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# THE EFFECT OF A TUMOUR NECROSIS FACTOR–ALPHA INHIBITOR AND A $\beta_1$–RECEPTOR ANTAGONIST ON DELAYED-ONSET MUSCLE SORENESS

**by**

***Tara-Lynne Rice***

*A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg in fulfillment of the requirements for the degree of Master of Science*

Johannesburg, 2006

## DECLARATION

I declare that this dissertation is my own, unaided work. This dissertation is being submitted for the Degree of Master of Science in the Faculty of Science at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other university.

_____

Tara-Lynne Rice

Signed in Johannesburg on this the _____ day of _____ 2006

## ABSTRACT

The involvement of the pro-inflammatory cytokine, tumour necrosis factor alpha (TNF-α) and the sympathetic nervous system in the development of delayed-onset muscle soreness has not been established. I assessed the effect of etanercept, a TNF-α inhibitor, and atenolol, a $\beta_1$-receptor antagonist, on DOMS induced in the quadriceps muscle. Thirteen male subjects reported to the exercise laboratory on three separate occasions, 6-15 weeks apart. In a randomised, double-blind cross-over format, I administered etanercept (25mg), atenolol (25mg) or placebo, one hour before the exercise. Subjects then completed four sets of 15 repetitions at 80% of their one repetition maximum (1RM) on a 45° inclined leg press machine. Muscle strength changes were detected by remeasuring the subject's 1RM 24h, 48h and 72h after the exercise. Sensitivity to pressure of the quadriceps muscle was measured using a pressure algometer before and 24h, 48h and 72h after exercise. The subject's perception of the pain was measured with the visual analogue scale and McGill Pain Questionnaire. Muscle tumour necrosis factor-alpha concentration was measured before exercise and then 2h and 24h after exercise in four subjects. Muscle strength was impaired 24h and 48h after exercise regardless of agent administered ($P < 0.001$). At 72h after exercise, muscle strength was significantly improved ($P < 0.01$) in subjects receiving etanercept and atenolol compared to those receiving placebo. The subject's were significantly more sensitive to pressure applied to the quadriceps 24h, 48h and 72h after exercise compared to before exercise, regardless of agent administered ($P < 0.001$). The VAS was elevated significantly at all three time intervals, with no difference after etanercept or atenolol administration compared to

that of placebo. There was no significant difference in the muscle TNF-α concentration between any of the time intervals or between subjects receiving placebo and etanercept ($P$=0.065). The administration of atenolol and etanercept, at the regimen used, had no effect on the soreness associated with DOMS.

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## CONFERENCE PROCEEDINGS

Part of the work in support of this dissertation has appeared in the proceedings of the 33rd meeting of The Physiological Society of South Africa (September 2005) and in the proceedings of the 11th Biennial Congress of the South African Sports Medicine Association (September 2005).

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| **ASIC** | acid sensing ion channels |
| **CK** | creatine kinase |
| **COX** | cyclo-oxygenase |
| **DOMS** | delayed-onset muscle soreness |
| **EDTA** | ethelene-diamine-tetra-acetic acid |
| **IL-1** | interleukin-1 |
| **IL-1β** | interleukin-1beta |
| **IL-1ra** | interleukin-1 receptor antagonist |
| **IL-4** | interleukin-4 |
| **IL-6** | interleukin-6 |
| **IL-8** | interleukin-8 |
| **IL-10** | interleukin-10 |
| **IL-13** | interleukin-13 |
| **kDa** | kiloDaltons |
| **LPS** | lipopolysaccharide |
| **MPQ** | McGill pain questionnaire |
| **NGF** | nerve growth factor |
| **NK cells** | natural killer cells |
| **NMDA** | N-methyl-D-aspartate |
| **PGE** | prostaglandin of the E series |
| **PPT** | pressure pain threshold |

| | |
|---|---|
| **PRI** | pain response index |
| **TNF-α** | Tumour necrosis factor-alpha |
| **VAS** | visual analogue scale |
| **1RM** | one repetition maximum |

# Chapter One

# Literature Review

## 1.1 Introduction

Pain is defined as an unpleasant sensory experience which causes or is able to cause tissue damage (O'Connor and Cook 1999) and is associated with hurting and soreness (Woolf 2004). Pain is experienced when nociceptors (sensory receptors), responding to a noxious stimulus, initiate an impulse which is carried to the cerebral cortex, where the sensation of pain is perceived (Basbaum et al. 2005, Woolf 2004). The intensity, duration and quality of pain is variable and depends on whether the pain is acute or chronic (Basbaum et al. 2005, Woolf 2004). Acute pain is classified as nociceptive pain occurring when a noxious stimulus (eg. mechanical, chemical or thermal) comes into contact with the skin or deep tissue (Basbaum et al. 2005, Woolf 2004). Chronic or neuropathic pain results from damage to the central nervous system (Basbaum et al. 2005, Woolf 2004). Inflammatory pain, which can either be acute or chronic, is defined as spontaneous pain with tenderness and hypersensitivity and is caused by tissue damage and inflammation (Basbaum et al. 2005, Woolf 2004).

Two mechanisms, peripheral and central sensitisation, can produce hyperalgesia. Hyperalgesia is an increased sensitivity to a noxious stimulus (Woolf 2004). Hyperalgesia occurs when the pain receptors become hypersensitive, and pain receptor stimulation causes the release of pro-inflammatory cyokines. Injury or inflammation of tissue causes changes to the surrounding chemical environment,

acting on the peripheral terminals of nociceptors (Aδ and C fibres), resulting in peripheral sensitisation (Basbaum et al. 2005, Woolf 2004). The injured cells release prostaglandins, cytokines, growth factors, adenosine triphosphate and potassium ions, which either directly activate nociceptors producing spontaneous pain or sensitise nociceptor terminals and cause hyperalgesia (Basbaum et al. 2005, Sachs et al. 2002, Woolf 2004). Central nociceptor neurones, in the spinal cord, also can be sensitised by responding to increased peripheral signals in a process known as central sensitisation (Basbaum et al. 2005, Woolf 2004). Central sensitisation causes hyperalgesia and in particular, secondary hyperalgesia, where tenderness is felt outside the injured site and is a result of molecular, cellular and circuit changes within the central nervous system (Woolf 2004).

During inflammation, some pro-inflammatory cytokines, specifically tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8), have been shown to induce hyperalgesia, in cutaneous tissue of rats (Cunha et al. 1992, Meyer et al. 2005, Woolf et al. 1997), and mice (Cunha et al. 2005). Tumour necrosis factor-alpha is a key cytokine that sensitises nociceptors, inducing hyperalgesia, in the hind paw of rodents (Cunha et al. 2005). When TNF-α is injected either systemically or locally into animals, mechanical hyperalgesia occurs, in cutaneous tissue (Meyer et al. 2005) and the hyperalgesia is abolished when a TNF-α antagonist is administered, which suggests that TNF-α is involved in hyperalgesia (Woolf et al. 1997). Inhibiting TNF-α is helpful in treating

inflammatory pain in both experimental animal models (Cunha et al. 2005) and in clinical inflammatory pain (Flagg et al. 2005, Inglis et al. 2005), such as musculoskeletal diseases.

## 1.2 Inflammatory hyperalgesia

The immune system is involved in repairing tissue following damage and infection and helps to protect the body from invading pathogens (Watkins et al. 1995b). Cytokines, small polypeptides, with a molecular weight ranging between 8 and 30 kDa, are immunomodulators that are released at the site of injury (Cannon 2000, Wieseler-Frank et al. 2005). Cytokines are secreted by numerous cell types, including macrophages, monocytes, endothelial cells, lymphocytes, smooth muscle cells and leukocytes (Gabay and Kushner 1999, Lundberg and Nyberg 1998). Cytokines have multiple targets and have more than one function (Cannon 2000, Gabay and Kushner 1999, Lundberg and Nyberg 1998), making it difficult to identify the role that cytokines play in pain. Cytokines are produced on demand, have low circulating concentrations and have a relatively short life-span (Janig and Levine 2005, Kress 2005, Turnbull and Rivier 1999). After binding to specific receptors in target cells (cells which are damaged or injured), cytokines can initiate a number of biological activities (Pyne 1994, Turnbull and Rivier 1999) and regulate the interaction between all cells associated with the inflammatory response (Nieman et al. 2001), with each cytokine playing a specific role in the acute inflammatory

response (Smith et al. 2000). Cytokines are involved in the communication between immune and non-immune cells as well as between various organs within the body (Northoff et al. 1994, Shephard 2002) such as between the immune system and the peripheral nervous system, central nervous system and the endocrine system (Janig and Levine 2005).

Two groups of cytokines exist, the pro-inflammatory cytokines and the anti-inflammatory cytokines. Trauma, infection and physical exercise, in particular eccentric exercise, have been shown to influence cytokine production, at the site of inflammation and also within the circulation (Cheung et al. 2003, Moldoveanu et al. 2001, Pedersen et al. 1998, Turnbull and Rivier 1999). The pro-inflammatory cytokines, interleukin-1β (IL-1β), IL-6 and TNF-α, cause leukocyte proliferation, cytotoxicity, produce prostaglandins, initiate the release of other cytokines and activate the acute phase response (Cannon 2000). The anti-inflammatory cytokines include interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1ra) and interleukin-13 (IL-13), and their function is to reduce the inflammation by preventing the production of the pro-inflammatory cytokines (Cheung et al. 2003, Moldoveanu et al. 2001, Turnbull and Rivier 1999). The function of the inflammatory response is to remove debris and invading pathogens and to aid in tissue repair (MacIntyre et al. 1995, Summan et al. 2003).

The role of cytokines in the inflammatory response is complicated since both pro- and anti-inflammatory cytokines are released, which act on a variety of cells causing a variety of changes (Woolf et al. 1997). Cytokines, which are released in response to inflammation, can induce the liver to release acute phase proteins in a process called the acute phase response (Pedersen and Hoffman-Goetz 2000, Watkins et al. 1995b). The acute phase response causes the activation and circulation of neutrophils (Fielding et al. 1993), which is followed by the infiltration of monocytes and macrophages in the tissue leading to the activation of cytokines (Camus et al. 1994).

A pro-inflammatory cytokine cascade is prominent during inflammation in cutaneous tissue and during fever, but whether it exists in muscle pain is not yet known. During cutaneous pain and fever, a pathogenic stimulus or injury activates the cells of the surrounding tissue and results in the release of bradykinin and other inflammatory mediators. Macrophages and monocytes are activated and release the pro-inflammatory cytokine, TNF-α. Tumour necrosis factor-alpha produces inflammatory hyperalgesia itself or by two pathways: the first is that TNF-α causes IL-6 and IL-1β to be produced and these two cytokines stimulate the cyclooxygenase-2 (COX2) enzyme which causes prostaglandin $E_2$ ($PGE_2$) to be released, resulting in peripheral sensitisation. The second pathway is where TNF-α induces the release of IL-8 from the immune cells; IL-8 then acts on the sympathetic nervous system via the hypothalamic-adrenal-pituitary axis or via the central nervous system directly. The release of sympathetic amines from the sympathetic neurons

sensitise nociceptors and cause hyperalgesia (Cunha et al. 1991, Janig and Levine 2005, Summan et al. 2003). Since TNF-α plays a key role in cutaneous hyperalgesia, it may play a role in muscle hyperalgesia also.

