THE EFFECT OF AN ANTI-INFLAMMATORY HOMEOPATHIC PRODUCT ON SYSTEMIC MARKERS OF INFLAMMATION FOLLOWING 90 MINUTES OF DOWNHILL RUNNING

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DECLARATION

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'The effect of an anti-inflammatory homeopathic product on systemic markers of inflammation following 90 minutes of downhill running'

in partial fulfilment of a Master of Medical Science in Sports Medicine (by Coursework).

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ABSTRACT

Background: The homeopathic preparation, Traumeel S, has been used as a valuable alternative to conventional non-steroidal anti-inflammatory drugs (NSAIDS) for over 30 years. This antihomotoxic, anti-phlogistic drug has been widely used by sportsmen and women in the treatment of lesions and inflammatory processes which result from exercise-induced skeletal muscle microtrauma. Although numerous randomised, double-blind placebo-controlled trials have confirmed the efficacy of Traumeel S as an anti-inflammatory agent, there are few *in vivo* studies which have specifically investigated the mechanism by which Traumeel S is effective in reducing inflammatory response to exercise-induced muscle cell damage.

Aim: To establish whether the administration of Traumeel S during the five days before participation and three days following participation, significantly attenuates the systemic markers of the inflammatory response, following a 90-minute downhill running trial.

Method: Twenty-four healthy athletes (14 men and 10 women), aged 20-50 years, were recruited for this study. Following baseline laboratory testing and familiarisation with the treadmill as well as a field test, subjects were matched according to gender, BMI, training age, training status, peak performance and foot strike patterns and randomised into Traumeel (TRS) and Control (PLAC) groups in a placebo-controlled, double-blind design. One Traumeel S or Placebo tablet was ingested three times per day for five days prior to and three days following a 90-minute exercise trial on a downhill (-6% gradient) at 75% $VO_{2\ max}$. Blood samples were collected prior to the 90-minute trial (PRE), immediately after the trial (IPE) and 24 hours (24 PE), 48 hours (48 PE) and 72 hours (72 PE) following the trial. Each subject was also requested to complete a training record prior to the trial and keep a record of the daily symptoms of delayed onset muscle soreness (DOMS) both at rest (general pain) and during walking (daily living). Full blood counts (FBC), serum creatine kinase (CK), lactate dehydrogenase (LDH) and cortisol concentrations were measured using standard haematological laboratory procedures and serum C-Reactive Protein (CRP) was determined by immunoturbidimetric assay. Sandwich ELISA's were used to determine myeloperoxidase (MPO) and plasma interleukin-6 (IL-6) concentrations. All results obtained were adjusted for changes in plasma volume as calculated from the red blood cell indices.

Results: Mean ± SD characteristics of the gender-matched subjects in the experimental (TRS) and placebo-control (PLAC) groups did not differ significantly in terms of BMI, age, % body fat, FVC, FEV_1, training age and status, foot strike pattern or peak running performance, maximal

Heart Rate, V_E, $VO_{2\,peak}$, RER, RPE during the maximal exercise test ($p > 0.05$). This indicated that the randomised pairs were well matched.

The 90-minute downhill running protocol resulted in significant elevations in total circulating white blood cell count (WBC), neutrophil, CK, LDH, cortisol, CPR, MPO and IL-6 concentrations ($p < 0.001$). When comparing the TRS and PLAC groups, mean ± SD total and differential WBC count, neutrophil count, CK, LDH, cortisol, CPR, MPO and IL-6 concentrations did not differ ($p > 0.05$) over the 5 time points. At 24 PE, MPO concentrations were significantly higher in the TRS group than in the PLAC group ($p = 0.03$). The lower mean ± SD post-trial DOMS scores reported by the TRS group were not significantly different from those reported by the PLAC group ($p > 0.05$).

Conclusion: Although the findings of this study did not identify differences in circulating CK, LDH, cortisol, CPR and IL-6 concentrations between the TRS and PLAC groups, the elevated MPO concentration at 24 PE did provide preliminary novel evidence of enhanced activation of neutrophil oxidative burst activity following exercise-induced muscle damage which is hypothesized to accelerate the recovery process.

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LIST OF ABBREVIATIONS

Δ A	Change in Absorbance
Δ PV	Change in Plasma Volume
24PE	24 Hours Post Exercise
48PE	48 Hours Post Exercise
72PE	72 Hours Post Exercise
ANOVA	Analysis of Variance
APR	Acute Phase Response
ATCH	Adenocorticotropic Hormone
BMI	Body Mass Index
BPM	Beats Per Minute
CD	Cluster of Differentiation
CK	Creatine Kinase
COX-1	Cyclo-oxygenase Type 1
COX-2	Cyclo-oxygenase Type 11
CO_2	Carbon Dioxide
CRP	C-Reactive Protein
DOMS	Delayed Onset Muscle Soreness
EDTA	Ethylene Diaminetetra-acetic acid
ELISA	Enzyme-linked Immunosorbent Assay
FABP	Plasma Fatty Acid Binding Protein
FEV_1	Forced Expiratory Volume
FVC	Forced Vital Capacity
H_2O_2	Hydrogen Peroxide
HB	Haemoglobin
HCT	Haematocrit
HOCℓ	Hypochlorus Acid
HR	Heart Rate
ICAM	Intracellular Adhesion Molecules
IL-1	Interleukin-1
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
IL-8	Interleukin-8
IPE	Immediate Post Exercise
LDH	Lactate Dehydrogenase
MACF	Macrophage Chemoattractant and Activating Factor
MPO	Myeloperoxidase

MRI	Magnetic Resonance Imaging
NaCl	Sodium Chloride
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
O_2	Oxygen
$O_2^{\cdot-}$	Superoxide Radical
$\cdot OH$	Hydroxyl Radical
OD	Optical Density
PGE-2	Prostaglandins of the E Series
PHA	Phytohemagglutinin
PLAC	Placebo Group
PMA	Phorbol Myristate Acetate
PMN	Polymorphonuclear Leukocytes
PRICE	Prevention, Rest, Ice, Compression, Elevation
PV	Plasma Volume
RBC	Red Blood Cells
RER	Respiratory Exchange Ratio
ROS	Reactive Oxygen Species
RPE	Rate of Perceived Exertion
RQ	Respiratory Quotient
SAA	Serum Amyloid A
SD	Standard Deviation
SOD	Superoxide Dismutase
sTnI	Skeletal Muscle Troponin I
TGF-β	Transforming Growth Factor Beta
T_H	T-Helper Lymphocyte
TNF-α	Tumour Necrosis Factor Alpha
TMB	Tetramethylbenzidine
TR	Traumeel S Group
URTI	Upper Respiratory Tract Infection
V_E	Pulmonary Ventilation
$VO_{2\,max}$	Maximal Oxygen Consumption
$VO_{2\,peak}$	Peak Oxygen Consumption
WBC	White Blood Cells
WCC	White Cell Count

CHAPTER 1

INTRODUCTION

1.1. INTRODUCTION TO THE PROBLEM

Traumeel S is a homeopathic formulation available in oral tablet form, as a subcutaneous injection, as oral drops, oral liquids in vials and as a topical ointment. It is an anti-homotoxic, anti-phlogistic drug which has been used as a valuable alternative to conventional non-steroidal anti-inflammatory (NSAIDS) drugs for over 30 years, due to its reported anti-inflammatory, analgesic, anti-oedematous and anti-exudate properties. It has been widely used by sportsmen and sportswomen in the treatment of lesions and inflammatory processes which result from exercise-induced skeletal muscle microtrauma. Although numerous randomised, double-blind, placebo-controlled trials have confirmed its efficacy as an anti-inflammatory agent, little is known of its scientific mechanisms of action.

In vitro studies have shown that Traumeel S is non-cytotoxic to granulocytes, lymphocytes, platelets and endothelial cells and that the functions of these cells are not impaired by Traumeel S (Porozov *et al.*, 2004; Conforti *et al.*, 1997) and to modulate the production of reactive oxygen species (ROS) during oxidative burst activity in the neutrophils (Conforti *et al.*, 1997). In studies on whole blood cultures, Traumeel S has been shown to elevate levels of Transforming Growth Factor Beta (TGF-β), which is an anti-inflammatory cytokine synthesised by T-Helper Type 3 (T_H3) lymphocytes (Heine and Adnrã, 2002).

In the most recent *in vitro* work, Porozov *et al.* (2004) examined the effects of Traumeel S on the ability of resting and activated phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) activated human T cells, monocytes, and gut epithelial cells, to secrete the pro-inflammatory mediators, tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-8 (IL-8) over a period of 24 to 72 hours. While IL-1β activation was reduced by up to 70% in both resting and active cells, TNF-α activation was reduced by up to 65% in resting cells and 54% in active cells and IL-8 activation was reduced by 50% in both resting and active cells ($p < 0.01$ for all cells). Traumeel S did not appear to affect T cell and monocyte proliferation and maximal inhibition was maintained with dilution of the Traumeel S stock material. In this particular study it was concluded that Traumeel S does not inhibit immune cell functions by exerting a toxic effect (Porozov *et al.*, 2004).

An *in vivo* study was conducted on 50 individuals (26 on Traumeel S and 24 on placebo) at the Sports Research Institute of Pretoria University which required the participants to run for 45 minutes on a treadmill at a downhill gradient of -10% (Jordaan, 2006). The full blood counts, rate of perceived exertion (RPE) and blood pressure recordings, as well as serum cortisol, lactate and creatine kinase (CK) concentrations were measured, but only RPE, heart rate (HR) and cortisol concentration showed significant differences between the two groups with the experimental group being significantly higher than the control group in all three measurements. As matching of the subjects, demographical details and training status of the subjects were not reported and few markers of exercise-induced muscle damage were assessed, further work is required and conclusions cannot be drawn from the available findings.

This study was therefore conducted to validate and extend upon the above-mentioned findings and to investigate whether the results of previous *in vitro* work could be replicated in an *in vivo* setting.

1.2. AIMS AND OBJECTIVES OF THE STUDY

- To confirm that 90 minutes of downhill treadmill running results in an elevation, within the circulation of leukocyte, neutrophil and monocyte numbers, selected muscle enzymes (creatine kinase, lactate dehydrogenase), acute phase proteins (C-Reactive Protein), cortisol, interleukin-6 and myeloperoxidase in the circulation.
- To establish whether administration of Traumeel S (during the five days before participation and three days following participation) significantly attenuates these systemic markers of inflammatory response, following a 90-minute downhill running trial.

1.3. HYPOTHESES

In keeping with consistent previous findings regarding inflammatory response to downhill running and eccentric exercise (Peake *et al.*, 2005a; Aoi *et al.*, 2004; Braun and Dutto, 2003; Malm *et al.*, 1999; Byrnes *et al.*, 1985), the following one-directional alternate hypothesis was set:

> *Downhill running will result in an elevation of neutrophil count and serum creatine kinase (CK), lactate dehydrogenase (LDH), C-Reactive Protein (CRP), myeloperoxidase (MPO), interleukin-6 (IL-6) and cortisol concentrations in the circulation.*

As the results of previous studies investigating the effect of Traumeel S on inflammatory response in an *in vitro* setting, are limited and inconclusive, the following non-directional, null hypothesis was set for this aspect of the study:

Traumeel S administration will not have an effect on systemic markers of an inflammatory response to 90 minutes of downhill running.

1.4. SCOPE AND LIMITATIONS

This double blind, placebo-controlled study was restricted to two sets of 12 ($n = 24$) experienced, well-trained athletes, who were matched for gender, body mass index (BMI), training status and training age as well as at least two of the following: foot strike, maximal oxygen consumption ($VO_{2\,max}$), 1609m running time and/or best performance over longer endurance running events within six months prior to the study trials.

After maximal exercise capacity was assessed in the Human Performance Laboratory and running biomechanics and 1609m running time were assessed during a field-side evaluation on an athletics track, each subject completed a -6% downhill 90-minute treadmill run at a workload which elicited 75% of $VO_{2\,max}$ on a level treadmill. Five days prior to the trial run, each subject ingested either a Traumeel S preparation or a placebo provided by Heel (Pty) Ltd. Red blood cell (RBC) indices and differential leukocyte counts as well as circulating concentrations of muscle enzymes, cortisol, CRP, IL-6 and MPO were measured from venous blood samples taken pre-, immediately post (IPE) 24 hours post (24 PE), 48 hours post (48 PE) and 72 hours post (72 PE) each exercise-trial, to establish whether a significant difference existed between TRS and placebo-control (PLAC) trials over this time-period. Subjective perceptions of post-exercise delayed onset muscle soreness (DOMS) were also monitored during the 72 hours following each exercise trial.

Due to the undisputed evidence which is now available regarding the repeated bout effect *viz.* the adaptive response to acute repeated exposure to similar bouts of eccentric exercise (Nosaka *et al.*, 2005; McHugh, 2003; Nosaka and Newton, 2002; Stupka *et al.*, 2001), it was not possible to conduct a crossover study. In order to eliminate a training effect as a potential confounder in the study, this double–blind, placebo-controlled study was therefore designed using pairs of runners who were matched according to gender, mass, training history, training age as well as athletic performance and/or foot strike in order to ensure sufficient external validity. As it was necessary to design this study within the framework of financial constraints as

well as difficulty in obtaining full compliance from the subjects, the sample size was restricted to 12 gender-matched couples (n = 24).

CHAPTER TWO

REVIEW OF THE RELATED LITERATURE

2.1. INTRODUCTION

Acute bouts of prolonged exposure to exercise have been shown to cause damage to muscle at the cellular level (Nieman, 1997). The degree of this damage is determined by the intensity and nature of the exercise causing disturbances to the contractile units inside the muscle (Stupka et al., 2000). This microscopic damage has also been associated with the release of inflammatory mediators (MacIntyre et al., 1995; Pyne 1994), which trigger an inflammatory response in an attempt to repair the damage and eliminate the toxic substances (Roitt et al., 2001).

Exercise-induced muscle inflammation has also been reported to result in the synthesis of large quantities of prostaglandins of the E series (PGE-2), which have been reported to sensitise Type II and IV pain nociceptors and activate afferents and are thought to be responsible for delayed onset muscle soreness (DOMS) (Aoi et al., 2004; Smith, 1991; Byrnes et al., 1985). This can negatively affect the intensity of subsequent training sessions and prevent adherence to exercise programmes. Muscle inflammation and DOMS has also been shown to adversely affect the running economy 48 hours following a downhill run (Braun and Dutto, 2003).

2.2. EXERCISE-INDUCED MUSCLE DAMAGE AND ACUTE INFLAMMATORY RESPONSE

2.2.1. Tissue Disruption

Exercise of high intensity and particularly exercise of an eccentric nature, has been shown to result in myofibrillar disruption and cell membrane damage (Friden and Lieber, 1992; Smith, 1991). Disturbances in the excitation-contraction coupling (Frennette et al., 2002) and calcium signalling have also been reported to be impaired owing to damage from exercise (Overgaard et al., 2002; Armstrong, 1990).

Damage to the sarcomere is thought to occur due to Z-line disruption (Magaudda et al., 2004). This disruption to the myocyte and its cell membrane is thought to be the result of a break down of desmin, a protein that connects the adjacent Z-lines (Figure 2.1) to the different myofibrils

(Boriek *et al.*, 2001). The breakdown has also been reported to cause subsequent streaming of the Z-lines (Grobler *et al.*, 2004; Stupka *et al.*, 2000).
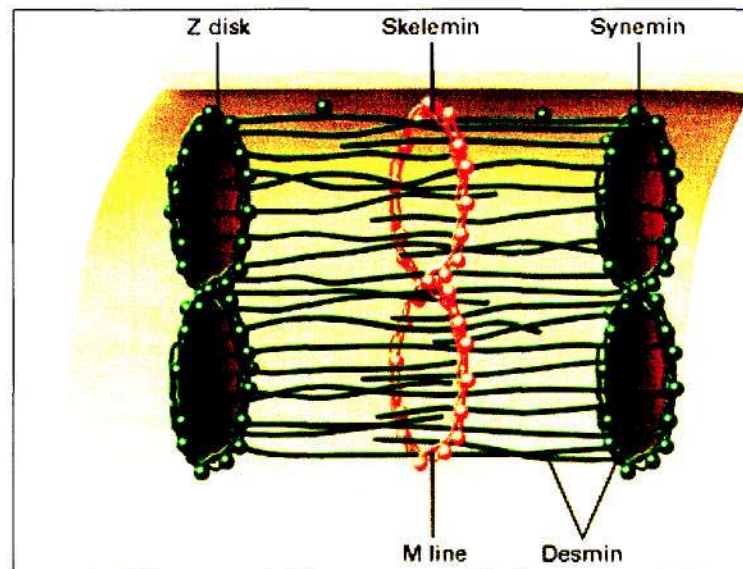
Figure 2.1:   Schematic illustration of muscle structure depicting the Z-line   (Lodish *et al.*, 2000) – Reproduced with permission from Dr. Harvey Lodish

The degree of Z-line disturbance is an indication of damage to the protein filaments within the sarcoplasm. Complex cellular and humoral responses, in conjunction with the connective tissue disruption and cell membrane damage, result in the release of various intracellular components into the blood stream (Smith, 2003; Lambert and Denis, 1994). Muscle proteins which have been found to leak from the compromised cell membrane, include creatine kinase (Totsuka *et al.*, 2002; Clarkson *et al.*, 1992), myoglobin (Croisier *et al.*, 1996), fatty acid binding protein (Sorichter *et al.*, 1998), lactate dehydrogenase (Overgaard *et al.*, 2002; Nosaka and Clarkson, 1996) and skeletal muscle troponin I (Sorichter *et al.*, 1997). The changes in the muscle structure and subsequent increase of muscle proteins and enzymes in the bloodstream have been shown to result in a loss of muscle strength and range of motion (Peake *et al.*, 2005a; Nosaka *et al.*, 2005).

2.2.2. Markers of Muscle Cell Damage

Serum creatine kinase (CK) and lactate dehydrogenase (LDH) have been the most commonly reported direct indicators of exercise-induced muscle cell membrane damage (Braun and Dutto, 2003; Overgaard *et al.*, 2002; Hortobagyi *et al.*, 1998). The literature is consistent in reporting peak LDH concentrations in serum immediately post-exercise, while a peak in the CK concentration has been shown to occur between 12-24 hours post-exercise (Semple *et al.*, 2007; Simpson *et al.*, 2006; Armstrong, 1990).

Conflicting evidence, however, exists as to which mode of exercise produces the greatest changes in CK activity (Sorichter *et al.*, 1997; Clarkson *et al.*, 1992). Sorichter *et al.* (1997) reported that changes in CK activity were relative to exercise intensity, duration and volume and that the subjects familiarity with eccentric exercise would also affect changes in serum CK, while Clarkson *et al.* (1992) reported that changes in CK activity after isometric exercise were similar to those that occur in downhill running but that the increase in CK activity in the blood tended to be substantially lower after downhill running (approximately 300 IU/ℓ), when compared with high force eccentric exercise of the forearm flexors (approximately 2,500 IU/ℓ). Clarkson *et al.* (1992) concluded that changes in CK activity differ relative to muscle mass recruitment during exercise. This was confirmed by Peters *et al.* (2001a, 2005) who reported CK concentrations as high as 3050 IU/ℓ following participation in the 1997 downhill Comrades Marathon and 20-fold increases in CK following the same 90km downhill event in 1999. Interestingly, the corresponding increases in serum LDH were only 2-fold and peaked immediately post exercise as opposed to the serum CK concentrations which peaked at 24 hours post-exercise.

As large intra- and inter-individual differences have been reported to occur in both LDH and CK (Peters *et al.*, 2005), investigators have sought other more reliable indicators. In 1997 Sorichter *et al.* conducted a study which revealed that skeletal muscle troponin I (sTnI) is an initial specific marker of exercise-induced muscle injury. In 1998 Sorichter *et al.* subsequently also found plasma fatty acid binding protein (FABP) to be a better indicator of muscle injury than CK. This finding was based on the rapid increase and decrease that was recorded in FABP following eccentric exercise and indicated that FABP is more practical in detecting early muscle damage.

Damage which occurs to the muscle cell membrane, also indirectly activates mast cells in the connective tissue to start degranulation and release their contents. These include heparin, histamine, serotonin and bradykinin. Vasodilatation, which results in an increased blood supply at the injured site, occurs as a result of the histamine release (Ganong, 2005). An increase in the permeability of the vascular walls will also occur, allowing easy release of fluids and defence cells and subsequent swelling. Increases in the vascular wall permeability will also accelerate diapedesis of neutrophils and monocytes through vessel walls and disrupt calcium homeostasis (Armstrong *et al.*, 1983).

An influx of calcium into the cell through the compromised cell membrane has also been shown to activate Phospholipase A (Gissel, 2000). As seen in Figure 2.2 below, this activation of phospholipase results in the release of membrane glycerophospholipids, from the activated mast cell (Ganong, 2005).

CELL DAMAGE

Activation of Phospholipase A

MEMBRANE GLYCEROPHOSPHOLIPIDS RELEASE

ARACHIDONIC ACID

Cyclo-oxygenase   Lipoxygenases   CYP mono-oxygenases

PROSTAGLANDINS   LEUKOTRIENES   EPOXYEICOSATRIENOIC
                                                ACID
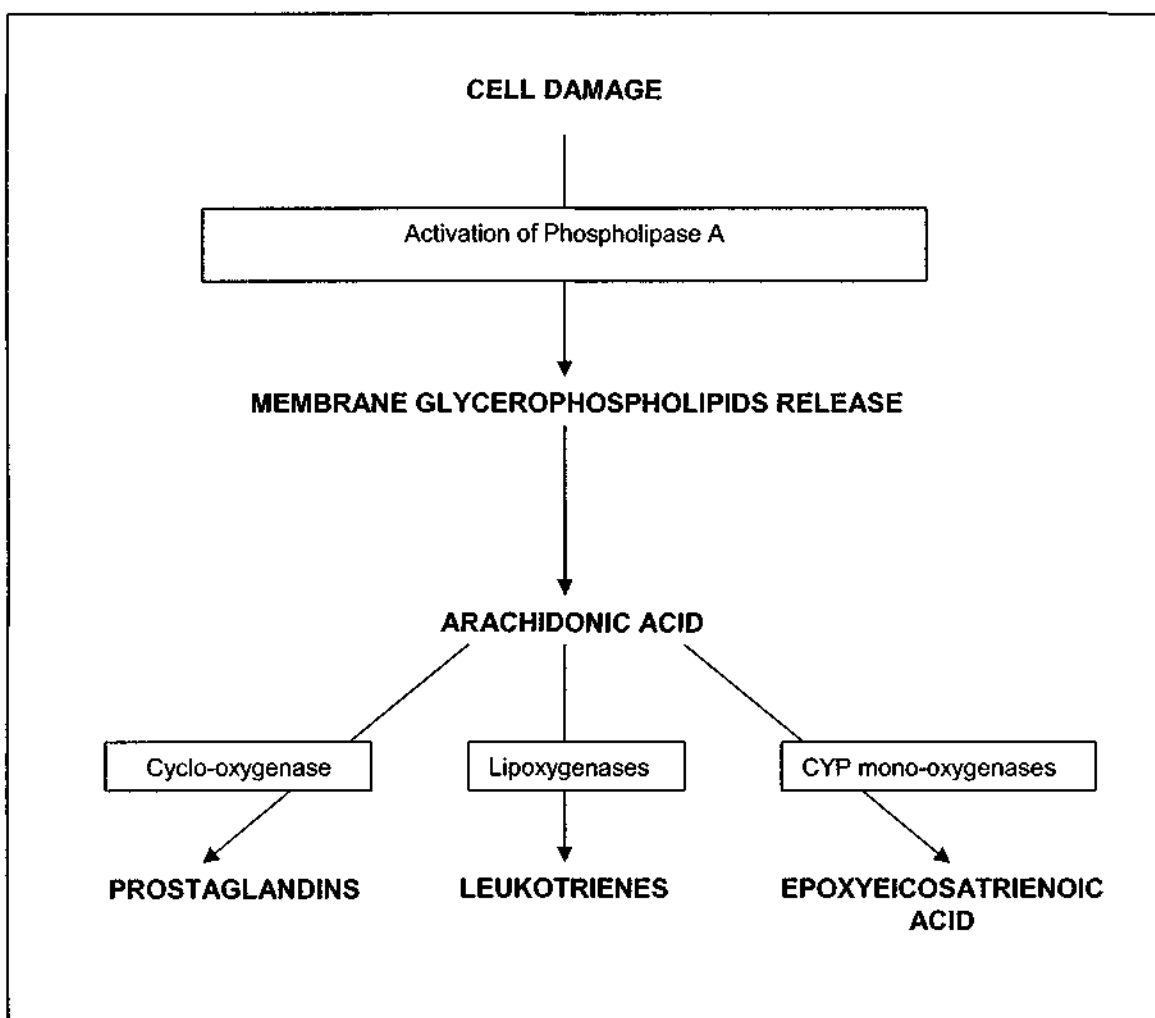
Figure 2.2:   Principal biologically active substances produced from arachidonic acid in response to exercise-
                     induced muscle cell damage & activation of mast cells (Ganong, 2005)

Arachidonic acid from the membrane glycerophospholipids is converted into prostaglandins by the enzymatic action of cyclo-oxygenase (COX) and into leukotrines by the enzymatic activity of lipoxygenase (Ganong, 2005; Van Brandt, 2002).

