced the following? (Please tick)

<input type="checkbox"/> Asthma	<input type="checkbox"/> Wheezing during exercise
<input type="checkbox"/> Cough during exercise	<input type="checkbox"/> Post nasal drip
<input type="checkbox"/> Allergies / hay fever	<input type="checkbox"/> Previous lung complaints
<input type="checkbox"/> Repeated infections in the respiratory tract (ear, nose, throat)	

Other respiratory complaints: _____

Comment: _____

5. Gastrointestinal history

Have you ever experienced the following? (Please tick)

- | | |
|---|---|
| <input type="checkbox"/> Heartburn | <input type="checkbox"/> Abdominal pain |
| <input type="checkbox"/> Weight loss/gain (>5kg) | <input type="checkbox"/> Change in bowel habits |
| <input type="checkbox"/> Liver / gall bladder disease | <input type="checkbox"/> Blood in stool |
| <input type="checkbox"/> Abdominal complaints during exercise | <input type="checkbox"/> Diarrhoea |

Other gastrointestinal complaints: _____

Comment: _____

6. Central Nervous System History

- | | |
|---|--|
| <input type="checkbox"/> Headache | <input type="checkbox"/> Epilepsy |
| <input type="checkbox"/> Blackouts | <input type="checkbox"/> Muscle weakness |
| <input type="checkbox"/> Muscle cramps during exercise | <input type="checkbox"/> Chronic fatigue |
| <input type="checkbox"/> Head injury with loss of consciousness | |

Other central nervous system complaints: _____

Comment: _____

7. Metabolic disease history

Have you ever experienced the following? (Please tick)

- | | |
|--|---|
| <input type="checkbox"/> Diabetes mellitus | <input type="checkbox"/> Thyroid complaints |
| <input type="checkbox"/> Hypoglycaemia (low blood sugar) | <input type="checkbox"/> Heat intolerance |

Other metabolic diseases: _____

Comment: _____

8. Dermatological history

Have you ever experienced the following? (Please tick)

 Skin rashes Allergies Skin cancer

Other skin complaints: _____

Comment: _____

_____**9. Gynaecological history (females)**

Have you ever experienced the following? (Please tick)

 No menstruation Irregular menstruation Excessive bleeding Dysmenorrhoea

Other gynaecological complaints: _____

Comment: _____

_____**10. Joint injury / disease history**

Have you ever experienced the following? (Please tick)

 Osteoarthritis Rheumatoid arthritis Gout Lower back pain

Other joint conditions: _____

Comment: _____

11. Running injuries

Have you suffered from any **injury caused by running** in the past that caused you to stop training for a time? (Please circle) **Yes** **No**

If yes, please describe the injury, date of injury and treatment you received.

Injury	Date	Treatment
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

12. Over-training

Do you **currently** suffer from any of the symptoms listed below? (Please tick)

- | | |
|-------------------------------|---|
| _____ 'Heavy legs' | _____ Increased waking pulse rate |
| _____ Lack of motivation | _____ Decreased enjoyment of exercise |
| _____ Sleep disorders | _____ Painful skeletal muscles |
| _____ Dizziness upon standing | _____ Frequent infections |
| _____ Weight loss | _____ Depression |
| _____ Decreased libido | _____ Increased effort during training (with no improvement in performance) |

Did you suffer from any of the symptoms listed below **prior to the onset of the symptom** that lead you to volunteer for this trial? (Please tick)

- | | |
|-------------------------------|---|
| _____ 'Heavy legs' | _____ Increased waking pulse rate |
| _____ Lack of motivation | _____ Decreased enjoyment of exercise |
| _____ Sleep disorders | _____ Painful skeletal muscles |
| _____ Dizziness upon standing | _____ Frequent infections |
| _____ Weight loss | _____ Depression |
| _____ Decreased libido | _____ Increased effort during training (with no improvement in performance) |

13. Previous operations

Please describe any previous operations you have had and the date that they took place.

Operation	Date
_____	_____
_____	_____
_____	_____

14. Diet

How many meals to you have per day? (Please circle)

1 2 3 4 5 6 7 >7

Do you ever skip meals? **Yes** **No**

If yes, how often do you skip meals? (Please circle)

<1 per week 3-4 times per week once per day >2 per day

How much water do you consume per day? (Please circle)

<0.5L 0.5-1L 1-2L 2-3L 3-4L 4-5L >5L

Do you **currently** take any nutritional supplements? **Yes** **No**

If yes, please describe the supplement, dose and length of time you have been taking it for.

Supplement taken	Dose	Time
_____	_____	_____
_____	_____	_____
_____	_____	_____

Have you taken any nutritional supplements in the **past** that you are no longer taking? **Yes** **No**

BODY COMPOSITION ASSESSMENT

ALL subjects to be assessed

Subject name: _____

Weight (kg) _____

Height (cm)

Stature _____

Subgluteal-knee _____

Skinfolds (mm)

	1	2	ave		1	2	ave
Triceps	_____	_____	_____	Calf	_____	_____	_____
Biceps	_____	_____	_____	Thigh	_____	_____	_____
Subscap	_____	_____	_____	Abdominal	_____	_____	_____
Suprailiac	_____	_____	_____	Sum of 7:	_____	_____	_____

Diameter (cm)

Humerus _____

Trans chest _____

Femur _____

A-P chest _____

Bi-acromial _____

Bi-iliac _____

Girth (cm)

Relaxed arm _____

Above knee _____

Con. arm _____

Abdominal _____

Calf _____

Forearm _____

Chest _____

Wrist _____

Sub-gluteal _____

Ankle _____

Mid-thigh _____

Bi-trochanter _____

Comments _____

SOCIAL SPORT / ACTIVITY PARTICIPATION

To be completed by **ALL** subjects

Please list any **activities** in which you have participated regularly in the past, and estimate for how long and how often you participated.