### 1.2.1 The role of tumour necrosis factor-alpha in pain

Tumour necrosis factor–alpha was discovered first in 1975 by Old and colleagues, who observed that a factor within the serum of animals, when injected with lipopolysaccharide, caused the necrosis of tumours (Bazzoni and Beutler 1996, Beutler 1995), and this factor was therefore named tumour necrosis factor-alpha (TNF-α). Tumour necrosis factor-alpha is a pro-inflammatory cytokine produced by a variety of cells (Cheung et al. 2003), is released during the inflammatory response, and has many biological functions (Bazzoni and Beutler 1996, Pedersen et al. 1998). Tumour necrosis factor-alpha has been shown to be both detrimental and helpful in disease and infection. Tumour necrosis factor-alpha is involved in the activation of the acute inflammatory response where it initiates fever and the infiltration of neutrophils and macrophages into the injured tissue (MacIntyre et al. 1995). Tumour necrosis factor-alpha is involved in skeletal muscle metabolism, by promoting muscle protein breakdown and by modulating myogenesis (Goodman 1991, Li and Reid 2001). Tumour necrosis factor-alpha also is involved in the anticoagulation of endothelial cells, apoptosis, growth and differentiation of many cells and T-cell proliferation (Bazzoni and Beutler 1996, Li 2003, MacIntyre et al. 1995, Shephard et al. 1994).

Two types of TNF exist, TNF-α and TNF-β, both of which are pathogenic mediators (Tracey and Cerami 1994). Tumour necrosis factor-alpha is a 26kDa transmembrane polypeptide released by both immune and non-immune cells (Tracey and Cerami 1994, Vandenabeele et al. 1995). Tumour necrosis factor-alpha is composed of three TNF monomers, which are non-covalently bound to form a trimeric molecule (Tracey and Cerami 1994, Vandenabeele et al. 1995) and it is this trimeric form of TNF-α which exists bioactively in the bodily fluids, including serum (Tracey and Cerami 1994).

There are two forms of TNF-α, soluble TNF-α and bound TNF-α, where a 26kDa TNF-α is bound to the surface of the cell membrane and a 17kDa TNF-α is soluble and secreted (Tracey and Cerami 1994, Vandenabeele et al. 1995). The soluble form of TNF-α is involved in mediating inflammation (Moldoveanu et al. 2001, Turnbull and Rivier 1999), and is able to bind to TNF-α receptors, TNFR1 and TNFR2 which are present on most nucleated cells within the body (Bazzoni and Beutler 1996, Vandenabeele et al. 1995). Both receptors are similar in structure but use different signaling pathways (Tracey and Cerami 1994, Turnbull and Rivier 1999). TNFR1 is involved in the production of hyperalgesia (Maihofner et al. 2005) and in the immune response to pathogens (Beutler 1995).

Tumour necrosis factor-alpha is able to both activate and sensitise nociceptors and so it is probably involved in the generation of both pain and hyperalgesia, within

muscle tissue (McMahon et al. 2005). In rats, primary hyperalgesia was induced within six hours, when TNF-α was injected into muscle and into the hind paw (Schafers et al. 2003). Tumour necrosis factor-alpha concentration in the hind paw is increased during inflammation, where the TNF-α concentration is elevated three hours following induced inflammation (Woolf et al. 1997). Therefore, TNF-α possibly plays an important role in the development of muscle pain (Capra and Ro 2004) since it is present in skeletal muscle during damage (Warren et al. 2002). Since TNF-α stimulates IL-8 release during cutaneous pain and IL-8 activates the sympathetic nervous system perhaps a component of inflammatory hyperalgesia is caused by the sympathetic nervous system.

## 1.3 Sympathetic pain

The autonomic nervous system controls the unconscious functions of the body such as heart rate and intestine activity (Elenkov et al. 2000, Martini 2001). The sympathetic nervous system releases noradrenaline and adrenaline into the blood stream (Elenkov et al. 2000, Martini 2001) and controls the body's response to stress, muscular exercise and emotion (Shepherd 1985). The effects of the sympathetic nervous system are from the interactions between both noradrenaline and adrenaline with their membrane sensitive receptors, of which there are two: alpha receptors and beta receptors (Elenkov et al. 2000, Martini 2001). Noradrenaline generally stimulates alpha receptors more than beta receptors whereas

adrenaline stimulates both alpha and beta receptors (Martini 2001). There are two types of alpha receptors: alpha-1 ($\alpha_1$) and alpha-2 ($\alpha_2$) receptors and three types of beta receptors: beta-1 ($\beta_1$), beta-2 ($\beta_2$) and beta-3 ($\beta_3$) receptors.

Beta$_2$-adrenergic receptors are found in skeletal muscle and when these receptors are stimulated by the sympathetic nervous system, an increase in the force of muscle contraction and glycogen and fat breakdown occurs, as well as regulation of glucose transport and muscle contractility (Liggett et al. 1988, Martin et al. 1989, Martini 2001). Blood vessels leading from the heart to the skeletal muscle contain $\beta_2$-receptors and when stimulated by the sympathetic nervous system cause the blood vessels to dilate, increasing blood flow to the muscles (Martini 2001, Shepherd 1985).

Slow-twitch muscle fibres have more $\beta_2$-adrenoreceptors than do fast-twitch muscle fibres and β-receptor density plays an important role in oxidative capacity of skeletal muscle and is also influenced by fibre type (Williams et al. 1984). Skeletal muscle may contain between 7-10% of $\beta_1$-adrenoreceptors (Navegantes et al. 1999). It has been suggested that β-receptors may also be involved in physiological adaptations to exercise training (Williams et al. 1984). Blocking $\beta_2$-receptors during exercise inhibits fat metabolism (Cleroux et al. 1989), which may impact on performance during exercise. Whether $\beta_1$-adrenoreceptors are involved in muscle pain and

hyperalgesia is unknown and more research needs to be conducted to determine the exact role of the receptors in skeletal muscle.

### 1.3.1 Role of beta-adrenergic receptors in hyperalgesia and pain

The sympathetic nervous system may be involved in inflammatory hyperalgesia where adrenoreceptors cause an increase in sympathetic activity which either activates nociceptors or increases cytokine concentrations (Meyer et al. 2005, Safieh-Garabedian et al. 2002). Bradykinin, when injected into cutaneous tissue of the rat, reacts with $\beta_2$-receptors which causes the release of prostaglandin from postganglionic neurones, and therefore prostaglandin sensitises nociceptors, causing hyperalgesia (Janig and Levine 2005, McMahon 1991). When the rats were given a sympathectomy, where nerve terminals of sympathetic nervous system are destroyed and therefore the nerve pathway is disrupted, the hyperalgesia was reversed (Janig and Levine 2005). Hyperalgesia also can be generated by injecting nerve growth factor into the skin, of both humans and animals (Janig and Levine 2005, Mense 1993). The sympathetic nervous system is possibly involved in the sensitisation of muscle nociceptors following administration of nerve growth factor, since sympathetic terminals have receptors for nerve growth factor and cause nociceptive mediators to be released, resulting in pain (Janig and Levine 2005).

Pain, which depends on sympathetic activity, is called sympathetically maintained pain and is usually treated by either a sympathectomy or receptor antagonist

(Martinez-Lavin 2004, Meyer et al. 2005). Under normal non-pathological conditions, noradrenaline and adrenaline cannot sensitise nociceptors but can cause an interaction between the sympathetic nervous system and the primary afferent nociceptors at both peripheral and central levels (Martinez-Lavin 2004). Sensory neurons which have been damaged can express more adrenoreceptors, which may explain the relationship between the sympathetic nervous system and pain (McMahon 1991).

Inflammatory pain and hyperalgesia can result from TNF-α releasing IL-8 from both endothelial cells and macrophages (Janig and Levine 2005, Safieh-Garabedian et al. 2002). Interleukin-8 acts on sympathetic terminals to release noradrenaline which stimulates $\beta_2$-adrenoreceptors to sensitise afferent nociceptors in the hind paw tissue of the rat, and thereby cause hyperalgesia and pain (Janig and Levine 2005, Safieh-Garabedian et al. 2002). Interleukin-8 is able to sensitise cutaneous nociceptors in animal models (Cunha et al. 1991) and IL-8 is increased in muscle during muscle contraction, in humans (Akerstrom et al. 2005). Therefore, in the presence of damage and inflammation, IL-8 may cause inflammatory hyperalgesia via a prostaglandin-independent mechanism involving the sympathetic nervous system (Cunha et al. 1991).

A study in rats, examined the involvement of sympathetic efferents in the production of inflammatory hyperalgesia by blocking the sympathetic nervous system during

endotoxin-induced hyperalgesia and concluded that sympathetic efferents are involved in inflammatory hyperalgesia (Safieh-Garabedian et al. 2002). The exact role of the sympathetic nervous system in the generation of inflammatory hyperalgesia and pain has not yet been fully elucidated and more research examining the relationships between the sympathetic nervous system, hyperalgesia and pain still needs to be conducted.

### 1.3.2 Beta-adrenergic receptors and exercise

Physical exercise causes an increase in sympathetic nervous system activity resulting in noradrenaline release from the sympathetic terminals (Pedersen and Hoffman-Goetz 2000) and changes in the inflammatory response (Kohut et al. 2004). The stimulation of the sympathetic nervous system increases adrenaline concentrations affecting the β-adrenoreceptors by increasing circulating neutrophil concentrations (Pyne 1994).

A study in humans, evaluated the response caused by a stressful situation, which is similar to a bout of exercise, on changes in blood leukocyte concentrations, in particular natural killer (NK) cells and the relationship between NK cells and β-adrenoreceptors (Klokker et al. 1997). Natural killer cells express β-adrenoreceptors and during exercise the number of β-receptors on NK cells is increased (Klokker et al. 1997), the $\beta_2$-receptors produce catecholamines which then cause NK cell

recruitment (Klokker et al. 1997) and this may be the link between the sympathetic nervous system and the immune response.

Catecholamines, released by the sympathetic nervous system, are involved in some immunomodulatory effects associated with exercise training, perhaps via the β-adrenergic receptors which are expressed on some immune cells (Kohut et al. 2004). Another study examined the effects of blocking β-receptors on skeletal muscle metabolism and exercise endurance, in humans (Cleroux et al. 1989). It has previously been shown that nonselective β-blockers decrease exercise endurance while $\beta_1$-selective blockers only have a small effect on exercise endurance (Cleroux et al. 1989). Non-selective β-blockers decrease fat and glycogen metabolism which may impact on endurance performance (Cleroux et al. 1989). No studies have examined what role, if any, β-adrenoreceptors have in the generation of DOMS.

## 1.4 Muscle pain

Deep tissue damage occurs frequently in sports injuries (Schaible 2005). Pain originating in muscle is one of the most presented symptoms clinically (Capra and Ro 2004, Schaible 2005), however, more is known about the mechanisms of cutaneous pain (Kehl et al. 2000, Tegeder et al. 2002). Little is known about muscle nociception and it may be because numerous inputs affect muscle sensation,

including muscle spindles, tendon, joint and skin receptors (Kehl and Fairbanks 2003, Tegeder et al. 2002).

Muscle pain, or myalgia, originates in skeletal muscle, tendons and surrounding fascia (Mense 1993) and is described as a widespread tender, sore, diffuse ache with associated muscle stiffness (Kidd 2005, Mills et al. 1989, Nie et al. 2005a). The quality of muscle pain is different from cutaneous pain, which is described as a superficial, sharp, pricking, stabbing and burning pain (Graven-Nielsen and Mense 2001, Mense 1993). Also, muscle pain is difficult to localise, since the pain is often referred to non-visceral structures, while cutaneous pain is localised easily (Mense 1993). Cutaneous and muscular pain may differ in that the input from nociceptors is processed differently within the spinal cord, resulting in different sensations being felt for the different types of pain (Mense 1993).

