TRAUMEEL S: THE SPORTSMAN'S ANSWER TO ENHANCHED EXERCISE PERFORMANCE AND THE OVERTRAINING SYNDROME?

by

DIRK PIETER JORDAAN

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DECLARATION

Full name: Dirk Pieter Jordaan

Student number: 97237681

Degree/Qualification: Magister Atrium (HMS)

Title of thesis/dissertation: Traumeel S: The Sportsman's Answer to Enhanced Exercise Performance and the Overtraining Syndrome?

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DEDICATION

I dedicate this research project to my wife Zelda and my daughter Gazelle, for their unconditional love, encouragement, time and understanding. Without your support I would not have been able to complete this study.

1 Chronicles 28:20

"Be strong and courageous and get to work. Don't be frightened by the size of the task, for the Lord your God is with you, He will not forsake you, He will see to it that everything is finished correctly."

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SYNOPSIS

TITLE	:	Traumeel S: The sportsman's answer to enhanced exercise performance and the overtraining syndrome?
CANDIDATE	:	Dirk Pieter Jordaan
PROMOTOR	:	Prof PE Krüger
DEPARTMENT	:	Biokinetics, Sport and Leisure Sciences
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Introduction

Research indicates that eccentric exercise is associated with delayed onset of muscle soreness (DOMS). The symptoms associated with DOMS is similar to other inflammatory conditions e.g. pain, swelling and tissue damage. The DOMS as a reaction to the muscle damage is accompanied by changes in cytokines, leukocytes and other markers of inflammation. Prolonged exercise training without adequate rest and nutrition can lead to chronic inflammation and altered cytokine production patterns, which could result in overtraining.

Methodology

The study included actively participating marathon runners and consisted of two groups, a control group (n = 24 athletes) and an experimental group taking Traumeel S (n = 26 athletes), assigned in a double-blind fashion. Subjects made use of the treatment protocol for a period of seven days and followed their normal training program with no additional training. DOMS was induced on day eight when subjects ran downhill at 75% of peak treadmill running speed (PTRS) for 45 minutes at a gradient of –10% with ratings of perceived exertion (RPE), perceived pain (RPP) and heart rate was measured during the run. After the DOMS was induced subjects reported for blood samples for serum creatine kinase (CK), serum cortisol and a differential white blood cell count was taken at the same time for four days after DOMS was induced.

Results

The t-test for independent groups was used to determine the statistical differences between the two groups and for inter-group analysis. The results showed minor and predominantly insignificant changes in CK-, basophil-, eosinophil- and lymphocyte-counts. The cortisol levels in the treated group were higher compared to the placebo group at 48-, 72- and 96-hours post-exercise. The treated subjects' mean monocyte count fell significantly on the first day of recovery and remained significantly lower for the four days post-exercise.

Conclusions

The increased cortisol concentrations can assist the immune system to shut of the acute inflammatory reaction associated with DOMS and in so doing reduce exercise induced muscle damage and inflammation. The decreased monocyte counts will reduce blood vessel permeability and swelling, fewer pain receptors will be stimulated because of lower PGE_2 levels associated with decreased monocyte activation. Secondary, muscle damage that can amongst others be associated with increased monocytic activity, will be limited. Additional blood tests and performance testing are needed to confirm and substantiate the findings of the research.

SAMEVATTING

TITEL	**:**	Traumeel S: Die sportman se antwoord tot verbeterde Oefenprestasie en die ooroefening sindroom?
KANDIDAAT	**:**	Dirk Pieter Jordaan
STUDIELEIER	**:**	Prof PE Krüger
DEPARTEMENT	**:**	Biokinetika, Sport- en Vryetydswetenskappe
GRAAD	**:**	MAGISTER ARTIUM (Menslike Bewegingskunde)

Inleiding

Navorsing dui aan dat eksentriese oefening geassosieer word met vertraagde spierpyn. Die simptome wat geassosieer word met vertraagde spierpyn is soortgelyk aan ander inflammatoriese kondisies en sluit in pyn, swelling en spierbeskadiging. Die vertraagde spierpyn is weens die reaksie op die spierskade en word vergesel deur veranderings in sitokene, leukosiete en ander tekens van inflammasie. Langdurige oefening sonder die nodige rus en voeding kan lei tot kroniese inflammasie en ‘n verandering in sitokeen produksie patrone wat kan aanleiding gee tot ooroefening.

Metodologie

Die studie het aktiewe marathon atlete ingesluit en het uit twee groepe bestaan, ‘n kontrole groep (n = 24 atlete) en ‘n eksperimentele groep (n = 26 atlete) wat in ‘n dubbel-blinde wyse aangewys is. Die proefpersone is onderwerp aan die behandeling vir ‘n periode van sewe dae en het hul normale oefenprogram gevolg met geen addisionele oefening nie. Die vertraagde spierpyn is geinduseer op dag agt deurdat die proefpersone afdraende teen 75% van die maksimale trapmeul snelheid gehardloop het vir 45 minute lank teen ‘n negatiewe helling van 10^{o}.

Die graad van waarneembare inspanning, waarneembare pyn en harttempo is gemeet gedurende die hardloop. Nadat die vertraagde spierpyn geinduseer is, is serum kreatien kinase (CK), serum kortisol en ‘n differensiële witbloedsel telling op dieselfde tyd van die dag vir vier dae lank geneem.

Resultate

Die t-toets vir onafhanklike groepe was gebruik om statistiese verskille tussen die twee groepe te bereken en om intra-groep verskille te bepaal. Die resultate het getoon dat daar klein maar meestal onbeduidende veranderings in kreatien kinase, basofiel, eosinofiel en limfosiet lesings was. Die kortisol vlakke in die eksperimentele groep was hoër as die van die kontrole groep. Die eksperimentele groep se gemiddelde monosiet tellings het beduidend afgeneem op die eerste dag van herstel en het beduidend laer gebly vir vier dae na die oefening.

Gevolgtrekking

Die verhoogde kortisol vlakke kan die immuniteit sisteem in staat stel om die akute inflammatoriese reaksie wat geassosieer word met uitgestelde spierpyn vinniger op te klaar en sodoende kan oefening geinduseerde spierbeskadiging en inflammasie verminder word. Die verlaagde monosiet tellings sal die bloedvate se deurlaatbaarheid en swelling verminder, minder pyn reseptore sal ook gestimuleer word met verminderde monosiet aktiwiteit. Addisionele bloedtoetse en prestasie gebasseerde toetse is egter nodig om die bevindinge van die navorsing te bevestig en te substansieer.

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LIST OF ABBREVIATIONS

5-HT	-	5-hydroxytryptamine
ACTH	-	Adrenocorticotropic hormone
ATA	-	Atmospheres of absolute pressure
CK	-	Creatine kinase
CMI	-	Cell-mediated immunity
COX	-	Cyclo-oxygenase
CRH	-	Corticotropin-releasing hormone
DOMS	-	Delayed onset of muscle soreness
EMG	-	Electromyography
FTryp	-	Free tryptophan
HBO	-	Hyperbaric oxygen therapy
HL	-	Hydroxylysine
HP	-	Hydroxyproline
HPA	-	Hypothalamic pituitary adrenal
HR Max	-	Maximal heart rate
IL-1β	-	Interleukin-1betha
IL-Ira	-	Iinterleukin-1 receptor antagonist
LDH	-	Lactate dehydrogenase
LHRH	-	Luteinizing-hormone-releasing hormone
MCL	-	Medial collateral ligaments
mmHg	-	Millimeter mercury
n	-	Sample size
NK	-	Natural killer cells
nmol/l	-	Nanomoles per liter
NSAID	-	Nonsteriodal anti-inflammatory drugs
^{o}C	-	Degrees Celsius
PaO_2	-	Oxygen content of arterial blood
PGE_2	-	Prostaglandin E_2
Po	-	Maximal load

PTRS	-	Peak treadmill running speed
RPE	-	Rating of perceived exertion
RPP	-	Rating of perceived pain
SSC	-	Stretch shortening cycle
T_h	-	T- helper lymphocyte
TNF -α	-	Tumor necrosis factor alpha
U/l	-	Units per liter
V_{max}	-	Mmaximum velocity
VO_2	-	Volume of oxygen consumption

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GLOSSSARY

Overtraining: Overtraining is the result of an imbalance between stress and recovery and is characterised by stagnation or deterioration in performance that may last several weeks or months despite rest or reduction in training volume.

Overtraining syndrome: A prolonged period of overtraining results in the development of the overtraining syndrome.

NSAID's: Non-steroidal anti-inflammatory drugs are frequently prescribed for treating DOMS related pain and inflammation through their ability to inhibit the synthesis of prostaglandin synthesis.

DOMS: Delayed onset of muscle soreness is the feeling of pain, tenderness, deep ache and stiffness in muscles that begins several hours after uncustomary and especially eccentric exercise.

Inflammation: The generalised response of the body to tissue injury, irrespective of the damaging stimulus. The inflammatory process is crucial to survival.

Eccentric exercise: Movement in which the muscle generates tension while lengthening. Eccentric exercise occurs primarily when the body stabilises itself against gravity and is associated with muscle fibre damage and inflammation and delayed muscle soreness.

Traumeel S: A complex homeopathic preparation comprising of twelve botanical, and two mineral substances in hydrophilic ointment and tablet form. The pharmacological constituents of Traumeel S are specific in their action as an anti-inflammatory.

Creatine Kinase: Creatine Kinase (CK) is an enzyme and a catalyst where the phosphate in creatine phosphate is donated to ADP to re-form ATP. The presence of CK in the bloodstream indicates injury to the sacrolemma.

Cortisol: A stress hormone, which conserves blood-sugar/insulin antagonist and has an anti-inflammatory effect.

Cytokine: A soluble, hormone-like protein produced by a variety of cells. Cytokines are involved in communication between immune cells and integrate systemic inflammatory events.

Leukocytes: Heterogeneous cells found in the blood and other tissues with different functions related to the immune system. The major leukocytes are granulocytes, monocytes and lymphocytes.

Monocytes: A circulating phagocytic leukocyte, which differentiates into a macrophage when migrating to tissue. Activated monocytes are representative of a systemic inflammatory response.

CHAPTER 1

INTRODUCTION

1.1 Problem Statement

Endurance performance is limited by the inability of athletes physiological systems to cope with frequent strenuous exercise, which is crucial for success in endurance events. The general adaptation theory of Hans Selye describes this situation, where instead of adapting positively to high volume and high intensity training the athlete's body tends to break down in response to the training stressors (Fry, 1998). The result of the long-term overexertion is the development of the overtraining syndrome. Early and accurate indicators of maladaptation to training stress is limited and the fine line between hard training and overtraining isn't always identifiable through current physiological, biochemical, immunological or psychological parameters (O'Toole, 1998). Once mal-adaptation to training is elicited, performance may deteriorate or at least stagnate for several weeks or months despite rest or reduction in training volume (Steinacker & Lehmann, 2002). The long-term effects of overtraining have potential medical consequences such a sports injuries, infection and depression, and prolonged deterioration in performance may have a serious impact on the career and longevity of the athlete (Fry *et al*., 1991; O'Toole, 1998; Steinacker and Lehmann, 2002).

The incidence of the overtraining syndrome is high in long distance runners and as many as 65% of elite runners experience staleness at some stage in their careers (O'Toole, 1998). Running downhill is an integral part of endurance athletes' training and competition regimes with eccentric muscle activity being the primary muscle action in downhill running. Eccentric muscle action lengthening of muscle tissue against resistance and is characterized by delayed onset of muscle soreness (DOMS). DOMS is a sensation of discomfort, which is most prevalent in skeletal muscle one to two days following eccentric or unaccustomed high repetition exercise. The high strain of active muscle lengthening causes cytoskeletal damage (MacIntyre *et al*., 1995). The initial mechanical damage does not seem to be the reason for the soreness but rather the subsequent inflammatory reaction to the cytoskeletal damage. Inflammation is characterized by movement of fluid, plasma proteins and leukocytes into tissues in response to injuries,

infection or antigens. The purpose of the response is to promote the clearance of damage tissue, to eliminate microbial invaders and prepare the tissues for repair (MacIntyre *et al*., 1995). Three of the five important signs of acute inflammation – pain, swelling and loss of function are observed during DOMS (Smith, 1991). The inflammatory response in DOMS is similar to inflammation seen after surgery or serious musculoskeletal injuries and is also sub-classified into acute and chronic inflammation.

The accumulation of neutrophils in damaged tissue is the histological marker of acute inflammation while chronic inflammation is characterized histologically by the presence of lymphocytes and monocytes. Monocytes and macrophages are primarily involved in the removal of neutrophils and necrotic tissue and are responsible for the resolution of the acute inflammatory response. If intense training is undertaken too early in the presence of unresolved inflammation, chronic inflammation may persist for an indefinite period of time (MacIntyre *et al*., 1995; Neumann *et al*., 2000). Chronic inflammation results in the release of inflammatory mediators. Cells and mediators of the immune system are always involved in the events following injury, various aspects of immune function are most likely changed as a consequence irrespective of the cause or initiating factors of the injury (Smith, 2003a). In response to muscle damage monocytes can easily be triggered to rapidly produce and release the eicosanoid, prostaglandin E_2 (PGE_2). PGE_2 is involved in post exercise suppression of natural killer cells (NK) for as long as seven days and this may contribute to a weakening of immune system defenses against viral infections.

When PGE_2 production is blocked by the nonsteriodal anti-inflammatory drugs (NSAID) after major surgery there is fewer incidence of opportunistic infections because PGE_2 secretion increases the development of T-helper lymphocyte (TH), subset T_H2 which down regulates T_H1-cell-mediated immunity (CMI) (Smith, 2003a). Nonsteriodal anti-inflammatory agents have been successfully used after eccentric exercise to suppress minor symptoms of muscle soreness, however the delayed recovery of function as seen for instance in strength losses is of concern and this could possibly predispose athletes, especially overtrained athletes, to musculoskeletal injury.

1.2 Importance of the Problem

Eccentric contraction-induced skeletal muscle injury and the effects of the injury for e.g. muscle soreness, inflammation and dysfunction are treated mostly with NSAID's. The mechanisms by which NSAID's treat inflammation and pain can be contributed to their ability to inhibit the synthesis of prostaglandins and in addition to interfere with certain aspects of inflammatory cell function (Peterson *et al*., 2003).

Some investigators have reported a reduction in either muscle soreness, muscle dysfunction or blood creatine kinase after contraction-induced muscle injury (Otto, 2002; Lanier, 2003), however the majority of studies failed to demonstrate a beneficial effect of NSAID's usage (Bourgeois *et al*., 1999; Trappe *et al*., 2002; Peterson *et al*., 2003). Each phase of the inflammatory process is necessary for the subsequent phase. The blockage of certain inflammatory mediators could therefore delay or disrupt the healing of musculoskeletal injuries (Stovitz & Johnson, 2003) through delayed protein synthesis (Trappe *et al*., 2002).

Lapointe *et al*. (2002a) found that the repeated use of anti-inflammatory drugs over extended periods to favour recovery of form and function may not be appropriate in the context that some inflammatory components are needed for both repair and adaptation in the long-term. NSAID's contribute to immune suppression because of their prolonged, intense counter-regulation effect on the immune system (Biffl *et al*., 1996). The known side-effects of NSAID's, such as gastro-intestinal bleeding, elevated mean arterial blood pressure, lung function decline in 10% of patients with asthma, and decreased renal blood flow in acute renal failure in marathoners, limits the usage of NSAID's in the treatment of exercise-induced muscle injury (Stovitz & Johnson, 2003).

Limited effectiveness and known side effects of NSAID's has led to renewed interest in the homeopathic treatment of exercise-induced muscle injuries and delayed onset of muscle soreness (Cheung *et al*., 2003). NSAID's appear to reduce muscle soreness and the symptomatic side-effects of DOMS, they do not seem to address the cause of the inflammatory reaction associated with DOMS (Bourgeois *et al*., 1999; Cheung *et al*., 2003).

Traumeel S, a homeopathic complex remedy has been used in Europe for over 50 years in the treatment of trauma, inflammation and degenerative processes (Oberbaum *et al*, 2001). If Traumeel S is deemed to be successful in the treatment of DOMS, the need for NSAID's could be decreased and Traumeel S could then be a relatively low cost alternative measure for the treatment of DOMS. The study could initiate further research into the use of Traumeel S and other homeopathic alternatives in treated DOMS and other related sports injuries.

1.3 Hypothesis

The prophylactic and therapeutic treatment with Traumeel S will reduce muscle damage and its associated inflammatory response and limit immuno-suppression in trained athletes.

1.4 Objectives of the Study

- To determine the effect of a prophylactic and therapeutic dose of Traumeel S on the markers of muscle damage.
- To determine the effect of a prophylactic and therapeutic dose of Traumeel S on the whitebloodcell count after DOMS has been induced.
- To determine the effect of a prophylactic and therapeutic dose of Traumeel S on the serum cortisol levels after DOMS has been induced.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Muscular exercise often results in micro injury to the active muscles when exercise is relatively intense, of a long duration, and includes eccentric contractions (Armstrong, 1990; Byrne *et al*., 2004).