	Age period								
	15 – 20 years			20 – 25 years			25 – 30 years		
Sport / Activity	years	month s / year	hours / week	years	month s / year	hours / week	years	month s / year	hours / week

	Age period								
	30 – 35 years			35 – 40 years			40 – 45 years		
Sport / Activity	years	month s / year	hours / week	years	month s / year	hours / week	years	month s / year	hours / week

	Age period								
	45 – 50 years			50 – 55 years			> 55 years		
Sport / Activity	years	month s / year	hours / week	years	month s / year	hours / week	years	month s / year	hours / week

Examples of sporting activities include:

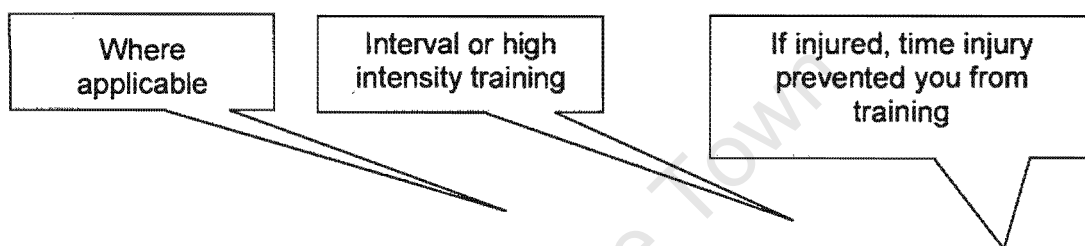
- Aerobic dance / step
- Badminton
- Canoeing
- Cycling
- Dance
- Football / soccer
- Golf
- Jogging
- Hiking
- Martial arts
- Netball
- Rock climbing
- Rugby
- Squash
- Strength and resistance training
- Swimming
- Tennis
- Volleyball
- Walking

COMPETITIVE SPORT TRAINING

To be completed by ALL subjects

Please complete the following table for each **competitive sport** that you have participated in regularly over the past **ten years**. If you have competed for **more than 10 years**, please use the next table for the additional years.

Sporting activity: _____ Age at which activity began: _____



Year	Months / year	Days / week	Average hours / week	Average distance / week (km)	Races or matches / year	Interval training		Injury (time no training)
						yes	no	
2004								
2003						yes	no	
2002						yes	no	
2001						yes	no	
2000						yes	no	
1999						yes	no	
1998						yes	no	
1997						yes	no	
1996						yes	no	
1995						yes	no	
1994						yes	no	

Comments: _____

Sporting activity: _____ Age at which activity began: _____

Year	Months / year	Days / week	Average hours / week	Average distance / week (km)	Races or matches / year	Interval training		Injury (time no training)
						yes	no	
2004								
2003						yes	no	
2002						yes	no	
2001						yes	no	
2000						yes	no	
1999						yes	no	
1998						yes	no	
1997						yes	no	
1996						yes	no	
1995						yes	no	
1994						yes	no	

Comments: _____

Sporting activity: _____ Age at which activity began: _____

Year	Months / year	Days / week	Average hours / week	Average distance / week (km)	Races or matches / year	Interval training		Injury (time no training)
						yes	no	
2004								
2003						yes	no	
2002						yes	no	
2001						yes	no	
2000						yes	no	
1999						yes	no	
1998						yes	no	
1997						yes	no	
1996						yes	no	
1995						yes	no	
1994						yes	no	

Comments: _____

APPENDIX 2

	<p>DEPARTMENT OF HUMAN BIOLOGY FACULTY OF HEALTH SCIENCES <i>UNIVERSITY OF CAPE TOWN</i></p>
	<p>UCT / MRC Research Unit FOR Exercise SCIENCE AND SPORTS MEDICINE</p> 

ACQUIRED TRAINING INTOLERANCE RESEARCH STUDY

<i>CONTENTS</i>	<i>ACTION</i>
Running Training	ATI and ATH subjects to complete
Running Performance	ATI and ATH subjects to complete
Racing Volume	ATI and ATH subjects to complete
Over-Training Diary	ATI and ATH subjects to complete
Retrospective Running Training Diary	ATI and ATH subjects to complete