2.2.3. Satellite Cells

Disruption of the myocyte and its membrane also activate the macrophages which reside in the muscle tissue and these are thought to contribute to the activation and proliferation of satellite cells within the damaged area (Grounds, 1991). These cells are known to play an important role in the repair and remodelling of the damaged muscle cells through the process of terminal differentiation, which lasts up to 10 days. They are myogenic precursor cells which later differentiate into myoblasts and fuse to form myotubes. The myotubes are reported to form myofibrils, which are then re-innervated to form mature muscle fibres (Hawke and Garry, 2001).

2.2.4. Pro-Inflammatory Cytokine Release

Activation of the macrophages residing in the damaged tissue will also result in the production and release of pro-inflammatory cytokines, specifically TNF-α, interleukin-1 (IL-1) and interleukin-6 (IL-6), which is currently better known for its inhibition of pro-inflammatory action via its activation of the adrenal cortex to release cortisol (Nieman, 1997). These cytokines have also been shown to increase cell permeability and together with the chemotactic cytokine, IL-8, and macrophage chemoattractant and activating factor (MACF) facilitate the migration of neutrophils and monocytes towards the site of tissue damage and inflammation. Through this influx of leukocytes and the clearance of antigens, healing can begin to occur (Roitt et al., 2001).

2.2.5. Adhesion Molecule Expression on Leukocytes

Within a few hours after tissue injury, there is a dramatic increase in the number of circulating polymorphonuclear leukocytes (PMNs), primarily neutrophils (Smith et al., 1998). These leukocytes then adhere to the venular wall at the site of inflammation due to the increased expression of adhesion molecules which arise from the activation of the endothelium lining the capillary venules, primarily by histamine, IL-1 and TNF-α. The extended period of vasodilation and increased cell wall permeability induced by histamine and heparin promotes diapedesis of the neutrophils, monocytes that occur with inflammation, a decrease in the activation of monocytes, and an increase in the expression of cell adhesion molecules on the monocytes (Pederson and Toft, 2000; Malm et al., 1999; Nieman, 1997).

This allows interaction to occur between the leukocyte and endothelial cell. This interaction may be broken down into three stages: rolling of the PMNs, activation of the PMNs and finally adhesion of the PMNs to the endothelial cell (Walzog & Gaehtgens, 2000; Van Eeden et al., 1999; Casadevall et al., 1999). Different adhesion molecules are responsible for these different stages and these have been divided into three basic categories: selectins, integrins and immunoglobins (Walzog and Gaehtgens, 2000; Van Eeden et al., 1999; Casadevall et al., 1999).

The role of selectins is in the capturing of leukocytes and the rolling of these leukocytes onto the endothelial cells and platelets. This is achieved by binding to carbohydrate ligands. Integrins, on the other hand, bind components of the immunoglobulin group of adhesion molecules and hence ensure the holding of leukocytes onto the endothelial cells. These integrins include the LFA-1 (CD 11a), Mac-1 (CD 11b) and p150,95 (CD 11c) which are expressed on monocytes and neutrophils. The third group of adhesion molecules, the immunoglobulins, as mentioned above, serve as ligands for the integrins. The most important of these immunoglobulins are the intracellular adhesion molecules (ICAMs), which once bound to the integrins play a key role in

ensuring recruitment of PMNs and the adhesion thereof to the site of inflammation (Casadevall et al., 1999).

The binding of leukocytes to endothelial cells is dependent on the binding of β_2 integrins (including CD 11a, b and c) to the adhesion molecules ICAM-1 or ICAM-2 on the endothelium. Less adhesion of leukocytes to endothelium will occur if the expression of adhesion molecules is low, or if the adhesion molecules have not been activated. Exercise with resultant increases in body temperature, induces the activation of adhesion molecules facilitating the adhesion of leukocytes to the endothelium and their movement to the site of inflammation.

In 1994 Gabriel and Kinderman showed that during intensive exercise, the cells that are preferentially mobilised from the marginal blood pool, expressed high levels of the β_2 integrin LFA-1 (CD11a) than cells that were not mobilised.

Studies which have examined the effect of exercise on the role and functioning of adhesion molecules have found the expression of adhesion molecules to be increased following exercise (Malm et al., 1999). Studies have also shown that PMNs, which were released from the marginated pool as a result of extensive exercise, expressed low levels of L-selectin and high levels of CD11b (Van Eeden et al., 1999). Further research findings suggested that the cell-surface expression of CD11a molecules is associated with granulocytosis following exercise (Kurokawa et al., 1995) and that a significant increase of leukocyte CD11b expression changes, negatively influence the ability of leukocytes to adhere to and actively transmigrate the endothelium to reach the tissues (Nielsen and Lyberg, 2004). The findings of a study conducted by Mills et al. (2006) indicated that immune cells that demarginate in response to exercise, have a reduced ability to adhere in individuals who are physically fit. Peake et al. (2004), however, showed that neutrophil activation remains unchanged after downhill running in well-trained runners, despite increases in plasma markers of muscle damage.

2.2.6. Neutrophil Activation

Neutrophils are involved in a number of inflammatory conditions and respond differently when exercise is performed at different intensities (Pederson and Hoffman-Goetz, 2000; Suzuki et al., 1999). There is consensus in the literature that neutrophil function is enhanced in response to moderate, sub-maximal, prolonged exercise, resulting in an increase in their chemotaxic activity and phagocytic and oxidative burst function (Peters, 1997a).

On arrival at the site of tissue damage, phagocytes extend their pseudopodia around the foreign particles, engulfing them and forming phagosomes which fuse with the neutrophil phagosome

granules and release digestive enzymes, including elastase. The membrane vesicles generate highly toxic and bacteriocidal reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydroxyl radicals ($^{\cdot}$OH) and hypochlorus acid (HOCℓ).

The generation of $O_2^{\cdot-}$ is catalysed by activated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase with the further production of hydrogen peroxide (H_2O_2) and ultimately HOCℓ. HOCℓ is cytotoxic and assists the neutrophils in removing foreign substances introduced into the cell following tissue damage. Its production from H_2O_2 is catalysed by myeloperoxidase (MPO) (Figure 2.3).

MPO is a peroxidase enzyme which is expressed in neutrophils during oxidative burst activity. Bury and Pirnay (1995) reported significant elevations of MPO concentrations in plasma following two hours of prolonged exercise at 75% $VO_{2\,max}$ which peaked at 722 $\pm$ 50 ng/mℓ immediately post exercise. They also found that intensity of exercise was an important parameter in explaining the magnitude of MPO increase ($p < 0.05$) and that despite significant increases in both the neutrophil count and MPO concentrations ($p < 0.001$) following exercise, MPO concentration and neutrophil number were not significantly correlated ($p < 0.05$). This inferred that neutrophil degranulation is independent of neutrophil mobilisation. However, Suzuki *et al.* produced contradictory evidence in their 1999 study, which showed that the increase in the MPO enzyme activity in plasma was proportional to the increase in the number of neutrophils after prolonged exercise.

Camus *et al.* (1992) compared plasma elastase and myeloperoxidase concentrations following uphill walking and downhill running. While both bouts of exercise were accompanied by similar increases of 33% above resting values in neutrophil concentration, uphill walking did not result in significant elevation in MPO or elastase concentration, but downhill running was followed by a 97% increase in MPO and a 70% increase in elastase above resting concentrations. These findings clearly demonstrate the importance of the eccentric component of muscle contractions in neutrophil activation during exercise.
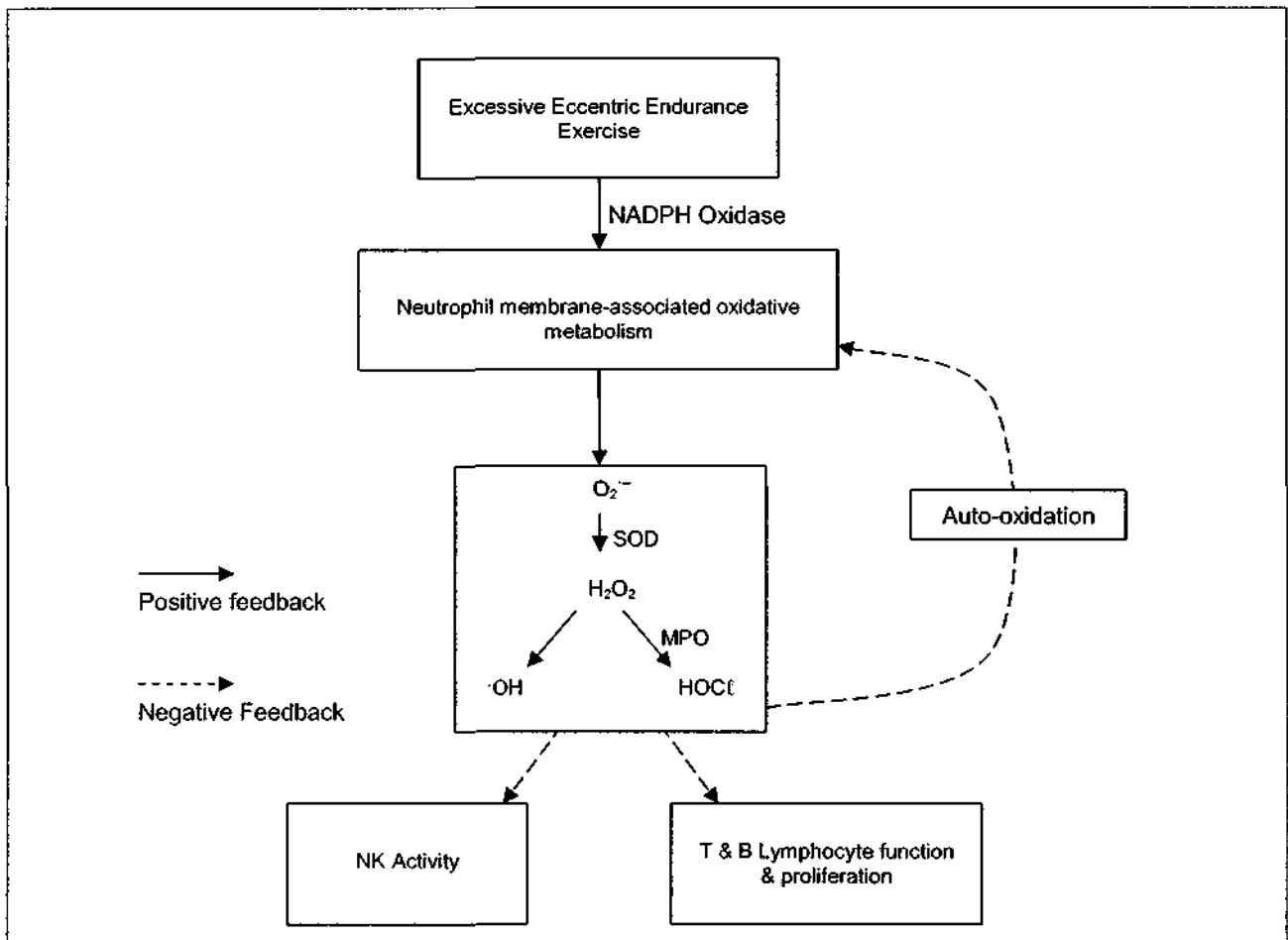
Figure 2.3:   Membrane-associated oxidative metabolic response to exercise in neutrophils (Modified from Peters, 1997a)

2.2.7. Post Exercise Neutrophilia and Monocytosis

During prolonged exercise, the absolute number of circulating leukocytes increases significantly towards a maximal value. It has been proposed that delayed release of cortisol during and after exercise is responsible for the continued exercise-induced increase in neutrophil count (Peters, 1997b). Pizza *et al.* (1995) reported a larger and more persistent elevation of total leukocyte and neutrophil number after a 60-minute downhill (-10% gradient) compared with level running. These remained significantly higher up to 12 hours after downhill running, suggesting a sustained recruitment into the circulation. The magnitude of recruitment of monocytes into the circulation during prolonged exercise is however, closely related to exercise intensity and duration (Nieman, 1997). Monocytes have been shown to localise to and infiltrate skeletal muscle damaged during exercise. It has been suggested that recruitment of these cells into the circulation during exercise, followed by their rapid removal after exercise, may reflect the homing of these cells to damaged tissue (MacKinnon, 1999).

2.2.8. Activation of Hypothalamic-Pituitary-Adrenal Axis

Inflammation may also be influenced at a higher level through the hormonal control by the hypothalamic-pituitary-adrenal axis when the tissue damage is of a large or more severe nature (Kushner, 1982). IL-6, which is produced by the activated macrophages, activates the pituitary release of adenocorticotropic hormone (ATCH), which then stimulates the adrenal cortex to release two cortical hormones, desoxicortisone and glucocorticoids (Figure 2.4). Glucocorticoids promote carbohydrate and protein catabolism, but also play a role in reducing swelling and restraining inflammation (Kushner, 1982)

The rise in serum cortisol concentration following prolonged eccentric exercise is well documented (Peters, 2001a, 2001b). Post exercise cortisol concentrations have been shown to rise by no more than 50% following 60 minutes of downhill treadmill running (gradient -13.5%) at 75% $VO_{2\ max}$ (Smith et al., 2007), but ± 200% increases to values of 1179 ± 93,2 nmol/ℓ have been reported following a 90km downhill ultramarathon run (Peters, 2001b). The exercise-induced rise in systemic cortisol concentration is a sequel to stimulation of the adrenal cortex by IL-6 and increased concentrations of circulating cortisol have been reported to act on the bone marrow, causing a further release of neutrophils into the blood stream (Steensberg et al., 2003; Pederson and Hoefman-Goetz, 2000, Suzuki et al., 1999). Due to the anti-inflammatory properties of cortisol, the inflammation has been theorised to be restrained once cortisol concentrations reach a critical level and the body is given the opportunity to repair the tissue (Van Brandt, 2002). Whether greater inhibition of the natural inflammatory response by administration of exogenous steroids is advisable, is therefore currently a matter of debate (Ritacca et al., 2002).

2.3. ACUTE PHASE RESPONSE

A further systemic response to exercise-induced skeletal muscle damage and subsequent inflammation is an acute phase response (APR), similar to that which occurs with infections, surgery and trauma (Fallon, 2001). While muscle damage results in elevated levels of CK, myoglobin, LDH and plasma FABP (Braun and Dutto, 2003; Overgaard et al., 2002; Hortobagyi et al., 1998), release of IL-6 from activated macrophages in the tissues and subsequent cortisol from the adrenal cortex stimulates hepatic production of acute phase proteins. These reactants include serum amyloid A inducer (SAA), CRP, fibrinogen, haptoglobulin, ceruloplasmin, transferrin, and a 2-macroglobulin (Pannen and Robotham, 1995; Weight et al., 1991) and form an essential part of host defence.
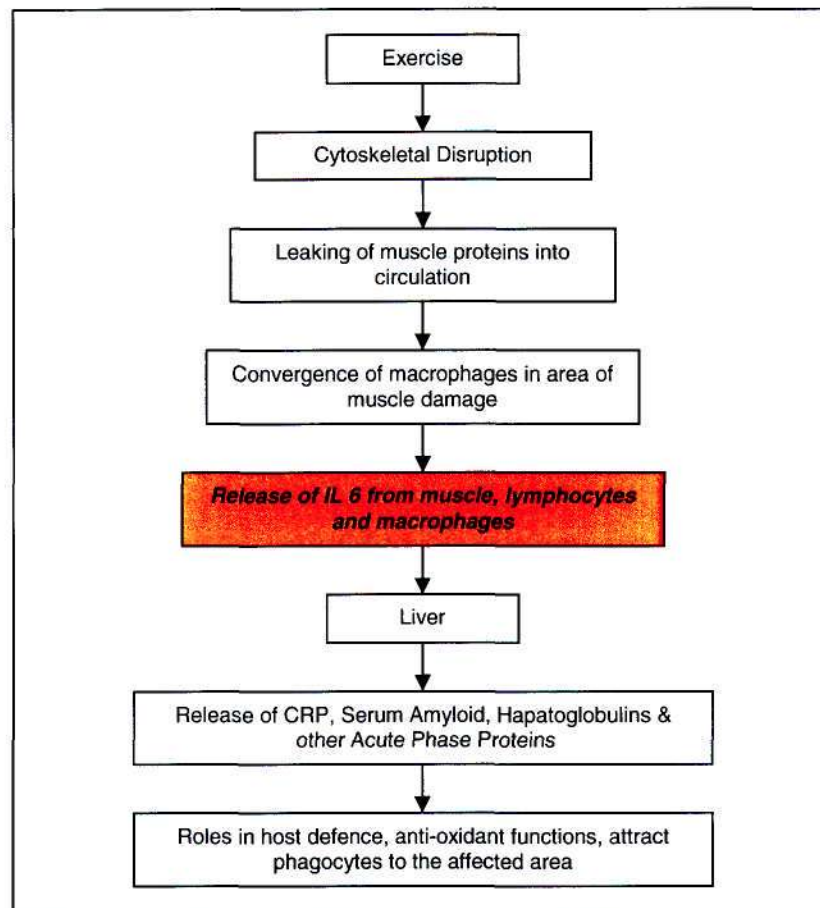
Figure 2.4:   The role of interleukin-6 in the Acute Phase Response

Fallon (2001) described the hepatic production of acute phase proteins following a track race of six days in duration. Of the 11 acute phase reactants measured, six responded as if an acute phase response was present. These included serum iron, ferritin, percent transferrin saturation, CRP and haptoglobin. The other five, transferrin, albumin, α-1 antitrypsin and complement components three and four (C3, C4), did not respond accordingly. Albumin, α-fetoprotein and transferrin which decrease during an acute phase reaction, have been labelled negative acute phase reactants. However, the complement components C3 and C4 usually increase during an APR, contradicting what was found in this study (Fallon, 2001). It is also expected that iron metabolism will be affected during exercise-induced inflammation as is seen by the increase in serum ferritin and decrease in transferrin.

Conflicting evidence exists as to whether the APR following exercise is analogous to that following surgery and trauma (Smith and Roberts, 1991; Weight *et al.*, 1991; Dufaux and Order, 1989; McCarthy and Dale, 1988; Taylor *et al.*, 1987; Dickson *et al.*, 1982; Liesen *et al.*, 1977). In the 1991 study conducted by Weight *et al.*, it was reported that the metabolic sequelae following a 42km marathon were similar, but could not be considered analogous, to the APR owing to inconsistencies which existed regarding iron metabolism and plasma protein levels.

Fallon (2001), also reported that the APR was not identical across a range of medical conditions and that there were differences in the cytokine milieu.

3.2.1. C-Reactive Protein

A sensitive indicator of the inflammatory response which is most commonly used in exercise studies on humans, is CRP. Studies have shown that CRP concentrations increase substantially 24 to 48 hours after acute exercise (Semple, 2006; Peters, 2005, 2001a) and that this increase is often more pronounced following exercise of greater intensity owing to the muscle damage which is incurred (Semple, 2006). The delay in the increase in serum CRP concentrations, which usually only peaks after 24 hours, occurs as hepatic release stimulated by an increase in circulating IL-6 concentrations, which occurs in response to its release by muscle tissue during exercise (Peters, 2004) and activated tissue macrophages following exercise (Nieman, 1997).

One of the primary functions of this exercise-induced increase in CRP is to assist in opsonisation and phagocytosis of bacteria, following injury, inflammation or infection, promoting the removal and uptake of the necrotised and damaged tissue (Semple, 2006). CRP also contributes to the repair process by activating the complement system as it binds to the first component of the cascade. This further enhances the process of opsonisation and phagocytosis (Dufaux *et al.*, 1994; Pepys, 1981).

Clyne and Olshaker (1999) have reported that the values of CRP do not differ between males and females with the averages of the general populations being considered normal when less than 5 mg/ℓ (Gabay and Kushner, 1999). Furthermore, no diurnal or seasonal variation has been observed in blood CRP values (Pepys, 1981) and it has been reported that the levels of CRP in humans are not affected by food intake (Semple, 2006). In addition to this, it has been shown that regular aerobic exercise does not reduce circulating CRP levels in adults (Kelley and Kelley, 2006; Marcell *et al.*, 2005).

2.4.   ECCENTRIC EXERCISE AND THE REPEATED BOUT EFFECT

In contrast to concentric exercise during which the muscles shorten while contracting, eccentric muscle contraction occurs when muscles lengthen while contracting. This eccentric concentration results in a greater load being placed on the muscle due to the recruitment of less muscle fibres than during a given amount of concentric contraction. Repeated stress is

therefore placed on a smaller number of active fibres, which is thought to result in a greater degree of damage, as well as possible necrosis, to the fibres (Edman, 1988; Friden et al., 1983, 1981). For example, it has been shown that the raised levels of IL-6 following eccentric muscle activity by far exceed the levels induced by concentric muscle activity (Pederson et al., 2001) when variables, such as duration of the exercise and the timing of the blood sample, are controlled.

Stupka et al. (2000) and Clarkson et al. (1992) have attributed the gender differences which occur in response to eccentric exercise, to less of an inflammatory response in women than men. Researchers have subsequently also consistently reported that women present with lower serum CK activity than men (Stupka et al., 2001; Clarkson and Hubal, 2001; Amelink et al., 1990) which has been suggested to be related to possible protection from circulating oestrogen (Amelink et al., 1990). Interestingly, gender differences in actual exercise-induced structural damage to muscle i.e. sarcomere impairment and Z-line streaming, have however, not yet been identified.