Delayed onset of muscle soreness is the feeling of pain, tenderness, deep ache and stiffness in muscles that begins several hours after exercise. Eccentric muscle actions involve actively resisting lengthening of the muscles as in lowering a weight slowly against gravity and running downhill (Miles & Clarkson, 1994).

Several definitive studies have shown that the eccentric component of exercise is the major stimulus that causes disruption or damage to muscle tissue and subsequently initiating Delayed Onset Of Muscle Soreness – (DOMS) (Miles & Clarkson, 1994; Morgan & Allen, 1999; Brooks & Faulkner, 2001; Warren *et al*., 2002).

During active muscle lengthening, the mechanical and energetic behavior is very different from isometric or shortening (concentric) contractions (Clarkson & Newham, 1995). Active muscles produce more force during stretch than when acting isometrically at the same length, however, the net energy produced and the chemical changes are smaller (Clarkson & Newham, 1995).

It is suggested that the underlying mechanism is that a part of the force enhancement during eccentric action is due to increased strain of attached cross bridges, possibly in combination with a slight increase in the number of attached bridges (Clarkson & Newham, 1995; Komi & Nicol, 2000).

In strenuous exercise micro injury to the muscle can be divided into four stages. The four stages are a combination of the muscle damage theory of DOMS as described by Armstrong (1990) and the inflammation theory of DOMS as described by Smith (1991).

2.2 Stage I – The Initial Stage

The initial stage includes the events that initiate the whole process.

The possible physical mechanisms for the initiation of muscle fibre injuries could be divided into two categories: mechanically induced and temperature induced (Armstrong *et al*., 1991).

2.2.1 Mechanically Induced Mechanisms

With eccentric exercise high specific tension could mechanically disrupt sacrolemma, sacroplasmic reticulum or myofilaments or permit phosphipase A_2 to physically come into contact with it's phospholipid substrates in the cell membrane and lyse structural components of the sacrolemma (Armstrong *et al*., 1991; Byrd, 1992; Fridén & Lieber, 1992; Clarkson & Newham, 1995; Komi & Nicol, 2000).

2.2.1.1 Myofibrillar Reactions to Eccentric Contractions

There is general agreement that muscle stress is high during eccentric contractions and that damage may not necessarily be a result of muscle stress. Research does however suggest that the muscle sacromere may have a physical threshold to damage, which is reached during eccentric contraction (Morgan & Allen, 1999; Komi & Nicol, 2000; Brooks & Faulkner, 2001).

Because muscles, which are forced to lengthen, can sustain high loads and because the lengthening contractions cause muscle injury, it could be speculated that the high tension causes the injury. Fridén & Lieber (1992) however found that high tension in itself does not appear to cause damage because passive stretching to tension approaching maximal load (Po) did not change maximum tetanic tension. Each adjacent group of sacromeres along the fibre length has a slightly different length and velocity, but generates the same tension.

Slight differences in length and velocity during shortening is not problematic for two adjacent sacromeres since the force-velocity relationship near zero velocity, is not very "steep". For a

0,5% V_{max} (maximum velocity) difference during shortening the forces sustained by the adjacent sacromeres varies by only 1-2 % Po (maximal load). However, during lengthening, due to the steepness of the lengthening portion of the force-velocity relationship, the force in the adjacent sacromeres (or even in adjacent overlap regions within a sacromere) may vary by more than 50% Po. This will lead to different forces on each end of an actin filament and the progressive "popping" or tearing of adjacent sacromeres as the fibre force exceeds their maximal tensile stress. This places excessive directional stress on the Z-disk. This stress imbalance could lead to Z-disk streaming (Fridén & Lieber, 1992).

The magnitude of damage is therefore partially dependent on velocity even though active muscle strain is the main predictor of muscle damage (Brooks & Faulkner, 2001).

2.2.1.2 Time Course of Myofibril Changes

Friden & Lieber (1992) reports that at various times post exercise, 16% of the fibres had focal changes and 16% and 8% of the fibres had extensive and very extensive changes, respectively, immediately after exercise.

It has been shown that there are focal disturbances of the striated band pattern in 32% of the fibres one-hour post-exercise. It has been postulated that lesions seen immediately post-exercise are precursors of more extensive damage seen one to three days after exercise (Friden & Lieber, 1992).

2.2.2 Metabolic Hypothesis of the Initial Events

2.2.2.1 Insufficient Mitochondrial Respiration

During exercise, mitochondrial respiration is elevated to match synthesis to ATP hydrolysis. During muscular activity there is always some reduction in the concentration of ATP and this reduction could occur within compartments of muscle fibres which could initiate the fibre injury (Armstrong *et al*., 1991).

Insufficient mitochondrial respiration could lead to decreased ATP levels in the vicinity of the CA^{2+}-ATP as in the sarcoplasmic reticulum (SR) or sacrolemma. Removal of Ca^{2+} from the cytoplasm could be limited, and this will allow an elevation in cytosolic Ca^{2+} levels. Inhibition of

Ca^{2+}-ATPase on the SR, sacrolemma and mitochondria causes rapid and dramatic damage to the ultrastructure of the muscle. A significant decrease in ATP levels however seems unlikely in eccentric exercise only because energy utilization is relatively low. Eccentric exercise combined with strenuous endurance based exercise could however affect Ca^{2+} extrusion from the cytoplasm by the ATP-dependent Ca^{2+} pumps in the sacrolemma, mitochondria and sarcoplasmic reticulum (Armstrong, 1990; Armstrong *et al*., 1991).

2.2.2.2 Free Radical Production

Free O_2 production and lipid peroxidation increase during exercise and potentially play a role in the initiation of muscle injury (McArdle *et al*., 2001).

In tissue that is highly active, oxygen free radicals are produced as a common metabolic intermediate of which superoxide anian (O_2) and hydrogen peroxide are most important. These substances can cause irreversible damage to many cellular structures (Armstrong *et al*., 1991; Byrd, 1992). It has been shown that with less than 15 minutes of exposure to superoxide, membrane lipids undergo rapid peroxidation and cell proteins can be broken down. Oxidation of the sulphydryl groups of the ATPase pump lead to a reduction in the rate of Ca^{2+} uptake by the SR (Byrd, 1992; McArdle *et al*., 2001).

2.2.2.3 Lowered Muscle pH

It is expected that exercises with concentric contractions would produce lower pH in the active muscle due to the greater metabolic cost of concentric exercises relative to eccentric exercise, indicating that a decrease in pH is not an initiating mechanism for muscle damage. It has also been shown that rapid myofibril damage can be induced by Ca^{2+} at a neutral pH with 3mmol/l ATP in the medium. This proves that muscle damage can occur in the presence of depleted ATP and low pH (Armstrong *et al*., 1991).

2.2.3 Temperature Induced Mechanisms

Heat production is greater during lengthening/eccentric contractions, except at very low velocities, than isometric contractions. Heat liberation during eccentric contractions is that which is produced by the metabolic processes and induced heat supplied by external energy. Muscle

blood flow is proportional to the metabolic rate and therefore inadequate to dispel the additional heat produced during eccentric exercise (Clarkson & Newham, 1995).

High local temperatures in the muscle could have an injury like effect on protein structures. Protein degradation rates are proportional to temperatures in the physiological range (24 – 42^{o}C) (Armstrong, 1990; Clarkson & Newham, 1995). As temperature rises above 25^{o}C, there is a progressive loss of cell membrane integrity and an increasing influx of extracellular Ca^{2+} (Warren *et al*., 2002). High temperatures may change the fluidity of the lipid membrane surrounding the ATPase pump and in doing so impair its ability to isolate Ca^{2+} (Byrd, 1992).

Studies show that temperature increases are the same in the muscles of trained and sedentary subjects even though muscle pain and damage is substantially less in the trained individuals. It is therefore assumed that the damage caused by high temperatures is not the only primary metabolic factor in muscle soreness (Friden & Lieber, 1992).

It is not always possible to categorize some of the events under one specific stage. There seems to be overlapping of certain events between the initial and autogenic stages and especially between the autogenic and phagocytic stages. Some of the metabolic events in the initial stage will therefore also be discussed in the autogenic stage.

It has been proposed that greater relative reduction of the respiratory pathways in the muscle during eccentric exercise could further increase production of O_2 radicals even though there is no clear evidence for this (Armstrong, 1990).

2.3 Stage II – Autogenic Stage

The autogenic stage of injury corresponds to the first three to four hours following the initiation of the injury and signals the beginning of the degradation of the lipid and protein structures in the injured cells (Armstrong, 1990; Komi & Nicol, 2000). Various studies have strongly implicated Ca^{2+} as playing a pivotal role in the injury process in skeletal muscle.

Calcium plays a vital role in the structure and function of the cell and it's membrane. Calcium helps in the activation of phosphorylase kinase, regulates calcium pumps and contractile proteins and helps maintain sacrolemmal integrity (Byrd, 1992).

A low intracellular free concentration ($[Ca^{2+}]f$) is needed for cell function, high $[Ca^{2+}]f$ is associated with cell necrosis and cell death because of the loss of Ca^{2+} homeostasis. Calcium induced cell damage is linked to ischemia in the myocardium and in some types of degenerative muscular dystrophies (Byrd, 1992). Ca^{2+} overload causes ultra structural changes in the muscle. These changes include swollen and disrupted mitochondria, dilated t-tubules and SR, a general cellular edema, and disruption of the myofilaments – particularly in the proteins making up the Z-lines (Byrd, 1992; Morgan & Allen, 1999).

Changes to the $[Ca^{2+}]$ stimulate compounds that are active in muscle autolysis, this includes Ca^{2+} sensitive proteases and phospholipases (Armstrong, 1990; Byrd, 1992; Tidball, 1995). These processes are believed to be initiated by increased intracellular calcium due to fatigue-induced mitochondrial inability to buffer calcium.

Ca^{2+} stimulated proteases (calpains) can be divided into that which are thought to act directly on the proteins in cell membranes e.g. myosin, α-actinin, talin and vinculin and proteases that act specifically on the z-lines (Byrd, 1992). While performing eccentric muscle contractions there is greater potential for damage to the sacrolemmal membrane. The damage to the sacrolemma membrane allows for an increase in $[Ca^{2+}]f$ from the extracellular pool and initiate muscle autolysis.

2.3.1 Potential Mechanisms Involved in the Autogenic Stage

2.3.1.1 Uncontrolled Contracture

Loss of Ca^{2+} homeostasis in the muscle could result in uncontrolled contraction of sacromeres in the affected area. The result of continuous contraction could be a local depletion of high-energy phosphates. Uncontrolled contractions could also produce mechanical forces in the fibres that further damage structural components in the membranes or contractile elements. ATP depletion

that precedes loss of Ca^{2+} homeostasis could also limit contracture of affected sacromeres (Armstrong *et al*., 1991).

2.3.1.2 Mitochondrial Calcium Accumulation

Evidence of elevated mitochondrial Ca^{2+} levels has been found in rats after downhill walking. Uptake of excessive amounts of Ca^{2+} by the mitochondria is accompanied by uptake of phosphate and calcium phosphate may be deposited in the intramitochondrial space. Accumulation of Ca^{2+} in the micromalar range depresses mitochondrial function (Armstrong, 1990; Armstrong *et al*., 1991).

2.3.1.3 Ca^{2+} Activated Neutral Proteases

Like lysosomal proteases, the enzymes responsible for Ca^{2+} dependent proteases have a neutral pH optima and their activation is associated with degradation of particular structures in the muscle cell (Friden & Lieber, 1992). Ca^{2+} activated proteases may specifically degrade Z-discs or contractile filament components and could also explain Z-line streaming or disruption of the A-band. Cytoskeletal proteins such as myosin, α-actin, talin and vinculin that anchor intracellular components to the sacrolemma are good substrates for Ca^{2+} activated proteases and could lead to increased sacrolemmal fragility (Armstrong *et al*, 1991; Byrd, 1992; Friden & Lieber, 1992; Farges *et al*., 2002).

2.3.1.4 The Phospholipase A_2 (PLA_2) Pathway

PLA_2 is the first enzyme in the pathway that uses membrane phospholipids as substrates for production of arachidonic acid and prostaglandins, leukotrienes and tromboxanes. Arachidonic acid and lysophospholipids can have a detergent effect on cell membranes, affecting stability of their structures.

Prostaglandin E_2 (PGE_2) produced in the cyclo-oxygenase pathway may activate lysosomal proteases and protein degradation. Muscles stimulated in the presence of dinitrophend and Ca^{2+} results in the release of lactate dehydrogenase (LDH), however when PLA_2 inhibitors and Ca^{2+} are present while muscles are being stimulated, LDH loss is attenuated. PLA_2 thus seems to be a contributing mechanism to the loss of intramuscular enzymes during muscle fibre injury (Armstrong, 1990; Armstrong *et al*., 1991).

2.3.1.5 Lysosomal Proteases

Proteolytic enzymes in the muscle fibres can degrade myobifibrillar proteins. Ca^{2+} could assist in activating proteases through exocytosis of the enzymes from the organelles (Armstrong *et al*., 1991; Toumi & Best, 2003).

Ca^{2+} can also stimulate lysosomal enzyme discharge in leukocytes. Elevated Ca^{2+} levels can possibly activate phospholipase A_2 (PLA_2) which increases production of particularly prostaglandin E_2. The involvement of lysosomal proteases in protein degradation is questioned because myofibrillar damage in muscles exposed to a Ca^{2+} ionophore is not blocked by inhibitors of various lysosomal proteases (Armstrong *et al*., 1991). Farges *et al*. (2002) were, however, able to elicit proteolysis in the absence of Ca^{2+} and proposed that 70 % of proteolysis occurs inside lysosomes and that a phagocytic process of mononuclear cell penetration inside the muscle fibre resulted in catabolism. According to Farges *et al*. (2002) liberation of free lysosomal enzymes accounted in large part for the increased protein catabolism associated with muscle trauma.

2.4 Stage III - Phagocytic – Inflammation Stage

The phagocytic stage is characterized by a typical inflammation response two to six hours after the initial injury and may last two to four days or more, with a peak on the third day post-exercise (Komi & Nicol, 2000).

The acute inflammation response begins when the vascular vessel wall structures are damaged, leading to structural and functional alterations of the basement membrane which includes vasodilatation and increased vascular permeability (Smith, 1991; Houglum, 2001). The cellular phase of inflammation involves the migration of neutrophils (polymorphs, PMN_s) and monocytes (Smith, 1991; Tidball, 1995).

Neutrophils are the first sub-population of leukocytes to appear at the injury site, representing 50 – 60 % of the total circulating leukocytes and are believed to play an important role in phagocytosis and degradation of damaged tissue, especially skeletal muscle damaged during eccentric exercise (Toumi & Best, 2003). In addition to phagocytosis, neutrophil invasion and

activation can lead to the release of oxygen free radicals, increase muscle permeability, proteases and phospholipase which could result in secondary damage, additional to the primary damage of mechanical stress (Macinnon, 1999; Pizza *et al*., 1995; Lapointe *et al*., 2002a)

Studies of muscle damage induced by eccentric exercise have highlighted that peak muscle damage occurs at the same time as maximal neutrophil invasion of the injured tissue (Toumi & Best, 2003). Neutrophils, once activated, provide a fresh supply of mediators, which may be partially responsible for amplifying and prolonging the inflammation. This is usually associated with cardinal signs and symptoms associated with acute inflammation (Komi & Nicol, 2000).

Monocytes are the predominant inflammatory cell type at all stages of inflammation following the first 12 hours post injury (Tidball, 1995). When monocytes leave the blood and enter the tissue compartment, they mature into macrophages. The presence of macrophages at the site of injury, as seen during acute inflammation and after eccentric exercise, is most likely to be responsible for the biosynthesis of PGE_2 (Smith, 1991).

Two sub-populations of macrophages, the $ED1^+$ and $ED2^+$ are present in the normal macrophage population in muscle (Tidball, 1995). The $ED1^+$ macrophages are mainly involved in the removal of cellular debris and are most likely responsible for secondary damage associated with local muscle inflammation (Tidball, 1995; Lapointe *et al*., 2002b). The $ED1^+$ populations decline in concentration in muscle after the phagocytic stage is completed.

The phagocytic stage is also characterized by the presence in the blood of indirect indicators of muscle injury, such as the muscular protein metabolites e.g. troponin I. Furthermore there is also an increase in specific muscle enzyme activity such as creatine kinase and lactate dehydrogenase. Troponin I is the first marker of cytoskeletal disruption and is indicative of the early degradation of the thin-filament troponin-tropomyosin complex (Sorichter *et al*., 1997).