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RUNNING TRAINING

To be completed by **ATI and ATH** subjects

- At what age did you start running > 40 km/week? _____ **years**
- For how many years did you train before you noticed problems which led you to volunteer for this trial? _____ **years**
- How long has it been since you were last able to run at your perceived optimal training **volume**? _____ **months** _____ **years**
- How long has it been since you were last able to run at your perceived optimal training **intensity**? _____ **months** _____ **years**
- On average, how many days a week did you train for the three months prior to your best marathon performance? (Please circle)
 1 2 3 4 5 6 7
- On average, how many days a week did you train for the three months prior to your performance deterioration? (Please circle)
 1 2 3 4 5 6 7
- How many days a week do you train at present? (Please circle)
 1 2 3 4 5 6 7
- What was your average weekly training distance for the three months prior to your best marathon performance? _____ **km**
- What was your average weekly training distance for the three months prior to your performance deterioration? _____ **km**
- What is your average weekly training distance at present? _____ **km**
- What was your average training speed for the three months prior to your best marathon performance? _____ **min/km**

- What was your average training speed for the three months prior to your performance deterioration? _____ **min/km**

- What is your average training speed prior at present? _____ **min/km**

- What was your best 5km time trial time prior to your performance deterioration? _____ **minutes**

- How did you rank yourself against the same aged 5 km runners? (Please circle)

Within the top:	20%	40%	60%	80%
-----------------	------------	------------	------------	------------

- When did you achieve this time? _____ **year**

- What was your best 5km time trial time in the month preceding the start of this trial? _____ **minutes**

- How did you rank yourself against the same aged 5 km runners? (Please circle)

Within the top:	20%	40%	60%	80%
-----------------	------------	------------	------------	------------

- When you were running at your peak, how many times a week did you train specifically for:

(Please circle)

• speed (fartlek / sprints)	1	2	3	4	5	6	7
• endurance (long runs)	1	2	3	4	5	6	7
• strength (gymnasium)	1	2	3	4	5	6	7
• flexibility (stretching)	1	2	3	4	5	6	7

- After your performance deterioration, how many times a week did you train specifically for:

(Please circle)

• speed (fartlek / sprints)	1	2	3	4	5	6	7
• endurance (long runs)	1	2	3	4	5	6	7
• strength (gymnasium)	1	2	3	4	5	6	7
• flexibility (stretching)	1	2	3	4	5	6	7

- In your opinion, what caused your decline in running performance? (Please tick)

- Too much training
- Viral infection
- Too much stress
- Poor nutrition
- Some unknown medical condition
- Other

If other, explain: _____

- When did you feel you were no longer capable of reaching your personal best time for a:

5km _____ (Date) or I still believe I can run a personal best

10km _____ (Date) or I still believe I can run a personal best

21.1km _____ (Date) or I still believe I can run a personal best

42.2km _____ (Date) or I still believe I can run a personal best

Two Oceans _____ (Date) or I still believe I can run a personal best

Comrades _____ (Date) or I still believe I can run a personal best

- Can you identify a race or event that seemed to be associated with your decline in running performance over the years?

No

Absolutely yes Year: _____ Race / event: _____

Maybe Year: _____ Race / event: _____

RUNNING PERFORMANCE

To be completed by ATI and ATH subjects

Year	Best 5km (min:sec)	Best 10km (min:sec)	Best 21.1km (hr:min:sec)	Best 42.2km (hr:min:sec)	Two Oceans (hr:min:sec)	Comrades (hr:min:sec)
2004						
2003						
2002						
2001						
2000						
1999						
1998						
1997						
1996						
1995						
1994						
1993						
1992						
1991						
1990						
1989						
1988						
1987						
1986						
1985						
1983						
1982						
1981						
1980						
1979						
1978						
1977						
1976						
1975						
1974						
1973						

RACING VOLUME

To be completed by ATI and ATH subjects

- Please complete the following table to document the number and distance of the races that you have participated in over your running career.
- Please distinguish between the **type** of race, for example, triathlon or cycle tour.
- If you took part in triathlons, biathlons or duathlons, document the total distance of each discipline (S = swim, R = run, C = cycle).
- If you are unsure, please do not guess but rather indicate that you are unsure or can't remember.

Year	Triathlons		Biathlons		Duathlons		Swimming		Cycling		Running	
	No.	Total dist.	No.	Total dist.	No.	Total dist.	No.	Total dist.	No.	Total dist.	No.	Total dist.
2004		S: C: R:		S: R:		R: C:						
2003		S: C: R:		S: R:		R: C:						
2002		S: C: R:		S: R:		R: C:						
2001		S: C: R:		S: R:		R: C:						
2000		S: C: R:		S: R:		R: C:						
1999		S: C: R:		S: R:		R: C:						
1998		S: C: R:		S: R:		R: C:						
1997		S: C: R:		S: R:		R: C:						
1996		S: C: R:		S: R:		R: C:						
1995		S: C: R:		S: R:		R: C:						

Year	Triathlons		Biathlons		Duathlons		Swimming		Cycling		Running	
	No.	Total dist.	No.	Total dist.	No.	Total dist.	No.	Total dist.	No.	Total dist.	No.	Total dist.
1994		S: C: R:		S: R:		R: C:						
1993		S: C: R:		S: R:		R: C:						
1992		S: C: R:		S: R:		R: C:						
1991		S: C: R:		S: R:		R: C:						
1990		S: C: R:		S: R:		R: C:						
1989		S: C: R:		S: R:		R: C:						
1988		S: C: R:		S: R:		R: C:						
1987		S: C: R:		S: R:		R: C:						
1986		S: C: R:		S: R:		R: C:						
1985		S: C: R:		S: R:		R: C:						
1984		S: C: R:		S: R:		R: C:						
1983		S: C: R:		S: R:		R: C:						
1982		S: C: R:		S: R:		R: C:						
1981		S: C: R:		S: R:		R: C:						
1980		S: C: R:		S: R:		R: C:						