In terms of the inflammatory response to eccentric exercise, Malm et al. (1999) showed that despite an increase in the number of circulating monocytes, leukocytes and neutrophils, monocyte activity decreased post exercise. It was also determined that an increase in the cell adhesion expression on monocytes, the specifically adhesion molecules 62L and CD 11b, existed following eccentric exercise. The observed phenotypic changes which occurred in this study, were attributed to the altered trafficking of cells between the blood and the lymphoid tissue, in response to eccentric exercise.

Recurring bouts of the same endurance exercise, however, cause less symptoms of muscle damage than the initial session. In the study conducted by McKune et al. (2006) on 11 untrained men, where two 60-minute downhill (-13.5% gradient) treadmill trials were conducted 14 days apart, there was a significant group-time interaction effect for CK. The findings of this study reported a significantly lower ($p < 0.001$) CK activity at six, nine, 12, and 24 hours after the second run than after the first run. This is referred to as "repeated bout effect" (McHugh, 2003) and is also characterized by less DOMS and torque deficit after the second of two separate eccentric exercise bouts (Pettitt et al., 2005).

Three theories which underlie the concept of repeat bout effect have been proposed by McHugh et al. (1999). Firstly, the neural theory postulates that there is a change in the motor unit recruitment (Golden and Dudley, 1992), which will result in the extent of damage being reduced in subsequent sessions of eccentric exercise. A study conducted by Hortobagyi et al. (1998) supports this theory in that their results revealed an increase in strength following repeated

bouts of exercise. According to the investigators of this study, these results indicated that better distribution of workload among fibres occurred, inferring that there was a more efficient recruitment of muscle fibres in the successive training sessions.

Secondly, the connective tissue theory suggests that the muscles become protected as a result of the initial damage (McHugh *et al.*, 1999). This protection is thought to be provided by the intermediate filaments within the muscle fibre, which consist of proteins, desmin, vimentin and synemin (Frieden and Lieber, 1992; Figure 2.1). These filaments play a role in maintaining the structural integrity of the sarcomere by preserving the sarcomere stretch and overlap during eccentric exercise. The protection of muscles is therefore thought to be due to an increase in intramuscular connective tissue following the initial bout of eccentric exercise (Frieden and Lieber, 1992).

Thirdly, the cellular theory suggests that adaptations occur within the muscle cell following the initial bout of eccentric exercise. The adaptations include strengthening of the cell membrane, removal of the weak fibres and the longitudinal addition of sarcomeres. By strengthening the cell membrane, it is proposed that the influx of calcium into the cell is reduced and therefore the cell necrosis, which occurs as a result of this calcium influx, is avoided. Furthermore, the addition of sarcomeres is thought to reduce the sarcomere strain and therefore maintain the myofilament overlap, which prevents cellular disruption (Byrnes, 1985).

The processes involved in repair and remodelling of skeletal muscle, following exercise-induced damage, ultimately allows the muscle to adapt in response to the mechanical and metabolic demands placed on it. The extent of this process of repair and adaptation is unknown, but evidence exists to suggest that there is a limit to this capacity for remodelling, which ultimately can result in the development of a pathological condition which has been described as "Acquired Training Intolerance" (Grobler *et al.*, 2004).

2.5.   DELAYED ONSET MUSCLE SORENESS (DOMS)

A distinguishing feature of DOMS is the delayed onset of pain within the muscle, which occurs 24 to 48 hours following exercise to which the muscles are not accustomed, with the pain being more pronounced following exercise of an eccentric nature (Aoi *et al.*, 2004). Stretching, contracting and palpating the affected muscle may elicit the pain associated with DOMS.

DOMS was originally attributed to a build up of lactate in previously active muscle fibres (Freund et al., 1989). However, the observed reduction in blood and muscle lactate concentration occurs within 30 minutes of cessation of exercise (Thomas et al., 2004; Apple and Rogers, 1986), while DOMS occurs 24 - 48 hours post exercise.

DOMS is now thought to be the result of microscopic tearing of the muscle fibres in response to the load under which they were worked (Peake et al., 2005b). This muscle degradation which has been shown to take place following extreme eccentric exercise, results in disruptions of the myofibrils and streaming of the Z-lines, as have been identified on electron micrographs (Grobler et al., 2004). In addition to this, swelling may also occur in and around the muscle, owing to the disruption of the intracellular calcium homeostasis and the release of muscle proteins and enzymes into the bloodstream (Armstrong et al., 1983). This swelling is thought to increase the pressure on the adjacent structures and will result in greater muscle pain and stiffness (Ross, 1999).

Other reasons for the delayed onset muscle soreness reported following eccentric exercise have also been proposed (Yu et al., 2002). As mentioned previously, with disruption to the myocyte, serum markers of inflammation increase. Both CK and LDH concentrations have been shown to increase (Byrnes et al., 1985; Schwane et al., 1983) as has prostaglandin E2 (PGE-2), which is released by blood vessel walls in response to inflammation (Smith, 1991) and is thought to sensitise nociceptors and activate Type II and IV pain afferents, decreasing the threshold of the pain receptors in the muscle (Tyers and Haywood, 1979).

Aoi et al. (2004) also postulated that DOMS could result from phagocyte infiltration and resultant production of ROS and other anti-oxidants, which attenuate inflammatory changes.

Researchers have frequently used downhill running to induce DOMS. Akimoto et al. (2002) used three different types of exercise in normal, healthy athletes. These included bicycle ergometry exercise at 80% $VO_{2\,max}$ for 16 minutes, 42km marathon running and 30 minutes of downhill running at an intensity of ventilation threshold. Close et al. (2004) used 30 minutes of running in physically active male subjects at 65% $VO_{2\,max}$ on a flat or a -15% downhill gradient. A 30-minute protocol at 60% $VO_{2\,max}$ and a gradient of 18% was used by Thompson et al. (2004) in testing healthy male students. Pizza et al. (1995) had runners complete a 60-minute downhill run at a -10% gradient at 70% $VO_{2\,max}$. The protocol used by Pizza et al. (1995) is similar to that of the present research in which a 90-minute downhill run at a –6% gradient at a speed which equated to 75% $VO_{2\,peak}$, was used.

In conclusion, the above-described factors which contribute to the occurrence of DOMS, compromise an athlete's maximum exercise capacity and ability to train (Boriek *et al.*, 2001). With the adaptation and repair that occurs following training and the repeated bout effect, which was discussed earlier, the occurrence and incidence of DOMS does, however, become less apparent with recurring bouts of the same type of endurance exercise.

2.6.   TREATMENT MODALITIES

Modalities which have been used to alleviate pain for soft tissue injuries include a variety of treatments, some are scientifically proven, whilst others remain merely a hypothesis. The one modality which is universally recognised is the principle of PRICE (prevention, rest, ice, compression and elevation) treatment immediately following injury. However, this in itself is surrounded in debate with regard to the icing and intermittent reflex vasodilatation, which occurs as seen in the Hunting Response (Guyton and Hall, 1996). This introduces the concept of the "Gate Control Theory", another contentious issue, whereby the application of cold 'gates' the pain impulse to the brain, acting as a counterirritant and breaking the pain cycle (Guyton and Hall, 1996).

Following the application of PRICE, different choices in pain and inflammation management are rather subjective with some athletes preferring massage, others receiving acupuncture, hyperbaric oxygen therapy, magnetic treatment, and relaxation techniques (White, 1998). However, a widely used and recommended form of inflammatory control is through anti-inflammatory drugs, predominantly those that are non-steroidal.
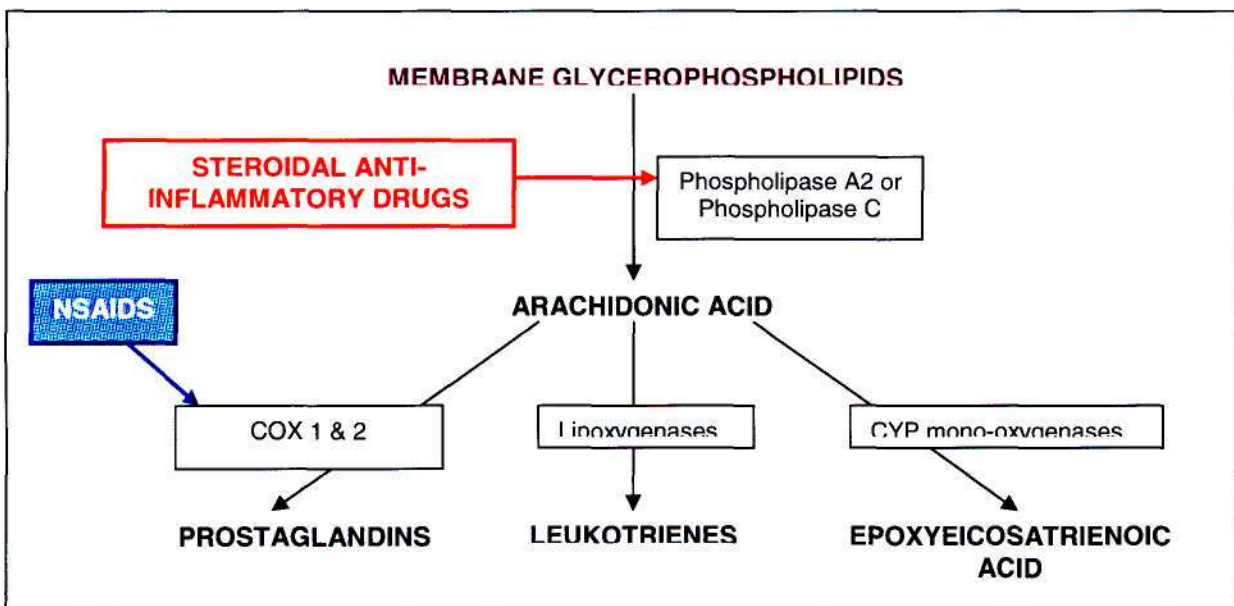
Figure 2.5:   Point of action of steroidal and non-steroidal anti-inflammatory drugs

While steroidal anti-inflammatory drugs act via inhibition of phospholipase and the formation of arachidonic acid, the NSAIDS act by inhibiting cyclooxygenase (COX) and hence do not interfere with the formation of lipoxygenase (Figure 2.5 above). There are two COX enzymes. Type 1 is responsible for the production of cytoprotective prostaglandins which function to produce the mucous that lines the stomach as well as to assist in platelet aggregation. COX-2 enzymes are responsible for the production of inflammatory prostaglandins through the conversion of arachidonic acid. Their function is primarily recruitment of inflammatory cells towards the damaged muscle (Stovitz and Johnson, 2003). Stovitz and Johnson (2003) are of the opinion that blocking the prostaglandins which mediate the inflammatory response however, causes a delay in the healing process.

More commonly reported, the disadvantages of the NSAID treatment regimes are the side effects, which occur as a result overuse of this class of drugs. These complications include gastrointestinal bleeding, renal and cardiovascular disturbances and respiratory symptoms (Stovitz and Johnson, 2003).

More recently, a more selective COX-2 inhibiting drug has been introduced. This drug inhibits the COX-2 enzyme whilst still allowing the functioning of the COX-1 enzymes. It thus allows the cytoprotective prostaglandins to be produced at the same time as eliminating the inflammatory prostaglandins, thereby reducing the side effects and complications which arise during the use of this drug (Stovitz and Johnson, 2003). This concept is yet to be fully evaluated and approved.

2.7.   HOMOTOXICOLOGY

As previously described, one of the mechanisms of action by which NSAIDS work is to block the prostaglandins, which mediate the inflammatory response, causing a delay in the healing of the injury. Concerns relating to blocking the inflammatory prostaglandins through the use of NSAIDS, has led scientists to examine alternative means of inflammatory control. One of the alternative means is the natural science of homotoxicology.

Homotoxicology focuses on the influence that toxic substances have on the human body. It is a combination of traditional eastern concepts including homeopathy, in which an illness is treated by administering diluted amounts of an infectious or toxic agent, which would normally cause illness in a healthy person, with the purpose of activating the production of specific memory β cells which are sensitive to the agent (Bellavite et al., 2005).

In contrast to previously described allopathic treatment which primarily involves the use of NSAIDS, naturalists believe that inflammation is necessary to eliminate the toxic substances in the body and to repair the injured tissues. They argue that by suppressing the COX-2 enzyme, the symptoms of inflammation may be reduced, but the actual cause of the inflammatory condition will not have been treated and hence, in their opinion, the allopathic treatment of inflammation is purely symptomatic, while the bio-therapeutic and homeopathic approach to inflammation is causal and aimed at accelerating the recovery process (Van Brandt, 2002).

The bio-therapeutic approach is centred on the extra cellular matrix and it aims to stimulate and regulate the different components of inflammation, which are proposed to ultimately "speed up" the process of healing. This type of treatment is thought to result in the eventual disappearance of symptoms and the "neutralisation" of the cause via the degradation of those toxins which are responsible for the inflammatory response (Kirkman, 2005).

Two models upon which homotoxicology is based, exist: Selye's model (1952) and the Immunological Bystander Reaction as described by Heine and Schmotz (1998).

Selye's model is based on the premise that because of damage, a change in pH will occur as a result of the release of the stimulating and inhibiting cytokines, within the tissue (Van Brandt, 2002). It views the process of inflammation as a self-regulating one, which will continue until the damaged tissue is restored to its original state. It describes the inflammatory response occurring in two stages, with the first stage being referred to as the "shock phase" and the second stage an "anti-shock phase".

The shock phase is identified as beginning when an increase in the activity of phagocytic cells occurs (Van Brandt, 2002). The shock phase continues as activation of the cellular immune system stimulates the release of IL-1 and TNF-α from the macrophages (antigen-presenting cells) and hence the pro-inflammatory and allergic responses are triggered (Van Brandt, 2002; Weiner, 2001).

A subsequent anti-shock phase has been described by Weiner (2001). This is characterised by an increase in the secretion of TGF-β from the T_H3 lymphocytes. This anti-inflammatory cytokine regulates inflammatory processes including cellular proliferation, -differentiation, -migration and -apoptosis (Weiner, 2001; Heine and Schmotz, 1998).

The Immunological Bystander Reaction, as described by Heine and Schmotz (1998), explains the effect of anti-inflammatory and anti-homotoxic preparations on inflammation. Traumeel S is one such preparation. The model focuses on the effect of the introduction of this anti-homotoxic

agent with low potentised protein into the cytoplasm of the damaged tissue, which is viewed by homotoxicologists as a biophysical filter between the capillary system, the lymph vessels and the inflammatory mediators (Van Brandt, 2002). Heine and Adnrã (2002) postulate that once the anti-homotoxic agent is inside the cytoplasm, it will be engulfed by macrophages to form "motives" on the macrophage. According to the model when these "motives" are recognised by the surrounding T-lymphocytes, transformation of the "motive" occurs and T_H3 cells are derived, which will result in the secretion of TGF-β and a subsequent decrease in the activity of T_H1 and T_H2 (Van Brandt, 2002). This is how the Immunological Bystander Reaction proposes that anti-homotoxic agents can regulate inflammation.

2.8. TRAUMEEL S

Traumeel S, a homeopathic formulation containing *Arnica Montana* and other plant extracts, has been used as a valuable alternative to conventional NSAIDS for over 30 years. It is a broad-spectrum agent, which has a regenerative action. It is composed of a combination of 12 botanical substances and 2 mineral substances. These include Arnica Montana, radix (mountain arnica), Calendula Officianlis (calendula), Hamamelis Virginia (with hazel) Millfolium (minfoil), Belladonna (deadly nightshade), Aconitum Napelus (monkshood), Chaomilla (chamomile), Symphytum Officinale (comfrey), Bellis Perennis (daisy), Echinea Angustifolia (narrow-leafed coneflower), Echinacea Purpea (purple corneflower), Hypericum Perforatum (St Johns Wort), Heparsulphuris Calcareum (calcium sulfide) and Mercurius Solubis (no common name).

Traumeel S is used in the treatment of primary and secondary inflammations and when compared with the conventional non-steroidal anti-inflammatory drugs, has both the effective antiphlogistic and analgesic characteristics. Unlike the conventional NSAID however, Traumeel S lacks the deleterious effects of gastro-intestinal toxicity (Arora *et al.*, 2002). Furthermore, Traumeel S does not inhibit platelet aggregation nor does it have any negative interactions with other medications.

Unfortunately, the exact mechanism of action is unknown and not fully understood. In addition to the previously described Immunological Bystander Reaction, Conforti *et al.* (1997) proposed the modulation of the release of oxygen radicals by activating neutrophils. This is thought to result in an increase in neutrophilic oxidative burst activity and lymphocyte function as well as subsequent inhibition of the release of inflammatory mediators, such as IL-1. Traumeel S is

therefore thought to accelerate the healing process and attenuate the inflammatory response by stimulating both innate and secondary adaptive immune responses to the tissue damage.

Despite its long-term use and availability, there are few sport-specific scientific studies, which support the preparation's anti-inflammatory effects. In 1989 Zell *et al.* conducted a placebo controlled, randomised, double-blind trial which tested the effectiveness of the Traumeel S topical ointment on joint mobility and subjective ratings of pain following sports related ankle sprains. Significant reductions in subjective ratings of pain ($p < 0.003$) and improvement in joint mobility ($p < 0.03$) were shown in patients in the Traumeel S group ($p = 33$) when compared to a placebo group ($p = 36$), 10 days after the injury. These findings were confirmed by Böhmer and Ambrus (1992) who investigated the effect of the topical ointment on recovery following sports related injuries in 102 subjects and found that Traumeel S patients resumed their sports activities after only 12.1 days in comparison to 13.5 days for patients in the placebo group. Wright-Carpenter *et al.* (2004) more recently followed this work up also showing a significant decrease in recovery time following muscle strains in sportsmen and using magnetic resonance imaging (MRI) analysis to support the observed acceleration of the lesion recovery time.

Besides the ointment studies (Böhmer and Ambrus, 1992; Zell, 1989) other studies which have been conducted, have used the intra-articular injection (Birnesser *et al.*, 2004; Thiel, 1987) and the mouth rinse (Oberbaum *et al.*, 2001) as experimental treatment, but none used the tablet form of the preparation. Positive findings that supported the use of Traumeel S as a well-tolerated alternative anti-inflammatory to NSAIDS were reported in these studies. These earlier studies used a variety of outcome measures, from pain scales to joint range of movement and mobility and oedema.

Lussignoli *et al.* (1999) conducted a study on rats to determine the effect of Traumeel S on blood-induced inflammation, whereby 0.1mℓ of homologous blood was injected into the hind paw, to facilitate the development of oedema. The results showed that Traumeel S appeared to speed up the healing process. This study was, however, designed for rats and the inflammation was induced through the re-admission of previously collected blood. The only inflammatory markers, which were measured and assessed were serum IL-6 and oedema both of which were found to be significantly reduced with Traumeel S use ($p < 0.05$).

In other studies on whole blood cultures, Traumeel S has been shown to elevate levels of TGF-β, an anti-inflammatory cytokine (Heine and Schmotz, 1998), while *in vitro* studies have shown that Traumeel S is non-cytotoxic to granulocytes, lymphocytes, platelets and endothelial cells and the defence mechanisms of these cells are not impaired by Traumeel S (Porozov *et al.*, 2004; Conforti *et al.*, 1997).

The most recent findings, relating to the efficacy of Traumeel S in attenuating systemic markers of a post-exercise inflammatory response, are provided by the Sports Research Institute of Pretoria University (Jordaan, 2006). This study compared an experimental group receiving a combination of Traumeel S tablets and ointment to a control group of subjects who received placebo treatment for seven days prior to 45 minutes of downhill running. Unfortunately, the only markers relevant to a systemic inflammatory response which were measured were blood cortisol, leukocyte counts and serum CK. The exercise protocol did also not appear to be of sufficient intensity to elicit a marked inflammatory response. Although serum CK was modestly raised in both the experimental and control subjects 24 hours following the trial, there was no significant difference between the two groups. The experimental group did, however, present with significantly higher concentrations of cortisol 48 hours after exercise than the control group. No significant difference was observed in leukocyte count between the experimental and control groups.

A more carefully designed *in vivo* study measuring appropriate indicators of systemic inflammatory response, following a more intense eccentric exercise protocol that induces muscle damage and an inflammatory response, is thus required.

2.9. CONCLUSION

For a drug that is so widely used by endurance athletes, so long in existence, with so little scientific evidence supporting its mechanism of action and alleged effects, a comprehensive study is required to confirm and validate its effectiveness.

As far as the researcher is aware, the majority of evidence in favour of the efficacy of Traumeel S is subjective in the case of sports and exercise related injuries and *in vitro* in the case of biochemical mechanistic studies. The need for an *in vivo* study on endurance athletes which includes a comprehensive analysis of blood inflammatory markers, therefore still exists.

CHAPTER 3

METHODS

3.1   ETHICAL APPROVAL AND INSURANCE

Prior to the commencement of this study, ethical clearance was obtained from the University of KwaZulu-Natal (UKZN), Nelson R. Mandela School of Medicine, Biomedical Research Ethics Committee, Clearance Number: - E017:05(1).

Insurance was obtained from Heel (Pty) Ltd. in Germany, to cover the UKZN in the event of possible research-related injury to the participant, which may have occurred due to the downhill running on the treadmill.

Each subject read and signed an informed consent form which had been approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Appendix A, B).

3.2   SAMPLE SELECTION

The study was limited to 24 men and women (n = 24), aged 20-50 years, who had covered a mean of 40-60 km per week in training or who were training a minimum of three times per week if participating in team sports, during the six weeks prior to participation in the study. All participants volunteered to participate in the study.

Subject exclusion criteria included:
- (i)   Pregnancy (in the case of ladies)
- (ii)   Smoking
- (iii)   Clinical signs of infection, cardiovascular impairments and diabetes (as verified by a medical practitioner)
- (iv)   Intake of medication (e.g. analgesics or anti-inflammatory) prior to the exercise testing

(v)   Habitual dietary vitamin intake without agreeing to a two-week washout period. Subjects were asked to avoid ingestion of caffeine, alcohol, or other drugs in the 24 hours before their visits to the laboratory.

Following baseline laboratory and field-testing, pairs of subjects were matched according to:

(i)   Gender

(ii)   BMI

(iii)   Current training profile

(iv)   Training age

as well as at least two of the following:

- Foot strike
- $VO_{2\ max}$
- 1609m running time and/or best performance over longer endurance running events (5km, 10km, half marathon or marathon), within six months prior to the study trials.

3.3   EXPERIMENTAL DESIGN

Following the provision of informed consent, subjects underwent exercise testing on three occasions: one introductory laboratory visit for baseline testing and familiarisation with the treadmill, one field visit and one subsequent 90-minute exercise trial (Appendix C). After baseline and field-testing, the subjects were matched as described above and then randomised to treatment sequences using random number tables. Interventions were coded by Heel (Pty) Ltd. in Baden-Baden, Germany prior to being dispatched to South Africa. Neither the subjects nor the researchers were aware of which treatment was active and which treatment was placebo. The coding was only revealed to the researchers once the study was complete and results were available.