In order to study the mechanisms and treatment of clinical muscle pain, experimental models have been developed to mimic muscle pain, including that which is induced by exercise (Slater et al. 2005). Muscle pain can be induced by endogenous methods such as exercise or ischaemia, or exogenously through the injection of algesic substances and electrical stimulation (Graven-Nielsen and Arendt-Nielsen 2003).

Muscle hyperalgesia is felt in response to either peripheral or central sensitisation. Sensitisation of the muscle nociceptors occurs during tissue damage from trauma and

inflammation, muscle spasms, ischemia and overuse (Graven-Nielsen and Mense 2001, Mense 1993). Peripheral sensitisation of muscle nociceptors results in a decreased mechanical excitation threshold resulting in an increased response to noxious stimuli (Arendt-Nielsen and Graven-Nielsen 2003). Muscle nociceptor sensitisation occurs in the presence of inflammatory substances (Kidd 2005, Mense 1993). To what extent each mediator contributes to the muscle hyperalgesia is yet to be determined (Tegeder et al. 2002).

Inflammatory cytokines (TNF-α and IL-6) and the neurotrophins (nerve growth factor and brain derived nerve factor, which induce neurone survival) are possibly involved in the peripheral mechanisms of muscle pain. These cytokines and neurotrophins change the impulse activity and mechanosensitivity of C-fibres, by either directly acting on receptors located on the fibre ends, or by stimulating the release of other agents which act on the C-fibres (Hoheisel et al. 2005).

Tumour necrosis factor-alpha, when injected into the muscle of rats causes primary hyperalgesia, measured by changes in forelimb grip force and withdrawal thresholds to a mechanical pressure, by increasing $PGE_2$ concentration at the site which sensitises nociceptors (Schafers et al. 2003). The development of muscle hyperalgesia may also involve acid sensing ion channels since an acidic saline injection into the muscle, induces hyperalgesia probably via acid sensing ion channels (ASIC) (Sluka et al. 2001). The three acid sensing ion channels (ASIC1,

ASIC2 and ASIC3) located in the muscle, are activated by a low pH (Sluka et al. 2003).

However, activation of ASIC3 is involved in the development of primary hyperalgesia within the muscle and also may be involved in secondary hyperalgesia and central sensitisation (Sluka et al. 2003).

Pain, including muscle pain, and hyperalgesia also have a central component, where input from peripheral nociceptors to the brain or spinal cord alters the function and connectivity of sensory neurons in the brain or spinal cord (Mense 2003). During this central sensitisation, the spinal nerves become hypersensitive and hyperexcitable resulting in secondary hyperalgesia, increased sensitivity to a noxious stimulus in adjacent, non-inflamed tissue, and referred pain, pain felt in a area different to the source of that pain (Arendt-Nielsen and Graven-Nielsen 2003, Mense 2003). Cellular changes result in hyperexcitable spinal neurons which allow ineffective synapses, within the spinal cord, to become effective causing referred pain (Arendt-Nielsen and Graven-Nielsen 2003, Mense 2003, Schaible 2005). The release of glutamate and substance P from the muscle afferent fibre, during nociceptor sensitisation, open N-methyl-D-aspartate (NMDA) receptors located on the cell surface of spinal neurons, causing intracellular calcium concentrations to increase in the spinal neurons, resulting in central sensitisation (Kidd 2005, Mense 2003). The exact mechanism in exercise-induced muscle pain and clinical muscle pain is yet to be

elucidated, however peripheral inflammation and central sensitisation are likely to be involved.

### 1.4.1 TNF-α inhibitor in musculoskeletal pain

Tumour necrosis factor-alpha antagonists are used when concentrations of TNF-α are elevated both systemically and at the area affected by inflammation, and the main purpose of the drug is to inhibit TNF-α (Nestorov 2005). Tumour necrosis factor-alpha antagonist drugs are used to treat rheumatoid arthritis and other autoimmune diseases and have successfully treated both the disease symptoms and the associated pain. One such drug is etanercept (Enbrel; Amgen Inc). Etanercept is a 150kDa recombinant human TNF-α receptor protein and has a half-life of about 5 days (Goffe and Cather 2003). Etanercept inhibits the activity of TNF-α by binding to both soluble and membrane bound forms of the cytokine and so prevents TNF-α from binding to its cell surface receptor (Goffe 2004, Goffe and Cather 2003, Nestorov 2005). Patients suffering from inflammatory autoimmune diseases such as psoriatic arthritis, chronic inflammatory polyneuropathy, ankylosing spondylitis, peripheral joint synovitis and rheumatoid arthritis have found a significant improvement in pain and a reduction in inflammation associated with the disease, following administration of etanercept (Chin et al. 2003, Flagg et al. 2005, Haraoui 2005, Kruithof et al. 2005).

A study examined the effects of etanercept on inflammatory diseases in rats and noted that etanercept reduces the mechanical and thermal hyperalgesia associated with inflammatory pain, in the joint (Inglis et al. 2005). Therefore, inhibiting TNF-α may attenuate other forms of inflammatory pain, such as inflammatory muscle pain occurring during DOMS and also inflammatory joint pain, such as rheumatoid arthritis.

## 1.5 Delayed-onset muscle soreness

### 1.5.1 Introduction

Unaccustomed exercise with a large eccentric component produces a sensation of pain and discomfort in the exercised muscle 24-48h after exercise, and is appropriately named delayed-onset muscle soreness (Allen 2001, Appell et al. 1992, Armstrong 1984, Newham 1988). Delayed-onset muscle soreness (DOMS) can be induced by downhill running (Braun and Dutto 2003, Byrnes et al. 1985, Friden and Lieber 2001, Simpson et al. 2005) or by eccentric exercises targeting specific muscle groups such as leg press and arm curls (Barlas et al. 2000, Clarkson and Tremblay 1988, Howell et al. 1993, Jones et al. 1987, Nie et al. 2005b). Eccentric exercise occurs when the muscle is lengthening during a muscle contraction (Cheung et al. 2003, Clarkson and Hubal 2002).

The muscles affected by DOMS, as with other clinical muscle pains, are described as being sore, tender, stiff and uncomfortable (Appell et al. 1992, Armstrong 1984, Brown et al. 1996, Friden and Lieber 2001). One of the main characteristics of DOMS is that the affected muscles become extremely sensitive to both muscle contraction, passive stretch and pressure (Appell et al. 1992, Armstrong 1984, Weerakkody et al. 2001), named mechanical hyperalgesia (Taguchi et al. 2005). DOMS also causes swelling, impairs proprioception (Cheung et al. 2003, Proske and Allen 2005, Saxton et al. 1995), results in a loss of range of movement (Cheung et al. 2003, Clarkson 1997) and a decrease in muscle strength (Armstrong 1990, Cheung et al. 2003). However, some researchers are uncertain whether DOMS and the hyperalgesia are related (Weerakkody et al. 2001).

Delayed-onset muscle soreness can occur in any skeletal muscle and is more prominent at the musculotendinous junctions, where a large collection of muscle nociceptors are present (Armstrong 1984). Since DOMS may affect movement patterns, by increasing sensitivity to pressure, it may impact on the performance of sportsmen such as impaired running economy (Braun and Dutto 2003), which may lead to further injury as the athletes place more stress on the injured muscle, ligaments and tendons as well as on those muscles which are compensating for the injured muscles (Cheung et al. 2003). It is therefore important to determine the exact mechanisms of DOMS.

### 1.5.2 Mechanisms of DOMS

Although the underlying mechanisms of DOMS have not yet been fully elucidated, two main mechanisms have been proposed in the production of DOMS; the soreness may be caused by damage to the muscle fibres themselves and to the connective tissue surrounding the muscle fibres, or by an acute inflammatory reaction releasing chemical mediators that contribute towards the pain (MacIntyre et al. 1995, Smith 1991). The most likely mechanism of the soreness includes a combination of both muscle fibre damage and inflammation.

Muscle, when subjected to high tension, such as eccentric muscle contractions, results in disruptive changes within the structural components of the muscle cells and causes a reduction in force generation, producing exercise-induced muscle damage (Kuipers 1994, Prasartwuth et al. 2005). Damage to the muscle fibres, following eccentric exercise, occurs immediately after exercise, with the damage becoming worse two to three days following exercise (Friden and Lieber 1998, Newham et al. 1983a, Newham et al. 1983b, Prasartwuth et al. 2005). The sarcomeres, which are the smallest functional units of the muscle fibre, are composed of thick and thin filaments (Martini 2001). The Z-lines are the junctions between adjacent sacromeres and have received much attention in the exercise-induced muscle damage literature (Cheung et al. 2003, Clarkson and Sayers 1999, Fielding et al. 1993, Lieber and Friden 1999, Morgan and Allen 1999, Newham 1988), since most studies report that eccentric exercise causes damage to and disruption of the Z-line. Most eccentric muscle damage affects fast-twitch (Type II) muscle fibres more than slow-twitch

(Type I) fibres, since there are weaker and more vulnerable Z-lines in type II muscle fibres (Jones et al. 1986, Takekura et al. 2001).

The initial damage to muscle during eccentric exercise is to the sarcomeres in the myofibrils which are overstretched and disrupted (Morgan and Allen 1999, Proske and Morgan 2001). The sarcoplasmic reticulum, a membrane complex surrounding a myofibril, may be damaged during eccentric exercise (Brown et al. 1996, Clarkson and Sayers 1999, Friden and Lieber 2001), resulting in increased intracellular calcium and disruptions to the Z-lines (Clarkson and Sayers 1999, Lieber and Friden 1999, Newham 1988). Eccentric exercise causes muscle fibre damage, however the relationship between muscle damage and pain is vague, since muscle fibre damage occurs immediately after exercise and the pain starts about 8h after exercise (Newham 1988).

If muscle fibre damage occurs, then some degree of inflammation must occur also. An inflammatory response may be involved in the development of some of the symptoms of exercise-induced muscle injury, which include pain and a decrement in muscle strength (MacIntyre et al. 2001, Smith 1991). Acute inflammation, like that seen after eccentric exercise, causes changes in both a local response resulting in pain, heat, redness and swelling, and a systemic response which results in the production of cytokines (Cheung et al. 2003, Smith 1991). The local response to

acute inflammation is caused by inflammatory mediators, released locally (MacIntyre et al. 1995).

During acute inflammation, as would occur during DOMS, fluid, plasma proteins and leukocytes move into the injured tissue to help in the repair process (Cheung et al. 2003, MacIntyre et al. 1995, Pedersen and Hoffman-Goetz 2000, Smith 1991). Tissue damage caused by exercise is followed by an increase in circulating neutrophils between one and four hours after the initial injury (Smith 1991). The infiltration of monocytes into the injured tissue, between six and 12h occurs next, with a peak in monocyte concentration occurring at 48h (Smith 1991). In an animal model of DOMS, plasma prostaglandin $E_2$ ($PGE_2$) and substance P concentrations were elevated after the muscle contractions, suggesting that the two mediators may play a role in the development of DOMS (Tegeder et al. 2002).

The inflammatory mediators, released during muscle damage, sensitise nociceptors so that the nociceptors respond to non-noxious stimuli (Proske and Morgan 2001). The muscle then becomes tender to touch, and muscle stretch and contraction produces pain (Proske and Morgan 2001). Therefore the pain is thought to be mediated by the changes taking place during inflammation. Inflammation is an important and essential response to muscle injury and is necessary for muscle regeneration (Collins and Grounds 2001, Tidball 1995).

### 1.5.3 Effects of eccentric exercise on muscle function

A number of studies have examined the effect that eccentric exercise and the associated DOMS has on biochemical and functional measures (Brown et al. 1996, Chleboun et al. 1998, Howell et al. 1993, Jones et al. 1986, MacIntyre et al. 1996, Rodenburg et al. 1993). Creatine kinase is one of the biochemical variables often measured in studies involving DOMS.