Creatine kinase (CK) is normally prevented from entering the extracellular space unless there is damage to the sacrolemma. The presence of CK in the bloodstream indicates injury of the sacrolemma (Lanier, 2003). The peak values of CK vary considerably between individuals and

do not reflect the amount of muscle damage and do not correlate well with functional performance decrements. Relative changes in CK might have some value to detect tissue inflammation and to comprehend the functional effect of stretch shortening cycle fatigue (Komi & Nicol, 2000).

A review by Smith (1991) provides some substantial evidence that the signs and symptoms associated with acute inflammation are similar to changes seen in DOMS.

2.4.1 The Five Signs of Inflammation

2.4.1.1 Pain

Muscle pain/soreness sets in at about six to eight hours after exercise and peaks at about 48 hours (Proske & Morgan, 2001). The generation of painful sensations involves activation of pain afferents, most likely in type III and IV fibres. Specific chemical substances such as histamine, bradikinin, serotonin, and prostaglandin (PGE) are released in response to muscle damage and generate the painful sensations. PGE appears to be the main source of painful sensation even though it does not directly cause pain but instead sensitizes nociceptors (pain receptors). There seems to be a similar time course for increase in PGE and DOMS and this could suggest a possible positive correlation between these two variables (Smith, 1991).

The presence of macrophage at the site of injury seen during acute inflammation and after eccentric exercise is most likely responsible for the biosynthesis of PGE_2 (Smith, 1991).

2.4.1.2 Swelling

Swelling or edema is a result of increased permeability of small blood vessels which allows protein rich fluid to escape into the tissue of the damaged area (Smith, 1991; Miles & Clarkson, 1994; Pizza *et al.*, 1995). Increases in limb volume have been measured at 24, 48 and 72 hours after eccentric muscle contraction with limb volume peaking at about five days. The increase in limb volume reflects synthesis of connective tissue associated with the process of regeneration and/or repair. The swelling within the muscle could produce pain and increase intramuscular pressure, as well as sensitize pain receptors to other noxious stimuli (Smith, 1991; Miles & Clarkson, 1994; Kibler & Chandler, 1998).

2.4.1.3 Loss of Function

Acute inflammation results in a loss of function in the involved area (Smith, 1991). A loss of function is the inability of an affected muscle to generate force. Loss of function could be due to mechanical interference in muscle length, force and velocity because of swelling and/or due to a reflex inhibition of the muscles experiencing pain (Smith, 1991; Clarkson & Newham, 1995; Proske & Morgan, 2001; Byrne *et al*., 2004).

Loss of function may return to baseline levels by 24 hours or may take as long as 89 days to recover after eccentric exercise. Recovery to baseline strength levels on average can be between two to six weeks (Byrne *et al*., 2004). Loss of function in DOMS does not only include strength losses, but can include loss of range of motion, flexibility and mobility (Clarkson & Newham, 1995; Cheung *et al*., 2003; Byrne *et al*., 2004).

Pain does not appear to be related to a loss of function and it is hypothesized that the sensation of DOMS appears during an important period of healing and the synthesis of new connective tissue (Miles & Clarkson, 1994). A reduction in the range of motion may place the injured muscle in a position for healing (Smith, 1991).

2.4.1.4 Heat and Redness

Local tissue temperature is at times associated with acute inflammation and is a direct result of a large increase in local blood flow (Houglam, 2001). Research on humans indicates that there is no increase in skin temperature in humans with DOMS (Smith, 1991). Smith (1991) reasons that an injury situated deep within the muscle is unlikely to produce changes in surface temperature.

Redness is caused by dilation of small blood vessels in the area of the injury. Redness is also not easily detected in deep muscle injury (Smith, 2000).

2.5 Stage IV - Regenerative Stage

The regeneration stage begins on days four to six after the initial injury and reflects the regeneration of muscle fibres (Komi & Nicol, 2000). The regenerative process is restricted to the

focal site of injury, except for the migration of satellite cells to the damaged area from uninjured parts. Tidball (1995) proposes that signaling occurs between the injured muscle cells and the mononucleated cells. Some of these cells remove the damaged material while other populations repair the injured site and generate new muscle fibres.

The late appearance of $ED2^+$ cells during the inflammation process when necrosis is nearly complete and muscle has begun to regenerate indicates $ED2^+$ macrophage involvement in regeneration. $ED2^+$ macrophages can regulate the regenerative process through the release of factors that can influence the regenerative factors of other cells, such as the damaged muscle fibre or satellite cells (Tidball, 1995; Lapointe *et al*., 2002b). Tidball (1995) reported that in each case where phagocytosis by macrophages was impaired, subsequent repair was also affected.

The fibroblast is another critical cell for wound healing. The fibroblast infiltrates the lesion after the monocyte and gradually replaces the white bloodcells. Some of the fibroblasts convert to myofibroblasts to decrease the wound size (Houglam, 2001). Fibroblasts are also involved in the synthesis and disposition of collagen and proteoglycons, the substance that form connective tissue (Smith, 1991).

Protein synthesis increases approximately 48 hours after injury, and remains elevated by 83% for five days post injury. By 10 to 14 days, muscle protein degradation and synthesis rates return to normal and phagocytic infiltration is no longer detected. Muscle mass, protein content and absolute force production are still lower than before at eight to 14 days post injury (Komi & Nicol, 2000; Cheung *et al*., 2003; Byrne *et al*., 2004).

2.6 Additional Theories of Muscle Damage

2.6.1 Lactic Acid Theory

The lactic acid theory is funded on the fact that lactic acid continues to be produced after exercise cessation. Lactic acid however returns to pre-exercise levels within one hour following exercise (Cheung *et al*., 2003). Individuals who suffer from McArdels's syndrome and do not produce lactic acid also suffer from DOMS. Concentric contractions produce higher levels of lactic acid than eccentric contractions at the same power output, however, DOMS is not associated with

concentric muscle contractions. Lactic acid may partially contribute to the acute pain associated with fatigue, however, it can not be attributed to the delayed pain that is experienced 24 to 48 hours post exercise (Plowman & Smith, 1997).

2.6.2 Muscle Spasm Theory

The muscle spasm theory was introduced after increased levels of resting muscle activity in eccentric exercise were observed. It is proposed that an increased resting muscle activation indicates a tonic localised spasm of motor units. This could lead to vasoconstriction of blood vessels, ischemia and the accumulation of pain substances, which in turn could further stimulate pain nerve endings causing further reflex spasms and prolonged ischaemic conditions. Bipolar and unipolar electromyography (EMG) has been used for studies and the lack of sensitivity to record the electrical activity in sore muscles makes this theory less credible (Cheung *et al*., 2003).

2.6.3 Connective Tissue Damage Theory

Type I (slow twitch) fibres display a more robust structure than type II (fast twitch) fibres. Fast twitch fibres may be more sensitive to stretch-induced injury and the excessive strain of the connective tissue may lead to muscle soreness (Cheung *et al*., 2003).

Hydroxyproline (HP) and hydroxylysine (HL) amino acids have been used to determine breakdown of connective tissue after eccentric exercise. There were statistically significant increases in the 48 hours post-exercises levels of hydroxyproline for eccentric exercised subjects compared to non-exercise subjects (McArdle *et el*., 1991; Cheung *et al*., 2003). A problem with the connective tissue damage theory is the fact that HP and HL excretion can reflect either increased collagen synthesis or collagen degradation. The specific mechanism leading to an increase in HP and HL is thus still to be determined (Cheung *et al*., 2003).

2.6.4 The Impact of Delayed Onset of Muscle Soreness on Athletic Performance

2.6.4.1 Power and Strength Generating Ability

Muscle strength and power can be significantly reduced after a bout of damage-inducing exercises (Clarkson *et al*., 1992; Nosaka & Clarkson, 1995; Clarkson & Newham 1995; Komi & Nicol, 2000). Eccentric strength losses seem to be influenced more than concentric and isometric

strength losses. Eccentric strength reductions following DOMS seem to be reduced for a longer time period, eight to ten days, while concentric and isometric strength are usually reduced for four days only (Cheung *et al*., 2003).

Isometric force production can decrease by as much as 50% of the pre-exercise level after muscle soreness was induced (Clarkson *et al*., 1992). Byrne *et al*. (2004) report that prolonged reduction in peak power and strength has been found immediately and for prolonged periods after a 30 seconds Wingate cycling test in the presence of muscle damage. Isometric strength demonstrated a linear recovery pattern, peak power demonstrated decrements immediately after exercise-induced muscle damage was induced, and even further decrements at one and two days post-exercise. These findings suggest that muscle power may be affected by the inflammatory response to exercise-induced muscle damage. The finding supports the notion that type II muscle fibres are mainly damaged with eccentric exercise.

2.6.4.2 Vertical Jump Performance

Komi & Nicol (2000) reported a reduction in vertical jump height when muscle damage was induced through plyometric exercise or through marathon running. The recovery process occurred in a bimodal pattern with an initial reduction in performance, which was followed by an early recovery before secondary reductions in performance two to three days post-exercise. The second decline in performance after the initial decline suggests once again that inflammation could have played a role in the performance.

2.6.4.3 Endurance Performance

Not all the physiological responses to endurance exercise are amplified in the presence of muscle damage. Post-exercise venous blood lactate and plasma cortisol levels were significantly higher when sub-maximal exercise was performed after eccentric versus concentric exercise. No differences in maximal oxygen consumption ($VO_{2\ max}$) or sub-maximal oxygen consumption were observed in response to incremental cycle ergometer exercise performed two days after eccentric exercise (Byrne *et al*., 2004).

Komi and Nicol (2000) indicated that the loss of muscle function and the stretch shortening cycle (SSC) efficiency through repetitive SSC actions, which occurs in the quadriceps and calf muscles during marathon running, could directly contribute to fatigue during prolonged exercise.

Muscle damage can alter thermoregulation during exercise in the heat. Core-temperature was elevated by 0.2 – 0.3 ^{o}C and heart rate by 12 beats per minute during treadmill walking at temperatures of 40 ^{o}C and 20% relative humidity, performed two and six to seven hours after eccentric exercises. Core-temperature increases of 0.4^{o}C and 12 beats per minute heart rate increases are equivalent to an individual that is hypo hydrated by 2-3% (Byrne *et al*., 2004).

2.6.4.4 Range of Motion

Clarkson *et al*. (1992) tested the range of motion in the elbow immediately after high force eccentric exercise and found that the subjects' ability to flex their elbows was dramatically impaired and the baseline angle of flexion was still not restored by 10 days after exercise.

The mechanism to explain increase muscle stiffness is not clearly understood. According to Clarkson & Newham (1995) stiffness could be from either spontaneous contraction of the elbow flexors or a shortening of the non-contractile tissue. Nosaka and Clarkson (1995) indicated by means of MRI that greatest swelling occurs in the forearm flexors at one to five days post exercise. Swelling may be one of the contributing factors to a decreased range of motion even though it is not the only factor. It has also been suggested that the spontaneous shortening could be due to an abnormal accumulation of calcium inside the muscle cell, due to either a loss of sarcolemmal integrity or a dysfunction of the sarcoplasmic reticulum (Byrd, 1992; Clarkson & Newham, 1995; Proske & Morgan, 2001).

2.7 NSAID's and Delayed Onset of Muscle Soreness

2.7.1 Mechanisms of Action of NSAID

Non-steroidal anti-inflammatory drugs (NSAID's) are frequently prescribed for musculoskeletal injuries – including delayed onset of muscle soreness, because DOMS is believed to be inflammatory in nature (Stovitz & Johnston, 2003). The mechanism for the therapeutic effect of NSAID's in treating pain and inflammation is their ability to inhibit the synthesis of

prostaglandin synthesis (Stovitz & Johnston, 2003; Wisemann, 2003). NSAID's have also been demonstrated to reduce both neutrophil chemotaxis and activation in vitro. NSAID's e.g. acetaminophen, aspirin (salicylic acid), indomethacin, ibuprofen and piroxicam perform their anti-inflammatory action by reversibly or irreversibly (in the case of aspirin) inhibiting the cyclo-oxygenase (COX) enzyme, responsible for converting arachidonic acid into the different forms of prostaglandins, in a non-selective manner (Huff & Prentice, 1999).

Research shows that there are at least two isoforms of the COX enzyme, namely COX I and COX II isoenzymes. COX I has an integral role in maintaining the integrity of the gastric and duodenal mucosa, regulation of blood flow and platelet aggregation. The COX II enzyme is an inducible enzyme, which is not produced under normal circumstance (Bogatov *et al*., 2003; Wisemann, 2003). COX II is greatly increased during states of inflammation. Recently the importance of inhibiting prostaglandin synthesis especially the COX II isoenzyme as a major mode of action has been highlighted (Wisemann, 2003). COX II catalyzes the conversion of arachidonic acid into the inflammatory prostaglandins that are involved in three key biological functions: sensitizing skin pain receptors, elevating body temperature through the hypothalamus, and recruiting inflammatory cells to injured body parts (Stovitz & Johnston, 2003).

2.7.2 The Effectiveness of NSAID in Improving Muscle Function and in Decreasing Delayed Onset of Muscle Soreness and Inflammation

Although a few investigators have reported a reduction in either muscle soreness, muscle dysfunction or blood creatine kinase activity after NSAID treatment for eccentrically induced muscle damage, the majority of studies failed to demonstrate a beneficial effect of NSAID's (Francis & Hoobler, 1987; Miles & Clarkson, 1994; Peterson *et al*., 2003).

Evidence suggests that there may be a long-term detrimental effect from NSAID use after muscle damage was seen in a study on rabbits. A study by Mishra et al. (as cited by Toumi & Best, 2003) found that NSAID administration attenuated myofibrillar protein loss and force deficits after three and seven days after repeated bouts of eccentric contraction. However the same study showed deficit in torque and force generation at 28 days.

A study making use of a COX I inhibitor to assess the beneficial effect of this NSAID on injured medial collateral ligaments in rabbit knees, showed no significant differences in the strength of the injured ligaments between the COX I inhibitor and the placebo treatment. Similar studies making use of COX II inhibitors impaired medial collateral ligament healing by 32%. It should be noted that there was a 22% increase in the load at failure and 27% increase in the energy to failure in the uninjured MCLs of the animals (Bogatov *et al*., 2003).

Trappe *et al*. (2002) also found that ibuprofen blunted the protein synthesis after exercise-induced muscle soreness and inflammation. He proposes that NSAID's block the prostaglandins, which have also been shown to regulate protein metabolism. The endothelium is a critical step in the sequence of events leading to muscle damage.

Significant increases in neutrophils together with macrophage concentrations after eccentric exercise have also been documented in literature (Peterson *et al*., 2003; Toumi & Best, 2003). A recent controlled laboratory study indicated that the NSAID ibuprofen did not influence inflammatory cell concentration of neutrophils and macrophages 24 hours after eccentric contraction in humans (Otto, 2002; Peterson *et al*., 2003). The above-mentioned indicates that pharmacological intervention may be better targeted against specific aspects of neutrophil function such as free radical production, while maintaining the steps necessary for phagocytosis and removal of cellular debris.

2.7.3 Possible Negative Effects Associated With NSAID Use

2.7.3.1 NSAID and the Inflammatory Healing Process

The general argument for using NSAID's in treatment of musculoskeletal injuries has been their anti-inflammatory quality. The viewpoint is that healthy tissue is not inflamed and if inflammation in an injured area is stopped, the tissue will be healthy. According to Stovitz & Johnston (2003) the concern with this viewpoint is that inflammation is seen as a necessary component of the healing process. It has been said that inflammation can occur without healing, but healing cannot occur without inflammation.

Inflammation is partly mediated by the same prostaglandins that are blocked by NSAID's. In the natural healing process, the proliferative phase, which consists of a mixture of inflammatory cells and fibroblasts, naturally follows the inflammatory phase. Each phase of repair is necessary for the following phase. By blocking or interfering with the inflammatory phase NSAID's can, at least in theory, delay the healing of musculoskeletal injuries (Stovitz & Johnston, 2003). Toumi & Best (2003) stresses the possibility that certain aspects of neutrophil function in a response to eccentric exercise cause damage to healing muscle or delay its regenerative capabilities. Because neutrophils can release oxygen free radicals during phagocytosis, it is possible that neutrophil derived oxidants worsen pre-existing muscle injury *in vivo* by damaging previously uninjured muscle. NSAID's can possibly decrease neutrophil activity, however delayed recovery and functional losses in the injured muscle remain problems.

2.7.3.2 NSAID's Effects on the Gastrointestinal Tract

In theory, COX II inhibitors should attenuate inflammation through prostaglandin inhibition, while keeping the COX I pathway intact- the pathway responsible for regulating the body's normal physiological function. The key issue with COX II inhibitors is that they are highly selective rather than specific inhibitors of COX II and that there will still be some inhibition of the COX I isoenzyme (Wisemann, 2003).