OVER-TRAINING DIARY

To be completed by ATI and ATH subjects

Instructions to athlete:

- a. Complete the over-training diary for a period of seven consecutive days
- b. Use one row per day
- c. The following explains how to measure or rate each component of the diary:

Waking heart rate:

Measure your heart rate each morning before getting out of bed. After waking, continue to lie in bed, relaxed for 5 min, count your pulse for 30 seconds. Multiply that number by 2 and record this value in your diary.

Sleep rating:

Document the quality of the previous nights sleep according to the following scale:

- 1 – uninterrupted sleep, feeling refreshed
- 2 – interrupted sleep, not requiring more sleep
- 3 – interrupted sleep, requiring more sleep
- 4 – interrupted sleep, feeling terrible

Postural dizziness score:

This refers to the dizziness experienced when getting up suddenly from a lying or sitting position. Monitor this each day as you get out of bed by standing up suddenly:

- 1 – no dizziness
- 2 – some dizziness, but disappears within 2 seconds
- 3 – marked dizziness, having to lie down again

Body mass:

Measure your body mass using the same scale every second morning after getting out of bed.

Symptom score:

Record the number if you have suffered from any of the following symptoms in the past 24 hours:

- 1 – sore throat
- 2 – cough
- 3 – nausea or vomiting
- 4 – diarrhoea
- 5 – stomach ache
- 6 – headache
- 7 – fatigue
- 8 – other

Muscle stiffness score:

Record any skeletal muscle stiffness or pain which you experience as a result of your training:

- 1 – no stiffness or pain
- 2 – mild stiffness and pain, not affecting workout
- 3 – moderate muscle stiffness and pain, able to complete workout with difficulty
- 4 – severe muscle stiffness and pain, unable to complete workout

Training detail record:

Record the duration and type of exercise.

Training effort:

- 1 – felt good during the entire workout
- 2 – neither hard nor easy, felt good during some parts of the workout
- 3 – somewhat difficult, felt a little tired
- 4 – very difficult, had difficulty completing the workout

General depression rating:

- 1 – no depression, good mood
- 2 – mild depression, feeling down or sad only at times
- 3 – moderate depression, feeling down or sad most times
- 4 – severe depression, feeling down or sad all of the time

RUNNING TRAINING DIARY

To be completed by **ATI** and **ATH** subjects

Instructions to athlete:

- Complete this retrospective running training diary for **two** 8 week periods.

Period 1: The running training that you did in the **8 weeks prior** to consenting to **participate in this study**.

Period 2: The running training you did in the **8 weeks before** you ran your **personal best marathon time**.

- Use one paragraph per training session.
- You may have more than one training session per day.
- Type of session refers to either a continuous run (C), an interval session (I) or a time trial (TT).
- Please record the duration of the session in minutes and estimate the distance covered during the session in kilometres.
- The effort of the session is the how hard you found the session according the perception of effort during training scale. This scale is at the back of this book.
- Write any comments you may have regarding the session in the space provided.

Example

Date: 01/01/02 (dd/mm/yy) Type of session: C C, I, TT)

Duration: 90 min (min) Approx. distance: 25 km (km)

Effort: 4 (according to effort scale)

Comments: _____

Period 1: The 8 weeks of running training done PRIOR to consenting TO PARTICIPATING in this study.

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)
Duration: _____ (min) Approx. distance: _____ (km)
Effort: _____ (according to effort scale)
Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)
Duration: _____ (min) Approx. distance: _____ (km)
Effort: _____ (according to effort scale)
Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)
Duration: _____ (min) Approx. distance: _____ (km)
Effort: _____ (according to effort scale)
Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)
Duration: _____ (min) Approx. distance: _____ (km)
Effort: _____ (according to effort scale)
Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)
Duration: _____ (min) Approx. distance: _____ (km)
Effort: _____ (according to effort scale)
Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)
Duration: _____ (min) Approx. distance: _____ (km)
Effort: _____ (according to effort scale)
Comments: _____

Period 2: The 8 weeks of running training done PRIOR to running your PERSONAL BEST marathon time

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)

Duration: _____ (min) Approx. distance: _____ (km)

Effort: _____ (according to effort scale)

Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)

Duration: _____ (min) Approx. distance: _____ (km)

Effort: _____ (according to effort scale)

Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)

Duration: _____ (min) Approx. distance: _____ (km)

Effort: _____ (according to effort scale)

Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)

Duration: _____ (min) Approx. distance: _____ (km)

Effort: _____ (according to effort scale)

Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)

Duration: _____ (min) Approx. distance: _____ (km)

Effort: _____ (according to effort scale)

Comments: _____

Date: _____ (dd/mm/yy) Type of session: _____ (C, I, TT)

Duration: _____ (min) Approx. distance: _____ (km)

Effort: _____ (according to effort scale)