Creatine kinase, a muscle protein and an indicator of muscle damage, is found in skeletal muscle and is released into the interstitial fluid when damage to the muscle membrane occurs (Cheung et al. 2003, Friden and Lieber 2001). Plasma creatine kinase concentration, before and after exercise, is extremely variable between people and is therefore used only as a general instrument to measure muscle damage (Saxton et al. 1995). Observation of the creatine kinase concentrations in trained athletes and in untrained men revealed that the resting creatine kinase concentrations were higher in the trained runners compared to the untrained men. However, creatine kinase concentrations in the untrained men increased significantly over the duration of the study compared to those of the trained athletes (Evans et al. 1986). The type and severity of exercise, and the previous exercise intensity and duration may therefore play a role in the amount of creatine kinase released (Evans et al. 1986, Schwane et al. 1983, Sorichter et al. 2001). However, despite its variability, many studies measure the concentrations of creatine kinase in the blood to determine whether muscle damage has occurred and also to determine the amount of muscle

damage (Brown et al. 1996, Clarkson and Tremblay 1988, Evans et al. 1986, Jones et al. 1986, Nosaka et al. 2002, Simpson et al. 2005).

The time course for increased plasma creatine kinase concentration, in humans, differs between studies, where some studies have shown creatine kinase concentration to peak between four and five days after exercise (Clarkson and Tremblay 1988, Evans et al. 1986, Rodenburg et al. 1993), while others have shown concentrations of creatine kinase to peak at 48h (Simpson et al. 2005) and 72h after exercise (Brown et al. 1996). In general, creatine kinase concentration increases between 24 and 48h after participating in eccentric exercise, with a peak occurring 96 and 120h (day 4-5) after exercise. Therefore, it appears that creatine kinase released from the muscle, also has a delayed-onset (Clarkson et al. 1992, Newham 1988), similar to that found in the muscle soreness.

Since plasma creatine kinase concentration is so variable between subjects it may not be the best measure of muscle damage. However, it does indicate that muscle damage has occurred and so perhaps it should only be used as an indicator of muscle damage together with other measurements such as plasma myoglobin concentrations. Also, functional measures examined during DOMS such as muscle strength, soreness, swelling and stiffness of the muscles may not correlate with the extent of the injury (Chleboun et al. 1998, MacIntyre et al. 1996, Rodenburg et al. 1993).

*Muscle stiffness and swelling*

Damage to muscle following eccentric exercise causes swelling which has a delayed-onset, starting two days after exercise and can last up to 11 days after exercise (Chleboun et al. 1998, Howell et al. 1993, Jones et al. 1987). Muscle stiffness is felt immediately after participating in unaccustomed or eccentric exercise, peaking between 48 and 96h after exercise and resolving four to six days later (Chleboun et al. 1998, Howell et al. 1993, Jones et al. 1987). However the swelling peaks between 72 and 96h after exercise and returns to pre-exercise values between 7 and 11 days after eccentric exercise (Chleboun et al. 1998, Hirose et al. 2004, Howell et al. 1993, Nosaka and Clarkson 1996). Muscle stiffness has been attributed either to connective tissue damage as measured by electrical activity (Jones et al. 1987), to a change in calcium homeostasis (Chleboun et al. 1998), or to oedema within the muscle as a result of muscle cell injury (Howell et al. 1993). Swelling after eccentric exercise is caused by either intracellular and extracellular oedema (Chleboun et al. 1998), muscle and connective tissue damage (Howell et al. 1993) or by the inflammatory response (Nosaka and Clarkson 1996). It is possible that swelling may cause muscle stiffness since swelling is a result of fluid accumulation in the damaged area. The resulting oedema, which may prevent movement, may lead to the sensation of muscle stiffness. However, muscle stiffness occurs before muscle swelling, indicating that swelling is probably not the cause of the muscle stiffness.

*Muscle strength*

Muscle strength has been shown to decrease immediately after eccentric exercise, with a peak in muscle function impairment between 24 to 48h after exercise. Furthermore, it can take more than 10 days for the damaged muscle to regain its pre-exercise strength (Brown et al. 1996, Chleboun et al. 1998, Hirose et al. 2004, Nosaka and Clarkson 1996). The loss in muscle strength has been ascribed to a failure in the excitation-contraction coupling system caused by changes to the intracellular calcium concentration (Brown et al. 1996). In another study, the loss in muscle strength during DOMS, was biphasic, where strength loss decreased immediately after exercise and then again 24h after the exercise (MacIntyre et al. 1996). The biphasic change in muscle loss is attributed, firstly, to mechanical damage to the muscle fibres and secondly to the inflammatory response (MacIntyre et al. 1996). Since the timing of peak muscle impairment correlates to peak muscle pain, it is possible that the pain is responsible for the muscle strength deficit.

*Muscle soreness*

DOMS is described as being sore and painful and many studies have examined the muscular soreness associated with DOMS (Jones et al. 1987, Jones et al. 1986, Nie et al. 2005b, Simpson et al. 2005). Muscle soreness can be measured using a visual analogue scale (VAS) (Hirose et al. 2004, Nie et al. 2005b, Nosaka and Clarkson 1996, Tegeder et al. 2002), which is usually a 100mm line, with "no pain" on the left

and “worst pain imaginable” on the right, and is a measure of pain intensity (O'Connor and Cook 1999). In other similar studies, subjects have scored their DOMS pain between 20-70mm (Hirose et al. 2004, Nosaka and Clarkson 1996, Tegeder et al. 2002). The McGill Pain Questionnaire measures the quality of pain, where subjects pick words to describe their pain and DOMS is described as “sore”, “tiring” and “tender” (Nie et al. 2005b, O'Connor and Cook 1999).

Pressure pain threshold is a valid and widely used test to measure muscle tenderness and hyperalgesia (Barlas et al. 2000, Jones et al. 1987, Jones et al. 1986, Nie et al. 2005b). The pressure pain threshold follows the same temporal pattern as that of the VAS, where subjects are most sensitive to pressure on the affected muscle and perceive the greatest pain 24h after exercise (Nie et al. 2005b). The intensity of muscle soreness may differ slightly between studies because of the type and intensity of exercise used to induce the muscle damage and according to the muscles involved (Clarkson and Hubal 2002, Newham 1988). Although numerous studies measure the soreness after eccentric or unaccustomed exercise, no conclusive pharmacological agent has been identified to attenuate the soreness (Bourgeois et al. 1999, Loram et al. 2005). Also, the mechanism of the soreness associated with DOMS is still to be determined.

### 1.5.4 Pro-inflammatory cytokines and muscle function

Many studies have been conducted in humans, to study the cytokine concentrations after eccentric exercise and whether the change in cytokine concentration plays any role in the production of DOMS (Brenner et al. 1999, Carmichael et al. 2005, Fielding et al. 1993, Hirose et al. 2004, MacIntyre et al. 2001, Malm et al. 2004, Nosaka and Clarkson 1996, Peake et al. 2005a, Peake et al. 2005b, Simpson et al. 2005). Numerous studies have focused on the involvement of IL-6 in exercise and have found that the cytokine plays both an anti- and pro-inflammatory role (MacIntyre et al. 2001, Ostrowski et al. 1999, Ostrowski et al. 1998). Cytokines such as IL-8, IL-1β and TNF-α also have been studied to better understand the role they play in the development of DOMS.

*Interleukin-6*

Following a bout of eccentric exercise, IL-6 may be involved in the early development of the acute inflammatory response (MacIntyre et al. 2001). Plasma IL-6 concentration peaked 6h following exercise that had induced DOMS (MacIntyre et al. 2001). However, a conflicting study found no change in plasma IL-6 concentration following a bout of downhill running, but IL-6 concentrations in muscle tissue did change (Malm et al. 2004). Other studies also have shown that plasma IL-6 concentrations did not increase following eccentric muscle contractions of the elbow flexors (Nosaka and Clarkson 1996), or following a variety of eccentric

exercises (Brenner et al. 1999). Plasma IL-6 concentration was elevated immediately, following a 45min downhill run, with a peak 1h after the exercise session, but the IL-6 concentration returned to pre-exercise concentrations 24h following exercise (Peake et al. 2005a), suggesting that IL-6 may not be involved in the development of pain during DOMS. Another study examined the cytokine response following a bout of eccentric exercises, where the subjects completed four sets of 12 repetitions of bench press and 12 repetitions of leg curls at 100% of their one repetition maximum, and found an increase in serum IL-6 concentration from 12h to 72h, after the exercise session (Smith et al. 2000). Therefore, it seems that plasma IL-6 concentration is not a good indicator, nor a likely contributor of DOMS since the temporal pattern is different to that of the pain experienced.

It may be, however, that muscle tissue IL-6 concentration is a better indicator of injury and that IL-6 in muscle may contribute towards the pain. Interleukin-6 concentration in muscle tissue usually is examined in endurance exercise studies and not in eccentric exercise studies. In muscle, IL-6 is involved in glucose metabolism, where it regulates the supply of carbohydrates to muscles (Shephard 2002). Since the concentration of IL-6 in muscle is elevated more during concentric muscle contractions compared to eccentric muscle contractions, IL-6 is more likely involved in muscle metabolism than in muscle pain (Shephard 2002, Steensberg et al. 2002). Therefore, perhaps another pro-inflammatory cytokine, such as IL-1β, is involved in muscle pain.

*Interleukin-1β*

An increase in the concentration of IL-1β was detected in muscle tissue, immediately after completing a downhill run, with a peak in IL-1β concentration occurring five days after the exercise (Fielding et al. 1993). However, following a downhill run or a bout of eccentric muscle contractions, no change in plasma IL-1β concentration was observed (Malm et al. 2004, Nosaka and Clarkson 1996). Interleukin-1β may be involved in the generation of pain during DOMS, since it has been detected in muscle tissue damaged during DOMS. Further research should determine whether IL-1β is involved in the development of DOMS.

*Tumour necrosis factor-alpha*

Many studies have examined the effects of exercise, both endurance and eccentric, on plasma TNF-α concentration (Brenner et al. 1999, Cannon et al. 1991, Nosaka and Clarkson 1996). No change in plasma TNF-α concentration, in response to a bout of eccentric contractions has been observed (Nosaka and Clarkson 1996, Smith et al. 2000), while another study noted an increase in plasma TNF-α concentration 24 to 48h following a 45 minute downhill run (Cannon et al. 1991). A decrease in plasma TNF-α concentration was observed, 24 to 72h following eccentric exercise of the elbow flexors (Hirose et al. 2004).

Many studies have examined the effect of endurance exercise on concentrations of TNF-α and have found TNF-α to increase following a long bout of exercise, such as

a marathon race (Nieman et al. 2001, Ostrowski et al. 1999, Starkie et al. 2001), a 5km run (Espersen et al. 1990) and 3 hours of cycling and walking (Moldoveanu et al. 2000). However, other studies found no change in plasma TNF-α concentration following endurance exercises (Suzuki et al. 2003, Suzuki et al. 1999, Suzuki et al. 2000). The amount of TNF-α, like other cytokines, in circulating blood following a bout of exercise seems to be closely related to the intensity and most importantly the duration of the exercise (Moldoveanu et al. 2001). Also, the method used to measure TNF-α concentration may play a role in whether the cytokine is detected or not. No studies have examined TNF-α concentrations in muscle tissue damaged by exercise and so in order to fully understand the role of TNF-α in DOMS, more research should be conducted. However, the role of TNF-α during other muscle injuries has been investigated.