Laboratory Testing

At the first laboratory visit, the subjects each reported to the laboratory for a pre-study evaluation. This included documenting details regarding exercise training status and measurements of basic anthropometry, lung function, resting heart rate and blood pressure (Appendix C). Percentage body fat was estimated from the sum of biceps, triceps, sub scapular and suprailiac skin folds using the technique described by Durnin and Womersley (1974). Lung function, including forced vital capacity (FVC) and forced expiratory volume (FEV_1) was tested using a Jaeger Mastercope

spirometer (Wuerzburg, Germany). A physician examined each subject in order to ensure that he was not in possession of any clinical symptoms of upper respiratory tract infection (URTI). An incremental exercise test to exhaustion was conducted on a level treadmill to determine their peak oxygen consumption ($VO_{2\,peak}$).

Subjects were familiarized with the motor driven treadmill (Powerjog GX100, Sport Engineering Limited, Birmingham, England) and warmed up for five minutes at a speed of 12 km/hr for men and 8 km/hr for women. Thereafter, the running speed was increased by 1 km/hour each minute until the subject reached fatigue. The test was terminated once the subjects reached two of the following: (i) a rating of 8-10 on the rate of perceived exertion (RPE) scale (ii) an estimated maximal age-predicted heart rate (iii) a respiratory quotient (RQ) of > 1.15. The highest VO_2 over a 30-second period was defined as $VO_{2\,peak}$.

To measure VO_2 during the treadmill tests, the Metamax Metabolic Analyser, (Jaeger, Netherlands) was used to measure respiratory and metabolic responses during exercise. The gas analysers were calibrated using a room air and gas cylinder containing 16% O_2 and 5% CO_2 in nitrogen (Afrox Gas, Durban). The flow volume sensors were calibrated using a 2ℓ calibration syringe (Jaeger, Netherlands). Mixed chamber analysis of the expired respiratory gases was conducted using a connector facemask and triple V transducer and twin tube. The volume of expired air was measured by the triple V sensors and a sample was fed to the gas analyzers via the twin tubes. In addition, heart rate was also recorded throughout the test using a polar heart-rate monitor (Polar Electro OY, Finland) and rating of perceived exertion, according to the 10-point scale described by Borg (1982), in addition to VO_2, Pulmonary Ventilation (V_E), VCO_2 and RQ, were recorded during the last 30 seconds of every phase of the incremental test.

A regression equation of oxygen consumption vs. power output was derived from the sub-maximal running speed to determine the running speed corresponding to 75 % $VO_{2\,peak}$.

Field Testing

A field visit was conducted at the University of KwaZulu-Natal's sports grounds approximately one week before the trial. Springbok athletics coach and consultant, Norrie Williamson conducted these assessments. Subjects were required to warm up at a relevant pace and thereafter ran a 1609m (four laps) time-trial on the track. Further recording and analysis of past and current training practices was conducted, as was analysis of running style in terms of foot strike, both while running on an athletics track and down a hill (gradient > -5%). Feedback was given to the subjects. Explanation of pre-intervention training and eating procedures was done.

The matching of subjects according to their gender, training age, baseline measurements taken at the first laboratory visit and their running style, training status and performance time in the 1609m field test, was completed after the completion of above-described baseline tests.

Traumeel S and Placebo Trials

- Five days prior to the trial, each subject was required to ingest three Traumeel S or Placebo tablets per day. Subjects were requested to dissolve the tablet under the tongue at least 30 minutes before mealtime (or on an empty stomach) and tablets were taken for the full duration of the trial until the last blood sample (72 PE) had been given.

- The day before the trial, subjects were instructed to avoid any heavy physical exercise.

- On the morning of the trial, the following breakfast was eaten three hours before the trial: 30g cornflakes, 150ml of low fat milk and 12-15g refined sugar.

- On trial day, the subjects were required to report to the laboratory in the morning three hours after consuming a prescribed breakfast. A pre-exercise venous blood sample was obtained from an antecubital vein. The exercise protocol consisted of a -6% downhill run for 90 minutes on a motorized treadmill, which had been adjusted to the appropriate downhill gradient. The running speed was set at that which corresponded to 75% of the individual maximal oxygen consumption (75% $VO_{2\,max}$), as determined during the baseline testing and field visit. During each actual trial run, the participants were required to wear a heart rate monitor to allow for continuous recording of their heart rate. At 30 minutes and at one hour into the run, the subjects were allowed a 2-minute reduction of workload to 50% $VO_{2\,max}$, ensuring an easy water intake from a bottle containing 500 ml of water. The subject was required to consume the total amount of water. Music was available to entertain the runners on the treadmill and free communication between runners and research assistants was encouraged.

- On completion of the exercise trial, a 30ml blood sample was drawn from an antecubital vein immediately after the trial.

Post-Trial Recovery

At 24, 48 and 72 hours following the trial, subjects were requested to return to the exercise laboratory, at the same time each day as their IPE blood sample was taken, to attain further blood samples and to record subjective ratings of DOMS. Subjects were also requested not to resume training during this post-trial period of three days.

Subjective ratings of muscle soreness were determined at the beginning of each day following the Traumeel S and Placebo exercise trials with a numerical 0-10 scale where 0 = no pain at all,

5 = moderate, 10 = severe/maximal pain (Appendix D). The quadriceps, hamstrings and calf muscles were assessed under four categories:

- General pain (subject seated and relaxed)
- Daily living (walking/moving)

These subjective ratings of discomfort in the groups of muscles in the four above-mentioned categories, as well as the peak rating in one particular muscle group, were expressed as a mean ± standard deviation (SD).

Haematological Analysis and Adjustments

All post-event blood samples were obtained at the same time of day to avoid the influence of diurnal rhythms on blood cortisol concentrations. The participants were asked to refrain from any strenuous exercise during the three days following the trial day.

Full blood counts including differential leukocyte and platelet counts were performed on K_3 - ethylene diaminetetra-acetic acid (EDTA) treated specimens using standard haematological procedures on an automated STKS model (Coulter Electronics Inc., Hialeah, Florida, USA). Plasma volume changes were determined from pre- and post-race haematocrit and haemoglobin concentrations according to the method of Dill and Costill (1974) in order to estimate changes in blood volume, which occurred following participation in the 90-minute downhill trial. All concentrations of the blood cell parameters were adjusted for exercise-induced changes in blood volume.

The following mathematical equation originally developed by Dill and Costill (1974) was used to calculate the percentage change in plasma volume (IPE versus PRE and 24 PE versus IPE):

$$\% \, \Delta PV = \left[\frac{Hb_{PRE}}{Hb_{PR}} \times \frac{1 - Hct_{PR}}{1 - Hct_{PRE}} - 1 \right] \times 100$$

Where Δ PV = Change in plasma volume

      PRE = pre-trial sample

      PR = post-trial sample

      Hb = Haemoglobin concentration

      Hct = Haematocrit

Five mℓ of the remaining blood was allowed to clot at ambient temperature, the serum separated and 0.5mℓ aliquots thereof stored in Eppendorf tubes at -70°C for later analysis of CRP, CK and

cortisol. The remaining blood was drawn into vacutainer tubes containing EDTA and the plasma separated. This was stored at -70°C for later measurement.

Determination of Serum CK, LDH, Cortisol and IL-6 Concentrations

Serum was sent to a local Chemical Pathology Laboratory (Global Clinical and Viral Laboratories, Jacobs, Durban), and analysed for CK and LDH concentration using a Synchron CX, X5Analyser (Beckman Coulter, SA (Pty) Ltd., Halfway House, South Africa).

Serum cortisol was assayed using the Gamma Coat radioimmunoassay procedure (AB Advia Centaur CPAdviacentaur, Siemens Medical Solutions Diagnostics, Deerfield, USA).

Plasma IL-6 concentration was determined by sandwich ELISA using a commercial kit (eBioScience, San Diego, CA, USA)

Determination of Serum CRP Concentrations

An immunoturbidimetric assay (Randox CRP Kit T, Cat No CP 7950, 2002 and CRP Standard, Cat No CP1599, Randox Laboratories Ltd., Antrim, UK) was used to determine the concentration of CRP in the serum samples. The standard was diluted with 0.9% sodium chloride (NaCl) solution in the following geometric series:

Table 3.1: Dilution factors for the CRP standard

Dilution	Neat	1:2	1:4	1:8	1:16	0.9% NaCl
Dilution Factor	1	0.5	0.25	0.13	0.06	0

- Well-thawed serum was used undiluted and 20 minutes prior to preparation, both the reagents and samples were equilibrated to room temperature.
- 1.0mℓ of buffer made up of 4% Polytheline Glycol, 20mmol/ℓ Tris/HCL (pH 7.4) and 150 mmol/ℓ NaCl buffer was added to 100$\mu\ell$/blank standard/undiluted sample and mixed well for three minutes to equilibriate.
- 150$\mu\ell$ 0.9% NaCl solution was added to the blank while 150$\mu\ell$ I anti-human CRP was added to the sample/standard. After mixing well and covering tubes with plastic cling film, they were left to incubate for 15 minutes. After mixing, the absorbance of each solution read on a spectrophotometer (Cary IE, Varian, Palo Alto, USA) at a wavelength of 340nm.
- The samples were read in a 1cm light path glass microcuvette at a temperature of 25°C

Standard Curve and Calculation of Results

In order to determine the CRP concentration of the sample, a standard curve, derived from the absorbance of standards, was constructed for the assay run. This curve was obtained from the following equations, which takes into consideration that the CRP concentration is dependant on the batch specific CRP standard, which is chosen.

CPR concentration of dilution series = assigned value of undiluted standard x dilution factor

$$\Delta \ A_{sample/standard} = \text{Test Absorbance} - \text{Blank Absorbance}$$
$$y = 0.017x$$
$$R^2 = 0.954$$

The $\Delta \ A_{standard}$ was plotted against the given CRP concentration of the dilution series and the $\Delta \ A$ of the samples was read off this graph to determine their concentration.
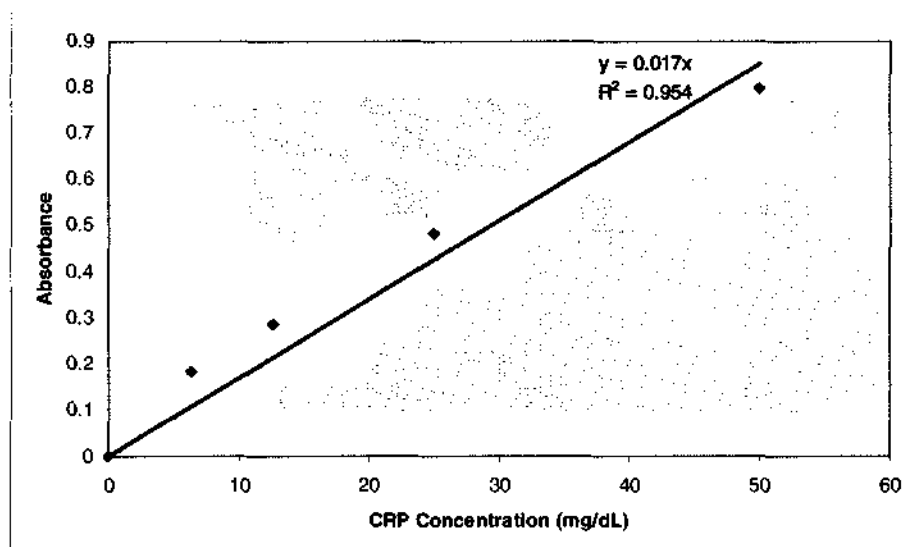
Figure 3.1:   Correlation between CRP concentration (mg/dL) and change in absorbance (nm)

Measurement of Serum Myeloperoxidase

An *in vitro* enzyme-linked immunosorbent assay (ELISA) (Immundiagnostik AG, Lot No K6631) was used to determine the presence of MPO in the serum samples. The assay utilised the two-site "sandwich" technique with two selected polyclonal anti-bodies that bind to human MPO.

Assay standards, controls and prediluted subject samples containing human MPO were added to the wells of the microplated that was coated with a high affine polyclonal anti-human MPO antibody. The MPO of the subject samples was captured by the antibody which was immobilised on the walls of the microtiter wells after the first incubation period of 60 minutes at room temperature. Peroxidase-conjugated polyclonal anti-human MPO antibody was then added to each microtiter well which formed a "sandwich" of captured antibody – human MPO –

Peroxidase conjugate. Tetramethylbenzidine (TMB) was used as a substrate for the peroxidase. To terminate the reaction, an acidic stop solution was added to the samples. The colour change from blue to yellow indicated the presence of MPO in the samples, as the intensity of the yellow colour is directly proportional to the concentration of MPO in the sample. The MPO present in the samples was directly determined from a dose response curve of the absorbance unit (optical density (OD) at 450nm) versus concentration. This curve was generated using the values obtained from the standard and the Δ A of the samples was read off this graph to determine their concentration.

MPO concentration of dilution series = assigned value of undiluted standard x dilution factor

$$\Delta A_{sample/standard} = \text{Test Absorbance} - \text{Blank Absorbance}$$

$$y = 12828x^2 + 1219.1x$$

$$R^2 = 0.9876$$

The $\Delta A_{standard}$ was plotted against the given MPO concentration of the dilution series and the Δ A of the samples was read off this graph to determine their concentration.
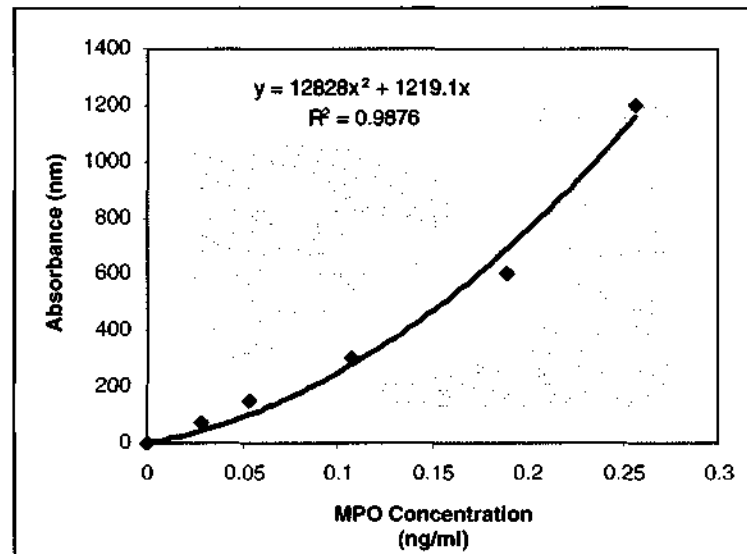
Figure 3.2:   Correlation between MPO concentration (ng/mℓ) and change in absorbance (nm)

3.4   STATISTICAL ANALYSIS

Data was analysed in SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA). A p value < 0.05 was considered as statistically significant. Baseline and field trial outcomes were compared between the two groups using paired t-tests as well as Wilcoxon Signed Rank Tests. Qualitative descriptions of foot strike pattern were categorised into three pattern types and the McNemar-

Bowker Chi Square Test was used for comparison of the paired foot strikes. Repeated measures analysis of variance (ANOVA) testing was used to compare quantitative normally distributed outcomes over time between the experimental (TRS) and placebo-control (PLAC) groups. Wilks' Lambda was used as test statistic with a Simple Contrasts *post hoc* correction to determine the time-point of the significant differences between treatments. Variables that were not normally distributed were log transformed. Significant time-group interactions indicated significant treatment effects. Profile plots were generated to examine trends. There was very little missing data, but for some outcomes, the final time point result was missing in a few (up to four) cases. In this case, repeated measures ANOVA was conducted on four time points as well as five and the results were compared. If there was no difference in the conclusion then the five time-point comparison was reported. For the DOMS analysis, the average of the three muscle types and modes were computed for each day. Repeated measures ANOVA was performed on these averaged measurements over time, which were normally distributed.

CHAPTER 4

RESULTS

4.1. SUBJECT CHARACTERISTICS

Of the 24 subjects who fully complied with all inclusion criteria, 10 were women and 14 were men with the age of the subjects ranging from 19 to 50 years. The BMI of the total sample ranged from 19.6 to 31.6 kg/m^2, while the body fat percentage ranged from 7.5 to 27.7%. Table 4.1 depicts the mean ($\pm$ SD) physical characteristics of the two gender-matched groups on Traumeel S (TRS) and Placebo (PLAC).

Table 4.1: Mean $\pm$ SD physical characteristics of subjects in the TRS (n = 12) and PLAC (n = 12) groups

Variable	TRS	PLAC	p value**
Age (years)	28.8 $\pm$ 7.8	31.1 $\pm$ 9.7	0.23
Stature (cm)	171.7 $\pm$ 11.5	170.7 $\pm$ 12.3	0.30
Mass (kg)	69.7 $\pm$ 13.4	68.6 $\pm$ 14.9	0.53
% Body Fat*	15.1 $\pm$ 4.2	16.4 $\pm$ 5.0	0.41
BMI (kg/m^2)	23.5 $\pm$ 3.1	23.3 $\pm$ 3.1	0.68

* Derived from the sum of triceps, biceps, suprailiac and subscapular skin folds
** Paired t-tests TRS vs. PLAC

Subject characteristics were analysed both parametrically using paired t-Tests and non-parametrically using Wilcoxon Signed Rank tests. Irrespective of the analysis used, the two groups did not differ significantly in terms of age, stature, mass, % body fat and BMI (p > 0.05).

4.2. BASELINE MEASUREMENTS

During the maximal exercise test, the maximal heart rate ranged from 170 to 196 beats per minute (bpm) and the VO_2 peak from 42.0 to 63.8 mℓ/kg/min. The maximum respiratory exchange ratio (RER) ranged from 1.02 to 1.28 and the $V_{E\,max}$ from 76 to 138 ℓ/min. The range of the FVC was from 2.57 to 7.91ℓ and the FEV_1 ranged from 2.05 to 5.91ℓ. The mean ($\pm$ SD) results of the above-mentioned measures in the TRS and PLAC groups are presented in Table 4.2.

Table 4.2: Mean ± SD baseline test measurements of subjects in the TRS (*n* = 12) and PLAC (*n* = 12) groups

Variable	TRS	PLAC	*p* value**
VO_2 peak (ml/kg/min)	54.00 ± 6.48	50.37 ± 4.66	0.06
RER	1.15 ± 0.07	1.14 ± 0.09	0.98
Maximum Heart Rate (bpm)	182 ± 7.85	185 ± 9.48	0.25
Resting Heart Rate (bpm)	62 ± 7.04	64 ± 8.34	0.53
V_E (l/min)	108.4 ± 18.0	104.6 ± 17.3	0.51
FVC (l)	4.67 ± 1.09	4.82 ± 1.36	0.69
FEV_1 (l)	4.00 ± 0.89	3.95 ± 1.03	0.86

** Paired *t*-tests TRS vs. PLAC

Data were analysed parametrically and non-parametrically and again, both confirmed that the gender-matched subjects within the two groups did not differ significantly in terms of the parameters listed in Table 4.2.

4.3. FIELD AND TRAINING PROFILE

Table 4.3 displays the comparative field and training profiles of the PLAC and TRS groups. Subjects within the two groups were also well matched in this regard as is depicted by marginal differences in the means ± SD, all of which were not statistically significant (*p* > 0.05).

Table 4.3: Mean ± SD field and training profile measurements of the subjects in the TRS (*n* = 12) and PLAC (*n* = 12) groups

Variable	TRS	PLAC	*p* value**
Ave Running Distance (km/week)	39.6 ± 13.5	40.5 ± 18.9	0.80
Number of Years Running	9.55 ± 4.13	10.72 ± 6.05	0.70
1609m Time (min)	6.16 ± 0.69	6.31 ± 0.97	0.37
5km Time (min)*	22.20 ± 5.22	20.00 ± 0.00	0.68
10km Time (min)*	43.8 ± 9.18	41.00 ± 1.41	0.71
Half Marathon Time (min)*	100.7 ± 9.99	102.0 ± 17.6	0.71
Marathon Time (min)*	233.0 ± 29.5	228.5 ± 23.0	0.66

* 5km: *n* = 8; 10km: *n* = 7; Half marathon: *n* = 15; Marathon: *n* = 8
** Paired *t*-tests TRS vs. PLAC

The different foot strike patterns and the prevalence (*n*; %) of each pattern in both the control and experimental groups, as well as over the entire sample is displayed in Table 4.4.

4.5. MARKERS OF MUSCLE CELL DAMAGE

4.5.1. Serum Lactate Dehydrogenase

The mean ± SD of the serum LDH concentrations obtained before and following the trial are shown in Figure 4.1. The mean ± SD LDH concentrations of all subjects ($n = 24$; top), adjusted for PV changes, peaked immediately post-trial reaching a significantly elevated mean value of 555.3 ± 137.3 IU/ℓ ($p < 0.001$) and ranged from 368 to 847 IU/ℓ. The LDH concentrations declined steadily for the duration of the recovery, returning to pre-exercise levels at 72 hours following the trial ($p > 0.05$).
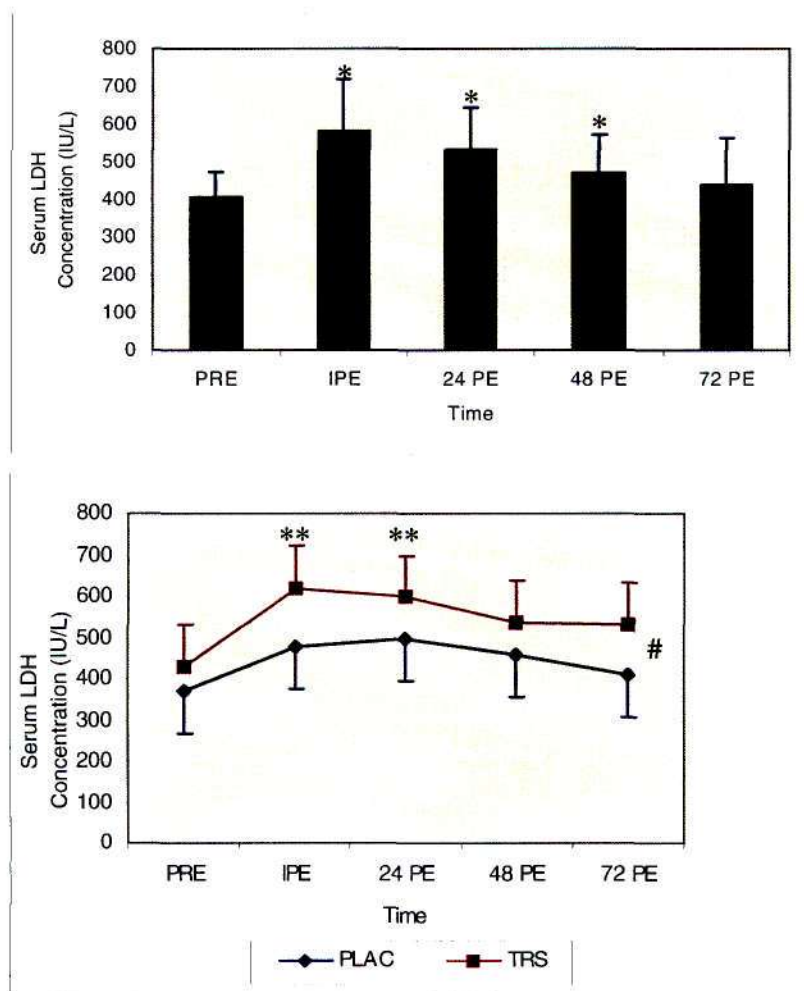
Figure 4.1:   Mean ± SD LDH concentrations of complete sample ($n = 24$; top) adjusted for PV changes and TRS ($n = 12$) and PLAC ($n = 12$) groups (bottom) prior to and following participation in the 90-minute downhill treadmill run
* $p < 0.05$ Paired t-tests vs PRE
# $p < 0.05$ Time-group interaction, repeated measures ANOVA, Wilks' Lambda Test Statistic
** $p < 0.05$ Simple contrasts *post hoc* comparison vs. PRE between groups at time point

The comparative serum LDH concentrations for both the TRS and PLAC groups are also shown in Figure 4.1 (bottom). In the TRS group, the LDH increased maximally immediately following the trial reaching a mean concentration of 620.2 ± 146.4 IU/ℓ, while the PLAC group reached a

mean concentration of 496.3 ± 107.8 IU/ℓ at 24 PE. When the plasma volume adjusted concentrations are expressed relative to the concentrations at the previous time point (Table 4.6) and baseline values (Figure 4.2), the differences between the two interventions are however, not statistically significant ($p = 0.536$).