Comments: _____

PERCEPTION OF EFFORT DURING TRAINING SCALE

Score	English	Afrikaans	Xhosa	Zulu
0	Rest	Rus	Ukuphumla	Phumula
1	Really easy	Baie maklik	ilula kakhulu	Kulula kakhulu
2	Easy	Maklik	(i)lula	Kulula
3	Moderate	Matig	Phakathi	Kulula kahle
4	Sort of hard	Effens moeilik	Inobonzima	Kululi – khuni nje!
5	Hard	Moeilik	Inzima	
6	HARD!	MOEILIK	INZIMA	Kulikhuni kabi
7	VERY HARD!	BAIE MOEILIK	INZIMA KAKHULU	KULIKHUNI KAKHULU
8	The coach tried to kill us!	Die afrigter wou ons doodmaak	Umqeqeshi ubezama ukusibulala	Umqeqeshi ubezama ukusibulala
9	I feel like death warmed over!	Ek voel asof 'n trein my getrap het	Ndiva ngathi ukufa kunifikele	Ngizwa sengathi ngiyafa
10	Oh!	Ai!		Ngiyafa manje!

- Use this scale to complete the running training diary.

APPENDIX 3**BORG'S RPE SCALE**

0	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

BORG'S RPE SCALE INSTRUCTIONS

While exercising we want you to rate your perception of exertion, i.e., how heavy and strenuous the exercise feels to you. The perception of exertion depends mainly on the strain and fatigue in your muscles and on your feeling of breathlessness or aches in the chest.

Look at this rating scale; we want you to use this scale from 6 to 20, where 6 means "no exertion at all" and 20 means "maximal exertion".

- 9 corresponds to "very light" exercise. For a normal, healthy person it is like walking slowly at his or her own pace for some minutes.
- 13 on the scale is "somewhat hard" exercise, but it still feels OK to continue.
- 17 "very hard" is very strenuous. A healthy person can still go on, but he or she really has to push him- or herself. It feels very heavy, and the person is very tired.
- 19 on the scale is an extremely strenuous exercise level. For most people this is the most strenuous exercise they have ever experienced.

Try to appraise your feeling of exertion as honestly as possible, without thinking about what the actual physical load is. Don't underestimate it, but don't overestimate it either. It's your own feeling of effort and exertion that's important, not how it compares to other people's. What other people think is not important either.

Look at the scale and the expressions and then give a number.

Any questions?

APPENDIX 4**Subjective Pain Scale**

0	No pain
1	Very very slight
2	Very slight
3	Slight
4	Mild
5	Moderate
6	Moderate-severe
7	Severe
8	Very severe
9	Very very severe
10	Maximal pain

APPENDIX 5

INFORMED CONSENT

ALL subjects to be read and sign

Delete and sign phrases not applicable

I, the undersigned, have been fully informed about the UCT/MRC Research Unit for Exercise Science and Sports Medicine within the Department of Human Biology, University of Cape Town study on the Acquired Training Intolerance (ATI). I agree to complete a questionnaire disclosing my personal details and information relating to my medical, racing and training history and psychological state. I understand that all the information that is collected during the study will be treated confidentially, will only be used for scientific research purposes and that my name and personal particulars will not be released under any circumstances.

I understand that the following measurements / tests **may** be conducted on me during this study, as described in the preceding General Information section:

All subjects (ATI, ATH, SED, ELD):

- Medical examination – If required, to be performed by a Sports Physician
- Body composition assessment - Height, weight, skinfold, girth and diameter measurements.
- Blood sample - Approximately 5 ml of blood will be drawn from the ante-cubital (forearm) vein during the medical examination.
- Muscle biopsy - Approximately 100 mg of muscle will be removed from the vastus lateralis (outer side of thigh) muscle.
- Muscle biopsy - Approximately 100 mg of muscle will be removed from the deltoid (shoulder) muscle.

I have been fully informed about the risks inherent in participation in this trial. I understand that the drawing of blood from the antecubital (forearm) vein and the taking of a muscle biopsy are invasive and have certain risks. I understand that my blood samples will only be used for the purposes explained to me, namely to measure DNA telomere length. My muscle sample will be used to examine the ultra-structure and pathology of the muscle, to determine the number of satellite cells

(precursor cells to muscle cells that are activated when the muscle cells are damaged) present, to measure DNA telomere (specialised structures at the ends of the chromosomes) length. I understand that my DNA sample will be destroyed on completion of the study on the Acquired Training Intolerance.

I agree to participate in the study and I have been informed that I will be free to withdraw from the study at any time if I so wish. I also understand that I will be free to request that my samples are destroyed before the completion of the study. I will be free to ask any questions about the procedures and results of the study. I understand that I will receive, where applicable, an estimate of my percentage body fat and fibre type upon completion of the study if I should desire it.