Tumour necrosis factor-alpha has effects on skeletal muscle metabolism, where it has been shown to cause protein lysis and catabolism (Charters and Grimble 1989, Garcia-Martinez et al. 1993, Zamir et al. 1992). The protein breakdown in skeletal muscle, increased by 28% in rats administered with TNF-α (Zamir et al. 1992). In a similar study, where TNF-α was injected into rats, muscle protein breakdown was accelerated and therefore TNF-α is involved in skeletal muscle lysis and catabolism.

*Interleukin-8*

Interleukin-8 concentration has been shown to increase, in skeletal muscle tissue, following exercise and therefore may be involved in the development of muscle pain. Plasma concentrations of IL-8 were elevated following downhill running (Peake et al. 2005a, Simpson et al. 2005). Concentric exercise causes muscle IL-8 concentration to increase 3-6h after exercise (Akerstrom et al. 2005). The exact role of IL-8 in the development of DOMS has not been fully elucidated and research should focus on the role of IL-8 and other pro-inflammatory cytokines in the development of muscle pain and inflammation.

### 1.5.5 Summary

Eccentric exercise may result in a different inflammatory response to that seen during inflammation due to infection or trauma, since small changes in plasma cytokine concentrations are observed following eccentric exercise (Nosaka and Clarkson 1996). Some studies find cytokine concentrations increase while in others, the cytokine concentrations remain unchanged and the possible reasons for this include: the type of DOMS-inducing exercise and the muscles affected by the exercise, where the cytokine is measured and the technique used to measure the cytokine. Studies use different exercises to induce DOMS, where some exercise protocols may induce pain at a much greater intensity than other exercise protocols. The different exercise protocols may then influence cytokine concentrations. Also,

the technique used to measure the concentration of the cytokines may differ between studies. In humans, exercise has been shown to exhibit an acute phase response which is similar to that induced by trauma and infection, however exercise-induced inflammation is of a smaller magnitude (Pyne 1994) and also has been shown to induce hyperalgesia in the muscle affected by the exercise. Most studies do not observe cytokine changes within the damaged muscle tissue and so little information regarding cytokine concentrations following eccentric or unaccustomed exercise in muscle tissue is available. In order to fully understand the involvement of cytokines in muscle pain, future research should focus on cytokine concentrations within damaged muscle. Research has been conducted on TNF-α in clinical forms of musculoskeletal pain (Petrovic-Rackov and Pejnovic 2005, Watkins et al. 1995a), however, the exact role of TNF-α in exercise-induced muscle pain still needs to be established.

## 1.6 Aims

1. To identify whether inhibiting the action of TNF-α attenuates DOMS.
2. To investigate whether blocking $\beta_1$-receptors, using atenolol, results in a reduction in the pain felt during DOMS.

# Chapter Two

## Materials and Methods

## 2.1 Subjects

Thirteen healthy male subjects, aged 24 ± 3 years (mean ± SD), height 1.78 ± 0.06 m and mass 72.5 ± 7.5 kg, participated in the study. Subjects were asked to refrain from exercise, massage, electrotherapy modalities, hot packs, caffeine and alcohol ingestion, four days before and for the duration of each trial. Any subject taking pain medication was excluded from the study. The experimental protocol was approved by the University of the Witwatersrand Committee for Research on Human Subjects (M050311) and all subjects gave written informed consent for participation.

## 2.2 Experimental procedures

Subjects reported to **my** exercise laboratory on three separate occasions. Each of the trials lasted four days and subjects completed each day of the three trials at the same time of the day. The time between the first and second trial was 7.6 ± 2.2 weeks (range, 6-14 weeks) and between the second and third trial was 9.8 ± 2.1 weeks (range, 8-15 weeks). In a random order and under double-blind conditions, subjects were assigned to one of three treatments, where by the end of the study, subjects had received all three treatments. Treatment one received the TNF-α inhibitor injection and placebo capsules, treatment two received atenolol capsules and a sterile water injection and treatment three, a sterile water injection and placebo capsules. The TNF-α inhibitor, etanercept (25mg, Enbrel, Wyeth, South Africa), was administered once as a 1ml subcutaneous injection, one hour before the exercise session. The time to peak concentration following the administration of 25mg etanercept, in serum is

69 ± 34h and etanercept has a half-life of 102 ± 30h (Goffe and Cather 2003). The corresponding placebo was a 1ml sterile water injection given one hour before exercise. The beta-adrenergic inhibitor, atenolol (Pharmacare Limited, South Africa) or placebo, was administered once daily, 25mg per mouth, starting one hour before the exercise session and thereafter one capsule per day for the subsequent three experimental days. Atenolol has a half-life of 6-8h. The corresponding placebo was a sugar-filled capsule, in identical gel capsules. The dose of atenolol (25mg per day for four days) is the lowest prescribed dose so that heart rate is not lowered excessively (Philipp et al. 1997) and etanercept (25mg once only) is the recommended dose given by the manufacturers for the treatment of rheumatoid arthritis (Moreland et al. 2006).

## 2.3 Exercise session

On the first day of each trial, each subject used a 45° incline leg-press machine (Cardio Genesis Fitness Systems, South Africa) to determine their lower limb one repetition maximum (1RM). The 1RM is the amount of weight (kg) that a subject is able to leg-press once only, and is an indication of the muscle strength of the hamstrings, quadriceps and gluteal muscle groups. In order to induce lower limb muscle damage, subjects completed four sets of 15 repetitions at 80% of their 1RM, using the leg-press machine, or until voluntary muscle fatigue. This unaccustomed exercise has a component of both concentric and eccentric muscle contraction, particularly in the quadriceps muscle group. Each subject's leg-press 1RM was

retested 24h, 48h and 72h after the initial exercise session and was expressed as a percentage of the 1RM measured before the exercise session.

## 2.4 Blood analysis

Immediately before the exercise session and 24h, 48h and 72h after the exercise, 5ml of blood from the brachial vein was collected by venous puncture and placed into EDTA collecting tubes. Blood was centrifuged at 2000g at 4°C for 10 minutes, the plasma removed and stored at -80°C until analysis. Plasma creatine kinase (CK) concentration was measured using a commercially available calorimetric assay kit (Roche Diagnostics, South Africa).

## 2.5 Pain measurements

Immediately before the exercise session, and 24h, 48h and 72h after exercise, I applied a pressure algometer (Somedic, AB, Sweden) with a $1cm^2$ probe to three points on the thigh: midway between the hip and the knee on the quadriceps femoris muscle, 5cm superior to the superior border of the patella, midway between the medial femoral epicondyle and the patella, and on the same point laterally. I applied the algometer at each point, at an increasing pressure, until the subject indicated the pressure stimulus was painful. Two measurements taken 30sec apart were done on each site and pressure pain threshold (PPT, kPa) was recorded as a mean of all six measurements. The PPT measured 24h, 48h and 72h after the exercise session was then expressed as a percentage of the pre-exercise PPT values.

In order to quantify the intensity of quadriceps muscle pain, after performing a simple squat (body weight only), a 100mm visual analogue scale (VAS) anchored with “no pain” on the left and “worst pain ever experienced” on the right was used. Each subject marked off on the VAS line the intensity of pain they felt after the squat. This procedure was done before, and then 24h, 48h and 72h after the exercise session. In addition, the subjects were asked to complete a McGill Pain Questionnaire (MPQ), after performing a squat, to measure the quality of the pain experienced. The MPQ was completed 24h and 48h after the exercise session. The subjects were asked to select which word or words best described the pain they were experiencing, and a numerical value, the pain rating index (PRI) was determined (Melzack 1975).

## 2.6 Muscle tissue

Muscle biopsies from the vastus lateralis muscle were obtained from four of the 13 subjects. The muscle biopsies were performed immediately before and 2h and 24h after the exercise session. Biopsies before the DOMS-inducing exercise were only performed at the first trial. In figure 4, the TNF-α concentration at time zero is therefore represented as one group and not as three separate groups. The muscle biopsies were not performed in the same site of the vastus lateralis muscle, at the different times in each trial. The biopsies were performed within a 2cm radius of each other. A local anaesthetic (0.5%, Macaine, Adcock Ingram Limited, South Africa) was injected into the dermis and subcutaneous tissue over the left vastus

lateralis muscle, 10min before the muscle tissue was excised. A 2cm incision was made through the skin and underlying fascia using a surgical blade. A biopsy needle (Stille Surgical AB, Sweden) with a diameter of 6mm was inserted into the muscle tissue, suction was applied and 100mg of muscle tissue was obtained. The muscle tissue was weighed, fresh frozen in liquid nitrogen and stored at -80°C for later analysis of cytokine concentrations.

### 2.6.1 Cytokine concentration

Each muscle tissue section was placed in a glass tissue homogenizer, together with 250µl of cell lysing solution (Bio-Plex cell lysis kit, Bio-Rad Laboratories, CA, USA), ground and then frozen at -80°C. After thawing, samples were sonicated (Cole-Palmer Instrument Company, Chicago, USA) on ice for 3min. Samples were then centrifuged at 12000g for 4min at 4°C. The supernatant was collected, and the protein concentration determined using a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, CA, USA).

Concentration of TNF-α was measured using a bead array analysis (Bio-Rad Laboratories, CA, USA). The pre-mixed beads, coated with TNF-α antibodies (50µl) were added to each well. After washing with wash dilution buffer, 50µl of standard or undiluted sample was added to the wells, with each sample measured in triplicate. The plate was incubated in the dark for 60min at room temperature. After washing

the plate, TNF-α detection antibodies (25µl) were added to each well, and incubated in the dark for 30min at room temperature. Each well was again washed and 50µl of steptavidin-PE was added to each well and incubated in the dark for 10min at 2000g at room temperature. The beads were then resuspended in 125µl of Bio-Plex assay buffer and the beads within the plate were read using the Bio-Plex suspension array system.

## 2.7 Statistical analysis

Data are shown as mean ± standard deviation unless otherwise stated. Visual analogue scale data were normalised using the arcsine transform. Two-way repeated measures analysis of variance, with time and agent administered as the main effects, was used to compare one repetition maximum, pressure pain threshold (PPT), visual analogue scale (VAS) and pain rating index (PRI). Tukey post-hoc tests were done where significant differences were found. The McGill Pain Questionnaire was analysed using the Chi-squared analysis for significant associations between words chosen. Chi-squared analysis was used to detect significant associations between the words selected and the three treatments, at the different time intervals. The words chosen by 45% of the subjects from the MPQ, to describe their pain, were included in the analysis. I used a Spearmans correlation to correlate 1RM to VAS, PPT to VAS and PRI to VAS and a Pearsons correlation to correlate 1RM to PPT, 1RM to PRI and PPT to PRI. The correlations were performed at 24h and 48h for each of the three treatments. A Pearsons correlation also was performed to correlate the TNF-α

concentration in muscle to the TNF-α concentration in protein. I used the non-parametric Friedman test, with a Bonferroni correction, to analyse for differences in plasma creatine kinase concentrations. Statistical significance was set at $P < 0.05$.

# Chapter Three

## Results

## 3.1 Muscle strength

There was no significant change in pre-exercise 1RM, between the three trials ($P$ = 0.16). Figure 1 shows the percentage change in one repetition maximum (1RM) of the quadriceps muscle from pre-exercise values at 24h, 48h and 72h following an unaccustomed exercise session, in groups receiving placebo, atenolol or etanercept, with a mean 1RM before exercise of 317 ± 6kg. There was a significant time effect ($F_{3,96}$ = 23.31, $P$ < 0.0001), with 24h ($P$ < 0.001) and 48h ($P$ < 0.001) 1RM lower than 72h and pre-exercise. There was a significant agent effect ($F_{2,32}$ = 3.59, $P$ = 0.039) in 1RM, with 1RM after atenolol ($P$ < 0.001) and etanercept administration ($P$ < 0.001) significantly different to that after placebo administration, regardless of time. There was a significant interaction, where 72h after the placebo administration, 1RM was significantly lower compared to the muscle strength in the groups receiving etanercept ($P$ < 0.01) and atenolol ($P$ < 0.01).