Table 4.6: Mean ± SD differences in LDH concentrations (IU/ℓ) prior to and following participation in the 90-minute downhill run in the TRS ($n = 12$) and PLAC ($n = 12$) groups

Time Difference	TRS	PLAC	p value**
IPE vs. Pre Trial	189.8 ± 141.4	107.4 ± 71.9	0.06
24 PE vs. IPE	-21.5 ± 129.7	18.4 ± 124.5	0.47
48 PE vs. 24 PE	-61.0 ± 182.8	-38.8 ± 49.2	0.64
72 PE vs. 48 PE	-4.23 ± 191.6	-50.1 ± 100.0	0.40

** Paired *t*-tests TRS vs. PLAC
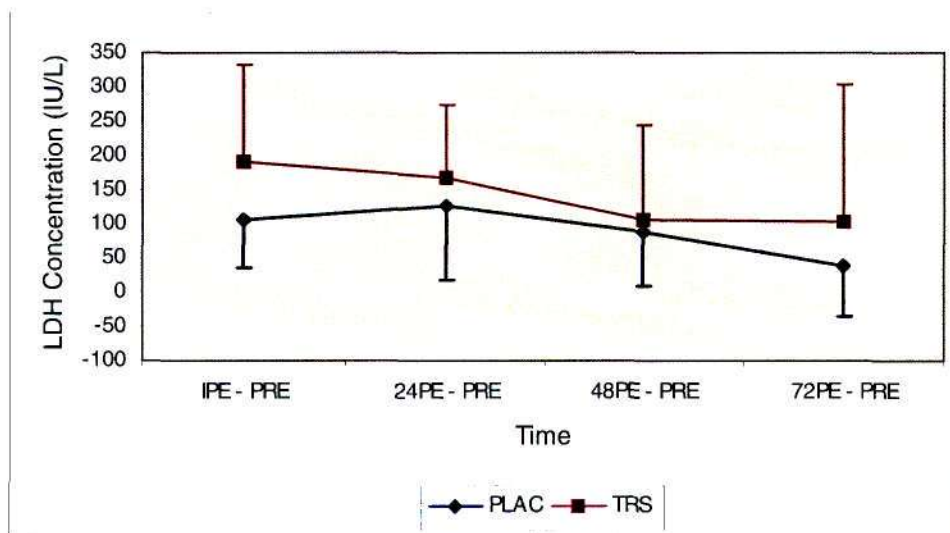
Figure 4.2:   Mean changes* in serum LDH concentrations in the TRS group ($n = 12$) and PLAC groups ($n = 12$) following participation in a 90-minute downhill run
* Adjusted for pre-trial concentration

4.5.2. Serum Creatine Kinase

The mean ± SD serum CK concentrations for the total sample ($n = 24$) is depicted in Figure 4.3 (top). The CK concentration rose significantly in response to 90 minutes of downhill running ($p < 0.001$), peaking at 24 hours post exercise (1474.2 ± 1172.3 IU/ℓ) and remained significantly elevated in the 48 PE and 72 PE serum samples ($p < 0.05$) despite relatively large variances within the groups.
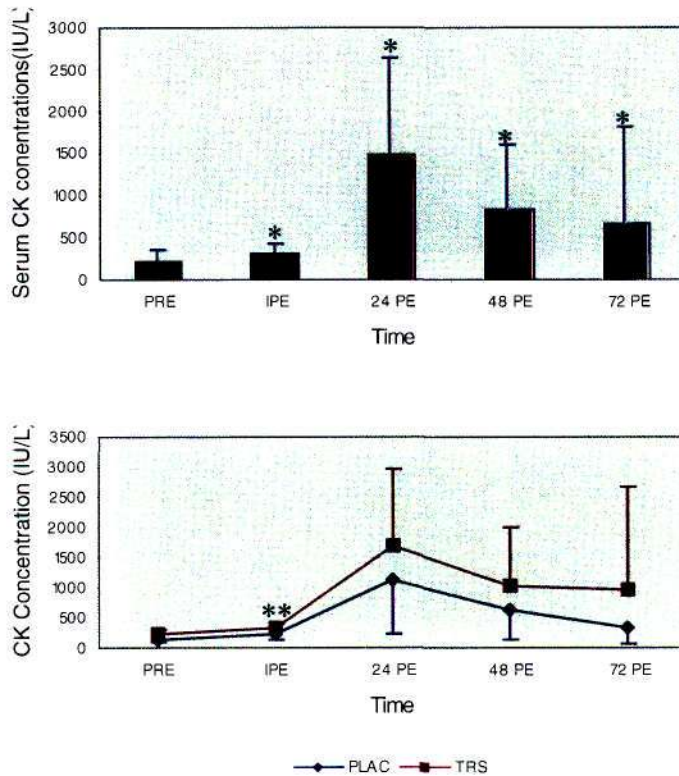
<u>Figure 4.3:</u>   Mean ± SD CK concentrations adjusted for plasma volume changes of complete sample ($n = 24$; above) and TRS ($n = 12$) and PLAC ($n = 12$) groups (below) prior to and following participation in the 90-minute downhill treadmill run
* $p < 0.05$ Paired t-tests vs PRE
** $p < 0.05$ Simple contrast *post hoc* comparison vs. PRE between groups at time point

As is shown in Figure 4.3 (bottom) the time effect between the TRS and PLAC groups was highly significant ($p < 0.001$), but the group-time interaction did not reach statistical significance ($p = 0.398$; repeated measures ANOVA, Wilks' Lambda test statistic). Simple contrasts *post-hoc* analyses revealed that the absolute CK concentration differences were only statistically significant at the IPE time point ($p = 0.04$).

When the concentrations are expressed relative to the concentrations at the previous time point (Table 4.7) and are adjusted for baseline values (Figure 4.4), the differences between the two interventions are also not statistically significant ($p > 0.05$).

<u>Table 4.7</u>: Mean ± SD differences in serum CK concentrations (IU/ℓ) adjusted for PV changes in the TRS ($n = 12$) and PLAC ($n = 12$) groups

Time Difference	TRS	PLAC	p value**
IPE vs. Pre Trial	88.4 ± 45.5	88.5 ± 61.9	0.99
24 PE vs. IPE	1394.8 ± 1224.6	915.9 ± 860.3	0.31
48 PE vs. 24 PE	-674.2 ± 449.2	-504.5 ± 456.4	0.33
72 PE vs. 48 PE	-71.27 ± 828.3	-319.7 ± 449.5	0.35

** Paired t-tests TRS vs. PLAC
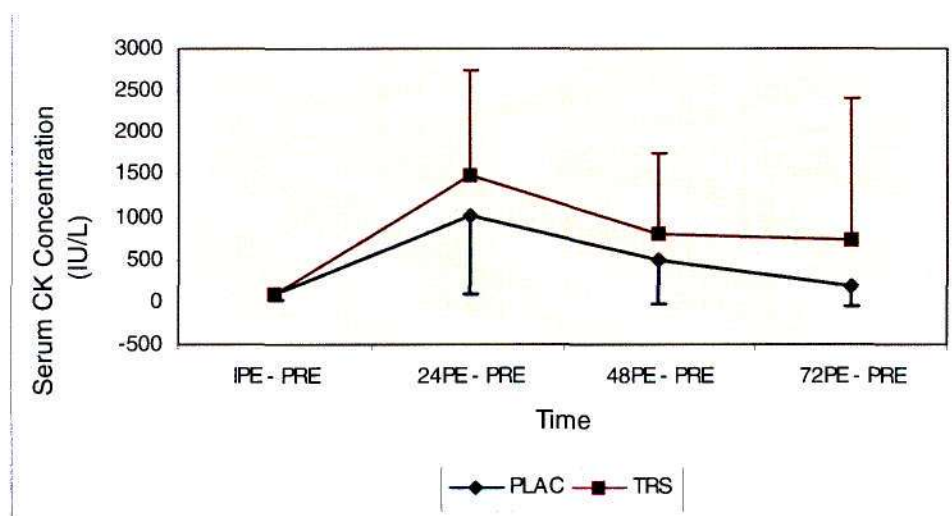
<u>Figure 4.4:</u>   Mean changes* in CK concentrations in the TRS ($n = 12$) and PLAC groups ($n = 12$) following
                      participation in a 90-minute downhill run
                      * Adjusted for pre-trial concentration

4.6.   DELAYED ONSET MUSCLE SORENESS

As depicted in Figure 4.5 the mean DOMS score peaked at Day 2 post trial and declined
steadily thereafter. The majority of subjects reported that pain was elicited during activities and
walking as opposed to at rest. The muscle groups most affected were the quadriceps muscles,
followed by the calf muscles. The hamstring muscle group was the least affected. Five subjects
reported pain in all muscle groups. The overall means of the DOMS scores over the four days
following the trial were analysed using repeated measures ANOVA tests not did not reveal a
significant group-time interaction over the three days post exercise ($p = 0.68$). The difference
between the two groups was only significant on the 2nd day post trial.
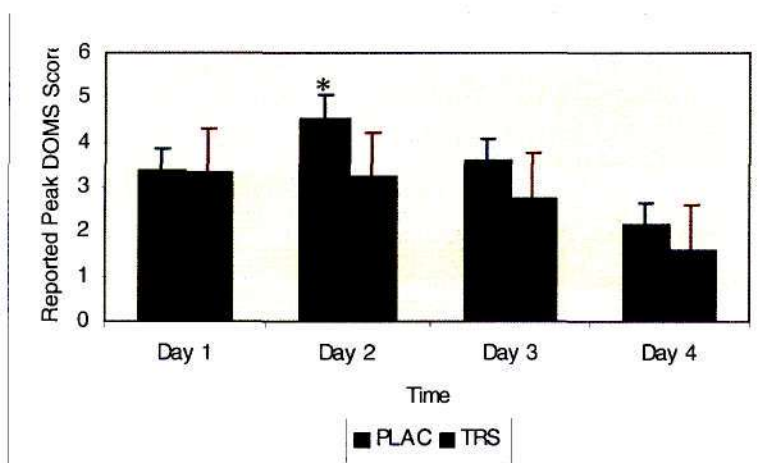
<u>Figure 4.5:</u>   Mean ± SD reported peak DOMS scores of the TRS ($n = 12$) and PLAC ($n = 12$) groups
                      following participation in a 90-minute downhill run
                      * $p < 0.05$ Paired t-tests, TRS vs PLAC

The peak DOMS score in each subject during the three-day recovery period the score was compared with the percentage change in measured serum CK concentrations from PRE to 24 PE (Figure 4.6). The correlation between these two indicators of exercise-induced muscle cell damage were statistically significant ($r = 0.33$, $p < 0.05$).

$$y = 0.0013x + 6.6607$$
$$r = 0.33$$
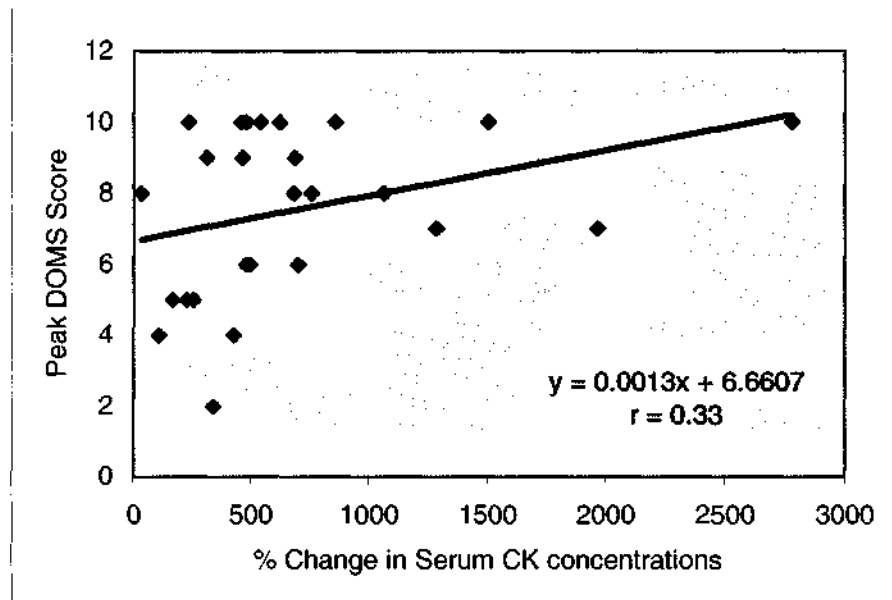
Figure 4.6:   Peak DOMS score versus % change in serum CK concentrations ($n = 26$) during the 24 hours after completion of the 90 minute running trial

4.7.   HAEMATOLOGICAL ANALYSES

The time effect in the red blood cell (RBC) indices before and after the 90-minute downhill run as well as the plasma volume changes between time-points are shown in Figure 4.7. There was a significant time-effect over the three days in each of the indices ($p < 0.05$).

4.7.1. Red Blood Cell Indices & Plasma Volume Changes*
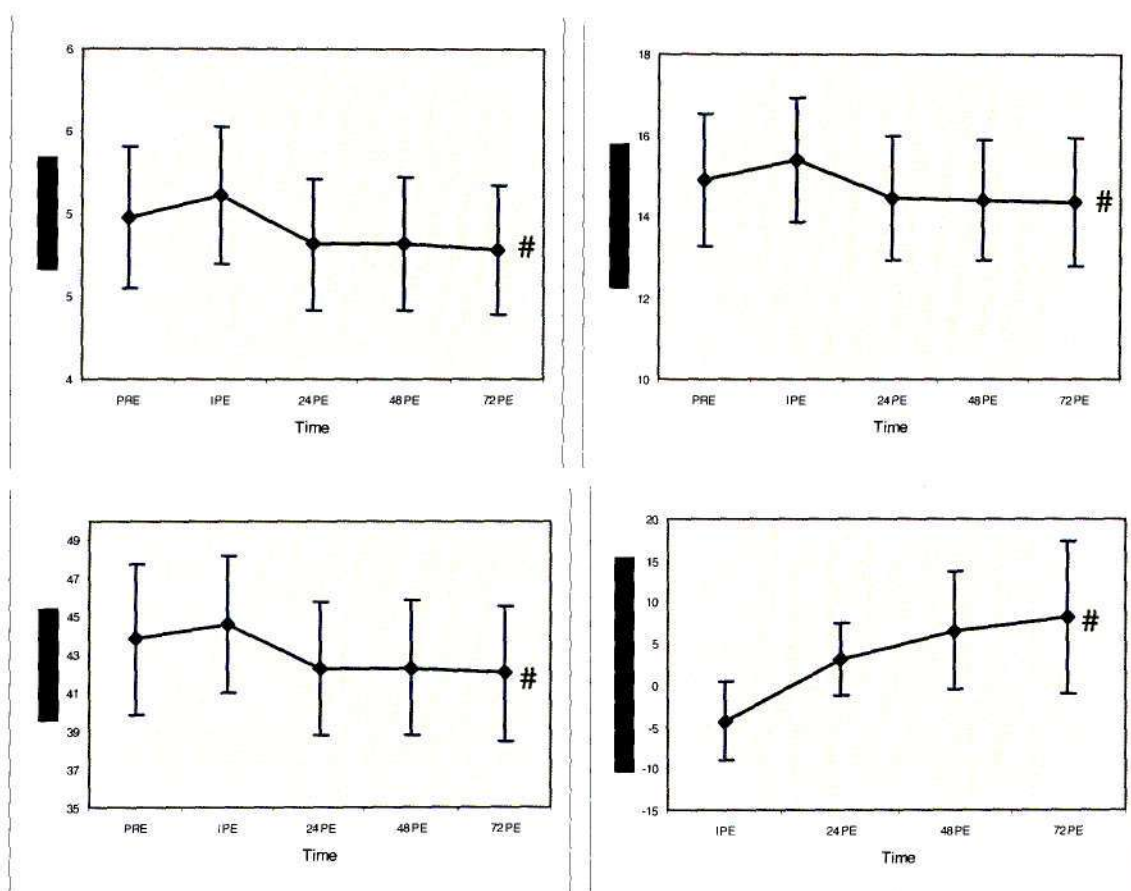
Figure 4.7:   Changes in RBC indices before and after 90 minutes of downhill running ($n = 24$)
$\# p < 0.01$; Repeated measures ANOVA, time effect
* Calculated using the formula of Dill and Costill (1974) vs.PRE

The comparative red blood cell indices for the experimental and placebo-control groups are presented in Table 4.8. No significant difference was found between the two groups in terms of RBC count, haemoglobin concentration (Hb), haematocrit (Hct) and plasma volume (PV) changes. Exercise-induced increases in these red blood cell indices were consistent between the two groups, resulting in significant decreases in PV following the 90-minute trial ($p < 0.001$; IPE) with subsequent increases at 24, 48 and 72 hours post-trial when compared to the pre-trial (Figure 4.7).

Table 4.8: Mean ± SD RBC indices in TRS (n = 12) and PLAC (n = 12) groups before and after 90 minutes of downhill treadmill running at the 5 time-points

	Pre – Trial	IPE	24 PE	48 PE	72 PE	Time Effect, Interaction Effect #
Red Blood Cell Count (10^{12} /µℓ)						
TRS Group	4.96 ± 0.40	5.09 ± 0.40	4.76 ± 0.38	4.78 ± 0.40	4.80 ± 0.25	$p < 0.001$
PLAC Group	4.89 ± 0.43	5.01 ± 0.41	4.75 ± 0.41	4.75 ± 0.40	4.65 ± 0.46	$p = 0.96$
Haematocrit (%)						
TRS Group	43.8 ± 3.62	44.6 ± 3.32	42.0 ± 3.07	42.2 ± 3.16	42.4 ± 2.20	$p < 0.001$
PLAC Group	43.7 ± 4.20	44.5 ± 3.92	42.3 ± 4.00	42.2 ± 4.11	41.4 ± 4.61	$p = 0.10$
Haemoglobin (g/dℓ)						
TRS Group	15.1 ± 1.42	15.5 ± 1.31	14.6 ± 1.29	14.5 ± 1.24	14.6 ± 1.02	$p < 0.001$
PLAC Group	14.6 ± 1.70	15.2 ± 1.76	14.3 ± 1.79	14.2 ± 1.75	14.0 ± 2.02	$p = 0.71$
Plasma Volume Changes (%)**						
TRS Group	-	- 3.93 ± 5.19	3.60 ± 5.03	7.20 ± 7.70	8.40 ± 9.80	$p < 0.001$
PLAC Group	-	- 4.80 ± 3.59	2.59 ± 3.78	5.94 ± 5.05	8.00 ± 9.12	$p = 0.98$

Repeated measures ANOVA, Wilks' Lambda Test Statistic
** Versus PRE – calculated using formula of Dill and Costill (1974)

4.7.2. White Blood Cell Indices

The time effect in the white blood cell (WBC) indices in the full study sample (n = 24) before and after the 90-minute downhill run are shown in Figure 4.8, while the comparative white blood cell indices for both the experimental and placebo-control groups are shown in Table 4.9.

The white cell count (WCC) increased significantly at both the IPE ($p < 0.001$) and at 24 PE ($p < 0.05$) when compared to the pre-trial WCC values. The neutrophil counts were significantly increased at the IPE interval ($p < 0.001$) and returned to near pre-trial values within 24 hours. No time or interaction effect was observed in the lymphocyte counts. Significant monocytosis occurred immediately following the trial ($p < 0.05$).

Figure 4.8: Mean WBC indices of subjects (n = 24) before and after participation in the 90-minute trial.
*p < 0.05 Paired t-tests vs PRE
# p < 0.05 Time-group interaction, repeated measures ANOVA
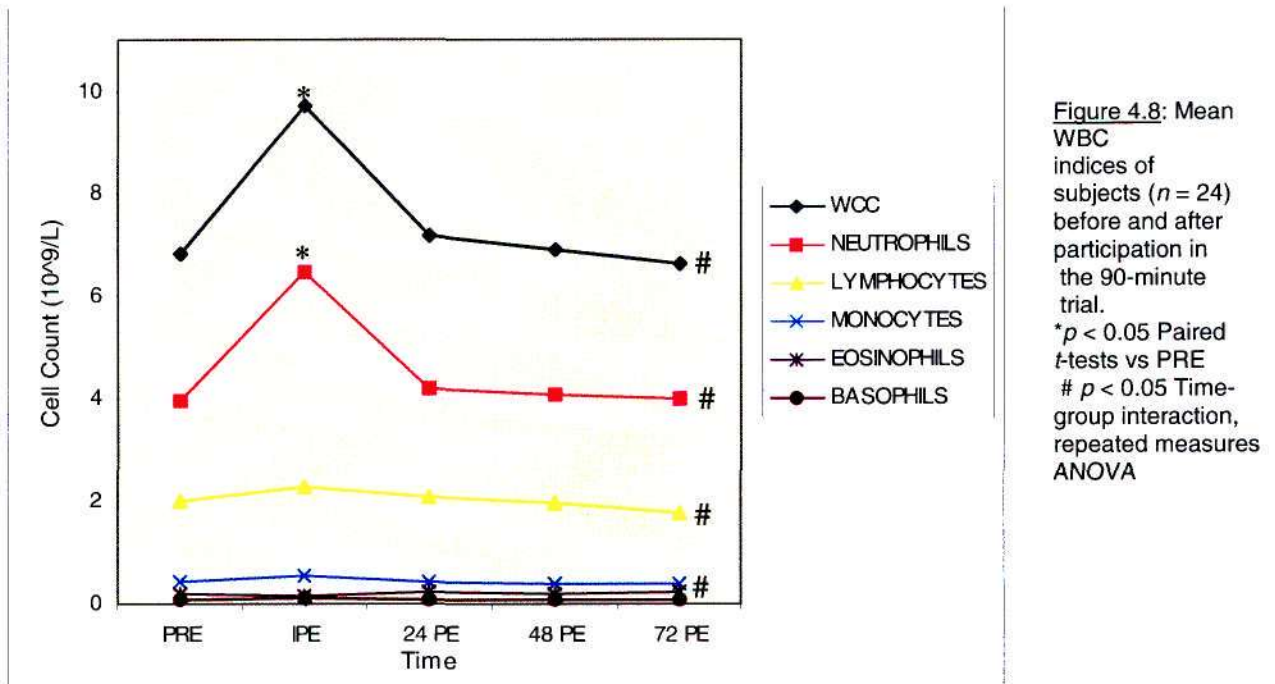
Table 4.9: Mean ± SD absolute differential WBC counts* in TRS (n = 12) and PLAC (n = 12) groups before and after the 90 minute downhill running trial at the 5 time points.