Subject's name: _____

Signature: _____

Investigator's name: _____

Signature: _____

Witness's name: _____

Signature: _____

Date: _____

APPENDIX 6

ADENOSINE TRIPHOSPHATASE

Solution A, pH 9.4 (Veronal Buffer)

0.1M Sodium Barbitone (MW 206.18; 20.62g/L)	12.5 ml
0.18M Calcium Chloride (fused granular MW 110.99; 9.99g/500ml)	12.5 ml
Distilled water	37.5 ml

Adjust to pH 9.4 with 0.1M NaOH or 0.1N HCL

**This is not a strong buffer so use quite a dilute HCL to bring the pH down.

Solution B

0.2N Acetic acid
(1.2 ml / 100ml distilled water)

Solution C

0.2M Sodium Acetate
(Anhydrous salt MW 82.03; 16.4g / 1L)

For 0.2M Acetate buffer at pH 4.3

20ml Solution B
10ml Solution C

For 0.2M Acetate buffer at pH 4.6

10ml solution B
20ml solution C

Adjust pH with NaOH or HCL. I find these buffers quite hardy so use quite concentrated HCL to bring them to the correct pH.

ATP solution, pH 9.4 (substrate incubating solution)

0.1M sodium barbitone	5ml
0.18M calcium chloride	2.5ml
Distilled water	17.5ml
ATP (disodium salt) (MW: 551.1)	0.075g

Adjust pH with NaOH. Use a relatively concentrated NaOH initially but once get closer to desired pH use very dilute. Solution will go milky as it nears pH 9.4. (**Place solution in oven at 37 degrees centigrade while cutting sections)

Method













1. Cut 10u cryostat section for the pH 9.4 ATPase and 20u for the pH 4.3 and pH 4.6 ATPase. Air-dry sections for at least 10 mins.
2. Pre-incubate sections at the specific buffers (ph 9.4, 4.3, 4.6) for 15 min.
3. Discard buffers and incubate all sections at pH 9.4 for 5 min.
4. Incubate all sections in the pre-warmed ATP solution at 37°C for the appropriate times:

pH 9.4	7 mins
pH 4.6	17 mins
pH 4.3	32 mins
5. Wash well in 0.09M calcium chloride
(stock CaCl₂ diluted 1:1 with distilled water)
6. Place in 2% Cobalt Chloride for:

pH 9.4	3 mins
pH 4.3 and 4.6	6 mins
7. Wash well in 0.01M Sodium Barbitone
(stock sodium barbitone diluted 1:10 with distilled water)
8. Rinse in distilled water
9. Develop the colour in dilute ammonium sulphide (MW: 68.14) in the fume cupboard.
(1% for pH 9.4 and 7% for pH 4.6 and 4.3)
10. Wash slides in running tap water for about 10 mins to get rid of excess ammonium sulphide

11. Dehydrate in various alcohol solutions, clear and mount in synthetic resin (DPX mounting stuff) and cover with coverslips.

Histochemical reactions in human muscle

MUSCLE FIBRE TYPE	1	2A	2B	2C
Routine Myosin ATPase at pH 9.4				
Myosin ATPase at pH 4.6				
Myosin ATPase at pH 4.3				

HAEMATOXYLIN AND EOSIN

Mayers haematoxylin

Haematoxylin (mw: 320.29)	4 g
Sodium iodate (mw: 197.89)	0.4 g
Aluminium potassium sulfate (CP) (mw: 474.39)	100 g
Distilled water	2 L
Chloral hydrate (mw: 165.5)	100 g
Citric acid (mw: 192.13)	2 g

1. Dissolve the haematoxylin, sodium iodate and potassium aluminium in distilled water overnight.
2. Add chloral hydrate and citric acid the next day.
3. Then boil the solution for 5 min in a well-ventilated room. It is ready for use once cool.

Eosin

Stock solution: 1% aqueous eosin (mw: 691.86)

1% aqueous phloxin B (mw: 829.64)

Mix 1 part phloxin B to 2 parts eosin (500 ml: 1000 ml).

Working solution: 150 ml stock solution
150 ml tap water

Method

1. Stain in haemotoxylin for 10 min.
2. Briefly wash in tap water to remove haemotoxylin.
3. Differentiate (3 dips) in 1% acid alcohol (99 ml 70% Alcohol + 1 ml conc HCL).
4. Blue sections in running tap water for 8 minutes.
5. Stain in eosin for 10 mins.
6. Wash sections briefly in tap water (just to wash excess eosin off slides and rack)
7. Dehydrate slides in alcohol: 1 minute in 70%, 90% and absolute alcohol.
8. Clear sections in xylol (3 dishes; one dip in each dish).
9. Place a drop of entellan mounting media on to a coverslip and place the slide, section side down directly on to the mounting media.
10. Allow to dry before turning over.

Results

Nuclei will stain blue and the cytoplasm, connective tissue and muscle will stain pinkish.

NADH (NICOTINAMIDE ADENINE DEHYDROGENASE)

Solutions

<u>0.2M Tris Buffer (ph 7.4)</u>	<u>100ml</u>
Tris hydroxymethyl amino methane (mw: 121.14)	0.606 g
Distilled water	58 ml
0.1M HCL	42 ml

Substrate

0.2M Tris Buffer (ph 7.4)	10 ml
NBT (Tetrazolium salt)	0.01 g
NADH (Reduced phosphopyridene nucleotide)	0.008 g
(β -NADH – reduced disodium salt) (mw: 709.4)	

Method

1. Cut sections at 7 μ m and air dry.
2. Incubate in the above substrate at 37°C for 30 – 45 minutes.
3. Rinse in distilled water.
4. Mount in glycerine jelly.