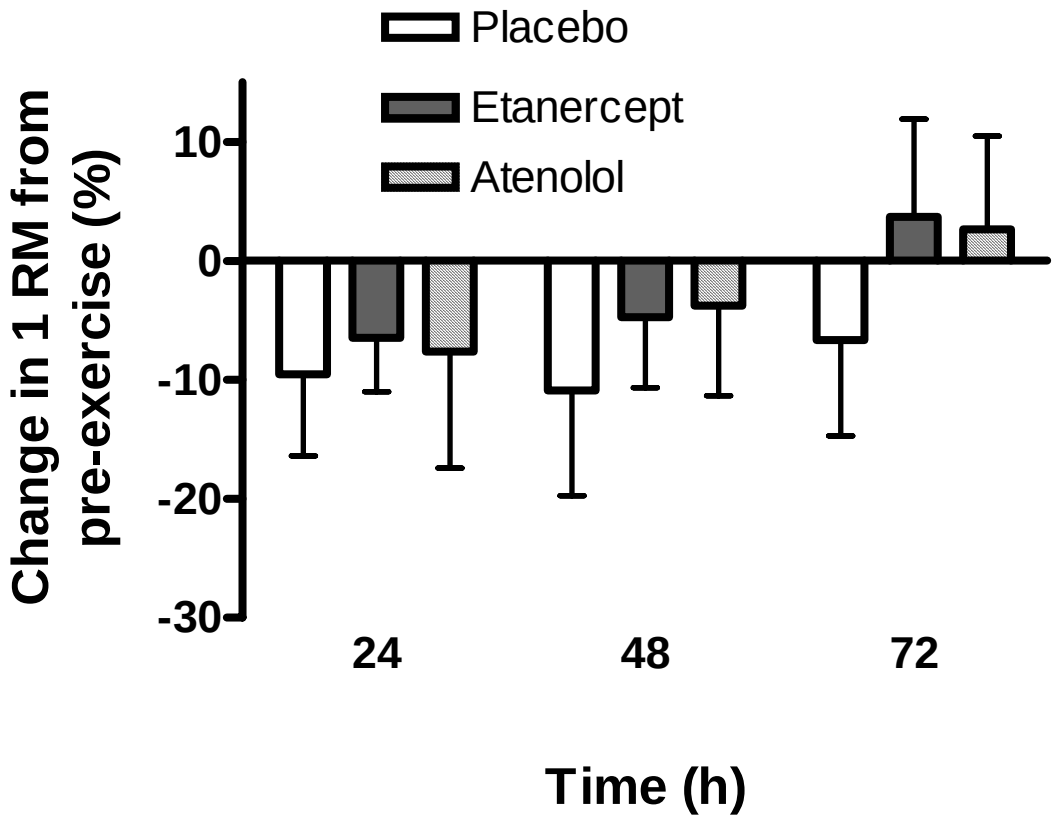



Figure 1. The percentage change (mean±SD) in one repetition maximum (1RM) at 24h, 48h and 72h following the DOMS-inducing exercise, after placebo, etanercept and atenolol administration, in 13 male subjects. The 1RM before the DOMS-inducing exercise was 317±6kg. A two-way repeated measures ANOVA revealed that muscle strength at 24h and 48h after exercise was significantly lower than pre-exercise and 72h after exercise ($P < 0.0001$). At 72h, the 1RM was significantly greater in groups receiving etanercept and atenolol compared to groups receiving placebo ($P < 0.05$).

## 3.2 Creatine kinase

There was no significant difference in the pre-exercise CK concentration, between the three trials ($P$ = 0.35). There was a significant difference in plasma creatine kinase concentration (Friedman statistic = 30.4, $P < 0.0001$) where the creatine kinase concentration at 24h ($P < 0.01$) was greater compared to the pre-exercise concentration. The creatine kinase concentration at 24h ($P < 0.001$) also was significantly greater compared to the concentration at 72h. Etanercept and atenolol had no effect on creatine kinase concentration, when compared to that after placebo administration. The mean creatine kinase concentration before exercise was 299 ± 144 $\mu mol.L^{-1}$, at 24h was 507 ± 97 $\mu mol.L^{-1}$, at 48h was 381 ± 58 $\mu mol.L^{-1}$ and at 72h was 341 ± 114 $\mu mol.L^{-1}$.

## 3.3 Pain measurements

### 3.3.1 Delayed-onset muscle soreness

The VAS score at 24h was 25mm (+21mm (+SD), +14mm,(-SD)) and at 48h was 35mm (+21mm, +18mm) indicating that the subjects were experiencing mild pain at 24h and moderate pain at 48h (O'Connor and Cook 1999). Figure 2 illustrates the visual analogue scale (VAS) and pain rating index (PRI) of the quadriceps muscle soreness after the exercise. Subjects reported significantly higher VAS scores at 24h, 48h and 72h compared to pre-exercise VAS scores ($F_{3,99}$ = 157.45, $P < 0.0001$). The VAS scores were significantly higher at 48h compared to that at 24h ($P < 0.009$) and

72h ($P < 0.009$), following the exercise, regardless of the agent administered. However, there were no significant differences in the PRI 24h or 48h following the exercise ($F_{1,33} = 0.13$, $P = 0.72$), although the PRI was greater than zero. Etanercept and atenolol did not significantly change the intensity of muscle pain, as seen by the VAS ($F_{2,33} = 0.35$, $P = 0.71$) and PRI ($F_{2,33} = 0.79$, $P = 0.46$), at any time, when compared to that after placebo administration.

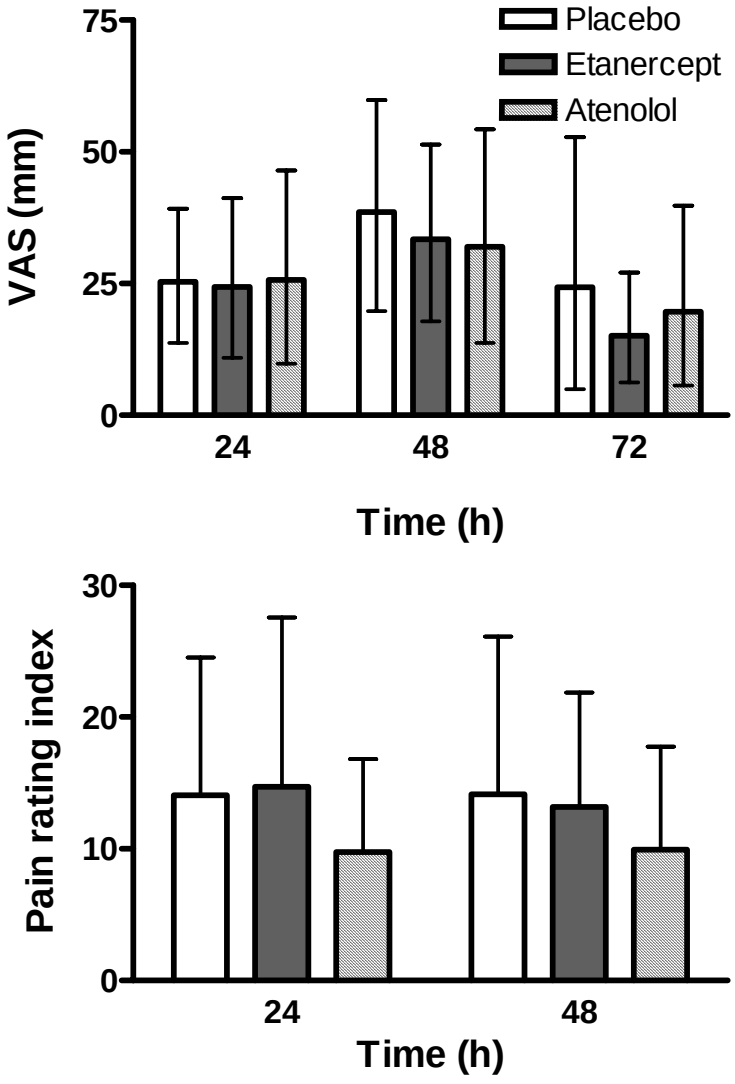



Figure 2. Pain intensity (mean±SD) of the muscle soreness in the quadriceps muscle, in 13 male subjects, measured on a 100mm visual analogue scale (VAS) 24h, 48h and 72h following exercise and on the pain rating index (PRI) 24h and 48h following exercise. The VAS data was normalized using the arcsine transform and both VAS and PRI were analysed using a two-way repeated measures ANOVA. The VAS scores were significantly higher ($P < 0.009$) at all three time intervals, compared to the VAS scores before exercise (0mm). The VAS was significantly higher 48h after the exercise compared to 24h ($P < 0.009$) and 72h ($P < 0.009$) after exercise, regardless of agent administered. There was no significant difference in the PRI at 24h and 48h, or between agents administered ($P > 0.05$).

### 3.3.2 Pressure pain threshold

There was no significant change in PPT before the DOMS-inducing exercise between the three trials ($P = 0.49$). Figure 3 shows the percentage change in pressure pain threshold (PPT) of the quadriceps muscle from pre-exercise, 24h, 48h and 72h after exercise, after placebo, etanercept and atenolol administration. The mean PPT of all three groups before exercise was 486 ± 29 kPa. The PPT, at all three time intervals, was significantly lower compared to pre-exercise PPT values ($F_{3,99} = 33.99$, $P < 0.0001$). Also, PPT was significantly lower 24h ($P < 0.003$) and 48h ($P < 0.001$) after exercise compared to that at 72h after exercise. There was no significant effect of etanercept or atenolol on PPT, at any time, compared to that after placebo administration ($F_{2,33} = 0.84$, $P = 0.44$).