	Pre – Trial	IPE	24 PE	48 PE	72 PE	Time Effect, Interaction Effect #
White Cell Count (10^9/ ℓ)						
TRS Group	7.02 ± 2.31	9.13 ± 3.08	7.61 ± 1.99	7.40 ± 2.45	6.92 ± 2.03	p = 0.008
PLAC Group	7.07 ± 1.85	9.27 ± 2.00	7.39 ± 1.88	7.35 ± 1.80	7.42 ± 2.21	p = 0.91
Neutrophil Count (10^9/ ℓ)						
TRS Group	4.19 ± 1.80	6.22 ± 2.76	4.65 ± 1.62	4.54 ± 1.92	4.19 ± 1.80	p = 0.003
PLAC Group	4.06 ± 1.63	6.09 ± 1.90	4.19 ± 1.53	4.21 ± 1.43	4.49 ± 1.79	p = 0.94
Lymphocyte Count (10^9/ ℓ)						
TRS Group	1.89 ± 0.64	2.00 ± 0.82	2.03 ± 0.72	1.92 ± 0.60	1.85 ± 0.43	p = 0.28
PLAC Group	2.12 ± 0.54	2.20 ± 0.59	2.21 ± 0.58	2.23 ± 0.77	1.94 ± 0.44	p = 0.94
Monocyte Count (10^9/ ℓ)						
TRS Group	0.43 ± 0.15	0.47 ± 0.14	0.43 ± 0.10	0.41 ± 0.10	0.37 ± 0.07	p = 0.04
PLAC Group	0.47 ± 0.17	0.54 ± 0.17	0.46 ± 0.19	0.43 ± 0.16	0.49 ± 0.19	p = 0.82
Eosinophil Count (10^9/ ℓ)						
TRS Group	0.21 ± 0.12	0.13 ± 0.12	0. 22 ± 0.13	0.20 ± 0.13	0.22 ± 0.10	p = 0.08
PLAC Group	0.19 ± 0.18	0.15 ± 0.16	0.23 ± 0.17	0.21 ± 0.08	0.23 ± 0.17	p = 0.12
Basophil Count (10^9/ ℓ)						
TRS Group	0.11 ± 0.09	0.11 ± 0.09	0.09 ± 0.05	0.10 ± 0.07	0.09 ± 0.06	p = 0.68
PLAC Group	0.11 ± 0.03	0.09 ± 0.05	0.08 ± 0.05	0.10 ± 0.06	0.12 ± 0.05	p = 0.40

* all post trial concentrations are adjusted for PV changes
# repeated measures ANOVA, Wilks Lambda Test Statistic
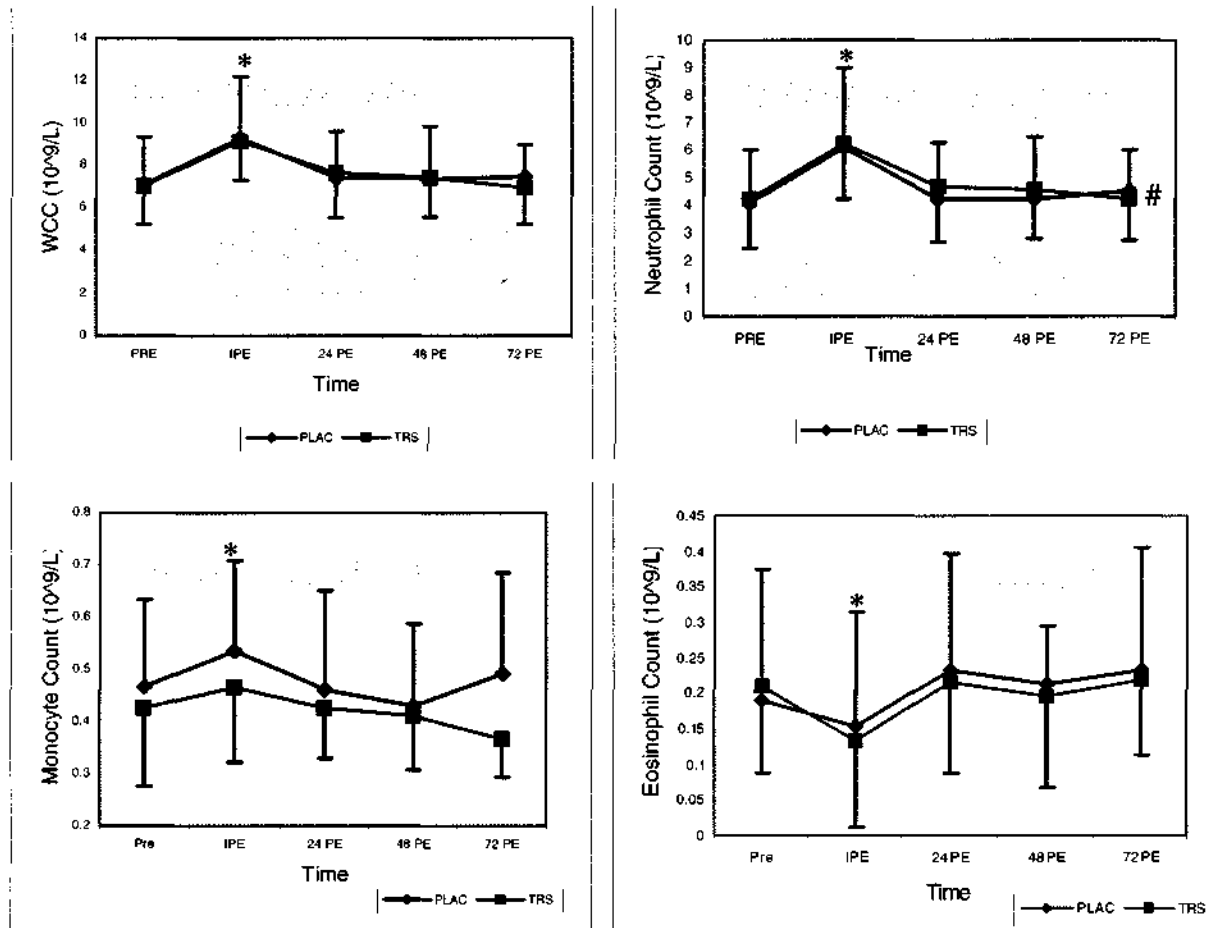
Figure 4.9: Changes in WCC (top left), neutrophil (top right) and monocyte (left bottom) and eosinophil (bottom left) concentrations in the TRS (n = 12) and PLAC groups (n = 12) before and after the 90-minute treadmill trial
* $p < 0.05$ Paired t-tests vs PRE
# $p < 0.05$ Time-group interaction, repeated measures ANOVA, Wilks Lambda Test Statistic

4.8. SERUM MYELOPEROXIDASE

The mean ± SD serum MPO concentrations for all subjects are depicted in Figure 4.10. MPO was significantly increased immediately post trial for both groups of subjects ($p < 0.05$) and reached a mean value of 155.8 ± 86.4 ng/ml at the 24 PE time point.
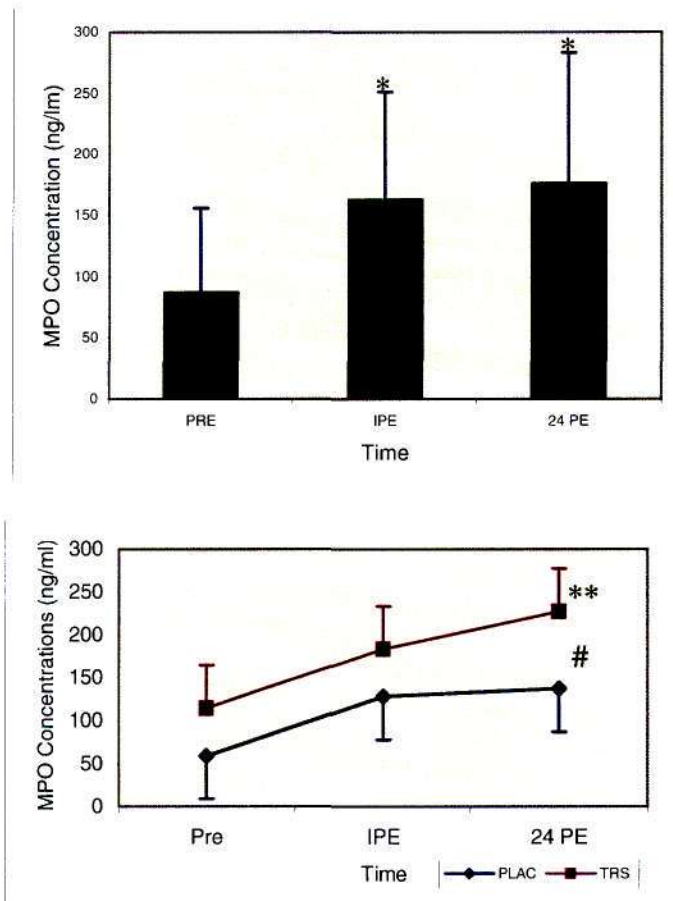
Figure 4.10:   Mean ± SD serum MPO concentrations of complete sample tested ($n = 14$; top) adjusted for PV changes and of the TRS ($n = 7$) and PLAC ($n = 7$) groups (bottom) before and after participation in the 90-minute trial
* $p < 0.05$ Paired t-tests vs PRE
\# $p < 0.05$ Time-group interaction, repeated measures ANOVA, Wilks' Lambda Test Statistic
** $p < 0.05$ Simple contrast *post hoc* comparison vs. PRE between groups at time point

As presented in Table 4.10, comparatively the TRS group reached a mean increase of 227.3 ± 130.7 ng/mℓ, which is significantly higher ($p > 0.05$) than the mean increase of 137.2 ± 71.9 ng/mℓ in the PLAC group.

Table 4.10: Mean ± SD serum MPO concentrations (ng/mℓ) in both the TRS ($n = 7$) and PLAC ($n = 7$) groups before and after 90 minutes of downhill running ($n = 14$)

Time	TRS	PLAC	p value**
Pre Trial	114.7 ± 89.6	59.4 ± 20.7	0.10
Immediately Post Trial	183.5 ± 99.9	128.1 ± 66.4	0.10
24 Hours Post Trial	227.3 ± 130.7	137.2 ± 71.9	0.04

** Paired t-tests TRS vs. PLAC

As is shown in Figure 4.11, when the data were, however, expressed relative to the concentrations at the previous time point, differences between the two interventions were only statistically significant at the 24 PE time point ($p < 0.05$).
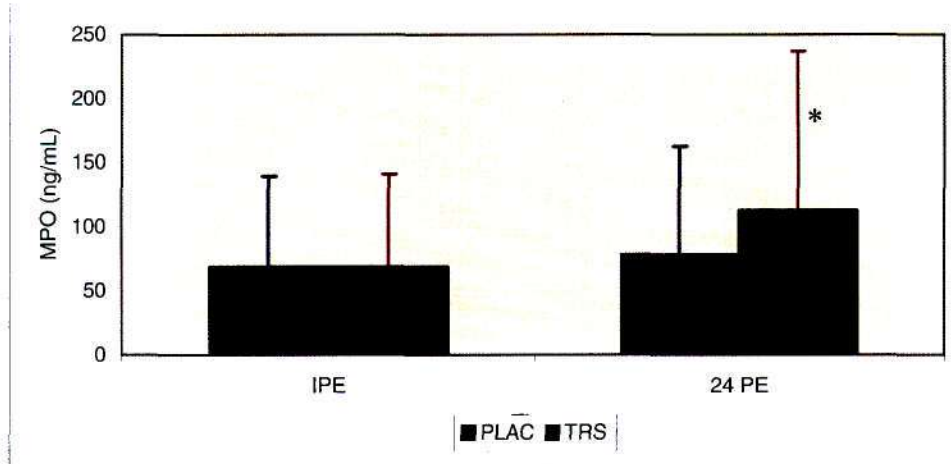
Figure 4.11:   Mean changes in MPO concentrations adjusted for pre-trial concentration in the TRS (n = 7) and PLAC groups (n = 7) following participation in a 90-minute downhill run
* p < 0.05 Simple contrast *post hoc* comparison between groups at time point

4.7.4. The relationship between Serum MPO and Absolute Neutrophil Concentrations
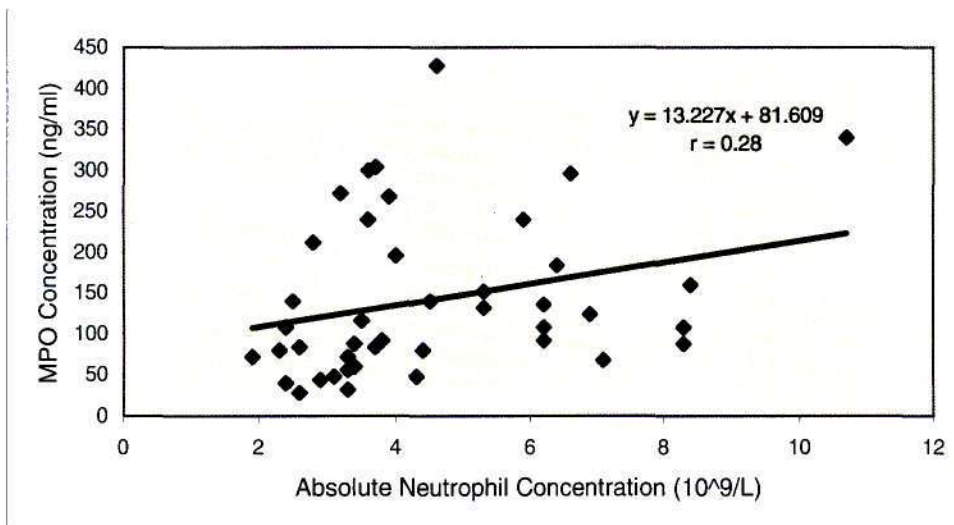
Figure 4.12:   Absolute neutrophil concentrations ($10^9/\ell$) in whole blood versus MPO concentration (ng/mℓ) in serum (n = 28).

The distribution and correlation between absolute neutrophil concentrations ($10^9/\ell$) in whole blood versus MPO concentration (ng/mℓ) in serum is graphically presented in Figure 4.12 (n = 28; r = 0.28; p = 0.07).

4.9.   INTERLEUKIN-6

The mean change in plasma IL-6 concentration for all subjects is shown in Figure 4.13. Plasma IL-6 concentrations were significantly increased immediately after the trial for all subjects ($p > 0.001$) and reached a mean value of 10.60 ± 10.77 mg/ℓ.
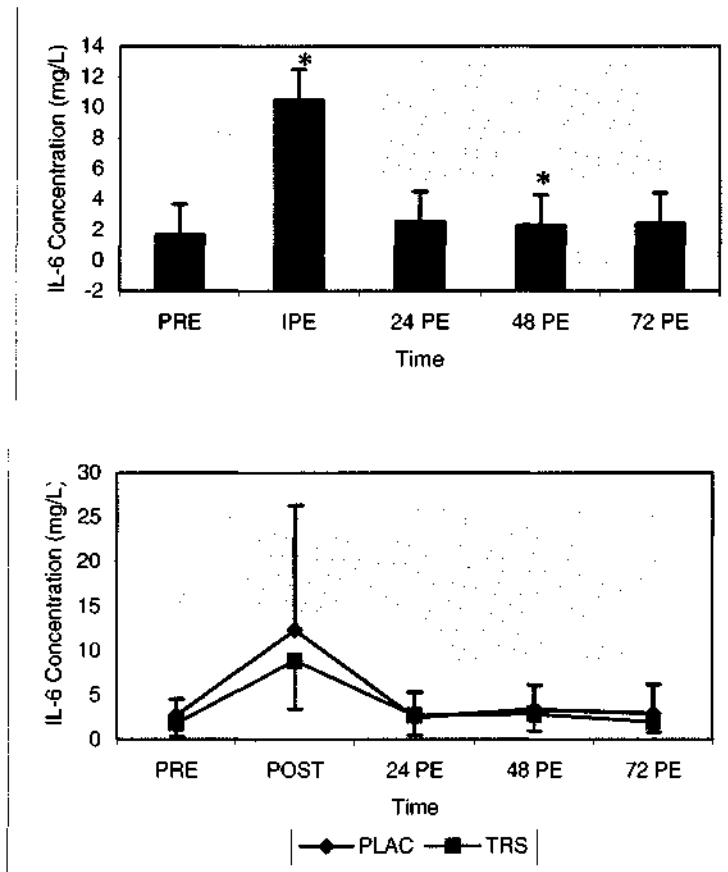
Figure 4.13:   Mean ± SD plasma IL-6 concentrations of subjects (n = 24; top) adjusted for PV changes and of the TRS (n = 12) and PLAC (n = 12) groups (bottom) before and after participation in the 90-minute trial at 5 time points
* $p < 0.05$ Paired t-tests vs PRE

As presented in Table 4.11, the TRS group reached a mean increase of 8.89 ± 5.43 mg/ℓ, while the PLAC group reached a mean increase of 12.31 ± 14.04 mg/ℓ. Both groups peaked immediately following their participation in the 90-minute trial and the difference between the two groups was not statistically significant ($p > 0.05$).

<u>Table 4.11:</u> Mean ± SD IL-6 concentrations (mg/ℓ) in the TRS (*n* = 12) and PLAC (*n* = 12) groups prior to and following 90 minutes of downhill running at the 5 time points

Time	TRS	PLAC	*p* value**
Pre Trial	1.82 ± 1.42	2.73 ± 1.85	0.16
Immediately Post Trial	8.89 ± 5.43	12.31 ± 14.04	0.39
24 Hours Post Trial	2.71 ± 2.30	2.52 ± 2.78	0.87
48 Hours Post Trial	2.84 ± 1.87	3.40 ± 2.70	0.55
72 Hours Post Trial	2.00 ± 1.23	2.95 ± 3.21	0.41

** Paired *t*-tests TRS vs. PLAC

4.10. SERUM CORTISOL

Figure 4.14 graphically displays changes in serum cortisol concentrations for the complete sample (top) as well as the TRS and PLAC groups (bottom). The TRS group increased to a peak of 754.2 ± 340.5 nmol/ℓ while the control group reached a peak of 600.6 ± 148.4 nmol/ℓ (*p* < 0.001). In both groups, the decline in serum cortisol concentrations was significant at 24 hours (*p* < 0.001), thereafter the decline tapered off and by 72 hours, both groups had returned to near-pre serum cortisol levels.
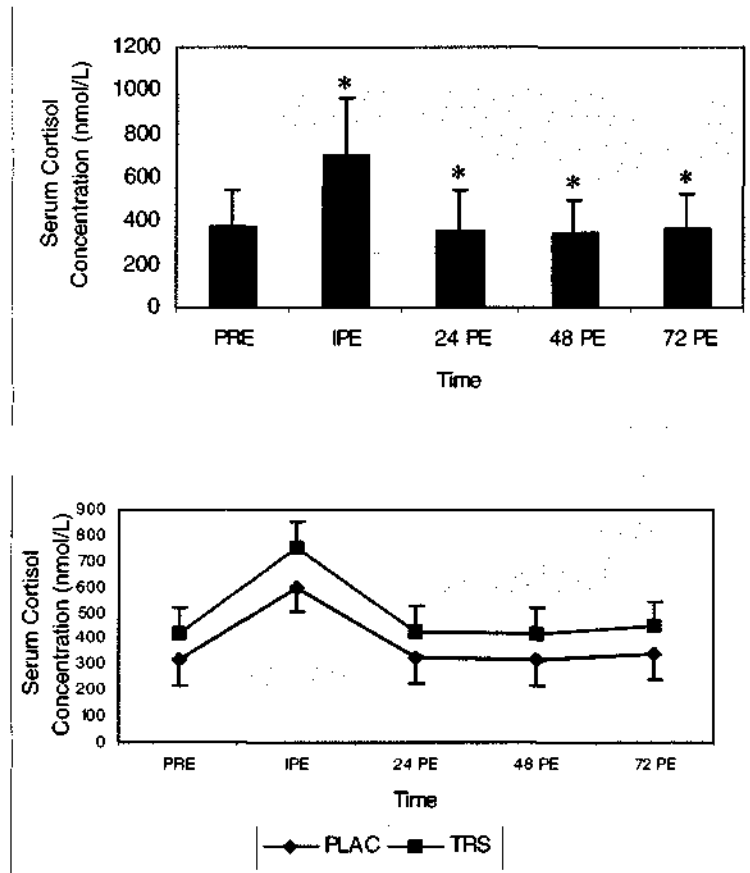
<u>Figure 4.14:</u>   Mean ± SD serum cortisol concentrations of the complete sample (*n* = 24; top) adjusted for PV changes and in the TRS (*n* = 12) and PLAC (*n* = 12) groups (bottom) before and after 90 minutes of downhill running on a treadmill * *p* < 0.05 Paired *t*-tests vs PRE

Repeated measures ANOVA revealed that the time effect was highly significant ($p < 0.001$) in both treatment groups, but the group-time interaction did not reach statistical significance ($p = 0.167$). Simple contrasts *post hoc* analyses verified these findings at each of the time points.

Table 4.12 presents the mean ± SD for the experimental and placebo-control groups in terms of the serum cortisol concentrations at each of the five time points.