Normal gastrointestinal function relies on a balance between the protective mechanisms of prostaglandins versus the damaging effects of peptic acid secretions. The impairment of this balance could allow chronic gastrointestinal injury to occur which is known as the dual insult mechanism: 1.) injury occurs locally, which is pH-dependent and varies greatly among different NSAID's, and 2.) systemic injury, which is less drug specific and does not depend on direct mucosal contact. Local injury is due to direct acid damage that occurs due to increased cell membrane permeability that can result in cell swelling and cell death and decreased mucus biosynthesis, which impairs the mucosal barrier (Wisemann, 2003).

Systemic injury is due to impaired prostaglandin synthesis especially prostaglandin E2 and decreases the antisecretory effect on gastric acid production, decreases bicarbonate synthesis, impairs gastric mucosal blood flow and the adaptive cytoprotective mechanism. Inhibition of

platelet aggregation also contributes to gastrointestinal complications (Stovitz & Johnston, 2003; Wisemann, 2003).

2.7.3.3 NSAID's Effects on the Renal and Cardiovascular System

Expert opinion is that COX II is found in renal tissue of all species. The COX II isoenzyme is proposed to be intimately involved in prostaglandin-dependent renal homeostatic processes. Prostaglandin is necessary for renal blood flow and the secretion of sodium and chloride. Studies on COX II inhibitors show qualitative changes in urinary prostaglandin excretion, glomerular filtration rate and sodium. Prostaglandin inhibitors have been shown to raise mean arterial blood pressure by an average of three to five mmHg. NSAID's can aggravate a decrease in renal blood flow due to hypo-hydration that is often experienced in endurance athletes (Stovitz & Johnston, 2003). Older individuals may be especially at risk because of the age-related decreased production of prostaglandins (Lanier, 2003).

The combination of hypo-hydration and the usage of NSAID's have been implicated in case reports of kidney ischemia and acute renal failure in marathoners (Huff & Prentice, 1999). Historically NSAID's have perceived to be either neutral or beneficial in regards to cardiovascular occlusive events. There is however some new evidence that NSAID's temporary platelet inhibition may limit the cardio-protective effects of aspirin by antagonizing aspirin's irreversible platelet inhibition (Stovitz & Johnston, 2003).

Evidence exists that the use of NSAID's significantly increases the risk of congestive heart failure in older patients, especially those with a prior history of heart disease. NSAID's presumably increases vascular resistance and may also interfere with the action of drugs, such as diuretics and ACE inhibitors, which are used to treat heart disease. It seems that NSAID's may not cause congestive heart failure but accelerate it in individuals who are at risk (Lanier, 2003).

2.8 Traumeel S as a Treatment for Inflammation

Traumeel S usage is indicated for muscle recovery in terms of its active ingredients. A study by Breedveld showed a significantly reduced myofascial pain of the upper trapezius muscle due to its anti-inflammatory and analgesic effects (as cited by Saunders, 2003).

Traumeel S is a complex homeopathic preparation comprising of 12 botanical, and two mineral substances in hydrophilic ointment and tablet form. The pharmacological constituents of Traumeel S are specific in their action as anti-inflammatories and include the following:

2.8.1 Anconitum Napellus

Anconitum napellus is a remedy for inflammations if muscle tissue and membranes are affected. Anconitum is also an important homeopathic fever remedy particularly if the patient presents with a rapid and irregular heartbeat and hyperthermia, (as cited by Saunders, 2003).

2.8.2 Arnica Montana

Arnica is a well-known remedy for wounds, injuries, haemotomas and contusions. Arnica usage is implicated in damage resulting from over-exertion e.g. in sports people and if patients complain of a sensation of weakness, tiredness and general exhaustion. A study by Synge *et al*. (2002) on the effectiveness of Arnica on muscle soreness after downhill running on adults showed that Arnica reduced soreness and assisted in maintaining running speed after DOMS was induced.

2.8.3 Bellis Perennis

Bellis perennis is implicated for treatment of sprains and bruises and for physical over-exertion such as sports injuries (Reckeweg, 1991). Bellis perennis helps to remove the exudations of swelling in many kinds of injuries (Vermeulen, 1997).

2.8.4 Chamomilla

Chamomilla is used for the reaction phase of inflammation and is used for the treatment of arthritis in the shoulder-joint (Reckeweg, 1991).

2.8.5 Echinacea Angustifolia

Echinacea is an “internal antiseptic” acting on the lymphatic system (Vermeulen, 1997). Echinacea is also an immune system stimulant and theoretically diminishes the effects of

immuno-suppressants (Smith, 2003b). The main indications to use echinacea angustifolia are fever, sepsis and inflammations of every kind.

2.8.6 Echinacea Purpurea

The indications are as for Echinacea Angustifolia (Reckeweg, 1991).

2.8.7 Hamamelis

The main indication is for haemorrhages, traumatic inflammations and the chronic effects of mechanical injuries (Reckeweg, 1991; Vermeulen, 1997).

2.8.8 Calendula

Calendula promotes healthy granulations and rapid healing of ruptured muscles, tendons and healing or reproduction of bone (Vermeulen, 1997).

2.8.9 Millefolium

It is indicated for haemorrhages, paralysis and contraction of limbs (Vermeulen, 1997).

2.8.10 Atropa Belladona

Belladona can be used in all localised inflammations in the first stage where no suppuration has taken place (Reckeweg, 1991).

2.8.11 Mercurius Solubilis Hahnemanni

Mercurius can be used for any kind of inflammation and especially for acute inflammatory conditions of the mucosa (Reckeweg, 1991).

2.8.12 Hepar Sulfuris

Hepar sulfuris helps to decrease swelling of the knee, ankle and foot (Vermeulen, 1997).

2.8.13 Symphytum

Symphytum is used in injuries and fractures of bones and injury to the periosteum. It can also be used in injuries to tendons and ligaments (Reckeweg, 1991).

2.8.14 Hypericum

Hypericum is one of the most widely used herbals worldwide. Hypericum has an antiviral action (Smith, 2003b) and is indicated for neuritis and muscle spasms.

2.9 Complementary Therapy Agents Options for Delayed Onset of Muscle Soreness

2.9.1 Cryotherapy

Superficial application of ice decreases tissue temperature and stimulates cutaneous receptors to excite the sympathetic adrenergic fibres causing the constriction of local arterioles and venules in acute muscle injuries. This results in the reduction of swelling and a decrease rate of metabolism, which then will reduce the inflammatory response, vascular permeability and the formation of oedema (Houglum, 2001; Cheung *et al*., 2003). Cheung *et al*. (2003) found in his review that subjects which were immersed in ice or received ice massages showed no significant reduction in muscle soreness, isokinetic torque or limb volume in treatment before, immediately after as well as 24 hours and 48 hours after exercise-induced muscle injury.

2.9.2 Stretching

Static stretching, pre- or post-exercise has been recommended to prevent DOMS as it is thought to relieve the muscle spasm as described in the muscle spasm theory. However, studies that have investigated the effect of stretching before, after or before and after eccentric exercise show no protective or limiting effects on the development of DOMS (Cheung *et al*., 2003). Contrary to the belief that stretching could reduce DOMS, research by Smith *et al*. (1993) showed significant amounts of DOMS and creatine kinase increases for up to five days after a static or ballistic stretching regime.

2.9.3 Electrical Current Techniques

Small electrical currents are used to accelerate healing of wounds and fractures. There is limited information available on the effectiveness of micro-current therapy on DOMS. A double blind, placebo-controlled study by Lambert *et al*. (2002) showed that electro-membrane current therapy reduced the signs and symptoms of DOMS. The treatment reduced muscle shortening, helped to maintain maximal force production and reduced CK activity in the blood. The mechanism of action is unknown but is likely due to the maintenance of intracellular Ca^{2+} homeostasis after damaging exercise.

2.9.4 Hyperbaric Oxygen Therapy

Hyperbaric oxygen therapy (HBO) inspiration of 100% oxygen combined with two to three atmospheres of absolute pressure (ATA), has been used as an effective tool in the treatment of crush, burn and trauma injuries, skeletal muscle compartment syndromes and treatment of soft tissue athletic injuries (Harrison *et al*., 2001). Appropriate HBO exposure results in an increase in the oxygen content of arterial blood (PaO_2). The increase in PaO_2 leads to an increase in the O_2 gradient between the blood and tissue and the secondary effect may be a reduction in the inflammatory response due to a PO_2-mediated vasoconstriction.

Research by Mekjavic *et al*. (2002) and Harrison *et al*. (2001) showed no benefit of treating DOMS with HBO in limiting oedema, in the recovery of isometric muscle strength, in lowering perception of muscle soreness or in lowering CK activity. HBO treatment may be more effective in treating injuries that involve more severe tissue damage or for injuries in which oxygen availability may be more of a limiting factor due to magnitude of oedema. Manipulating the number of treatments within the first 24-hour period may prove to be more beneficial in treating DOMS with HBO. The risk of oxygen toxicity must be considered before altering the treatment frequency (Harrison *et al*., 2001).

2.9.5 Vitamin C and E Supplementation

Vitamin C and E has been proposed to limit exercise-induced muscle damage, which results in DOMS through inhibition of free radical formation (Vitamin C & E) and through stabilisation of the cell membrane (Vitamin E) (Peters *et al*., 2001; Beaton *et al*, 2002). The support for a protective role of Vitamin C and E after exercise-induced muscle damage is however inconsistent.

Beaton *et al.* (2002) concluded in a study that Vitamin E supplementation for 30 days at 1200 $IU.d^{-1}$ had no effect in reducing post-exercise torque deficits, Z-band streaming and neutrophils activation. The Vitamin E-treated group did however show attenuated CK release at three days

post-exercise, which could indirectly indicate a reduction in membrane damage. Peters *et al*. (2001) observed a Vitamin C-associated attenuation of the cortisol response in 15 ultra-marathon runners immediately after a 90 kilometre ultra-marathon. Cortisol is known to increase as a result of exercise-induced damage. Vitamin C could therefore be effective in reducing the long-term immuno suppressive response associated with DOMS by limiting cortisol increases after muscle damaging exercise.

2.9.6 Exercise

Exercise is one of the most effective strategies for alleviating DOMS (Cheung *et al*., 2003). After a repeated bout of the same exercise, performed one to 10 weeks later, the recovery of the strength and range of motion is significantly faster than that found after the first bout, soreness development is less, and muscle protein increases in the blood are greatly reduced (Nosaka & Clarkson, 1995).

2.9.7 Massage

It has been suggested that an increased blood flow during vigorous massage hinders the margination of neutrophils and reduces subsequent prostaglandin production, and in so doing, any further damage associated with inflammation (Cheung *et al*., 2003). A study by Hilbert *et al*. (2003) however did not find an alteration in circulating neutrophils when massage was administered two hours after exercised induced injury. Muscle function in the study of Hilbert *et al*. (2003) did not improve muscle function, soreness and mood in treated subjects when compared to the control group. Inconsistent findings on the effectiveness of massage on DOMS is problematic because many studies differ in the massage technique, duration of the massage and the timing of the massage (Cheung *et al*., 2003; Robertson *et al*., 2004).

2.10 Overtraining

2.10.1 The Effect of Overtraining on Exercise Performance

In endurance sport there is often a small difference between optimal improvement in performance and maladaptation that can lead to deterioration in performance (O'Toole, 1998). The maladaptations are often the result of an imbalance between stimulus – the combination of

exercise intensity, volume and recovery. Unfortunately little scientific data exist about the optimal training volume and intensity for peak performance (Kuipers, 1996).

The generally used training model is based on the idea that physical exercise leads to a disturbance in cellular homeostasis. The exercise-induced changes are the stimulus for initiating physiological responses to restore homeostasis (Kuipers, 1998). It is however important to note that the recovery process does not stop when homeostasis is restored, but will continue until a small overcompensation is attained and performance improves (Krüger, 1995; Kuipers, 1998).

The prevalence of overtraining has been reported in various sporting codes. A study of top endurance athletes found that 64% of the women and 66% of the men have been overtrained at least once during their running careers (Morgan *et al*., 1987). Hooper *et al*. (1995) studied 19 swimmers during six months preparation for national team selection. Five swimmers were unable to complete the six-month training period, predominantly because of viral infections. At the end of the six months three of the 14 (21%) remaining swimmers were classified as stale. Overtraining has also been seen in 33% of Indian national-level basketball players and in more than 50% of semiprofessional soccer players after a five-month competitive season (Kellmann, 2002).

2.10.2 Overtraining Defined

Most researchers agree that the general definition of overtraining by Lehmann *et al*. (1997) is one of the most accurate descriptions of overtraining (Kellmann, 2002). According to Lehmann *et al*. (1997) overtraining is the result of an imbalance between stress and recovery, stress that is too much combined with too little regeneration. It is however important to note that stress also includes some non-training and competition factors, for example social, educational, occupational, economical, nutritional and travel factors, time stress and the monotony of training (Lehmann *et al*., 1997).

A clear description of overtraining is made difficult because some authors clearly differentiate between overtraining and staleness, where others do not differentiate or equate overtraining and staleness with burnout. An additional problem exists due to the fact that scientists use different

terms to explain the overtraining phenomena. In North America scientists use the term ***staleness*** while in Europe the term ***overtraining*** is used (Krüger, 1995; Kellmann, 2002). Krüger (1995) and Kellmann (2002) differentiate between overtraining as a process and staleness as the undesirable consequence of overtraining.

Lehmann *et al*. (1997) developed a terminology that integrates the two main approaches in defining overtraining. The first part of the combined approach is called short-term overtraining or overreaching. Overreaching is a common part of athlete training which is characterized by under performance, which is reversible within a recovery period of one to two weeks. The time following the recovery period is characterized by super compensation.

When short-term overtraining or overreaching is too profound or continues for too long a period, and there are too many competitions and non-training stress factors, then short-term overtraining turns into long-term overtraining. The overtraining syndrome or staleness will be the result if long-term overtraining continues (Krüger, 1995; Kellmann, 2002).

2.10.3 Overtraining Syndrome Theories

There are currently no conclusive indicators to prevent or identify overtraining. An overview of the various theories on overtraining does however give a very good perspective on the diverse contributing factors to overtraining.

2.10.3.1 Autonomic Imbalance Theory

Lehmann *et al*. (1998a) reports an approximately 60-80% higher pituitary corticotropin-releasing hormone (CRH) stimulated adrenocorticotropic hormone (ACTH) response in the early stage of the overtraining syndrome. The higher ACTH response could however no longer prevent a significant reduction in adrenal cortisol response when compared to baseline. The condition still existed after two weeks of incomplete recovery and the decreased adrenal cortisol responsiveness was no longer completely compensated by increased pituitary ACTH response (Lehmann *et al*, 1998a). The response of the ACTH is contrary to the primary response of ACTH on the adrenal cortex, which is to increase cortisol secretion by increasing its synthesis. A decrease exercise-

related maximum cortisol level was also observed in overtrained distance runners compared to the baseline.

A decreased cortisol release in overtraining athletes to insulin-induced hypoglycemia indicates that adrenal sensitivity to ACTH can decrease in overtrained athletes (Uusitalo, 2001). The decreased cortisol release is paralleled by a significantly decreased pituitary ACTH response to hypoglycemia (Lehmann *et al*., 1998b). An additional decreased hypothalamic and/or pituitary responsiveness, in addition to reduced adrenal responsiveness to ACTH, also occurs. A decreased pituitary release of growth hormone has also been documented. The above-mentioned pattern could be characteristic of an advanced stage in the overtraining process (Lehmann *et al*., 1998a).

A study by Duclos *et al*. (2003) provides evidence that the sensitivity of monocytes to cortisol is reduced when individuals are submitted to repeated stimulation of the hypothalamic pituitary adrenal (HPA) axis, due to endurance training. The restrained inflammatory response may, on the one hand, decrease exercise-induced muscle damage or muscle inflammatory reactions, while on the other hand it may lead to increased susceptibility to bacterial and viral infections immediately after post-exercise recovery.

In addition to reduced adrenal sensitivity to ACTH, Hooper *et al*. (1993; 1995) observed increased sub-maximal plasma norepinephrine levels at rest and at sub-maximal workloads, in overtrained elite swimmers. Increased resting plasma norepinephrine levels in overtrained athletes are accompanied by decreased basal catecholmine excretion (Hooper *et al*., 1993; Lehmann *et al*., 1992, 1998b).

The elevated sub-maximal plasma epinephrine stress response is accompanied by lower heart rate, blood glucose, lactate and free fatty acid response, which indicate a reduced sensitivity of the organism to catecholamines. The elevated plasma norepinephrine stress-response can possibly be interpreted as an attempt to compensate for the reduced catecholamine sensitivity (Lehmann *et al*., 1997). Increased plasma norepinephrine could be a response that indicates the attempt to overcome overtraining-related peripheral or muscular fatigue. The increased plasma

norepinephrine stress-response can be a consequence of decreased β-adrenoreceptor density on blood cells. The decrease in β-adrenoreceptor density seems to be a protective mechanism of the target organs against overload–dependent irreversible cellular damage (Lehmann *et al*., 1998b). The complete pattern of decreased intrinsic sympathetic activity, decreased β-adrenoreceptor density, decreased β-adrenoreceptor mediated effects, and increased norepinephrine levels occur only after prolonged periods of daily training of two to three hours but not at a training load of less than one hour per day (Lehmann *et al*., 1998a).