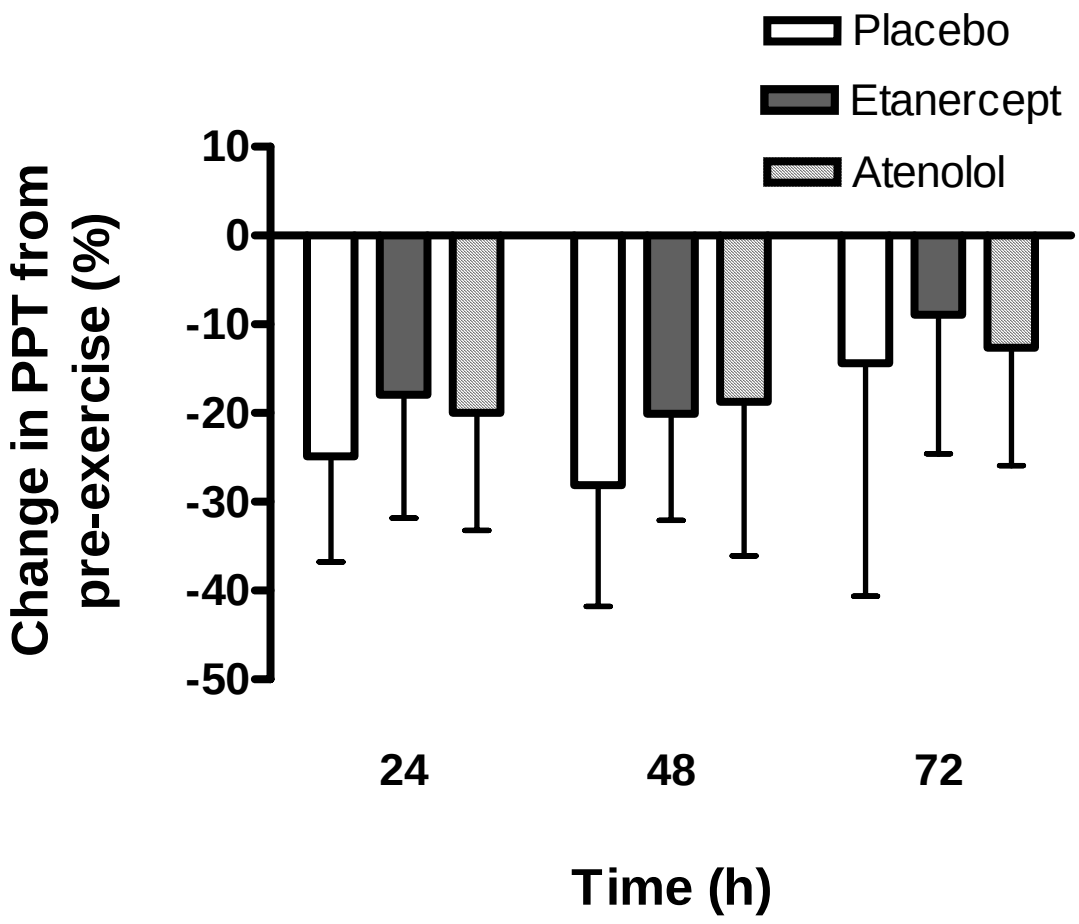



Figure 3. Percentage change (mean±SD) in pressure pain threshold (PPT), in 13 male subjects, calculated as the average pressure pain threshold following the application of the pressure algometer to the vastus medialis, vastus lateralis and rectus femoris muscles, 24h, 48h and 72h after exercise, in groups receiving placebo, etanercept and atenolol. The algometer was applied twice at each point and the mean of all six measurements was recorded as PPT. The PPT before the DOMS-inducing exercise was 486±29kPa. A Two-way repeated measures ANOVA revealed that the PPT, at all time intervals, was significantly lower compared to PPT before exercise ($P < 0.0001$) and PPT was significantly lower 24h ($P < 0.003$) and 48h ($P < 0.001$), following exercise, compared to 72h after exercise.

### 3.3.3 Words chosen from the McGill Pain Questionnaire

Administration of etanercept and atenolol did not significantly affect the words frequently chosen by the subjects to describe the muscle pain ($\chi^2 = 2.54$, $P = 0.86$), 24h or 48h after exercise. However there was a significant association between the words used to describe the pain 24h after exercise compared to the words used 48h after exercise ($\chi^2 = 26.37$, $P < 0.0001$). Table 1 shows that the muscle pain associated with DOMS was most commonly described as "tender", "tight" and "annoying" at 24h while at 48h was described as "tender", "tight" and "sore".

## 3.4 Cytokine concentrations

Cytokine concentrations are reported as concentration of TNF-α per wet weight of muscle ($pg.mg^{-1}$) and per protein concentration in the muscle ($pg.mg\ protein^{-1}$). The detection limit of the assay for TNF-α was $6pg.ml^{-1}$, or $0.17pg.mg^{-1}$ tissue and 0.33 $pg.mg^{-1}$ protein. The muscle TNF-α concentration before exercise or drug administration was $0.72 \pm 0.58pg.mg\ tissue^{-1}$ and $1.34 \pm 1.36pg.mg\ protein^{-1}$. No TNF-α was detected in muscle 2h after exercise in the subjects receiving etanercept or 24h after atenolol administration. Figure 4 shows the muscle TNF-α concentration per wet weight of tissue and per protein content before exercise and 2h and 24h after administration of placebo, etanercept and atenolol.

Table 1 Words selected from the McGill Pain Questionnaire, by 45% or more of the subjects, 24h and 48h after the DOMS-inducing exercise and following placebo, atenolol and etanercept administration, to describe the pain experienced during DOMS

| Time | Words chosen | Placebo | Atenolol | Etanercept |
|---|---|---|---|---|
| **24h** | Tender | 69 | 46 | 46 |
| | Tight | 62 | 38 | 54 |
| | Annoying | 46 | 31 | 46 |
| | Sore | 31 | 31 | 23 |
| **48h** | Tender | 62 | 62 | 69 |
| | Tight | 46 | 62 | 69 |
| | Annoying | 15 | 31 | 23 |
| | Sore | 54 | 31 | 31 |

*A significant association was found between the words used to describe the pain 24h after the DOMS-inducing exercise compared to 48h after the exercise ($\chi^2 = 26.37$, $P < 0.0001$). Placebo, atenolol and etanercept did not significantly affect the words chosen to describe the exercise-induced pain ($\chi^2 = 2.54$, $P = 0.86$).*

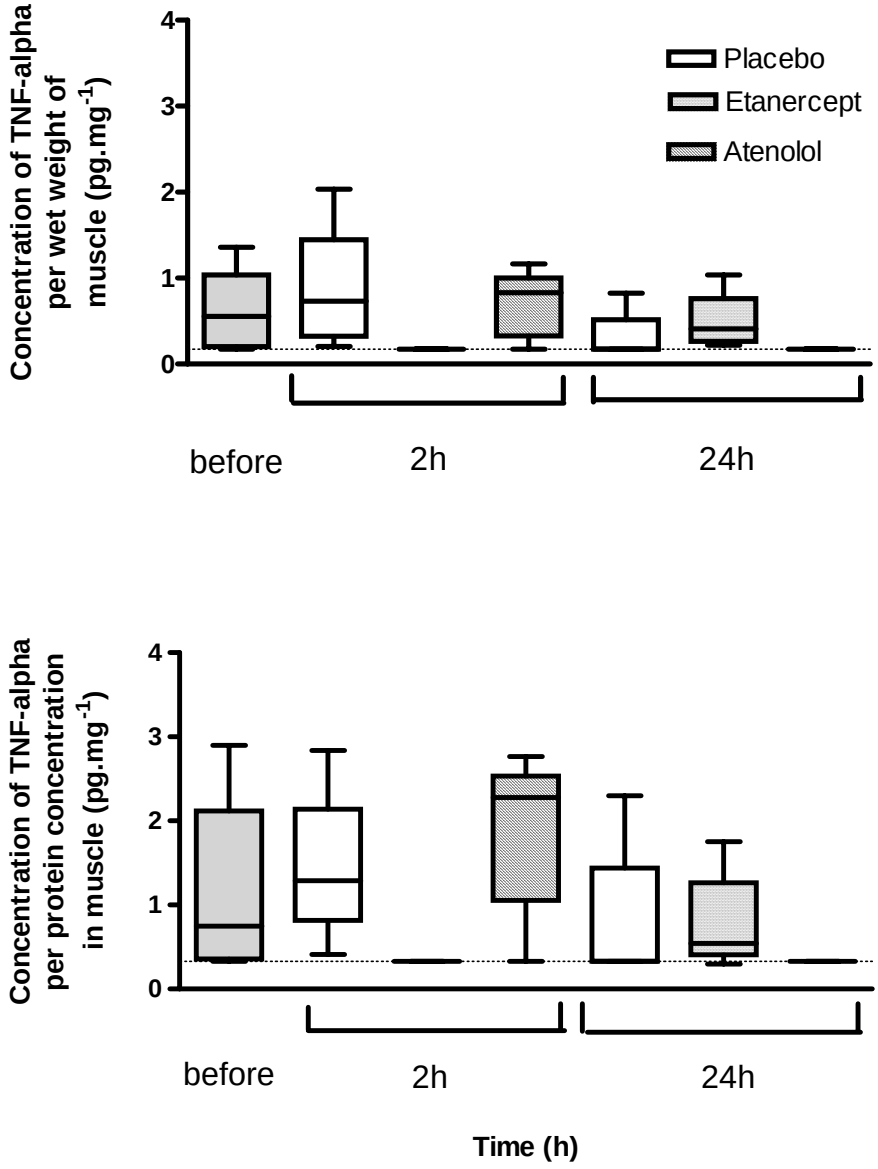



Figure 4. Concentration of TNF-α per wet weight of muscle ($pg.ml^{-1}$) and per protein concentration within the muscle ($pg.ml^{-1}$), from four subjects. The dashed line indicates the detection limit of the assay. The bars with a TNF-α concentration of zero represent a concentration which is below the detection limit of the assay. The concentration of TNF-α was measured before exercise and 2h and 24h after administration of placebo, etanercept and atenolol.

## 3.5 Correlations

There was no significant correlation between 1RM and VAS, 24h ($r^2$ = -0.11, $P$ = 0.73) and 48h ($r^2$ = -0.36, $P$ = 0.28) following placebo administration and 24h ($r^2$ = -0.52, $P$ = 0.08) and 48h ($r^2$ = -0.28, $P$ = 0.37) following etanercept administration. However, 1RM was significantly correlated to VAS, 24h ($r^2$ = -0.86, $P$ = 0.0004) and 48h ($r^2$ = -0.66, $P$ = 0.02), following the administration of atenolol. There was no significant correlation between PPT and VAS, 24h ($r^2$ = -0.33, $P$ = 0.29) and 48h ($r^2$ = -0.30, $P$ = 0.34) following placebo administration, 24h ($r^2$ = -0.17, $P$ = 0.60) and 48h ($r^2$ = -0.18, $P$ = 0.34) following etanercept administration, or 24h ($r^2$ = -0.36, $P$ = 0.26) and 48h ($r^2$ = -0.01, $P$ = 0.97) following atenolol administration. There was no significant correlation between PRI and VAS, 24h ($r^2$ = 0.24, $P$ = 0.45) and 48h ($r^2$ = 0.007, $P$ = 0.98) following placebo administration, 24h ($r^2$ = 0.19, $P$ = 0.56) and 48h ($r^2$ = 0.47, $P$ = 0.12) following etanercept administration, or 24h ($r^2$ = 0.24, $P$ = 0.44) and 48h ($r^2$ = 0.44, $P$ = 0.15) following atenolol administration. There was a significant correlation between PPT and 1RM, 48h ($r^2$ = 0.39, $P$ = 0.04) following placebo administration and at 24h ($r^2$ = 0.37, $P$ = 0.04) and 48h ($r^2$ = 0.41, $P$ = 0.03) following atenolol administration. There was no significant correlation between 1RM and PRI, 24h ($r^2$ = 0.12, $P$ = 0.26) and 48h ($r^2$ = 0.14, $P$ = 0.26) following placebo administration, 24h ($r^2$ = 0.0003, $P$ = 0.96) and 48h ($r^2$ = 0.05, $P$ = 0.49) following etanercept administration, or 24h ($r^2$ = 0.32, $P$ = 0.06) and 48h ($r^2$ = 0.08, $P$ = 0.36) following atenolol administration. There was no significant correlation between PPT and PRI, 24h ($r^2$ = 0.05, $P$ = 0.48) and 48h ($r^2$ = 0.006, $P$ = 0.80)

following placebo administration, 24h ($r^2 = 0.06$, $P = 0.43$) and 48h ($r^2 = 0.09$, $P = 0.34$) following etanercept administration, or 24h ($r^2 = 0.04$, $P = 0.53$) and 48h ($r^2 = 0.006$, $P = 0.81$) following atenolol administration.

# Chapter Four

# Discussion

I successfully induced DOMS in the subject's quadriceps muscle after a bout of unaccustomed exercise on a leg-press machine. The subject's muscle soreness was identified by an increased subjective pain score, as measured by the VAS and PRI and also an increased sensitivity to pressure applied to the thigh muscle (PPT). The subject's quadriceps muscle was significantly more sensitive to pressure 48h following a bout of unaccustomed exercise on the leg press machine, regardless of the agent administered. In my study, etanercept and atenolol, at the doses I used, did not attenuate the soreness associated with DOMS or the sensitivity to pressure as seen by the pressure pain threshold.