Table 4.12: Mean ± SD serum cortisol concentrations (nmol/ℓ) in the TRS (n = 12) and PLAC (n = 12) groups before and after a 90-minute downhill run at the 5 time points

Time	TRS	PLAC	p value**
Pre Trial	419.8 ± 209.8	320.3 ± 88.7	0.10
Immediately Post Trial	754.2 ± 340.5	600.6 ± 148.4	0.18
24 Hours Post Trial	423.9 ± 210.2	322.5 ± 171.6	0.85
48 Hours Post Trial	418.2 ± 201.5	318.2 ± 105.1	0.12
72 Hours Post Trial	446.6 ± 210.3	341.4 ± 141.2	0.09

** Paired t-tests TRS vs. PLAC

When the data are expressed relative to the concentrations at the pre-trial time point, differences between the two interventions are not statistically significant ($p > 0.05$), as shown in Figure 4.15.
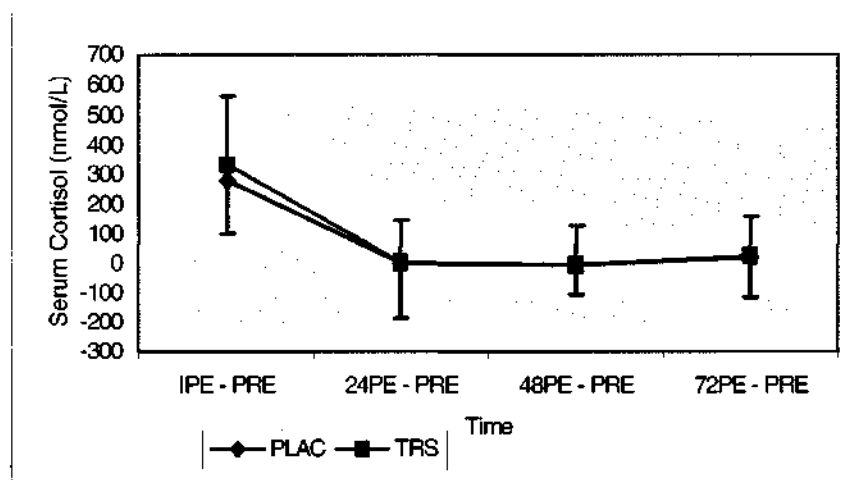
Figure 4.15: Mean changes* in serum cortisol concentrations in the TRS group (n = 12) and PLAC groups (n = 12) following participation in a 90-minute downhill run
* Adjusted for pre-trial concentration

4.11.   C-REACTIVE PROTEIN

The mean and standard deviations of the CRP concentrations for all subjects ($n = 24$) obtained before and following the trial are shown in Figure 4.16. The CRP concentrations peaked at 24 hours following the trial reaching a mean value of 10.9 ± 4.0 ng/mℓ. Thereafter the CRP concentrations declined steadily, returning to the pre-trial levels by 72 hours following the trial.
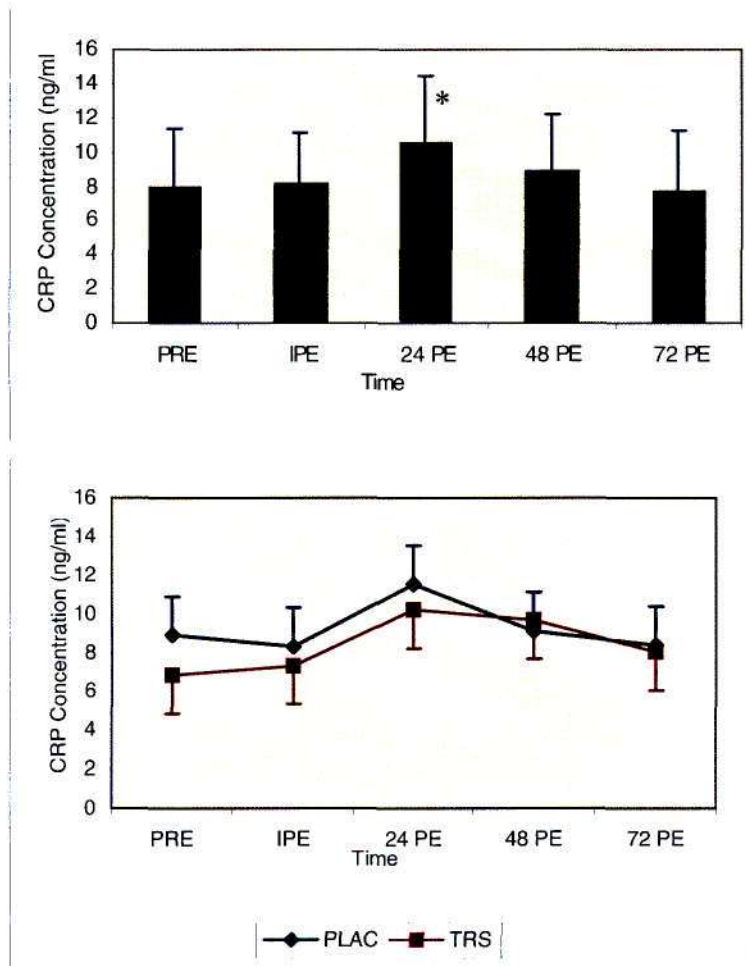
Figure 4.16:   Mean ± SD CRP concentrations of subjects ($n = 24$; top) adjusted for PV changes and of TRS ($n =12$) and PLAC ($n = 12$) groups (bottom) before and after participation in a 90-minute downhill run in the 5 time points.
* $p < 0.05$ Paired t-tests vs PRE

The comparative CRP concentrations for both the experimental and control groups are also shown in Figure 4.16 and these values are presented Table 4.13. In both groups, the CRP concentrations increase maximally at 24 hours post trial with the experimental group reaching a mean increase of 10.2 ± 3.3 ng/mℓ while the placebo-control group reached a mean increase of 11.5 ± 4.6 ng/mℓ. The difference between these two groups was not statistically significant ($p > 0.05$).

Table 4.13: Mean ± SD CRP concentrations (ng/mℓ) in the TRS (n = 12) and PLAC (n = 12) groups before and after participation in the 90-minute downhill treadmill trial

Time	TRS	PLAC	p value**
Pre Trial	6.9 ± 3.4	8.9 ± 3.5	0.16
Immediately Post Trial	7.3 ± 2.7	8.3 ± 2.8	0.36
24 Hours Post Trial	10.2 ± 3.3	11.5 ± 4.6	0.49
48 Hours Post Trial	9.7 ± 3.6	9.1 ± 3.2	0.69
72 Hours Post Trial	8.1 ± 4.6	8.4 ± 3.1	0.90

** Paired t-tests TRS vs. PLAC

4.12. THE RELATIONSHIP BETWEEN SERUM CORTISOL, CRP AND IL-6 CONCENTRATIONS
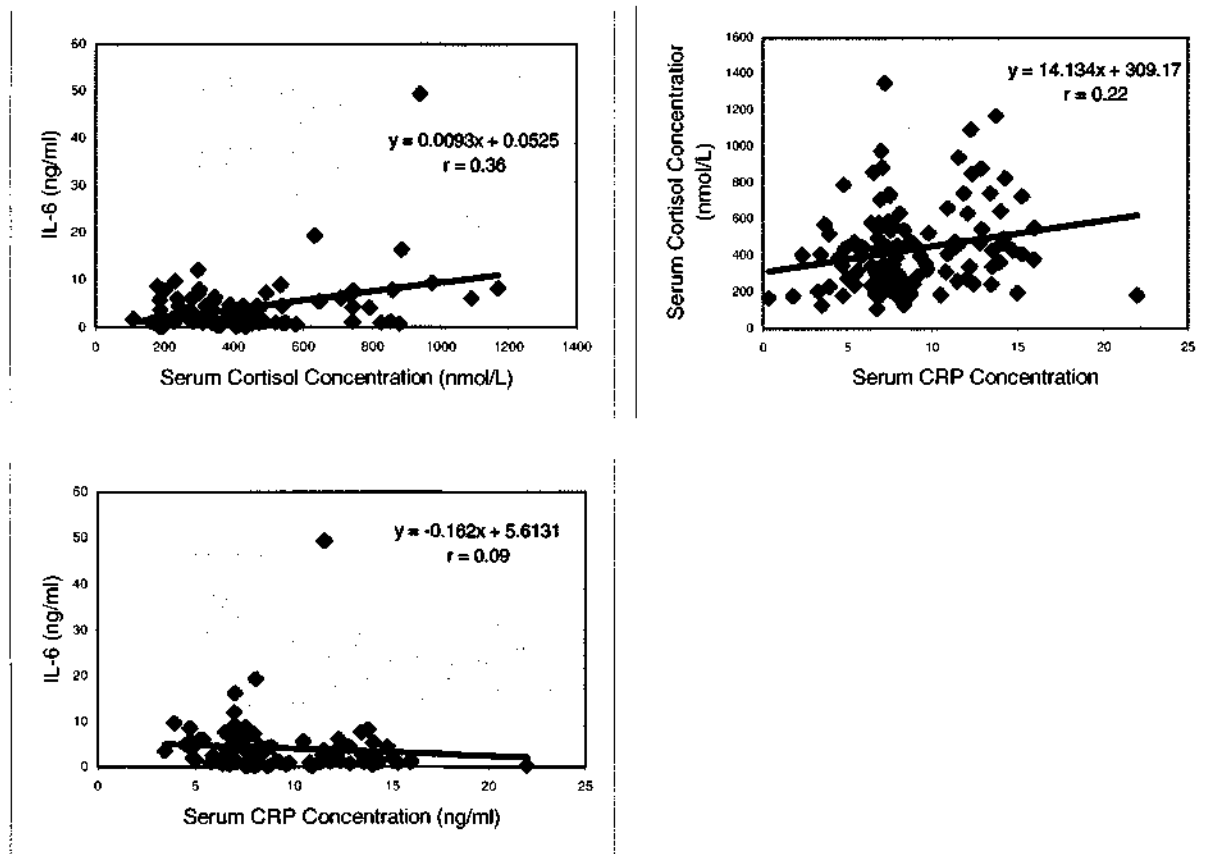
Figure 4.17: Plasma interleukin-6 concentrations (ng/mℓ) versus serum cortisol concentration (nmol/ℓ) (n = 120; top left).
Serum cortisol concentrations (nmol/ℓ) versus serum CRP concentration (ng/mℓ) (n = 120; top right).
Plasma interleukin-6 concentrations (ng/mℓ) versus serum CRP concentration (ng/mℓ) (n = 120; bottom left).

The distribution and correlation between plasma IL-6 concentrations (ng/mℓ) and serum cortisol concentration (nmol/ℓ); serum cortisol concentrations (nmol/ℓ) and serum CRP concentration (ng/mℓ); and plasma interleukin-6 concentrations (ng/mℓ) and serum CRP concentration (ng/mℓ)

is graphically presented in Figure 4.18 (n = 120). Table 4.14 presents the r-values for these correlations between IL-6, serum cortisol and serum CRP.

Table 4.14: Co-efficients of correlation between plasma IL-6, serum cortisol and serum CRP

Relationship	r – value	p value**
Plasma IL-6 versus serum cortisol	0.358	$p > 0.05$
Serum cortisol versus serum CRP	0.22	$p > 0.05$
Plasma IL-6 versus serum CRP	0.094	$p > 0.05$

** Pearsons Product Moment Correlation Coefficient: Upper Critical Values

CHAPTER 5

DISCUSSION

The aim of the study was to establish whether the administration of Traumeel S during the five days before participation and three days following participation, significantly attenuates the systemic markers of the inflammatory response, following a 90-minute downhill running trial. This was achieved by measuring and analysing DOMS, WBC indices, serum CK, LDH, cortisol, CRP, MPO and IL-6 concentrations immediately before and during the three day post-trial period.

As decoding of the prescribed tablets only occurred after completion of the trial and provision of the results to Heel (Pty) Ltd. in Germany, the study was strictly a double-blinded one. The design of the study is therefore a strength of the work.

Due to the possibility of the repeated bout effect being a large confounder following the first exercise trial which involved downhill running and a large eccentric component (Nosaka *et al.*, 2005; McHugh, 2003; Nosaka and Newton, 2002; Stupka *et al.*, 2001), it was, unfortunately, not possible to conduct a cross-over study. In order to eliminate a training effect as a potential confounder in the study, this double-blind, placebo controlled study was therefore designed using pairs of runners who were matched according to gender, BMI, training history, training age as well as performance level (i.e. best times for the 1609m time trial, 5km, 10km, half marathon, marathon and ultramarathon) and/or foot strike in order to ensure sufficient external validity.

The success of this matching was confirmed by parametric and non-parametric statistical analyses which revealed the absence of a significant difference between the two sets of gender-matched pairs in each of the parameters assessed during the baseline tests, field tests and treadmill trials. In terms of study design, it was felt that the matching of the athletes was superior to the alternative option of using two randomly selected samples, which in the case of the small sample size, may have resulted in large differences between the experimental and placebo-control groups and hence threatened the validity of the results.

5.1.   SUBJECT CHARACTERISTICS AND MATCHING

This is the first study of this nature to have been completed on endurance athletes. Due to financial constraints, the data are however limited to 12 runners on the active substance and 12 runners on the placebo.

This trial required a large amount of commitment from the subjects; they needed to adapt their training programmes, take their tablets, complete both a laboratory and baseline field-testing as well come into the laboratory on four occasions to provide blood samples following the 90-minute downhill run on the treadmill. As the subjects were not being paid to participate in the study (minimal funding was available), it was difficult to obtain full compliance from the subjects. The feedback which they received from the field and laboratory test results were the only incentives to participate in the study (Appendix E).

In terms of anthropometric measurements, the overall mean ($\pm$ SD) BMI of the 24 subjects was 23.6 ($\pm$ 2.9) kg/m^2 which falls within the acceptable range of 20 – 25 kg/m^2. The mean ($\pm$ SD) percentage body fat of 16.7 ($\pm$ 4.5) was slightly above the range of 6% - 15% of body fat which is reported by Wilmore (1983) for male endurance athletes. The lack of statistical significance in the difference between the subject characteristics of the groups (Table 4.1), confirms that the two gender-matched groups were also well-paired in terms of age, stature, mass, BMI and percentage body fat. Gender matching was regarded as imperative in order to control for the possibility of a protective effect of oestrogen (Amelink *et al.*, 1990).

5.2.   BASELINE MEASUREMENTS

Although the participants of this study cannot be classified as elite athletes, who have been described by MacFarlane *et al.* (1991) as possessing a VO$_2$ $_{max}$ in excess of 74.7 ($\pm$ 1.43) mℓ/kg/min in elite athletes, a combination of the results of the above-average lung function, low resting heart rate, V$_E$ max, maximum RER as well as VO$_2$ $_{peak}$ achieved during the incremental maximal test to exhaustion on the treadmill confirms that the subjects used in this study were healthy, well-trained athletes of above-average endurance capacity. The mean ($\pm$ SD) VO$_2$ $_{peak}$ of the subjects was 52.7 ($\pm$ 5.7) mℓ/kg/min, which is lower than the VO$_2$ $_{max}$ previously reported in elite endurance athletes. It is, however, substantially higher than those published for non-athletes (Wilmore, 1983).

5.3. FIELD AND TRAINING PROFILE

The mean average training distance and number of years of running (training age) were marginally higher in the PLAC group, as was the mean performance time for the 1609m time-trial. However, as was the case in the 5km, 10km and marathon times, there was no significant statistical difference with respect to the performance profile of the PLAC and TRS groups (Table 4.3).

In terms of training status, the subjects were matched according to average training distance and distance and training age and this is reflected in the means ± SD of the two groups which are not statistically different (Table 4.3).

It can therefore be concluded that the PLAC (n = 12) and TRS groups (n = 12) were well matched in terms of physical characteristics, foot strike, and training and performance profiles.

5.4. TREADMILL TRIAL

This indicates that although the subjects in the TRS group ran faster (p = 0.03), there was no difference between the groups in terms of their RPE, HR, or their mean RPE, mean heart rate, % heart rate max and max RPE, indicating that the subjects were undergoing the same relative intensity of exercise and making the responses to the two 90-minute treadmill trials comparable.

5.5. MARKERS OF MUSCLE CELL DAMAGE

Muscle proteins which have been found to leak from the compromised cell membrane, include CK (Totsuka *et al.*, 2002; Clarkson *et al.*, 1992), myoglobin (Croisier *et al.*, 1996), FABP (Sorichter *et al.*, 1998), LDH (Overgaard *et al.*, 2002; Nosaka and Clarkson, 1996) and skeletal muscle troponin I (Sorichter *et al.*, 1997). The present study focussed only on serum LDH and serum CK concentrations as representative of this protein group.

5.5.1. Lactate Dehydrogenase

In all athletes evidence of exercise-induced muscle damage was provided by an elevation in serum LDH concentrations which was recorded in the IPE samples. This reached a mean peak

value of 555.3 ± 137.3 IU/ℓ that was outside of the normal clinical range (200 – 500 IU/ℓ) and constituted a 44% mean increase from PRE ($p < 0.001$). This confirms the findings in the literature which are consistent in reporting peak LDH concentrations in serum immediately post-exercise (Semple *et al.*, 2007; Simpson *et al.*, 2006; Armstrong, 1990).

The response to the downhill running protocol, however, differed between athletes. It can be deduced from the IPE samples that more damage was produced when the running speed and impact loading on the eccentrically contracted muscles was greater. The 24 PE samples, however, provided evidence of significant recovery (time effect, $p < 0.05$) despite the statistically insignificant difference in the treatment effect (time-group interaction, $p > 0.05$).

When comparing the experimental and placebo-control group relative to the LDH concentrations at the previous time points (Table 4. 6) and adjusted for baseline values (Figure 4.2), no significant difference was observed ($p > 0.05$), despite the significant time effect ($p < 0.001$) in both groups.

5.5.2. Serum Creatine Kinase

Pre-exercise CK values in the current study fell within the normal laboratory ranges (70 – 270 IU/ℓ) which were in the range of 66 – 350 IU/ℓ, only one subject fell below the 70 IU/ℓ and four subjects were above the 270 IU/ℓ range, which indicates that the majority of the athlete's entered the trial well rested.

In the 24 PE samples, serum CK peaked in all of the 24 runners with a mean of 1339.6 IU/ℓ (almost 6-fold). This mean peak of serum CK concentration at 24 PE is consistent with previous findings of studies examining the effects of exercise-induced muscle damage following downhill running (Semple *et al.*, 2007; Jordaan, 2006; Simpson *et al.*, 2006; Peters *et al.*, 2001b). Findings of this study also confirmed that peak CK concentrations occur between 12 and 24 hours post exercise (Semple *et al.* 2007; Simpson *et al.*, 2006; Peters *et al.*, 2001b; Armstrong, 1990) as well as large inter-individual differences (Peters *et al.*, 2005). In contrast to the findings of Clarkson *et al.* (1992) who showed that CK concentrations after downhill running only increased by approximately 300 IU/ℓ and those of Jordaan (2006) in which mean serum CK concentrations increased only by a mean of 599 ± 448 IU/ℓ, the downhill running protocol used in this study resulted in exercise-induced rises as high as 3243 IU/ℓ. This indicates that the exercise intensity used for this trial exceeds that used by Clarkson *et al.* (1992) and Jordaan (2006) and demonstrates that it was appropriate in inducing substantial muscle damage in the participants.

Although the pre- and immediate post exercise concentrations of serum CK were higher in the TRS group, no difference between these two groups was apparent 24 hours following the exercise trial and the group-time interaction effect was not statistically significant ($p = 0.398$). It could therefore be inferred that there had been more leakage of CK into the blood stream prior to the trial, which raises the question of whether the TRS group subjects were as well rested as the subjects in the PLAC group. Interestingly the post exercise concentration was highest in the TRS group, which confirms the faster running speed and greater impact loading during the downhill running (although metabolic response seems to indicate no difference). However, the results adjusted for baseline values and presented relative to the previous time point (Figure 4.4) indicate no statistically significant overall group-time interaction between the two groups.

In interpreting these results, it is however, important to take the small sample sizes and heterogeneity of the samples into account as these would have lowered the power of the study and resulted in a possible type 2 error. Statisticians concur (personal communication, 2008) that the faster recovery trend in the TRS group may, however, be meaningful and recommend use of a larger, more homogenous sample to verify this.

Furthermore, previous research findings conducted on the effect of gender differences on CK concentrations, report that women had a lower serum CK activity than men (Stupka *et al.*, 2001; Clarkson and Hubal, 2001; Amelink *et al.*, 1990). Our findings did not support this as the female subjects had lower mean pre-trial CK concentrations which increased almost 8-fold to reach peak CK concentrations above those in the men, whose mean CK concentrations only increased 5.6-fold. Statistically, the differences were, however, not significant ($p = 0.70$) which is most likely due to the large variation in values of the subjects.

5.6. DELAYED ONSET MUSCLE SORENESS

Aoi *et al.* (2004) described the delayed onset of muscle pain which occurs 24 to 48 hours following exercise to which the muscles are not accustomed within the muscle, as a distinguishing feature of DOMS. Following the downhill treadmill trial, the majority of athletes complained of pain within the 24 to 48 hour timeframe following the trial. Two subjects complained of pain immediately after and again 48 hours following the trial, which may indicate that they did not comprehend the DOMS concept and most likely initially confused it with muscle fatigue following their treadmill trial.

As is to be expected, the muscle groups which were reported to be the most affected from the downhill trial were the quadriceps muscles and to a lesser extent, the calf muscles. The eccentric loading of quadriceps muscle group over a period of 90-minutes supports the work of Peake *et al.* (2005), which proposes that DOMS is the result of microscopic tearing of the muscle fibres in response to the load under which they were worked. The hamstrings muscle group was the least affected with only a minority of the athletes reporting pain in this group. The symptom of DOMS within these muscle groups was elicited predominantly while subjects were active, with only two athletes reporting pain while at rest.

Interestingly, there appeared to be no relationship between the foot strike pattern and the muscle group which was affected. However in order to fully investigate the relationship between DOMS and foot strike pattern, a comprehensive biomechanical assessment of the lower limb and lumbar spine would need to be conducted. In addition to this, complaints of knee, hip or lumbar pain, made by the athletes during their trial may have been a result of certain biomechanical predispositions, which were not documented.

It was also interesting to note that although the correlation which exists between the peak DOMS score in each subject and the percentage change in measured serum CK concentrations from PRE to 24 PE in each subject was relatively low, it did reach statistical significance and revealed a trend in that TRS group reported lower DOMS scores from the onset of the trial. A shortcoming of this aspect of the research was that pressure pain was not used to measure DOMS.

The findings in this study of significant increases in circulating MPO also supports the theory that DOMS could result from phagocyte infiltration and resultant production of ROS and other anti-oxidants (Aoi *et al.*, 2005).

5.7.   HAEMATOLOGICAL ANALYSES

5.7.1. Red Blood Cell Indices

Should euhydration have been maintained during the 90-minute run, little change in red blood cell indices would have been expected. The findings of this study however confirm significant increases in RBC, Hb and Hct concentrations in the IPE samples, indicating a degree of haemoconcentration. This would imply that the 500 mℓ of water that each subject was given to drink during the 90-minute run, was not sufficient to maintain hydration status. This was confirmed by the mean reduction in plasma volume of 4.4% which occurred in the combined

sample (n = 24). The changes of 4.8% in PLAC group and 3.9% in TRS group therefore necessitated adjustment of all post exercise concentrations of circulating cells, enzymes, hormones, and other systemic markers of inflammation.

Interestingly, the subjects also appeared to have increased their fluid intake post trial as is indicated by the increase in their plasma volumes during the three days post exercise. Once again, adjustment for plasma volume changes was imperative prior to meaningful comparison of circulating concentrations of solutes (including WBC's, hormones, cytokines and enzymes) within the two treatment groups.

5.7.2. White Blood Cell Indices

As is shown in Figure 4.8 the findings of this study revealed a significant increase (p < 0.001) in the PV-adjusted absolute WBC and neutrophil concentrations when comparing pre-trial concentrations to immediate post-trial concentrations. This confirms the immediate post exercise peak in total WBC, neutrophil and monocyte counts as observed by Smith (1998), Nieman (1997) and Pizza et al. (1995). No significant lymphocytosis, monocytosis, eosinophilia or basophilia was, however, induced by the downhill running protocol (p > 0.05; Figure 4.8).

These results confirm the previously described exercise-induced neutrophilia which may be related to increasing serum cortisol concentrations (Pedersen, 1997) and would appear to also be indicative of an acute reaction to the microscopic muscle damage inflicted by the downhill running protocol. The absence of lymphocytosis is to be expected due to failure of adapted immune responses to be triggered immediately post exercise. Interestingly the exercise-induced monocytosis only reached border-line significance (time effect: p = 0.51) which indicates relatively low mobilisation of monocytes from the bone marrow while the absence of eosinophilic and basophilia are to be expected in this relative healthy sample of athletes who would have been unlikely to present with asthmatic or parasitic conditions.

No significant time-group interaction was, however, shown between the TRS and PLAC groups in terms of WBC and neutrophil response to the exercise protocol over the three-day period. This appears to indicate that TRS does not act by inducing the mobilisation of neutrophils in response to microscopic muscle damage. As will be discussed later, the adrenal release of cortisol, well described as trigger of exercise-induced neutrophilia, was also not significantly different between the two groups.

Circulating monocytes have been reported to increase in response to exercise-induced muscle injury for up to 48 hours after exercise (Peters et al., 2001a, Malm et al., 1999). However,

findings of this study only showed borderline significance ($p < 0.051$) of the elevated mean monocyte concentrations immediately after the exercise trial, and these values had returned to pre-trial concentrations within 24 hours. There was no significant ($p > 0.05$) difference between the TRS and PLAC groups with regard to the monocyte concentration response to the exercise trial. These findings conflict with those of Jordaan (2006) who reported a significant decrease in monocyte activity in the TRS group, but not the control group within 24 hours of recovery.