2.10.3.2 Glutamine Theory

Evidence suggests that a single bout of exercise can decrease the ability of cells of the immune system to respond to mitogenic stimulation (Parry-Billings *et al*., 1992; Lagranha *et al*., 2004). Glutamine is the most abundant amino acid in the body and is utilized at a very high rate by cells of the immune system and is considered to be an important fuel for these cells (Field *et al*., 2000).

Glutamine is also the most important nitrogen carrier that is required as a nitrogen donor for the synthesis of purine and pyrimidine nucleotides and is therefore essential for protein synthesis and cell proliferation (Parry Billings *et al*., 1992; Rodhe *et al*., 1998).

Skeletal muscle is the major tissue involved in glutamine production and is known to release glutamine into the blood stream at high rate; it has been suggested that the skeletal muscles play a vital role in the maintenance of the key process rate of glutamine utilization in the immune cells (Rodhe *et al*., 1998; Field *et al*., 2000; Hiscock & Pederson, 2002). The activity of the skeletal muscles may directly influence the immune system. The hypothesis exists that during intense physical exercise the demands on muscle and other organs for glutamine are so high that the immune system may suffer from a lack of glutamine that temporarily affects its function (Parry-Billings *et al*., 1992; Hiscock & Pederson, 2002). Parry-Billings *et al*. (1992) reported marginal lower plasma glutamine concentrations of nine percent in athletes showing symptoms of overtraining syndrome compared with well-trained athletes.

The apparent requirement of lymphocytes for glutamine suggests that low plasma glutamine levels associated with overtraining may compromise lymphocyte function and possibly contribute

to an increased risk of infections in competitive athletes (Parry-Billings *et al*., 1992). Recent studies however report normal proliferative responses to mitogenic challenge in overtrained athletes despite lower glutamine concentrations (Field *et al*., 2000; Hiscock & Pederson, 2002). A possible weak point in the glutamine hypothesis is that when lymphocytes are cultured in a glutamine concentration identical to the lowest plasma glutamine concentration, measured post-exercise (300 – 400 μM), these cells will function equally well as when glutamine is added at a concentration identical to that of the resting level (600 μM). It is hypothesized that a low glutamine concentration does not suggest overtraining but could indicate that the volume of training has exceeded an athlete's capacity to tolerate work (Smith & Norris, 2000).

2.10.3.3 Glycogen Depletion Theory

Muscle glycogen is an important energy source for endurance performance, and there is a close relationship between muscle glycogen depletion and fatigue (Sherman *et al*., 1998). Low muscle glycogen levels can impair exercise performance at intensities primarily between 65% and 85% of maximal oxygen uptake, the exercise intensity at which most endurance athletes train (Snyder *et al*., 1995). Costill *et al*. (1988) have noted that low dietary intake and resting muscle glycogen levels were associated with athletes unable to tolerate an increase in training. The men in the study conducted by Costill *et al*. (1988) did not show any losses in sprint or endurance performance tests and could therefore not be classified as overtrained.

In contrast to the lowered glycogen levels of swimmers who were not overtrained, Snyder (1998) found normal resting glycogen levels in competitive cyclists who were diagnosed as overtrained. Because overtraining appears to occur even when resting muscle glycogen levels are normal, other mechanisms except for muscle glycogen levels seem to play a role in overtraining (Snyder, 1998). Plasma norepinephrine concentrations and maximal heart rates were higher in subjects consuming a low carbohydrate diet compared to subjects consuming a high carbohydrate diet, which suggests that chronic training while consuming less than optimal amounts of carbohydrate over time may contribute to overtraining (Sherman *et al*., 1998).

2.10.3.4 Amino Acid Imbalance / Central Fatigue Theory

Amino acids are the foundation of protein in the body and are essential for the synthesis of tissue, specific proteins, hormones, enzymes and neurotransmitters (Kreider, 1998; Little & Volpe, 2002). Exercise induced changes in specific amino acids have been associated with chronic fatigue, overtraining and immunosuppression (Kreider, 1998; Halson & Jones, 2002). During prolonged exercise, the oxidation of the branched chain amino (BCAA) acids leucine, isoleucine, valine and glutamine is increased and contributes to the total energy expenditure (Calders *et al*., 1999). The oxidation of BCAA and glutamine in the muscle could exceed the catabolic capacity to increase BCAA and glutamine availability, plasma BCAA and glutamine levels may therefore decrease during prolonged endurance training.

A decrease in carbohydrates during prolonged exercise or initiating exercise with low glycogen availability increases the utilization of free fatty acids (FFA) and BCAA as metabolic fuels (Kreider, 1998). The levels of FFA in blood correlates with the concentration of free tryptophan (fTryp). FTryp and BCAA compete for entry into the brain through the same amino acid carrier. A decrease in BCAA levels during exercise or an increase in the level of fTryp increases the ratio of fTryp to BCAA, and increases the entry of tryptophan in the brain (Parry-Billings, 1990; Kreider *et al*, 1993).

Increased concentrations of tryptophan in the brain promote the formation of the neurotransmitter 5-hydroxytryptamine (5-HT) or serotonin (Kreider, 1998; Uusitalo, 2001). Exercise-related increases in 5-HT have been linked to tiredness, psychological perception of fatigue, decreased muscle power output and altered hormonal regulation during exercise (Parry-Billings *et al*., 1990; Kreider, 1998). A decrease in 5-HT in some part of the brain could play a role in central fatigue (Parry-Billings *et al*., 1990, 1992). Chronically elevated 5-HT concentrations may explain some of the signs and symptoms of the overtraining syndrome including postural hypotension, anemia, immunosupression, weight loss, depression and decreased performance capacity.

2.10.3.5 Cytokine Theory of Overtraining

The cytokine hypothesis of overtraining proposes that muscular, skeletal and/or joint systems could be the initiator of the overtraining syndrome. Extensive muscle damage has been reported

in biopsies of overtrained athletes and serves as direct evidence to the cytokine hypothesis (Kibler & Chandler, 1998; Smith, 2003a).

Neutrophils and monocytes are regarded as the primary cells in the inflammatory response, however coordination of these cells and amplification of various aspects of inflammation are accomplished by molecules collectively known as cytokines. Cytokines are soluble hormone-like proteins. Cytokines are produced by a variety of cells such as immune cells, endothelial cells and fat-storing cells (Smith, 2003a). The cytokines central to the proposed theory of overtraining are the pro-inflammatory interleukin-1β (1L-1β), tumor necrosis factor (TNF)-α and the anti-inflammatory interleukin –IL6 and interleukin-1 receptor antagonist (IL-Ira) (Tidball, 1995; Pederson & Toft, 2000; Smith, 2000).

Changes in psycho-behavior are a well-documented finding in overtrained athletes. Evidence demonstrates a relationship between systemic cytokine and psychological depression. Depressed individuals also seem to exhibit a systemic inflammatory-like condition, which includes elevated serum cytokines. Systemic infection or inflammation may also lead to alterations in cognition that have been observed in overtrained individuals (Smith, 2000).

Smith (2000), in her discussion of the cytokine theory of overtraining, discusses the link between systemic inflammation and current overtraining theories:

2.10.3.5.1 Cytokine Theory - Glutamine

Glutamine is needed as a fuel for the activation of lymphocytes and macrophages during inflammation (Pederson & Toft, 2000). Systemic inflammation is associated with a catabolic state in which glutamine is needed for gluconeogenesis and to maintain blood glucose levels. During systemic inflammation, synthesis of inflammatory-related acute-phase proteins by the liver, such as C-reactive and heptoglobin, are crucial to contain inflammation and glutamine is a primary precursor for many of these protein molecules. The cytokines IL-6 and TNF-α work with glucocorticoids to stimulate amino acid (glutamine and alanine) uptake in human hepatocytes. The increased need for amino acids during a catabolic state accelerates muscle

protein degradation, which contributes to a negative nitrogen balance and lean muscle mass losses (Smith, 2000).

2.10.3.5.2 Cytokine Theory – Glycogen Depletion

Even though muscle glycogen seemingly isn't the main reason for overtraining, it is frequently observed in overtrained athletes. Smith (2000) suggests the large volumes of training, systemic inflammation and elevated levels of pro-inflammatory cytokines directly and/or indirectly, induce anorexia, resulting in a reduced calorie intake. A decreased movement of glucose into cells for glycogen re-synthesis has been seen secondary to eccentric exercise-induced muscle membrane injury and reduced availability of the glucose transporter protein, GLUT-4. In addition it is speculated that whole-body insulin resistance could contribute to reduced glycogen synthesis which is most likely mediated by TNF-α.

2.10.3.5.3 Cytokine Theory – Hypothalamic Related Hormones

During systemic inflammation pro-inflammatory cytokines IL-1 and IL-6 appear to interact with specific hypothalamic receptors, resulting in the release of corticotropin releasing hormone (CRH). CRH stimulates release of pituitary adrenocorticotropin releasing hormone (ACTH), with subsequent release of cortisol from the adrenal cortex (Smith, 2003a). IL-6 may also control the release of steroid hormones by direct action on adrenal cells. Elevated cytokines and systemic inflammation could therefore be the reason for elevated cortisol levels in the overtraining syndrome (Smith, 2003a).

It has also been shown that cytokines influence the hypothalamic-pituitary gonadal axis (HPG) by suppressing reproductive function via inhibition of the luteinizing-hormone-releasing hormone (LHRH) (Smith, 2000).

2.10.3.5.4 Cytokine Theory – Amino Acid Imbalance

Smith (2000) proposes that if serum tryptophan (TRY) is reduced in the overtraining syndrome (OTS) and OTS does reflect systemic inflammation, the low serum TRY levels could be due to reduced availability of the TRY transporter, serum albumin which is reduced during systemic inflammation. The low serum TRY levels could also be due to increased TRY usage for

leukocyte activity and synthesis of lower proteins and increased degradation. Lowered TRY and branched chain amino acids levels are related to systemic inflammatory events, which are evident in clinical depression. Depression has also been diagnosed in overtrained athletes (Smith, 2000; Gould & Dieffenbach, 2002).

Studies on overreached athletes (Halson *et al*., 2003) and on overtrained athletes (Parry- Billings et al., 1992) found unchanged systemic levels of IL – 1,IL – 6 and TNF-α and did not fully support the cytokine theory of overtraining. The overreached study was on cyclists and the lack of exercise-induced muscle injury could have accounted for the low levels of IL-6 and TNF-α.

CHAPTER 3

METHODOLOGY

3.1 Subjects

Subject inclusion criteria

Actively competing endurance runners were recruited from clubs, on the grounds of their best 42.2 km running performance (completing a marathon in three to four hours) in the year 2002/2003.

Subject exclusion criteria and discontinuation criteria

- Failure to comply with testing procedures.
- Applying/consuming any treatment not approved by the researcher.
- Injury.
- In event of illness (e.g. the flu) the decision lies with the subject whether or not he wants to continue.
- Making drastic changes in training programmes.
- Subjects that were ill within seven days prior to the start of the trial was also excluded.

Expected duration of subject participation

13 days

The total number of 50 subjects was split into an experimental (26 subjects) and a control group (24 subjects).

3.2 Test Protocol

3.2.1 Medical Screening

On the first day that subjects reported for testing, full medical screening was done on each subject to ensure that he was healthy and had no undetected medical conditions. The screening included a full medical history and basic evaluations e.g. blood pressure and heart rate.

3.2.2 Familiarization Trial

Subjects were familiarized prior to the start of the trial with a short incremental run on the treadmill while wearing the facial mask that was worn during the $VO_{2\ max}$ test.

3.2.3 Specific Trial Procedures

Day 0: $VO_{2\ max}$ testing

The subjects reported to the laboratory well rested, having refrained from strenuous exercise for 24 hours, and having fasted for a period of at least three hours. Subjects performed a continuous incremental test for maximal aerobic capacity ($VO_{2\ max}$) on a motorized treadmill. The starting workload was set at eight km/h at a one-degree gradient and the treadmill speed was increased by two km/h every three minutes until exhaustion. Peak treadmill running speed (PTRS) was determined. The greatest treadmill speed that the subject could maintain for three minutes was defined as the peak treadmill-running speed.

Cardio-respiratory measurements were recorded by means of the Schiller CS-200 combined VO_2/ECG exercise system. A mask covering the mouth and nose was constantly worn during the test and subjects expired through a bi-directional differential pressure pre-vent pneumotach. Expired air was collected by means of a sample line and analyzed for fractions of O_2 and CO_2. The gas samples were taken at the end of each 20-second exercise period using a patented gas drying sample circuit with a warm-up time of 30 minutes from a cold start. Heart rate response was measured throughout the test with a 12 lead electrocardiograph and the test was terminated

when the subject could no longer maintain the treadmill speed, there was no further increase in the heart rate, or the respiratory exchange ratio (RER) values exceeded 1.15.

Expiratory gas volumes collected by the Shiller CS-200 spirometer were analysed for $VO_{2\ max}$ and RER. After completion of the $VO_{2\ max}$ tests, subjects were paired according to PTRS and in a double-blind fashion assigned to either an active or placebo group.

The following variables were measured during the treadmill run **:** $VO_{2\ max}$, maximal lactate, peak treadmill running speed (PTRS), maximal rating of perceived exertion (RPE Max) (using the twenty point original Borg scale), maximal rating of perceived pain (RPP Max) (as determined by a ten point pain scale (0-no pain, 10-maximal pain)), maximal heart rate (HR max), rating of perceived exertion at 75% (RPE 75%), rating of perceived pain at 75% (RPP 75%) and heart rate at 75% (HR 75%).

Day 1:

Subjects were paired according to PTRS and in a double-blind fashion and collected the Traumeel S or placebo from the laboratory and started the treatment protocol.

Day 2 – 7:

Subjects followed their normal training program with no additional training allowed.

Day 8 – 12: (Day 0 –Day 4 Blood Tests)

Day 8: (Day 0 – Blood Test)

Subjects reported to the laboratory well rested having fasted for at least three hours. Blood samples for the measurement of blood lactate, serum creatine kinase activity, serum cortisol concentration and a differential white blood cell count were taken between 05h00 and 06h00. Thereafter muscle damage was induced *via* downhill running on the treadmill at a 10° (degree) gradient. Subjects ran at 75% of PTRS for 45 min.

The following variables were measurements at different time intervals (zero minutes to 45 minutes) on day eight: rating of perceived exertion (RPE), rating of perceived pain (RPP) and HR at zero to 45 minutes. The measurements were taken at three-minute intervals.

Day 9: (Day 1 – Blood Test)

Subjects reported to the laboratory exactly 24 hours after muscle damage was induced for the collection of blood samples.

The following variables were measured on day eight to twelve: serum creatine kinase (SCK), blood lactate (LA), Cortisol, Neutrophils Relative, Neutrophils Absolute, Lymphocytes Relative, Lymphocytes Absolute, Monocytes Relative, Monocytes Absolute, Eusinophil Relative, Eusinophil Absolute, Basophils Relative, Basophils Absolute.

Day 10: (Day 2 – Blood Test)

Subjects reported to the laboratory exactly 48 hours after muscle damage was induced for the collection of blood samples.

Day 11: (Day 3 – Blood Test)

Subjects reported to the laboratory for the collection of blood samples.

Day 12: (Day 4 – Blood Test)

Subjects reported to the laboratory for the collection of blood samples.

3.2.4 Measures To Minimize/Avoid Bias

Both the researchers and subjects were blind as to which group were the active group and which the placebo group. The supplier appointed which group would receive which treatment in a randomized fashion. The research was thus a double-blind, placebo controlled study.

3.2.5 Treatment of Subjects

The dosage and dosage regimen of the investigational products:

Subjects were required to apply Traumeel S cream to their quadriceps, hamstrings and calves before training and to put the Traumeel S pill under their tongue three times daily.

Treatment period: 12 days

3.2.6 Medication(s)/Treatment(s) Permitted and Not Permitted Before and/or During the Trial

Subjects were permitted to continue taking any vitamin/mineral supplements that were taken prior to the start of the trial, but were not allowed to take any new supplements during the trial.

3.3 Blood Sample Analysis

Blood samples were collected from the antecubital vein between 5h00 and 6h00 to limit cortisol diurnal variations. Samples were collected in tubes containing sodium fluoride and potassium. The plasma was separated and frozen at -20°C until analysis. Lactate Reagent, in conjunction with SYNCHRON System MULTI^{tm} Calibrator, and Creatine Kinase Reagent were used for the quantitative determination of lactate and creatine kinase concentrations in the plasma on the SYNCHRON LX20 Clinical System (Beckham Coulter, Inc.Fullerton, CA).

The plasma white blood cell differential count was determined by the Advia 120 Hematological Analyzer, which makes use of the peroxidase method (Bayer, Tarrytown, New York). Cortisol was determined by making use of the competitive immunoassay procedure on the IMMULITE 2000 Cortisol Analyzer (Diagnostic Production Corporation, Los Angeles, CA).