Not only did I induce muscle pain, but also a loss in muscle strength, of about 10%, was observed at both 24h and 48h following the unaccustomed exercise session, regardless of the agent administered, confirming that my subjects experienced DOMS. Therefore, both etanercept and atenolol, at 24h and 48h following exercise, did not significantly improve muscle strength, compared to that of placebo. However, 72h after the exercise session, the muscle strength in the subjects receiving either etanercept or atenolol showed a significant improvement, compared to those given the placebo. Both drugs resulted in the subjects' strength exceeding pre-exercise muscle strength, which may be related to the change in muscle TNF-α concentration.

Two hours after the exercise, the muscle TNF-α concentration was not different to that measured before the exercise. However, 2h after the exercise in the etanercept trial, the TNF-α concentration, in the muscle, was below the detection limit of the assay. My results show that the dose of etanercept was effective in decreasing TNF-α concentration in the muscle. However, regardless of the agent administered there was no significant increase in muscle TNF-α concentration following the DOMS-inducing exercise.

The two drugs, etanercept and atenolol, may not have attenuated the DOMS because their doses were too low to diminish the muscle pain generated by the unaccustomed exercise. However, etanercept is successfully used to pharmacologically treat rheumatoid arthritis, at the same dose used in our study (Flagg et al. 2005). Patients with rheumatoid arthritis rate their pain intensity at 59 mm on the VAS while my subjects rated the pain intensity of the DOMS lower and therefore less severe at 32 mm. Also, TNF-α concentration was lower in the muscle after administration of the TNF-α inhibitor than the TNF-α concentration before exercise, which suggests that the drug did inhibit TNF-α.

Also, I believe that my dose of atenolol was adequate, since subject's heart rate decreased from pre-drug levels of 71 ± 12bpm to 52 ± 11bpm after drug administration. Also, my subject's blood pressure dropped from 131/71 ± 6/9mmHg before administration to 114/57 ± 9/8mmHg after atenolol administration. Therefore

the lack of attenuation of the muscle soreness associated with DOMS was not likely attributed to the dose of either drug.

My study employed a cross-over design format, where subjects participated in all three trials. Cross-over design studies to investigate the mechanisms of DOMS may be obstructed by training adaptations if trials are done too close together. However, I found no changes in the subjects' pre-exercise values of any of the measurements between the three trials. My trials were separated by between 6-15 weeks which was sufficient time between trials (Byrnes et al. 1985), since no differences between subjects were observed at the start of each trial in 1RM or PPT.

A limitation of my study is that I did not measure the concentration of TNF-α 48h and 72h after the DOMS-inducing exercise, which is when most of the changes in muscle strength and muscle hyperalgesia occurred.

The features of DOMS I produced in my study were similar to other studies (Table 2), with an increased sensitivity to pressure of the muscles affected by the DOMS (Barlas et al. 2000, Nie et al. 2005b), a similar intensity of muscle pain, as measured on the VAS (Barlas et al. 2000, Bobbert et al. 1986, Byrnes et al. 1985) and a loss in muscle strength, as measured by the 1RM (Brown et al. 1996, MacIntyre et al. 1996). My study is the first study to show that TNF-α does not contribute to the muscle pain

during DOMS, or that β-adrenergic receptors are not involved in DOMS muscle soreness.

Studies have examined the effects of eccentric exercise on plasma TNF-α concentrations (MacIntyre et al. 2001, Malm et al. 2004, Moldoveanu et al. 2000, Peake et al. 2005b) and found conflicting results with some studies reporting no change in plasma TNF-α concentrations, while others found an increase in plasma TNF-α concentration, following eccentric muscle contractions (Brenner et al. 1999, Cannon et al. 1991, Nosaka and Clarkson 1996, Simpson et al. 2005, Smith et al. 2000). However, the studies targeted different muscle groups and used different exercise protocols to induce DOMS. No study, to my knowledge, has investigated TNF-α concentrations in skeletal muscle tissue following eccentric exercise, however, studies have shown that TNF-α is present in normal skeletal muscle cells (Reid and Li 2001) and is produced following muscle damage in an animal model, by either a freeze injury (Warren et al. 2002) or a crush injury (Collins and Grounds 2001). However, it is still not clear whether TNF-α is released in the muscle during DOMS or whether the TNF-α contributes towards the associated muscle hyperalgesia.

Table 2. Studies examining DOMS in various muscles and the percentage change in VAS and in muscle strength, 24h and 48h following DOMS-inducing exercise. The studies did not use drugs as an intervention.

| Author | Targeted muscle group | VAS | Muscle Strength |
|---|---|---|---|
| Barlas et al, 2000 | Elbow flexors | 24h - ↑ 20%<br>48h - ↑ 35% | -------- |
| Brown et al,1996 | Knee extensor muscles | 24h - ↑ 19%<br>48h - ↑32% | -------- |
| Byrnes et al, 1985 | Quadriceps | 24h - ↑ 20%<br>48h - ↑ 30% | -------- |
| Clarkson et al, 1988 | Forearm flexors | 24h - ↑ 55%<br>48h - ↑ 60% | 24h - ↓ 50%<br>48h - ↓ 60% |
| Nie et al, 2005 | Neck and shoulder muscles | 24h - ↑ 75%<br>48h - ↑ 50% | -------- |
| Gleeson et al, 1995 | Quadriceps | 24h - ↑ 35%<br>48h - ↑ 41% | -------- |
| Hirose et al, 2004 | Elbow flexors | 24h - ↑ 60%<br>48h - ↑ 70% | 24h - ↓ 90%<br>48h - ↓ 50% |

Tumour necrosis factor-alpha injected into the quadriceps muscle of rats causes primary mechanical hyperalgesia, but does not cause tissue damage (Schafers et al. 2003). Tumour necrosis factor-alpha is upregulated in other musculoskeletal diseases such as rheumatoid arthritis (Petrovic-Rackov and Pejnovic 2005) where it causes inflammation and hyperalgesia (Cunha et al. 1992, Junger and Sorkin 2000). Etanercept, the TNF-α inhibitor, successfully reduces the hyperalgesia in rheumatoid arthritis patients by blocking TNF-α receptors and therefore inhibiting the actions of TNF-α, reducing inflammation and hyperalgesia (Goffe and Cather 2003, Haraoui 2005). Since TNF-α is involved in clinical inflammatory musculoskeletal diseases, it may be that TNF-α is involved in DOMS. I propose that during DOMS, there is a degenerative phase (0-8h), where the muscle is damaged, followed by a regenerative phase (8-72h, or longer), resulting in the sensation of pain. During muscle damage, it is thought that TNF-α would be released as part of the inflammatory process. Blocking TNF-α following a freeze injury, only mildly affected the muscle damage process but did delay the regeneration process (Warren et al. 2002).

However, in my study I found no increase in muscle TNF-α concentration 2h or 24h after the exercise. Therefore, TNF-α does not appear to be released from muscle during DOMS or that TNF-α contributes to the soreness associated with DOMS, in the early stages of DOMS. Also, in my study, subjects receiving etanercept did not show an improvement in the muscle pain, as seen by the VAS and PRI scores, caused by DOMS. Also, when an inflammatory agent, carrageenan, is injected into

the muscle of rats no increase in TNF-α concentration is found during muscle inflammation (Loram et al. 2006). Therefore, when TNF-α is administered, it is able to induce muscle hyperalgesia, however it may not be released during mild muscle damage, but rather during extensive muscle damage, such as a freeze injury (Warren et al. 2002). Also, it may be that TNF-α is only released from muscle when muscle homeostasis has been extensively disturbed (Petersen and Pedersen 2005).

Studies have shown that TNF-α is involved in skeletal muscle fibre proteolysis, following injury. A toxin-induced necrosis in rat skeletal muscles, causes muscle TNF-α concentrations to increase immediately and for three days following the injury (Zador et al. 2001). Also, by the fourth day after muscle injury, TNF-α is not detected in the regenerating areas of the muscle (Zador et al. 2001). Tumour necrosis factor-alpha when injected into rats causes skeletal muscle breakdown and therefore muscle weakness (Garcia-Martinez et al. 1993, Goodman 1991, Zamir et al. 1992). Blocking TNF-α would therefore inhibit skeletal muscle breakdown and improve muscle strength. This may explain the improvement in muscle strength at 72h, seen in my study.

If the hyperalgesia associated with DOMS is not TNF-α and therefore cytokine mediated, it may be sympathetically-mediated. Beta-adrenoreceptors are involved in both pain and hyperalgesia, where $\beta_2$-receptors, stimulated by inflammatory mediators, such as bradykinin and nerve growth factor, sensitise nociceptors and

cause mechanical hyperalgesia (Janig and Levine 2005, Meyer et al. 2005). Very little is known regarding the involvement of $\beta_1$-adrenoreceptors in muscle pain and hyperalgesia. A study examining the effect of a $\beta_2$-adrenoreceptor agonist on hyperalgesia in the muscles of mastication and the neck and shoulder muscles did not find any change in pressure pain threshold (PPT) from pre-drug values (Reid et al. 1996). However, an injury must be present before sympathetically maintained pain can occur (Rivner 2001). The stimulus in the above study may not be strong enough to induce a sympathetic response. However, the hyperalgesia from DOMS observed in our subjects may be a large enough stimulus, observed by the decrease in PPT in my subjects, to exert sympathetically mediated pain, except the pain during DOMS, as seen by the VAS and PRI scores, was not attenuated in subjects receiving the $\beta_1$-adrenoreceptor antagonist, atenolol. Perhaps $\beta_1$-adrenoreceptors are involved in sympathetically maintained pain, where once stimulated, the $\beta_1$-receptors cause pain. However, no studies, to my knowledge, have been conducted to determine whether $\beta_1$-adrenoreceptors are involved in sympathetically maintained pain.

Since my subjects received a $\beta_1$-adrenoreceptor antagonist and $\beta_2$-adrenoreceptors are found in skeletal muscle, perhaps a $\beta_2$-adrenoreceptor antagonist may have a greater effect on the pain during DOMS. Also, type I muscle fibres have more β-adrenoreceptors compared to type II muscle fibres (Martin et al. 1989). Perhaps atenolol improves muscle function by decreasing blood flow to the skeletal muscles damaged by exercise. The lowered blood flow to the muscles would possibly lower

the concentration of inflammatory mediators moving into the muscle resulting in less damage to the muscle.

It is possible that sympathetic fibres may play a role in muscle pain via integration with muscle spindles and the associated large fibre afferents. Muscle spindles have some innervation by sympathetic fibres which causes a depression in muscle spindle activity (Rivner 2001). Muscle spindles regulate skeletal muscle length and since DOMS does not cause pain at rest, but during muscle contraction and passive stretch it may be that large fibre mechanoreceptors are involved in the generation of pain associated with DOMS (Weerakkody et al. 2003, Weerakkody et al. 2001).

The $\beta_1$-receptor antagonist, atenolol, did not attenuate the pain felt during DOMS, however research has shown that the sympathetic nervous system is involved in inflammatory hyperalgesia (Cunha et al. 1991, Safieh-Garabedian et al. 2002) and so perhaps the α-adrenoreceptor, rather than the β-adrenoreceptor, is involved in the generation of muscle pain, following eccentric exercise.

In conclusion, my study has shown that the pain during DOMS was not attenuated by inhibiting TNF-α or β-adrenergic receptors, suggesting that DOMS may not be an inflammatory mediated pain or a pain mediated by beta adrenergic receptors but that other mechanisms such as central sensitisation must be involved. **I** have shown, however, that the mechanism behind the strength impairment during DOMS is

independent of the hyperalgesia induced during DOMS, since both agents attenuated the muscle strength deficit but not the pain.

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