Results

NADH will stain blue. The darker the blue hue the greater the oxidative potential of the cell.

SUCCINATE DEHYDROGENASE

Reagents

Solution A: Stock succinate solution

0.6 M Sodium succinate (mw: 270.15)	4.05 g
Distilled water	20 ml
1M HCl (mw: 36.46)	0.13 ml

Adjust to ph 7 and make total volume of 25 ml. Store at -4°C.

Solution B: Tetrazolium solution (NBT)

Nitroblue tetrazolium (4mg/ml)	5 ml
0.2M Tris buffer, ph 7.4	5 ml
Distilled water	7 ml

Final incubating solution

Add 1 ml of solution A to 9 ml solution B just before use.

Bakers Formal Calcium

0.1 M Calcium acetate (mw: 158.2?) 8.8 g / 500ml

Formaldehyde (mw: 30.03) 20 ml

Weigh out 8.8 g Calcium acetate, add 400 ml distilled water and mix well. When dissolved add the formaldehyde and finally make up to volume of 500 ml.

Method

1. Cut 15 μ m sections and allow to air dry.
2. Incubate sections in final incubating solution at 37°C for about 45 minutes.
3. Drain slides and place in Baker's formal calcium at room temperature for 15 minutes.
4. Wash well in distilled water.
5. Mount in glycerine jelly.

Results

Mitochondria will stain up purple.

MODIFIED GOMORI TRICHROME**Reagents****Gomori stain**

Chromotrope 2R	0.6 g
Fast green FCF	0.3 g
Phosphotungstic acid	0.6 g
Glacial acetic acid	1 ml
Distilled water	100 ml

Adjust the ph to 3.4 with 1M NaoH. Prepare fresh each week or when pale.

Method

1. Cut cryostat frozen sections 7 μ m and allow to air dry at least 10 minutes.
2. Stain in haemotoxylin for 3-5 minutes
3. Blue in running water.
4. Stain in Gomori solution for about 10 minutes.
5. Rinse in water
6. Differentiate in 0.2% acetic acid.
7. Dehydrate in alcohol, clear in xylol and mount in entellan media.

Results

Nuclei will stain blackish. Connective tissue will stain green. Cytoplasm will stain reddy/pink.

APPENDIX 7**HISTOLOGY SCORING****Categories:**

The incidence / degree of skeletal muscle pathology will be determined according to the following six categories:

Category 1: Internal nuclei

0 – Absent (less than 3 %)

1 – Present (greater than 3 %)

Category 2: Fibre size variation

0 – None

1 – Some – Mild

2 – Some – Severe

Category 3: Necrosis and degeneration

0 – Absent

1 – Present – Mild

2 – Present – Severe

Category 4: Inflammation

(must include lymphocytic inflammation, not just phagocytes in necrotic fibres)

0 – Absent

1 – Present – Mild

2 – Present – Severe

Category 5: Regeneration

0 – Absent

1 – Present – Mild

2 – Present – Severe

Category 6: Accumulation of subsarcolemmal mitochondria.

0 – Normal

1 – Abnormal – Mild

2 – Abnormal – Severe

APPENDIX 8**DNA EXTRACTION FROM MUSCLE**

(Decary, S. et al. 2000)

MUSCLE SAMPLE STORAGE

- Place freeze fresh muscle sample into cryotube and freeze immediately in liquid nitrogen
- Store at $-80\text{ }^{\circ}\text{C}$

DNA EXTRACTION

- Isolation of genomic DNA from muscle samples

Reagents

- Proteinase K digestion buffer
 - 100 mM NaCl
 - 10 mM tris HCl, pH 8
 - 100 mM EDTA, pH 8
 - 1% Triton X-100
- Proteinase K
 - final concentration in buffer 20 U/ml
 - our specific activity is 30U/mg
 - ∴ need 0.67mg Proteinase K per ml of buffer
- Liquid N₂
- Phenol / chloroform / isoamyl alcohol (25:24:1 vol/vol/vol)
 - For 25 ml tot vol in sterile bottle: 12.5 ml TE-saturated phenol
 - 12 ml chloroform
 - 0.5 ml iso-amyl alcohol
- Chloroform / isoamyl alcohol (24:1 vol/vol)
 - For 12 ml tot vol in sterile bottle: 12 ml chloroform
 - 0.5 ml iso-amyl alcohol
- 7.5 M Ammonium acetate / 100% ethanol (1:4 vol/vol)
 - For 10 ml total vol in sterile bottle: 2 ml 7.5 M ammonium acetate
 - 8 ml 100% alcohol

- 70% Ethanol
- TE buffer - 10 mM Tris-HCl
- 1 mM EDTA pH 8

Procedure

1. Weigh 1mg of Prot K into a sterile eppie
2. Add 1.5 ml Prot K buffer
3. Invert to mix
4. Keep on ice

Muscle digestion

5. Weigh approximately 10mg of muscle into a sterile eppie
6. Grind muscle to a powder in a liquid nitrogen chilled mortar (or grind in liquid N₂ chilled cryotube with glass rod)
7. Add 650 µl of proteinase K digestion buffer (containing prot K) to ground muscle
8. Incubate in a shaking water bath overnight (4 hours) at 55 °C