The above-mentioned procedures are routine standard laboratory methods that were conducted by the Clinical Research Unit of the Department of Pathology at the University of Pretoria Medical School.

3.4 Data Analysis

The data was captured onto Excel and converted to SPSS in order to do the analysis. The data analysis had the following aims:

- to determine whether significant differences existed between the two groups on all variables measured; and

- to determine whether there were significant changes in the measurements taken at different time intervals and days within the same group.

Since the sample was relatively small, non-parametric statistics were used to analyze the data. Non-parametric tests, also known as distribution-free tests, are a class of tests that does not rely on a parameter estimation and/or distribution assumptions. The major advantage of these tests is that they do not rely on any seriously restrictive assumptions concerning the shape of the sampled populations and thus accommodates small samples as in the case of this study.

3.4.1 The Following Statistical Data Analysis Procedures Were Used:

3.4.1.1 Descriptive Statistics

Descriptive statistics are primarily aimed at describing the data. The mean, standard deviation, minimum and maximum scores for each measurement per group were determined for reference purposes.

3.4.1.2 Inferential Statistics

Test hypotheses about differences in populations on the basis of measurements made on samples of subjects.

3.4.1.3 Two Independent Samples t-test

One of the most common uses of the t-test involves testing the difference between the means of two independent groups. In conducting any experiment with two independent groups, we would most likely find that the two sample means differed by some amount. The important question, however, is whether this difference is sufficiently large to justify the conclusion that the two samples were drawn from different populations (Howell, 1992). In this study the t-test for

independent groups will be used to determine whether there are any statistically significant differences between the mean scores of the experimental and control group on all measurements taken during the experiment. The aim is to determine whether the two groups were equivalent at the onset of the experiment. If so statistically significant differences at the post-test could give an indication of the effect of the intervention.

3.4.1.4 Matched-Samples t-test

Situations, in which we will use the matched-sample t-test, will have two sets of data for the same subjects (Howell, 1992). In this experiment each subject will have a pre- and post-test score. The analysis will be aimed at determining whether statistically significant differences existed between the pre- and post-test scores within the same group. In this way it can be determined whether the intervention led to changes within the experimental group that could not be detected in the control group.

All significant differences will be reported at the five-percent level of significance. All graphs reflect the mean ranks based on the statistical analysis used. To see the actual mean scores of all measurements per group please refer to the descriptive statistics section. It is important to note that statistically significant differences in scores do not necessarily reflect clinical significance.

CHAPTER 4

RESULTS

There were no statistically significant differences in age, stature and body mass between subjects in the experimental and control group.

Table 1. General characteristics of the Traumeel S and placebo subjects. Values are expressed as x ± SD

Variable	Experimental	Control

Age(years)	31.3 ± 10	34.8 ± 11.0
Stature(cm)	173 ± 10.1	173 ± 9.18
Mass(kg)	68.5 ± 12.8	70.1 ± 9.64

Table 2. Descriptive Statistics for Initial Measurements taken

Group		N	Minimum	Maximum	Mean	Std. Deviation
Experimental Group (Group A)	VO_2 Max ($ml.kg^{-1}.min^{-1}$)	26	35	56	44.61	6.249
	Lactate Max (mmol/l)	26	6	16	10.14	2.584
	Speed Max (km/h)	26	12	20	16.54	1.693
	RPE Max	26	16	20	19.04	1.334
	RPP Max	26	.0	10.0	1.542	2.5998
	HR Max (bpm)	26	163	203	185.88	11.035
	RPE (75%)	26	6	16	11.46	2.303
	RPP (75%)	26	.0	2.0	.167	.4341
	HR (75%)	26	134	185	162.50	12.247
	Valid N (listwise)	26				
Control Group (Group B)	VO_2 Max ($ml.kg^{-1}.min^{-1}$)	24	32	58	43.76	6.922
	Lactate Max (mmol/l)	24	5	19	11.25	3.342
	Speed Max (km/h)	24	12	19	15.90	1.832
	RPE Max	24	13	20	17.75	2.049
	RPP Max	24	.0	10.0	.600	2.2219
	HR Max (bpm)	24	165	204	181.25	11.729
	RPE (75%)	24	8	15	12.06	2.231
	RPP (75%)	24	.0	7.5	.390	1.6749
	HR (75%)	24	123	180	141.93	14.818
	Valid N (listwise)	24				

Only one statistically significant difference was found on the measurements taken at the onslaught of the experiment. There was a statistically significant difference between the experimental and control groups with regards to their RPE Max scores. The experimental group's score was significantly higher than the control group's.

4.1 Results of the Analysis of the Comparison of the Same Group Across Various Measurements at Different Time Intervals. This Analysis Was Repeated for Both Groups

The results of the analysis will be presented in figures 1 to 5.

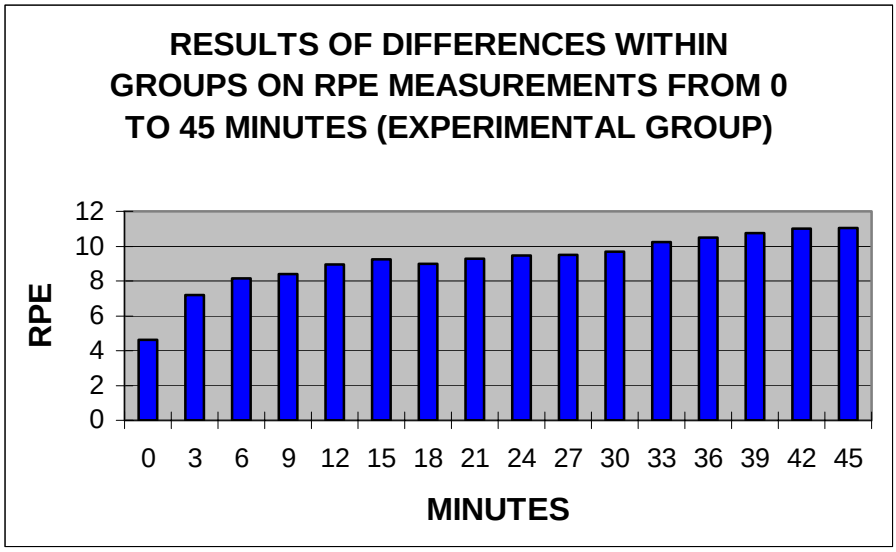

Figure 1: Results of differences within groups on RPE measurements from 0 to 45 minutes (experimental group)

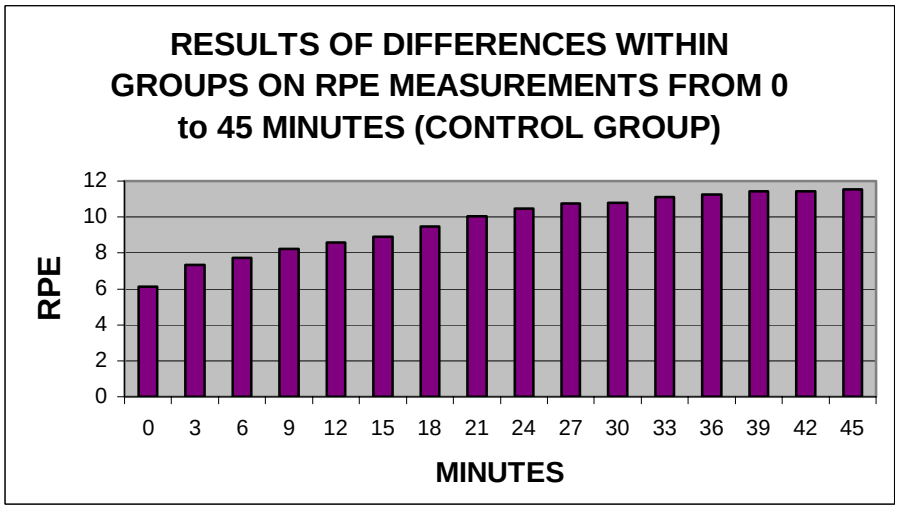

Figure 2: Results of differences within groups on RPE measurements from 0 to 45 minutes (control group)

The results in figures 1 and 2 indicate the following:

Statistically significant differences were found between the RPE measurements from zero to 45 minutes for the experimental group (see figure 1). There seems to be a steady increase in scores with the biggest increases occurring between zero and three minutes, three to six minutes and nine to 12 minutes.

The same tendency was found for the control group with statistically significant differences between measurements taken from zero to 45 minutes (see figure 2). The biggest increases occurred between zero to three minutes, three to six minutes and nine to 12 minutes.

The control group had a statistically significantly higher RPE score at zero minutes than the experimental group. However, in the consecutive measurements from three to 45 minutes no statistically significant differences were found.

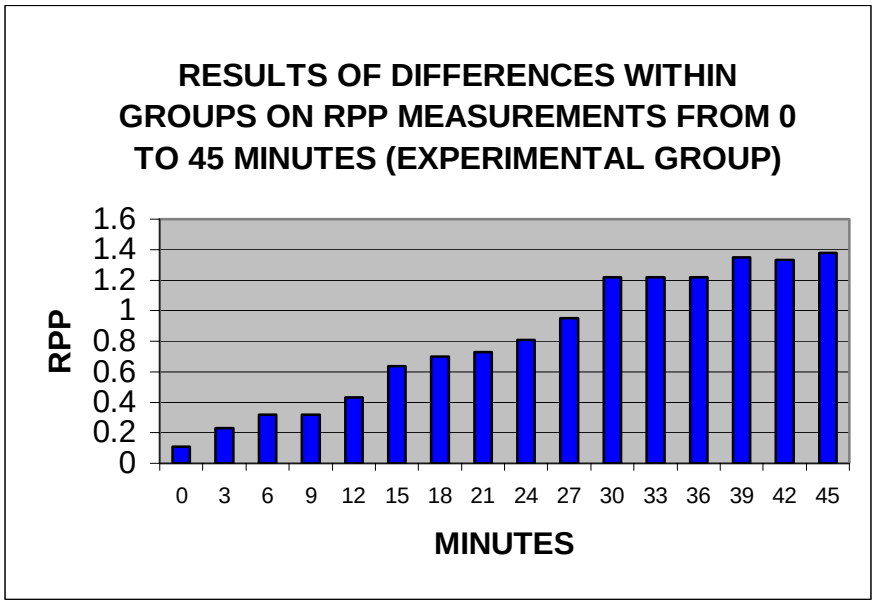

Figure 3: Results of differences within groups on RPP measurements from 0 to 45 minutes (experimental group)

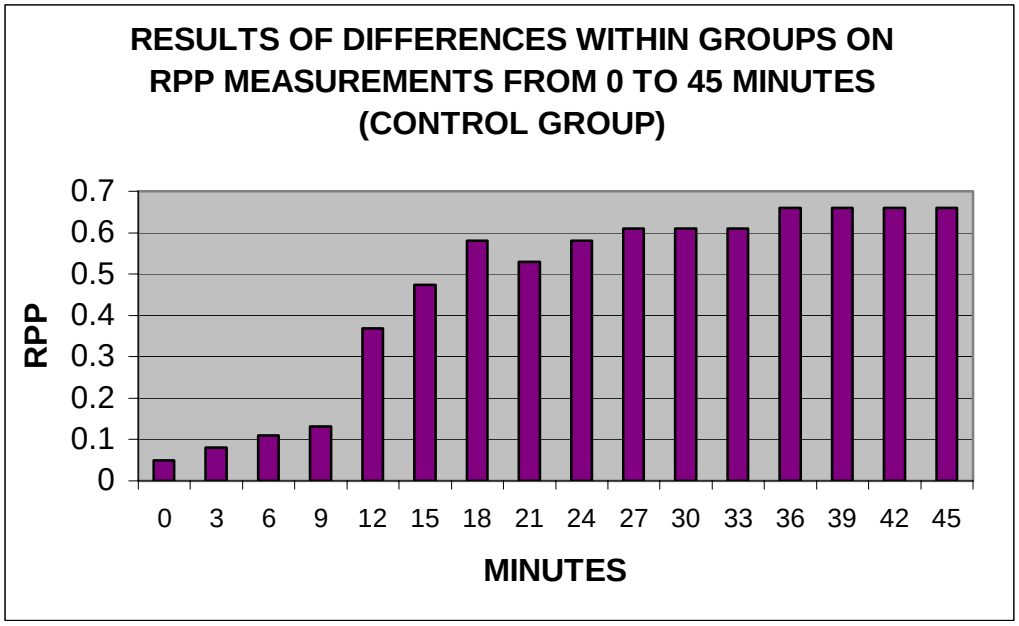

Figure 4: Results of differences within groups on RPP measurements from 0 to 45 minutes (control group)

The results in figures 3 and 4 indicate the following:

Statistically significant differences were found between the RPP measurements from zero to 45 minutes for the experimental group (see figure 3). There seems to be a steady increase in scores with the biggest increases occurring between 12 to 15 minutes, 15 to 18 minutes and 21 to 24 minutes.

The same tendency was found for the control group with statistically significant differences between measurements taken from zero to 45 minutes (see figure 4). The biggest increase occurred between nine to 12 minutes. There was even a slight decrease from 21 to 24 minutes and the measurements stabilized from 36 to 45 minutes.

No statistically significant differences were found between the experimental and control group on any of the RPP measurements.

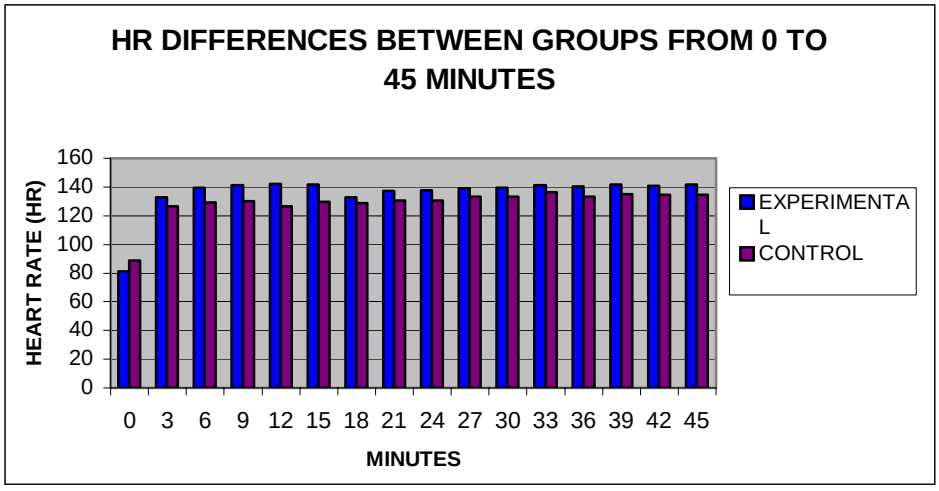

Figure 5: HR differences between groups from 0 to 45 minutes

The results in figures 5 indicate the following:

Statistically significant differences were found between the HR measurements from zero to 45 minutes for the experimental group (see figure 5). Even though the scores seem to fluctuate up and down at times there seems to be a steady increase in scores with the biggest increases occurring between zero to three minutes, three to six minutes and 24 to 27 minutes and 36 to 39 minutes. Decreases in HR scores were found between 15 to 18 minutes, 33 to 36 minutes and 39 to 42 minutes.

The same tendency was found for the control group with statistically significant differences between measurements taken from zero to 45 minutes (see figure 5). The biggest increases occurred between zero to three minutes, 12 to 15 minutes, 18 to 21 minutes and 30 to 33 minutes. Decreases in HR scores were found between three to six minutes, 15 to 18 minutes, 21 to 24 minutes and 33 to 36 minutes.

Statistically significant differences were found between the experimental and control group on the HR measurements at six, nine, 12 and 15 minutes respectively (see figure 2). In all cases the experimental group had significantly higher scores. This seemed to be the trend throughout this exercise, even though the differences at the other time intervals were not statistically significant.

At zero minutes the HR scores of the two groups were almost identical with a smaller difference at three minutes.

4.2 The Following Results Were Obtained for Intra Group and Inter Group Differences for the Days After Muscle Damage Was Induced

4.2.1 Creatine Kinase

Mean change in serum creatine kinase is shown in figure 6. Serum creatine kinase was raised significantly in both the experimental and control subjects 24 hours following the training intervention (experimental Pre 251 ± 171 u/l, cont 324 ± 439 u/l), one day post-treated 422 ± 424 u/l, cont 599 ± 448 u/l), however returned to near pre-training levels by 48 hours (experimental 288 ± 141 u/l, cont 363 ± 246 u/l) and continued to drop, all be it at a slower rate, through to day four (experimental 181 ± 77 u/l, cont 306 ± 166 u/l).