Unfortunately differentiation between the monocyte and macrophage sub-populations and inclusion of testing for serum prostaglandin levels was not within the scope of this study. This information would have been of great interest in interpreting the pro-inflammatory responses to the exercise interventions.

5.8.   SERUM MPO

MPO is a hemoprotein that is abundantly expressed in neutrophils and secreted during their activation. As is shown in Figure 2.3, it plays an important role in neutrophil microbiocidal action by catalysing chloride ion oxidation to $HOCl$, a potent antimicrobial agent. In view of the muscle damage or expected presence of necrotic/ foreign tissue in primarily the quadriceps muscles (as is confirmed by the ± 44% elevation in mean LDH and ± 600% elevation in circulating mean CK concentrations following the 90-minute downhill run), increased migration of neutrophils to the site of inflammation would be expected. While it was unfortunate that actual muscle biopsy samples were not available, the significantly elevated post exercise serum MPO concentrations of the group as a whole ($n = 24$) confirm previous findings of Bury and Pirnay (1995) who reported significant elevations of MPO concentrations in plasma following two hours of prolonged exercise at 75% $VO_{2\,max}$.

Although Suzuki et al. (1999) have shown that the increase in the MPO enzyme activity in plasma was proportional to the increase in the number of neutrophils after prolonged exercise, our findings did not support this. The low correlation between serum MPO and neutrophil concentration is, however, to be expected as post–exercise neutrophil concentrations peak IPE whereas serum MPO concentrations peaked 24 hours post exercise. Interestingly when the co-efficient of correlation between IPE samples was determined, the r-value rose to 0.28 ($p = 0.07$). MPO release into the serum may therefore be a delayed response to exercise-induced neutrophilia and activation of the neutrophils which have been drawn to the site of the damaged tissue.

The key finding of this study is the significantly higher mean ± SD concentration of MPO in serum in the 24 PE samples. This is indicative of greater neutrophil activation by MPO which is responsible for the catalysis of H_2O_2 to $HOCl$ in the TRS group after 24 hours. Although the immediate post exercise values were not significantly different, the immunological effect may be a delayed one which is not apparent in the IPE blood samples, but in only in the 24 PE blood samples. This confirms the findings of Conforti *et al.* (1997) who hypothesised that the mechanism by which Traumeel S is effective in improving recovery rates from muscle cell damage is via modulation of neutrophilic production of free radicals during oxidative burst activity of the neutrophils. This is the first report of confirmation of this hypothesis via measurement of serum MPO concentrations, an indirect marker of neutrophilic oxidative burst activity.

5.9.   INTERLEUKIN-6 CONCENTRATIONS

The results of this study showed that the IL-6 concentrations significantly increase ($p < 0.05$) from pre-trial values of 2.27 ± 1.67 mg/l to 10.60 ± 10.77 mg/l IL-6 immediately after the exercise invention. However, when comparing the experimental and placebo-control groups, the difference was not statistically significant ($p > 0.05$).

IL-6, in conjunction with other pro-inflammatory cytokines, has been shown to increase blood vessel permeability which facilitates the migration of neutrophils and monocytes towards the site of tissue damage and inflammation (Roitt *et al.*, 2001). In addition to this, IL-6 is well known for its inhibition of pro-inflammatory action via its activation of the adrenal cortex to release cortisol (Nieman, 1997) and stimulation of CRP release (Nieman, 1997), which is evident from the delayed increase in CRP concentrations as observed in this study.

The findings of this study confirm a significant time-effect ($p > 0.001$). While this was to be expected following the downhill running protocol, the peak in post-exercise IL-6 concentrations may not have been recorded as, if induced by muscle damage following eccentric exercise, previous work has shown that this occurs at between 3-6 hours post-exercise (Peters, 2004). A shortcoming of the current study design was that it was not possible to include further blood sampling at these time-points.

The results of this study are in contrast to the findings of Lussignoli *et al.* (1999) or Conforti *et al.* (1997), which showed an attenuation of the IL-6 response to an inflammatory stimulus in rodents following administration of Traumeel S. The failure to confirm a significant time-treatment

interaction of this cytokine between the two groups over the three day time-span, may however, have been due to the heterogenous response within the two groups and large standard deviations, that could have masked any significant effect.

5.10. SERUM CORTISOL CONCENTRATIONS

Analysis of the complete sample data (n = 24) showed a 46.9% increase of cortisol concentration to a peak of 706.84 ± 257.81 nmol/ℓ at the 24 hour time point which is lower than the findings of Smith (2007) who reported that post-exercise cortisol concentrations have been shown to rise by more than 50% following 60 minutes of downhill treadmill running (gradient - 13.5%) at 75% VO_2 $_{max}$ and Peters et al. (2001b) who reported increases in cortisol concentrations of ± 200% to mean concentrations of 1179 ± 93,2 nmol/ℓ, following a 90km downhill ultramarathon run.

The increase in serum CK did, however, indicate that the downhill running induced damage within the muscles in both the TRS and PLAC groups. Neutrophil activation, oxidative stress and the activation of the macrophages with subsequent release of IL-6, is thought to trigger the release of cortisol (Peters et al., 2001a) which has well described anti-inflammatory action.

Although a greater anti-inflammatory response in the TRS group was observed (754.2 ± 340.5 nmol/ℓ) when compared to the PLAC group (600.6 ± 148.4 nmol/ℓ) the difference between the two groups was not statistically significant (p = 0.97). The most likely reason for the lack of significant differences in these largely divergent means are the large intra-group variances as reflected by the large standard deviations. It also confirms the results of the IL-6 concentrations supporting the suggestion that the treatment effect of Traumeel did not appear to manifest via this pathway of anti-inflammatory response.

5.11. C-REACTIVE PROTEIN CONCENTRATIONS

The findings of this study are in agreement with those of Semple (2006) and Peters et al. (2005, 2001a) which showed that CRP concentrations increase substantially 24 to 48 hours after acute exercise and that this increase is often more pronounced following exercise of greater intensity owing to the muscle damage which is incurred (Semple, 2006).

Clyne and Olshaker (1999) have reported that the values of CRP do not differ between males and females and the averages of the general populations are considered normal when less than 5 mg/ℓ (Gabay & Kushner, 1999). Based on this premise, the pre-exercise CRP concentrations for our sample were high (7.88 ± 3.52 mg/ℓ). However, the anti-serum used for the purpose of the immunoturbimetric assay is mono-specific for CRP. It has been shown not to cross react with other serum proteins under the conditions of the assay, and it is therefore unlikely that residual fibrinogen or other plasma proteins that may have remained in the serum, were responsible for the unusually high concentrations detected using the commercial kit (described in Chapter 3).

Furthermore, no diurnal or seasonal variation has been observed in CRP values (Pepys, 2003) and it has been reported that the levels of CRP in humans are not affected by food intake (Semple, 2006). On the other hand, the high general training status of the subjects should also, according to the findings of Kelley and Kelley (2006) and Marcell (2005) not have resulted in an elevation of baseline CRP concentrations. That the subjects have not rested sufficiently prior to the exercise trial is also not likely as this was a requirement of participation in the trial and resting cortisol, IL-6, CK and LDH were low.

The discrepancy in terms of the high CRP values is therefore difficult to account for.

The significant time-effect ($p > 0.001$) with a slight increase in immediate post exercise CRP concentration with a peak at 24 hours does however, confirm previous findings of Peters *et al.* (2005, 2001b) and Semple (2006) and indicate the presence of an acute phase response following the downhill running protocol. As hepatic release of CRP is a sequel to the release of cortisol by the adrenal cortex and the release of IL-6 by macrophages and muscle tissue (Peters *et al.*, 2005), the absence of a group-time interaction in CRP would also be expected.

5.12. CONCLUSION

While the IL-6-cortisol-CRP pathway does not appear to be affected by the Traumeel intervention in this study, preliminary findings of significantly elevated MPO concentrations at the 24PE time-point, do suggest evidence of a positive effect in the clearance of debris and increased neutrophil activity through enhanced oxidative burst activity.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS OF FUTURE STUDIES

Despite shortcomings of this study which include the relatively small sample sizes, low power of the study and greater likelihood of type 2 errors occurring, as well as the failure to ensure "perfect" matching with minimal genetic differences between pairs and homogeneity within each treatment group, this study did highlight an important novel finding.

Although no significant treatment effects were identified in terms of differential white blood cell, IL-6, cortisol or CRP response to the exercise–induced damage which appeared to be greatest in the TRS group, the primary finding of this study is that it provides novel preliminary evidence of enhanced serum MPO concentrations at the 24 PE time-point. These are indirect indicators of enhanced neutrophil activation and oxidative burst activity with increased production of cytotoxic HOCℓ which is instrumental in facilitating the removal of foreign substances from the muscle tissue. Ultimately, this would facilitate healing and accelerate the recovery process.

The exact mechanism by which this enhanced MPO activation is induced in the Traumeel treated group is, however, not clear. On the one hand, it could be due to a direct effect of one of the 12 different herbal extracts of which Traumeel S is made up, on the activity of MPO. On the other hand, the stimulation may occur at the level of NADPH oxidase or superoxide dismutase (SOD).

Future directions for research would therefore include examining the transcription factors for NADPH oxidase, which our research team are currently in the process of undertaking. Measurement of SOD concentrations would another research option which would provide an independent means of confirming the possible enhancement of pro-inflammatory action of Traumeel S, which the findings of this study appear to support.

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APPENDIX A

SUBJECT INFORMATION SHEET

Study Title: The effect of an anti-inflammatory homeopathic product on systemic markers of inflammation following downhill running.

Introduction: This study will investigate the effect of Traumeel, an homeopathic anti-inflammatory product, on the delayed muscle soreness (DOMS) which occurs following prolonged exercise. As we are interested in establishing the mechanisms which result in this muscle soreness and whether this can be minimized by the use of homeopathic preparations, we shall be doing a variety of analyses on your blood samples before and after exercise.

Who will qualify to participate in this study? Thirty healthy long-distance athletes
- aged 20 –50 years
- non-smokers, non-diabetic, free of cardiovascular impairments (as verified by medical practitioner)
- not showing clinical signs of infection (as verified by medical practitioner)
- taking no medication (e.g. analgesics or anti-inflammatories)
- covering a minimum of 30 km per week in training

What will be expected from volunteers in this study?
You will be asked to:
- not use any analgesics, anti-infective or anti-inflammatory or Vitamin C preparations in addition to the prescribed study supplements for 2 weeks before and for the duration of the trials.
- maintain your usual diet for the duration of the trials
- maintain the same type of training during the trial period
- avoid any heavy physical exercise the day before each exercise trial.

Outline of tests and trials:
- **First Laboratory Visit** –Exercise Laboratory, Physiology Department, Nelson Mandela Medical School: approx two weeks before trial)
- Interview regarding exercise training status, medical status
  - Assessment of height, mass and percentage body fat (to be estimated from skinfold thicknesses)
  - Lung function test
  - Resting heart rate and blood pressure measurement
  - VO₂ peak test on treadmill: After a 5 minute warm-up at a relevant pace, expired air will be collected and analysed as running speed will increase by

A furthering of the knowledge regarding supplementation procedures which will optimize both your performance and the stiffness you feel following exercise, will be obtained from this work. You will be the first to be notified of the results of this study.

Will you be exposed to adverse effects of the exercise or side effects from Traumeel?

As with all exercise exposure, it is possible that an accident may occur (e.g. that you may perhaps fall). In the event of your heart or one of your other organs having a disorder which has to date, not been diagnosed, the increased blood flow to the heart and increased mean blood pressure during exercise may also result in a heart problem or other organ damage occurring while you are exercising. This is the reason why we have been so careful in terms of who we have allowed to participate in the study. Do, however, also be assured that there will be medical personnel present or on call throughout all aspects of the testing and that the laboratory is fully equipped with first aid and emergency equipment.

In terms of possible side-effects from the Traumeel supplementation that you will be using, although some people do sometimes develop mild headaches, diarrhoea or nausea, recent studies have shown that these resolve without discontinuation of the treatment and it has not been proven that these are directly related to ingestion of Traumeel.

As with all prolonged exercise involving downhill running, you should expect a certain amount of muscle soreness 48-72 hours post-exercise. This will be carefully monitored using a 0-10 numerical scale recording your levels of discomfort. **It is also worth noting that previous studies and coaches suggest that the downhill training session will assist in protecting you from muscle soreness and muscle inflammation during other downhill sessions over the following 3 months** (Mosake K, Sakamoto K, Newton M, Sacco P. How long does the protective effect on eccentric exercise-induced muscle damage last? Med.Sci.Sports Exerc, 33: 1490-1495). **Should you be participating in a competitive event involving downhill running following participation in this trial, you are therefore assured of a beneficial training effect for at least 3 months thereafter.**

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

Will your individual results remain confidential?
Yes. The records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the study are published, the participant's identity will remain confidential

Sponsorship of the study/Financial compensation
The Traumeel and placebo products to be used during the study will be manufactured in Germany, and packaged and distributed to you by Heel Pty Ltd, South Africa in Johannesburg.

- **Field visit – University of KZN – Sports grounds Francois Rd Track (approx 1 week before trial)**
  - Warm up at relevant pace – subject will be timed over 4 laps (1609m) on track with heart rate and time recorded.
  - Recording and analysis of past and current training processes
  - Analysis of running style and feedback (as above)
  - Race schedule analysis for past month and following month (as above)
  - Explanation of pre- intervention training and eating procedures.

- **Intervention Trial to be conducted in the Exercise Laboratory, Nelson R. Mandela School of Medicine.**

  The protocol of each of the intervention trial will be the following:
  - **5 days prior to the trial** – 1 x TRAUMEEL tablets 3 X per day (provided) sublingually before meals.
  - **The morning of the trial** – the following breakfast is to be eaten 3 hrs before the trial :30g cornflakes, 150ml of low fat milk and 12-15 g refined sugar
  - **The exercise trial** – 30ml blood sample to be taken on arrival
    - exercise protocol – wearing a heart rate monitor, six subjects to run on treadmill in the exercise laboratory for 90 minutes in a relaxed atmosphere. TV screens/music will be available to entertain the runners on the treadmill and free communication between runners and research assistants will be encouraged. The running speed will be individualized according to the results of the VO₂ max tests and the 1609m time trial.
    - 30ml blood sample will be drawn 1, 24, 48 and 72 hours after the trial.

The order of the trials is to be **randomized**. By this we mean that you will be placed into treatment sequences using random number tables. You may therefore not be receiving the treatment in the same order as the other subject(s). As it is to be a **double blind study,** neither the researchers or you will be informed regarding which treatment you are on. The products are to be coded as placebo or active substance in the chemical laboratory before distribution to the subjects.

How can you benefit from participation in this study?

Following completion of all aspects of the trial, you will receive
- the results of your VO₂ peak test
- the results of your full blood counts and other biochemical/ laboratory tests which were run on your blood samples.
- a prediction of your current ability / potential at various race distances from 5km to Comrades marathon (as relevant to your goals / Training)
- a list of training paces relevant to your current ability
- information on your running style and relevance of your current running shoes to this style.
- an indication, based on the information supplied, as to the relevance of your current training to running goals

Further queries?

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

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

Study Director
Department of Physiology,
Faculty of Health Sciences
University of KwaZulu-Natal

Athletics Coach and Consultant: Norrie Williamson – 031-201 7687
www.coachnorrie.co.za

Post-graduate research assistants:
Dr Aadil Docrat, Department of Physiology
Ms Megan Smith, Department of Physiology
Ms Smith and Dr Docrat will be assisting with the implementation of this study and writing up sections of the results as part of their M (Med) Sc Degree by coursework requirements.

APPENDIX B

I, ... hereby agree to participate in a research study to be performed by Edith Peters-Futre and co-workers in the Department of Physiology at the University of KwaZulu-Natal Medical School.

I understand that the basic procedures involved in this study are to include:
- Assessments of training history, anthropometric measures, lung function, selected baseline clinical measures and an exercise test (VO₂ peak) during 1ˢᵗ Laboratory visit (approx 2 weeks before 90-minute trial in the laboratory).
- Field test conducted by Norrie Williamson which will consist of a 4 lap (1600m) time-trial on the KZN athletics track with heart rate and time recorded (1 week before 90-minute trial in the laboratory).
- Sublingual intake of 1 x TRAUMEEL or placebo tablet 3 x per day 30 minutes before meals at each meal (i.e. 3 x per day), for 5 days prior to the exercise trial (to be held in the Exercise Laboratory, Nelson R Mandela School of Medicine.
- Having a 30ml blood sample taken on arrival at the 90-minute exercise trial.
- Laboratory Exercise Trial: 90minutes of treadmill at an intensity of 75% VO₂ max. Ad libitum ingestion of water will be permitted 30 and 60 minutes into the run.
- Having a 30ml blood sample drawn 1, 24, 48 and 72 hours after both trials.
- Completing a numerical rating of muscle soreness experienced during the 72 hours after both exercise trials.

The details of these procedures have been explained to me in full. I am aware that a certain level of discomfort may occur when the blood is taken and that the exercise trials will involve strenuous prolonged exercise which may be accompanied by certain medical risks and result in muscle soreness in the 48-72 hours post test. I also understand that I do not have recourse against the University in the case of an acute medical event occurring while I am running on the treadmill.

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

Signed...................................

Date...................................

Principal Investigators signature...................................

UNIVERSITY OF
KWAZULU-NATAL

Verification from General Practitioner

Dear Dr _____

_____ is being considered as a potential subject in the following research project which is to take place on trained endurance athletes in our Department in May 2005.

Title of Project: The effect of an anti-inflammatory homeopathic product on systemic markers of inflammation following downhill running.

As a precaution we would like you to verify that you have examined this patient recently and are not aware that he has any cardiovascular or metabolic problems e.g. diabetes which may result in it being hazardous for him to participate in the study.

With sincerest appreciation,

Dr E M Peters-Futre

Exercise Physiologist
Department of Physiology
Nelson Mandela School of Medicine
University of KwaZulu Natal
Private Bag 7, Congella,
4013, South Africa
Tel: +27(0)31-2604237
Fax: +27(0)31-2604455

I hereby confirm that Mr _____ has been examined by me and I regard him to be free of cardiovascular impairments which may render participation in a 1.5 hr treadmill run hazardous to his health.

I also confirm that Mr _____ is not a diabetic and is medically fit to participate in this research.

Signed _____

Qualification _____

Date

Day, Date and Time of Session	Training Mileage	Intensity Sessions	Rating of Perceived Exertion and General Comments
		Type: Duration: % of max (eg. 50%):	
		Type: Duration: % of max (eg. 50%):	
		Type: Duration: % of max (eg. 50%):	
		Type: Duration: % of max (eg. 50%):	
Rate of perceived exertion	1= Very easy 4= Fairly Exhausting	2= Easy 5=Exhausted	3= Moderate

APPENDIX C

ATHLETE'S QUESTIONNAIRE

Name:
_____

Date of Birth:
_____/_____/_____ (dd/mm/yyyy)

Age: _____

Address:
_____
_____
_____
_____

Phone Numbers
Home: _____

Work: _____

Cell: _____

Athletics Club:
_____

Occupation:
_____

TRAINING DETAILS

Average weekly running distance: _____ km

Running History
   Serious\Social : _____
   Age started running: _____ (....yrs)
   Level of training during these years.........................
   Total number of marathons run: _____

Describe normal terrain used for training (to be discussed / confirmed by athletics coach) ....
....
....
(Take into consideration – number of days on flat – number on hills, distance on hills, group training, etc)

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

List peak event(s) for each year of training age (or last 5 years)
....
....
....
....
....

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

List races planned for next 2 months all sports
....
....
Any training group partner in study? If so who?
....

Describe your running shoes (make model and age / distance covered)

Describe your running foot strike (heel-toe / Forefoot / pronation)
....

Do you use orthotics?

....

Describe the wear pattern on your shoes?

....

Personal Best times in last 12 months:
5 km time trial .... 8km time trial .... 10km ....

15 km .... 21.1km .... 32km ....

42.2km .... 56 km .... Comrades ....

1609m time trial time (will be tested as part of study) ....

Pace to be used for test (will be filled in after test) ....

Please sign: _____ Date: _____

Principal investigator's signature: _____

MEASUREMENTS

Pregnancy Test(if female only)...

Lung Function Tests

FVC: _____   FEV₁: _____

FVC/FEV₁: _____

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

Skin – fold Measurements

Biceps: _____ _____ _____ ave: _____

Triceps: _____ _____ _____ ave: _____

Suprailliac: _____ _____ _____ ave: _____

Subscapular: _____ _____ _____ ave: _____

Body fat percentage: _____

Resting heart rate: _____

Resting blood pressure: _____

Results of VO_2 max test:

Speed Km/hr	VO₂ ml/kg/min	RQ	VE	VO	₂	HR	RPE

Running Speed at 75% VO2 peak:

APPENDIX D

Trial Record

Trial No: _____

Name: _____   Date: _____

Breakfast: _____   Time: _____

Resting HR: _____   Resting BP: _____

Ambient Temperature:

Dry Bulb: _____ Wet Bulb: _____ Black Bulb: _____

Pre-Trial Mass: _____ Fan: On / Off _____ Stretches: _____

Time	Workload	Distance	H R	VO2	RPE
30 min					
60 min					
90 min					

Post-Trial Mass: _____   Post BP: _____

Training Record: _____   Tablets: _____

DOMS Record:

Study Title: The effects of an anti-inflammatory homeopathic product on systemic markers of exercise-induced inflammation

Name.............................   Date of Trial: ....

DAY	General post-trial pain seated and relaxed			Post-trial pain during daily living while walking		
	Quads	Hamstrings	Calf	Quads	Hamstrings	Calf
Day 1: immediately after the run						
Day 2: after the run						
Day 3: after the trial						
Day 4: after the trial						
Day 5: after the trial						

0 = no pain at all
5 = moderate
10 = severe/maximal

Do you use Traumeel? Wanting to know more about it?
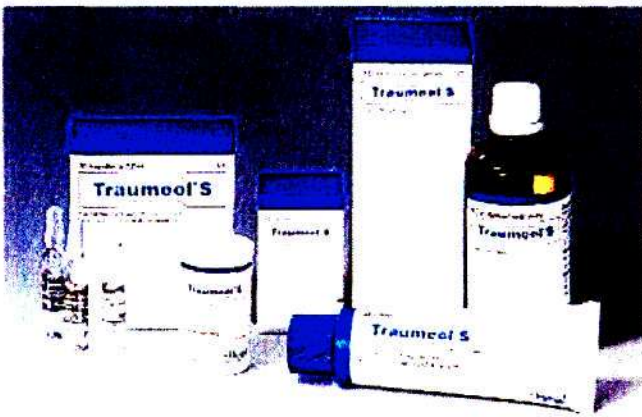
At UKZN we are conducting a study on the effect of Traumeel on exercise–induced muscle damage and inflammation …

What will you get out of it?
•Professional training advice from Norrie Williamson
•Free VO$_2$ max test
•Free blood test results
•Study results

What will it involve?
•One mile field test on UKZN athletics track
•VO$_2$ max test
•One 90-minute downhill run following ingestion of Traumeel/placebo

If interested, please contact Edith Futre @ 073759797 or 2604237(w) or Megan Smith @ 0824887557 for further details