Nucleic acid extraction

9. Add 1 volume phenol / chloroform / isoamyl alcohol (650 µl) to sample
10. Extract by gently inverting tube for 5 minutes
11. Centrifuge for 5 min
12. Carefully transfer the top aqueous phase to a new sterile eppie
13. Repeat 9-12
14. Add 1 volume chloroform / isoamyl alcohol (650 µl)
15. Extract by gently inverting tube for 5 minutes
16. Centrifuge for 5 min
17. Carefully transfer the top aqueous phase to a new sterile eppie

DNA precipitation

18. Add 1 volume 7.5 M ammonium acetate / 100% ethanol
19. Invert tube to ppt DNA
20. Spin at 13 000RPM for 3 min (room temperature)
21. Pour off supernatant

Washing DNA

22. Add 0.5ml 70 % ethanol
23. Invert to wash
24. Spin at 13 000RPM for 2 min (room temperature)
25. Repeat 22 – 24
26. Dry DNA (approximately 30 min) under light

Storage of DNA

27. Add 100µl TE buffer
28. If DNA does not completely dissolve, incubate on bench top
29. Store at 4 °C

University of Cape Town

APPENDIX 9

TELOMERE LENGTH ANALYSIS

(Allsopp, R. 1992, Decary, S et al. 1996, Decary, S. et al. 1997, Decary, S. et al. 2000, Renault, V. et al. 2002)

RESTRICTION DIGESTION OF TOTAL GENOMIC DNA

- To generate TRF's containing the TTAGGG tandem-repeat sequence and a subtelomeric fragment of non-TTAGGG DNA.

Reagents

- Hinf1 (10U/ μ l)
- Buffer H (10x)
- dH₂O
- Extracted genomic DNA

Procedure

1. Set heating block to 37 °C
2. Spin all components
3. Prepare cocktail

Component	[Stock]	[Rxn]	1 rxn (μ l)
Buffer H	10x	1x	2
dH ₂ O	-	-	6
<i>Hinf1</i>	10U	20U	2
Total volume			10

4. Pipette 10 μ l cocktail into each reaction eppie
5. Pipette 10 μ l DNA into respective eppies
6. Spin eppies
7. Incubate for 4 hours at 37 °C

GEL ELECTROPHORESIS

Reagents

- Digested DNA
- Agarose
- 1x TAE buffer
- ³²P-DNA ladder (1kb and HMW)

Procedure

1. 30 ml of a 0.7% agarose gel
0.21g agarose + 30 ml 1x TAE
2. Run each sample on three separate gels
3. Load 3 µg (1 - 5 µg?) of digested DNA and a ³²P-DNA ladder (1kb and HMW)
4. Load 3 µg of undigested DNA sample (to verify the absence of DNA degradation.)
5. Run for ? (for 1400 Vh or 600-700 Vh?)

DRYING, DENATURING AND NEUTRALISING GEL

- To avoid loss of high-molecular-weight DNA

Reagents

- 0.5 M NaOH / 1.5 M NaCl
- 0.5 M Tris, pH 8 / 1.5 M NaCl

Procedure

1. Dry gel under vacuum at 60 °C for 45 – 60 minutes (to dry gel)
2. Soak gel in 0.5 M NaOH / 1.5 M NaCl for 10 min (to denature gel)
3. Soak gel in 0.5 M Tris, pH 8 / 1.5 M NaCl for 10 min (to neutralise gel)

HYBRIDISATION OF OLIGONUCLEOTIDE PROBES (Southern Blotting)

- The TRF's are detected by hybridisation to a ^{32}P -(TTAGGG)₄ probe

Reagents

- 5 x standard saline / citrate and 3 x standard saline / citrate
- ^{32}P -end-labelled (CCCTAA)₃

Procedure

1. Incubate gel in 5x standard saline / citrate (SSC) at 37 °C with ^{32}P -end-labelled (CCCTAA)₃ for 8-12 hours
2. Wash gel 3 times in 3 X SSC at 48 °C (10 min each)

AUTORADIOGRAPHY PROCEDURE

1. Expose gel to preflashed Kodak XAR film for 1-2 days (*varies from 96 – 120 hours*)
2. Each of the three gels are exposed several times so that all of the samples have equal intensities within the linear response range of the X-ray film.

(Value for each sample is the mean of these 3) (Decary 2000)

SIGNAL RESPONSE ANALYSIS

Analysed using NIH image 1.62 and ProFit software.

1. The mean and minimal value of TRF length (in Kbp) is determined 3 times for each sample on 3 independent gels
2. The mean TRF is calculated by integrating the signal intensity above background over the entire TRF distribution as a function of TRF length using the formula:

$$L = \Sigma(\text{OD}_i \times L_i) / \Sigma(\text{OD}_i)$$

where OD_i and L_i are the signal intensity and TRF length respectively at position i on the gel image

3. The min TRF corresponds to the value at which 95% of the densitometric profile had been integrated according to the distance of migration
4. The minimal values of TRF length (kbp) was determined from the minimal signal intensity at the lower side of the peak three times for each sample on different gels. (Decary 2000)