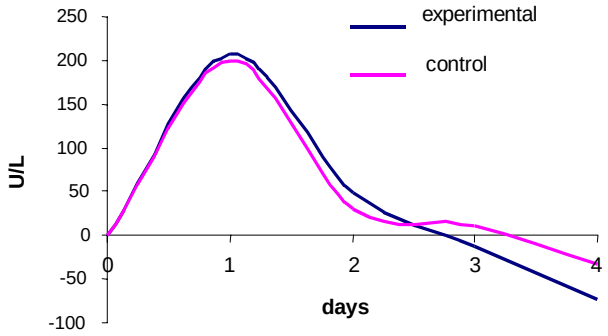

Figure 6: Mean change in serum creatine kinase following eccentric running

4.2.2 Cortisol

The means and standard deviation of the change in cortisol concentration between the experimental and control subjects is shown in table 3.

Table 3. Blood cortisol concentration in experimental and control subjects pre- and four days post-eccentric running

	Days after exercise.				
Experimental	**0**	**1**	**2**	**3**	**4**
Mean (nmol/l)	**485.62**	**518.33**	**590.26**	**541.50**	**549.57**

St Dev	**208.32**	**188.81**	**180.17**	**288.03**	**154.58**
Control					
Mean (nmol/l)	**505.65**	**461.65**	**529.47**	**449.20**	**440.20**
St Dev	**135.11**	**102.61**	**116.48**	**96.23**	**58.45**

Cortisol did not change significantly in control subjects throughout the observation period. Experimental subjects showed a significant rise in cortisol 48 hours after exercise when compared to the control group ($p < 0.001$) and remained significantly raised above the controls on day three ($p < 0.04$) and day four ($p < 0.03$) post-exercise.

4.2.3 Basophils

Mean basophil reaction to eccentric running is shown in figure 7. Basophils rose significantly in the experimental subjects from base levels on the first ($p<0.02$) and second day ($p<0.01$) following exercise, although it dropped again on day three, it remained significantly above the pre-exercise level on day four ($p<0.03$). Control subjects showed no significant change on any of the four days post-exercise.

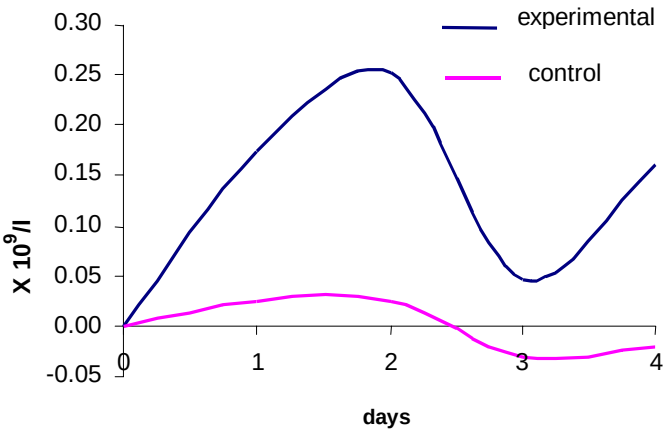

Figure 7: Mean basophil response for four days following eccentric down hill running

The experimental subjects basophil concentration was significantly above the control subjects on day two ($p<0.03$) but not on any other day.

4.2.4 Monocytes

The degree of change in monocytes is shown in figure 8. Mean monocytes concentration in control subjects was 6.73 + 1.34 pre-exercise and did not change significantly throughout the four days following eccentric running. Experimental subjects mean monocytes were 7.29 + 1.88 prior to the exercise intervention and fell significantly (6.57 + 1.68, $p> 0.02$) on the first day of recovery and remained significantly lower than the pre-exercise level through to the fourth day post-exercise.

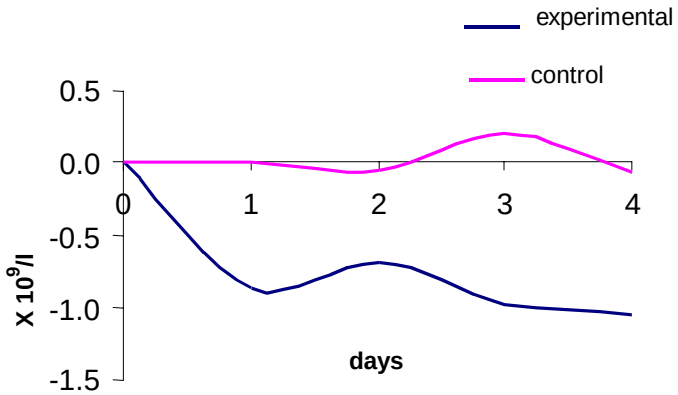

Figure 8. The degree of change in monocytes following eccentric running

4.2.5 Eosinophils

The variation in eosinophil response between treated and control subjects are shown in table 4.

Table 4. Mean and standard deviation of eosinophil response for experimental and control subjects pre- and 4 days post-eccentric running

	Days after exercise.				
experimental	0	1	2	3	4
Mean (nmol/l)	3.03	3.04	2.67	3.06	3.15
St Dev	1.70	1.90	1.50	2.13	2.14
Control					
Mean (nmol/l)	3.61	3.48	3.83	2.73	2.87
St Dev	1.83	1.82	1.77	1.61	2.18

Eosinophil concentration rose significantly above pre-exercise levels ($p < 0.02$) in control subjects two days post-exercise, but fell significantly on day three ($p<0.01$, but was not significantly below the pre-running level ($p < 0.3$). The mean of the experimental subjects fell significantly below the pre-exercise level two days after exercise ($p<0.02$) and was significantly below the control group on this day ($p<0.002$). The eosinophil level for the experimental group gradually rose on day three and day four but remained below the pre-exercise level.

4.2.6 Lymphocytes

Mean lymphocyte response in control and treated subjects is shown in figure 9. A mean upward pulsative effect appears in the treated subjects whereas a mean downward pulsative effect is noted in the control subjects. There was no intra significant difference between the pre- and following days in either experimental or control subjects, nor in the inter-group relationship.

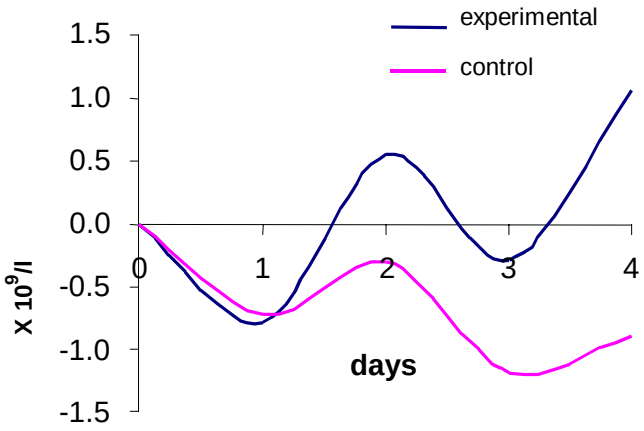

Figure 9: Mean daily variation in lymphocytes in experimental and control subjects following eccentric running

4.2.7 Leukocytes

Although mean leukocytes showed a small rise in treated subjects (fig 10) through day two to day four post-exercise, the control subjects showed a larger rise in leukocyte concentration on day four. Mean leukocyte rise in experimental subjects was significantly above pre-exercise levels on days three and four. Mean leukocytes of control subjects were only significantly above the pre-exercise level on day four.

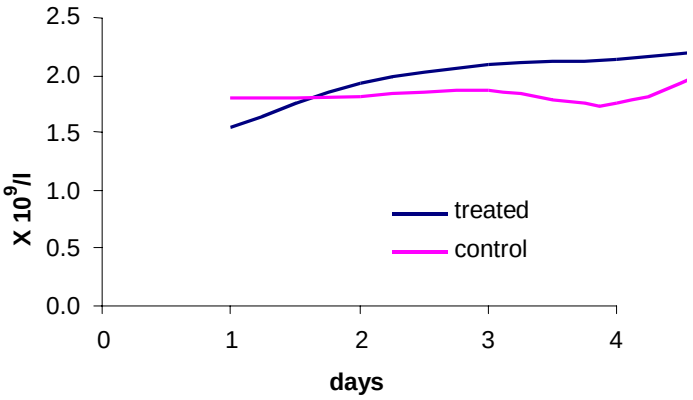

Figure 10: Mean leukocyte response

There was no significant difference between experimental or control group changes in the mean leukocyte values from the pre-exercise through the four days post-exercise ($p<0.05$).

CHAPTER 5

DISCUSSION

The aim of the study was to determine whether treatment with the homeopathic product Traumeel S could decrease the markers of muscle damage and the associated inflammatory response after exercise.

Researchers have used various exercise protocols to study the effect of eccentric exercise and the subsequent DOMS. Clarkson *et al*. (1992) used two sets of 35 maximal eccentric contractions of the forearm flexors every 15 seconds and five minutes rest between sets. The contractions were performed on a modified arm curl machine. Lee and Clarkson (2003) made use of a similar arm curl machine and the untrained subjects performed one set of 50 maximal eccentric contractions of the forearm flexors where each contraction lasted three seconds with 12 seconds of rest between contractions. A study by Dolezal *et al*. (2000) made use of a trained and an untrained group- each subject performed leg presses that emphasized the eccentric movement for eight sets at six repetition maximum. Sorichter *et al*. (1997) used four different exercise regimes in normal physical education teacher trainees, 20 minutes of level or downhill (16% decline) running at 70% $VO_{2\ max}$, 70 maximal eccentric contractions of the quadriceps femoris on a specially designed exercise rack and 40 maximal continuous concentric contractions of the quadriceps femoris on an isokinetic dynamometer at an angular velocity of 3.14 rad/s.

Downhill running has also been used by researchers to induce DOMS. Akimoto *et al*. (2002) used three different types of exercise in normal healthy athletes, bicycle ergometry exercise at 80% $VO_{2\ max}$ for 16 minutes, 42 km marathon running and 30 minutes 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% of level VO_{2max}. The protocol used by Pizza *et al*. (1995) is similar to the protocol used in the present research. The protocol in our study was a 45-minute downhill run at a 10% gradient at 75% of peak treadmill running speed (PTRS). Daniels (1998) indicates that an intensity of 70% $VO_{2\ max}$ is equal to 75% $vVO_{2\ max}$ (velocity at $VO_{2\ max}$). Peak treadmill running speed should differ very little if at all from $vVO_{2\ max}$, thus the intensity of 70% $VO_{2\ max}$ used by Pizza *et al*. (1995) should equal our PTRS of 75%.

5.1 Creatine Kinase (CK)

According to Clarkson *et al*. (1992), increase in CK activity in the blood tends to be substantially lower after downhill running (about 300 $\mu.l^{-1}$) compared with high-force eccentric exercise of the forearm flexors (about 2,500 $\mu.l^{-1}$). The changes in CK activity after isometric exercise are similar to those for downhill running. Contra-indications in the research as to which mode of exercise produces the greatest increases in CK activity could be a result of various factors. The research protocols differ relative to muscle mass, (Clarkson *et al*., 1992) intensity, duration and volume of exercise and whether the subjects are accustomed to eccentric exercise (Sorichter *et al*., 1997).

Marathon running produces significant increases in CK activity, but the increase is much more rapid than after various repetitions of high force eccentric actions (Clarkson *et al*., 1992). Research by Sorichter *et al*. (1997) showed, however, that CK plasma levels increase 20 minutes after downhill running and eccentric quadriceps contractions compared to level running and concentric quadriceps contractions. Research indicates that peak CK activity occurs at about 24 hours after downhill running.

Pre-exercise CK values in the current study were similar to those found in the study of Thompson et al. (2004) which were in the 200-300 μ/l range, but much higher than the resting values found in Pizza *et al*.'s (1995) study (100μ/l).

The mean peak CK response in the current study occurred after 24 hours and is consistent with data from other studies that found peak CK increases at the same time period following downhill

running (Clarkson *et al*., 1992; Close *et al*., 2004). The CK values of the control subjects in this study are similar to values found by Pizza *et al*. (1995) - ± 600 μ/l, while the peak values found for the treated group (422 μ/l) are lower than those found by Pizza *et al*. (1995). The CK values at 24 hours in the study conducted by Thompson *et al*. (2004) - ± 1000μ/l, were much higher than the CK values in our study. A possible explanation for the lower values in our study is the fact that our subjects were marathon runners who ran at a –10% gradient. The subjects in the research of Thompson *et al*. (2004) took part in a variety of activities but were unfamiliar with the eccentric exercise protocol and the subjects ran at a much steeper gradient of –18%.

The CK levels at 48 and 72 hours in the current study were consistent with the reported time course of CK response after downhill running (Akimoto *et al*., 2002; Close *et al*., 2004). The CK levels continued to decrease in the study and fell below the pre-exercise values three days post-exercise for the treated subjects and at four days post-exercise for the control subjects. The consistently lower CK activity post-exercise for the treated group compared to the control group indicates that Traumeel S could possibly limit CK response to eccentric exercise by limiting the release of CK by muscles or through increased clearance of CK by the reticuloendothelial system. Traumeel S seems to work by modulating the generation of reactive oxygen by activated neutrophils. Neutrophils have been implicated as a mediator of tissue damage and as a mechanism for the release of CK following eccentric exercise (Pizza *et al*., 1995).

5.2 Cortisol

The rise in serum creatine kinase indicates that the downhill running induced damage within the muscles in the treated and control subjects. Oxidative stress, secondary to neutrophil activation is presumably the trigger for the release of, amongst others, cortisol. Cortisol protects the body against inflammatory mediated tissue damage (Peters *et al*., 2001). The resting pre-exercise cortisol values for the study were similar but slightly higher than found by Pizza *et al*. (1995), and higher than the pre-marathon values found by Peters *et al*. (2001). The cortisol levels in the study by Pizza *et al*. (1995) decreased at 24 hours post exercise and continued to decrease even further at 48 hours post-exercise. The cortisol levels for the treated subjects in the current study were increased for 96 hours post-exercise relative to the pre-exercise values and were higher by ±

200 nmol/l at 24 hours and 48 hours post-exercise for the treated subjects compared to the studies of Pizza *et al*. (1995) and Peters *et al*. (2001).

According to Mackinnon (1999) cortisol is usually released only during rigorous exercise, especially in well-trained subjects and results in lower responses of circulating cortisol to the same amount of exercise. In the current study the endurance-trained treated subjects showed an increase in cortisol from the pre-exercise levels over the four days. Atlaoui *et al*. (2004) indicated that significantly elevated resting cortisol levels have been observed in overtrained runners with impaired performance whereas declines or increases in plasma cortisol have been reported in athletes with stable performances. The increased cortisol levels measured within the treated group can have an anabolic or catabolic effect. The anabolic effect involves the stimulation of gluconeogenesis and glycogen synthesis in the liver. Eccentric muscle contractions are known to decrease muscle glycogen levels and the increased cortisol concentrations could assist with the resynthesis of muscle glycogen stores. The catabolic effects of cortisol involve a decreased glucose uptake in peripheral tissue and increased protein degradation in skeletal muscle (Keizer, 1998).

Cortisol also has the ability to cause stabilization of the intracellular lysosomal membranes. Cortisol makes it much more difficult than normal for the membranes of the lysosomes to rupture. The result is that most of the proteolytic enzymes released by the damaged cells that cause inflammation and that are mainly formed in the lysosomes are released in greatly decreased quantities (Guyton, 1992). Increased proteolytic activity is associated with an increase in aseptic inflammation and activation of the immune system (Neumann *et al*., 2000). The lower, even though not significantly reduced, CK values in the treated group could mean that cortisol limited the initial reaction to mechanical muscle damage due to improved lysosomal phagocytic activity and thereby reduced secondary muscle damage because less monocytes were attracted to the injured site.

Cortisol has been proven to reduce the accumulation of leukocytes from the circulation into extra-vascular fluid space, to reduce the accumulation of monocytes and granulocytes at inflammatory cites, and to suppress the production of and/or actions of cytokines and inflammatory mediators

by immune cells. It also inhibits lymphocyte and leukocyte proliferation, migration and cytotoxicity. Because of these responses researchers indicated that stress and cortisol are immunosuppressive. Evidence exist however that stress and the related hormones do not suppress all aspects of immunity but changes immunocompetence and can exaggerate the response of specific components of immune/inflammatory reactions (Smith, 2003a).

The increased cortisol levels at rest in the treated group is therefore not a direct indication that Traumeel S supplementation will lead to immunosuppression because cortisol can suppress cellular-mediated immunity but boost humoral immunity. Upregulated humoral activity includes antibody activation, which is important for antigen recognition and antigen-antibody binding. Antigen-antibody binding is an important step in initiating the adaptive immune response. Antibodies bind to and neutralize bacterial toxins, inhibit nutrient uptake by bacteria and movement of bacteria, and inhibit bacterial access to host cells. Anti-bodies also play a vital role in host defense against viral infections (Mackinnon, 1999).

Cortisol tends to suppress cellular mediated immunity (CMI) and T-helper-1 cells (T_H1) by suppressing production of IL-12. The primary role of CMI is the elimination of intracellular pathogens such as viruses and bacteria. A decreased activation of CMI function can increase an organism susceptibility to infection (Smith, 2003a).

If the increased cortisol production in the treated group is deemed to be of a permanent nature, cytokine production can be influenced and an altered T_H1/T_H2 balance can develop. An increased PGE_2 secretion is however needed for the down regulation of T_H1 immunity and it is questionable whether that will be the situation in the current study because a major producer of PGE_2 monocytes were greatly reduced in the treated group.

5.3 Monocytes

The anti-inflammatory effects of cortisol have been shown to reduce the accumulation of monocytes at inflammatory sites. Neutrophils are the main leukocyte during the initial phase of

acute inflammation but are no longer active at 24 hours. Monocytes form the next line of defense in the immune system and are the predominant cell type at the injury site 24 to 48 hours after injury. When monocytes move from the blood into the tissue they are transformed into macrophages.

Circulating monocytes tend to increase in response to exercise-induced muscle injury for up to 48 hours after exercise (Malm *et al*., 1999; Peters *et al*., 2001). Subjects treated with Traumeel S showed a significant reduction in monocytes in the first 24 hours and remained significantly below those of the control subjects up to 96 hours post exercise. Monocytes produce a number of inflammatory mediators such as IL-1 and prostaglandin. IL-1 is pyrogenic (induces fever) and is involved in prostaglandin releasing proteolysis. IL-1 is one of the pro-inflammatory cytokines central to the development of overtraining (Halson *et al*., 2003). Prostaglandins are important for recruiting inflammatory cells toward injured tissue (Stovitz & Johnson, 2003) and have a pro-inflammatory action that could exacerbate the initial injury and/or create new damage (Lapointe *et al*., 2002a).

When ED_1+ cells, present in circulating monocytes, enter into the damage tissue, non-specific secondary damage can be induced. Lapointe *et al*. (2002a) indicated that decreased ED_1+ activity could result in improved preservation of a muscle's ability to produce isometric force during the first few days post-injury because inflammation is repressed. It should however be noted that a decrease in ED_1+ macrophages could result in slower removal of necrotic fibers, which could significantly impair and/or prolong the repair phase necessary for the adaptive response to eccentrically-induced muscle injury. The possibility exists that because Traumeel S reduced circulating monocyte values post-exercise, secondary muscle damage was reduced because ED_1+ macrophages and prostaglandins were possibly attenuated.

Echinacea purpurea is one of the constituents of Traumeel S and a study on triathletes indicated that the substance could be effective in treating and preventing acute upper respiratory tract infections. The proposed mechanism of action of echinae treatment seems to be due to an increased cytokine-synthesizing capacity of monocytes. The increased synthesizing of these immune cell chemical messengers enables the immune system to communicate the presence of

infection to the units of the immune system which react to infection (Gleeson, 2002). If the cytokine-synthesizing capacity of monocytes increase the possibility exists that fewer monocytes are recruited into the blood because the activated monocyte function improved.

The risk of long term (longer than seven days post-exercise) Traumeel S usage is that it could probably impair the repeated bout effect and the important subsequent adaptation to eccentric exercise as seen with NSAID usage. The reason for this is that certain monocytes e.g. ED_{2+} cells are needed for regeneration processes. It can not be concluded that lower monocyte levels early on, after muscle injury, will limit ED_{2+} cells because these cells appear late in the inflammation process when necrosis is nearly complete and muscles have begun to regenerate. Traumeel S should therefore possibly be used in a cyclic manner and possible only after intense exercise and not longer than seven days continuously to enable natural regeneration through ED_{2+} macrophages. Whether this is indeed the case with Traumeel S would have to be researched in other long term studies.

The reduction in circulating monocyte values in the treated group could on the other hand be due to the migration of monocytes to the inflamed area because monocytes are known to reduce infection in inflamed muscles and to clear up necrotic tissue. The possibility that monocytes in the treated group migrated to the inflamed area is small because CK in the treated group was consistently lower than the control group and the cortisol levels were higher for the treated group, which should have limited the initial inflammation in the treated group.

The control subjects only had slightly increased circulating monocyte values. This could indicate that the protocol used was ineffective to induce muscle injury because the subjects who participated in the study were actively participating marathon runners and the repeated bout effect associated with repetitive running could have limited the inflammatory response of the monocytes.

Chronic inflammation appears to be monocytic in nature (MacIntyre *et al*., 1995). Increased circulating monocyte activity could result in the inability to switch off local inflammatory signals leading to inappropriate survival of inflammatory signals within the inflamed area (Smith,

2003a). The continued inflammatory signals may therefore induce immunosuppression. Due to the decreased monocyte activity in the Traumeel S group the possibility exists that Traumeel S could limit immunosuppression in athletes.

The experimental design did not differentiate between the monocyte or macrophage sub-populations and did not include testing for serum prostaglandin levels. The discussion of the monocyte results can only be seen as possible events that led to decreased resting monocyte activity and the effect that the decreased monocyte levels could possibly have on the immune system. Immunologists do however indicate that lower monocyte activation can be beneficial in limiting production of vaso-active substances (e.g. leukotrines, prostaglandins, thromboxane A_2, lysosomal enzymes), which may result in less capillary permeability and outward shift of fluid and cells in response to exercise.

5.4 Eosinophils

Research on the role of eosinophils and basophils in exercise induced muscle injury is very limited. Diurnal variations of blood eosinophil count (lowest in the morning, highest at night) may be as much as 40% and is possibly related to reciprocal variations in cortisol levels (Bridgen, 1999). Eccentric exercise does not seem to alter the number of circulating eosinophils at 24 hours and 48 hours post-exercise (Malm *et al*., 1999). The result for the eosinophil concentrations in both the control and treated groups was inconsistent with research. The eosinophil levels in the control group increased significantly relative to pre-exercise levels, while in the treated group, eosinophil levels fell significantly below the pre-exercise levels two days after exercise and were also significantly below the control levels on this day.

The treated subjects had a significant rise in cortisol levels at 48 hours post exercise and this coincided with a significant decrease of the eosinophil value at 48 hours post exercise.

Eosinophils are moderately effective as a phagocyte of bacteria and eosinophils are believed to detoxify some inflammatory-induced substances and in so doing prevent the spreading of local inflammatory processes particularly within the lungs and the intestinal tract (Guyton, 1992; Bridgen, 1999). The lower eosinophil levels at the onset of the study and at 48 hours post-

exercise suggests that the potential of increased risk of infection within these two organs should be limited with Traumeel S supplementation.

5.5 Basophils

The possible impact of the lower eosinophil values on the immune system cannot be evaluated in isolation. Basophil concentrations increased significantly within the treated group 24 and 48 hours after exercise and were significantly raised above the control group 48 hours post exercise. Basophils play an important role in certain types of allergic reaction because the antibody immunoglobulin E (IgE) that caused allergic reaction has a special ability to become attached to basophils. When the antigen binds to a basophil the membrane of the cell changes and many of the basophils will rupture and release substances like histamine, eosinophil- and neutrophil-attracting substances and tissue protease. The decreased circulating eosinophil level at 48 hours, which coincided with the increased basophil level in the treated group, could have been due to the eosinophil chemotactic substance release which is mediated by basophils.

The significance of the eosinophil and basophil changes in both the control and treated subjects relative to exercise immunology is however unclear. If the basic function of these sub-populations of white blood cells is analyzed it is doubtful that the observed changes will have a major influence on the immune system.

5.6 Lymphocytes

Mackinnon (1999) indicates that exercise with an eccentric bias appears to induce greater mobilization of lymphocytes into the circulation during and efflux of cells after prolonged exercise. Observed significant increases in circulating lymphocytes occur mainly immediately post-exercise and lymphocyte counts return to or decrease slightly below pre-exercise values by 24 hours. After the initial 24 hours post-exercise the lymphocyte count stabilizes at pre-exercise levels or decreases slightly below the pre-exercise levels (Pizza *et al*., 1995; Close *et al*., 2004).

The values for the control and treated subjects were in contrast to the studies of Pizza *et al*. (1995) and Close *et al*. (2004). The lymphocyte count of the control subjects decreased 24 hours post-exercise and remained below the pre-exercise levels. The lymphocyte count for the treated subjects also decreased 24 hours post-exercise but were above, even though not significantly above, the pre-exercise lymphocyte count on days two and four. Peak lymphocyte increases occur during exercise and the possibility exists that significant changes to the lymphocyte count could have occurred during the exercise. The limit increases in lymphocyte count can possibly be attributed to the fact that actively competing marathon runners were used in the study and lymphocyte counts increases more in untrained subjects (Mackinnon, 1999). The fact that lymphocyte counts were mainly unaltered after exercise in this study cannot be accounted to increased cellular infiltration of lymphocytes to the injured area because lymphocytes continuously enter and exit the circulation (Pizza *et al*., 1995).

5.7 Leukocytes

The major types of leukocytes found in circulation are granulocytes (neutrophils, eosinophils and basophils), monocytes and lymphocytes. Leukocyte numbers increase greatly during downhill running and immediately post- and up to 12 hours post-exercise (Pizza *et al*., 1995; Close *et al*., 2004). Leukocytocis are mainly due to neutrophil and lymphocyte increases. Observed leukocyte variables usually return to pre-exercise levels by 24 hours (Close *et al*., 2004). The significant increases in leukocytes, as seen in the treated group on days three and four and in the control group on day four, are therefore inconsistent with the majority of the research.

The significant increases in leukocytes on days two, three and four is most likely due to increased neutrophil activation because neutrophils make up ± 60% of leukocytes. Monocytes were significantly reduced on days three and four and the lymphocytes showed no significant increases on these days. Basophils and eosinophils only represent a small segment of leukocytes and the changes in these leukocytes should not lead to leukocytosis.

Recruitment of neutrophils into circulation is associated with cortisol release during exercise, which is prolonged (< 30 minutes). It is debatable whether cortisol influenced the neutrophil and leukocyte count in this study because increased cortisol levels were found on days two, three and

four while there was only an increase in leukocyte values in the treated group in days three and four. The increased leukocyte count in the control group on the fourth day can almost certainly not be associated with cortisol because cortisol levels was at their lowest values during the four days of testing on the day. Correlation coefficient statistics will be needed to confirm or refute the role of cortisol on the leukocyte count. The unavailability of the neutrophil count also limits the explanations of the possible events leading to leukocytocis in either the treated or control groups.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The results of the research indicate that supplementation with Traumeel S tablets and the applying of Traumeel S cream to the lower limbs increases the production of cortisol at 48, 72 and 98 hours after eccentric exercise compared to the control group. Traumeel S also significantly decreases circulating monocyte counts for up to 96 hours post-exercise.

Basophils increased significantly 24 and especially 48 hours after exercise within the Traumeel S group and eosinophils decreased significantly compared to the control group 48 hours after exercise. The implications of the basophil and eosinophil changes relating to delayed onset of muscle soreness is however unclear and the changes in cortisol and monocytes should have a much greater impact on the inflammation found in muscle tissue after exercise induced injury.

Overtrained endurance athletes may develop decreased cortisol production (Lehmann *et al*., 1998b) and decreased monocyte sensitivity to cortisol (Duclos *et al*., 2003). The cortisol increases in the treated subjects could therefore help to increase the sensitivity of monocytes and the increased cortisol can enable the immune system to shut off the acute inflammatory reaction and cytokine synthesis. The cortisol may thus decrease exercise-induced muscle damage and muscle inflammation reactions. The cortisol response associated with Traumeel S supplementation should therefore limit inflammation over the first four days after exercise and in so doing limit late immunosuppression due to an attenuation of the early inflammatory response. Longitudinal studies are however necessary to determine whether Traumeel S supplementation is associated with prolonged exercise-induced cortisol secretion, which can predispose athletes to the subsequent development of immunosuppression.

The lower phagocytic and oxidative burst activity associated with lower monocyte levels may alter the body's systemic defenses against microbial infection. T_H1 cells, which may cause excessive tissue damage and develop into an auto-immune reaction, are phagocytic-dependent and T_H1 cell activity will therefore be limited. The lower PGE_2 levels will prevent PGE_2 associated suppression of the cellular immune system and decrease the synthesis of pain receptors. The mentioned benefits of decreased circulating monocyte have been thoroughly researched in immunological research and will also be applicable to the current research and include the following: reduced swelling associated with DOMS through reduced permeability of small blood vessels through limited production of vaso-active substances, and secondary muscle damage which can, amongst, others be linked to monocytic activation will be reduced. Swelling, pain and muscle damage are all symptoms of DOMS and it can therefore be concluded that DOMS should be greatly reduced with Traumeel S supplementation. Instead of making some suppositional extrapolations to the impact that decreased monocyte activity will have on DOMS and the immune system circumferences, PGE_2 levels and monocyte sub-populations should be measured in future research. Slightly suppressed natural immunity as found in the current study may reflect a normal down regulation of inflammation in response to chronic tissue injury due to intense daily exercise. The down regulation of phagocytic activity (e.g. a lower monocyte count) may be compensated for by changes in other, possibly later occurring immune functions.

6.1 Recommendations for Future Research

The following recommendations could be helpful to direct future research designs when studying the effect of Traumeel S on the immune system:

6.1.1 Exercise Protocols:

Downhill running is effective in inducing delayed onset of muscle soreness. The metabolic effects of downhill running could however have additional influences on the immune system and hormonal levels, which aren't the case with more isolated eccentric protocols. Multiple eccentric contractions of the quadriceps or biceps brachi muscles on an isokinetic dynamometer will be more effective in inducing greater levels of muscle damage as compared to downhill running. Multiple eccentric contractions of the quadriceps or biceps brachi muscles on an isokinetic dynamometer will be excellent to induce DOMS. The proposed protocol will limit the possible

metabolic influence that eccentric running could have on cortisol because of decreased blood glucose and muscle glycogen levels. Cardiovascular exercise such as downhill running is known to increase stroke volume and the increased stroke volume is associated with an increased circulating white blood cell count.

The effectiveness of Traumeel S can be more thoroughly studied if markers of muscle damage are not studied in isolation but jointly with other markers of overtraining e.g. plasma glutamine levels, reductions in peak torque and heart rate and blood pressure changes. The exercise protocol required to produce the needed changes associated with the overtraining syndrome would have to incorporate a weight bearing, high volume training regime extending over a time period of at least six months. High performance ultra marathon athletes preparing for an important competition should fit this framework.

6.1.2 Blood markers

6.1.2.1 Timing of Blood Tests

Neutrophils are the first sub-population of leukocytes to appear at the injury site and Traumeel S seems to work by modulating the generation of reactive oxygen produced by activated neutrophils. The testing of neutrophil levels is important to assess the effectiveness of Traumeel S on muscle damage. Neutrophil and lymphocyte activity are altered during and soon after (± two and a half-hours after) exercise. To accurately assess the influence of Traumeel S on these two leukocytes, blood tests would have to be performed during the mentioned time periods. By assessing neutrophil levels soon after cessation of exercise statistical relationships between post-exercise neutrophil levels and peak CK and perceived pain levels at 24 hours or 48 hours can be made.

6.1.2.2 Additional Hematological Testing

Additional blood tests together with the differential white blood cell count used in this study can more accurately determine Traumeel S effectiveness in limiting immunosuppression.

Hematological testing should look at the balance between pro-inflammatory Th_1 products –IL-2, IL-6, IL-12, TNF-α and IFN-γ and anti-inflammatory T_H2-products – IL-4, IL-5, IL-6, IL-8 and

IL-10. Testing for transforming growth factor (TGF)-β is of utmost importance because it modulates the down regulation of Th_1 cells which is involved in the initiation of an excessive inflammatory reaction and studies have shown that Traumeel S increases TGF-β levels.

6.1.2 Performance and Overtraining Related Tests

The results of immunological blood tests in isolation cannot confirm or disprove the positive relationship between an altered immune response and the overtraining syndrome. Performance and overtraining related tests should be performed throughout the different training phases of endurance athlete's preparing for a major competition. The following tests can be used to confirm an overtrained state in subjects:

- a reduction in peak treadmill running speed;
- a reduced maximal heart rate of greater than five beats per minute;
- a reduction on heart rate variability (HRV);
- a reduced plasma cortisol level of greater than 60 nmol/l;
- a reduced maximal lactate to rate of perceived exertion (HLa: RPE) ratio of greater than 20 points; and
- a significant change in the profile of mood state (POMS) questionnaire.

The above mentioned tests should be statistically correlated with changes in the immune system to determine the impact that immune system changes will have on performance and overtraining.

CHAPTER 7

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APPENDICE A

RATING OF PERCEIVED PAIN

0 No Pain

0.5 Just noticeable

1 Light discomfort

5 Heavy Discomfort

10 Maximal pain

APPENDICE B

RATING OF PERCEIVED EXERTION (BORG SCALE)

6 No exertion at all

7

Extremely light

8

9 Very light

10

11 Light

12

13 Somewhat hard

14

15 Hard (heavy)

16

17 Very hard

18

19 Extremely hard

20 Maximal exertion